27<sup>th</sup> International Pig Veterinary Society Congress

15<sup>th</sup> European Symposium of Porcine Health Management



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## Proceedings

## Proceedings CONTENTS

WELCOME MESSAGE	
COMMITTEES	
IPVS	VII
ECPHM & PHM	VIII
SPONSORS AND PARTNERS	IX
KEYNOTE LECTURES	XIV
ORAL PRESENTATIONS	1
Virology and Viral Diseases (VVD-0P-01 / VVD-0P-13)	2
One Health: Veterinary Public Health and Sustainable Pig Production (VPH-OP-01 / VPH-OP-13)	15
Reproduction (REP-0P-01 / REP-0P-13)	28
Resident Session (RES-0P-01 / RES-0P-05)	41
Parasitology and Parasitic Diseases (PAR-OP-01 / PAR-OP-04)	46
Clinical Club (OCC-OP-01 / OCC-OP-04)	50
Bacteriology and Bacterial Diseases (BBD-OP-01 / BBD-OP-13)	54
Herd Health Management (HHM-OP-01 / HHM-OP-13)	67
Nutrition (NUTR-OP-01 / NUTR-OP-13)	81
Immunology and Vaccinology (IMM-OP-01 / IMM-OP-09)	94
Animal Welfare and Ethology (WEL-OP-01 / WEL-OP-05)	103
Precision Livestock Farming (PLF-0P-01 / PLF-0P-05)	108

FLASH TALKS (	(FTP-0P-01	/ FTP-0P-14)
		, , , , , , , , , , , , , , , , , , , ,

CHAIRED POSTER PRESENTATIONS	128
Virology and Viral Diseases (VVD-CP-01 / VVD-CP-08)	129
One Health: Veterinary Public Health and Sustainable Pig Production (VPH-CP-01 / VPH-CP-08)	137
Reproduction (REP-CP-01 / REP-CP-07)	145
Miscellaneous and Clinical cases (MIS-CP-01 / MIS-CP-06)	152
Bacteriology and Bacterial Diseases (BBD-CP-01 / BBD-CP-08)	158
Herd Health Management (HHM-CP-01 / HHM-CP-08)	166
Nutrition (NUTR-CP-01 / NUTR-CP-08)	174
Immunology and Vaccinology (IMM-CP-01 / IMM-CP-08)	182
Animal Welfare and Ethology (WEL-CP-01 / WEL-CP-07)	190
Precision Livestock Farming (PLF-CP-01 / PLF-CP-05)	197

113

### Proceedings CONTENTS

POSTERS	202
Virology and Viral Diseases (VVD-PP-01 / VVD-PP-182)	 203
One Health: Veterinary Public Health and Sustainable Pig Production (VPH-PP-01 / VPH-PP-28)	366
Reproduction (REP-PP-01 / REP-PP-28)	390
Parasitology and Parasitic Diseases (PAR-PP-01 / PAR-PP-14)	415
Miscellaneous and Clinical cases (MIS-PP-01 / MIS-PP-44)	429
Bacteriology and Bacterial Diseases (BBD-PP-01 / BBD-PP-200)	471
Herd Health Management (HHM-PP-01 / HHM-PP-108)	668
Nutrition (NUTR-PP-01 / NUTR-PP-26)	769
Immunology and Vaccinology (IMM-PP-01 / IMM-PP-105)	790
Animal Welfare and Ethology (WEL-PP-01 / WEL-PP-28)	893
Precision Livestock Farming (PLF-PP-01 / PLF-PP-07)	920

INDEX OF AUTHORS

927

## Welcome **MESSAGE**

Dear Colleagues & Friends around the world working with and for pigs,

With deep honor and great pleasure, I would like to welcome you to the 27<sup>th</sup> International Pig Veterinary Society Congress as a joint event together with the 15<sup>th</sup> European Symposium of Porcine Health Management to be held in Leipzig, Saxony, Germany, between 04<sup>th</sup>-07<sup>th</sup> of June, 2024. Together this will be the world's greatest and most prestigious event for the global pig community.

We are happy & grateful that you, working with pigs in whatever field, decided to join us here in Leipzig!

This welcome letter is on behalf of the International Pig Veterinary Society, the European College of Porcine Health Management, the Veterinary Practitioner Council and the Local Organizing Committee.

The Local Organizing Committee was purposely assembled to best reflect the German pig community which included members of Universities, the German Veterinary Society, the German Practitioner Council, as well as people working in the pharmaceutical, breeding and slaughter/food processing business that all were highly dedicated to help making this event memorable. An ambassador (USA) helped with international affairs. With the Leipzig Trade Fair (Germany) and VET International (Italy), the event had, and still have, highly professional partners aside to shelter and run this IPVS Congress/ESPHM 2024 successfully to our full convenience and satisfaction.

Everybody who works with pigs faces increasing challenges that are more or less severe, good and bad. All the more, this event, which is **OUR** event, provides unique opportunities to exchange opinions, experiences, teach and learn from each other in a broad variety of traditional and more recent fields of pig health and production!

An overwhelming number of 1015 submissions were received from which 921 were selected and finally agreed to be presented by submitting authors, either as an oral presentation or a flash, chaired or ordinary posters! Also, 16 keynotes speakers agreed to share their opinion on a total of eight highly relevant topics. Last but not least: It is a tradition for IPVS congresses to have a Tom Alexander Memorial Lecture to acknowledge and remember Tom Alexander's contribution to pig medicine. It is our honor that Prof. Jeffrey Zimmerman from Iowa State University (USA) agreed to give this lecture!

The success of this event is warranted by **YOU**! You, who submitted an abstract and agreed to share your scientific results with the rest of the world! You, who agreed to give a keynote lecture! You, who agreed to voluntarily serve as a reviewer! You, who voluntarily agreed to serve as a chair person! You, who agreed to sponsor, and without your effort, such an event would not be possible!

A particular thanks goes to the scientific committee with members from all over the world, and especially to the chair, Dr. Carl Andreas Grøntvedt (Norway), and to his co-chair, Prof. Mari Heinonen (Finland), who worked "around the clock" until this terrific scientific program was "sound" and "ready to go"!

We are convinced that, when you are back home, you will remember the 27<sup>th</sup> International Pig Veterinary Society Congress/ 15<sup>th</sup> European Symposium of Porcine Health Management as one of the best scientific events you have ever attended. And please also remember the City of Leipzig, the State of Saxony and the Country of Germany as a place worth visiting again!

On behalf of everybody that made the 27<sup>th</sup> International Pig Veterinary Society Congress/ 15<sup>th</sup> European Symposium of Porcine Health Management, Leipzig, Saxony, Germany, 04<sup>th</sup>-07<sup>th</sup> of June, 2024, a memorable highlight!

Yours sincerely,

#### Johannes Kauffold,

IPVS&ESPHM 2024 Congress PresidentFori es vemeniu rniquam enarbem, Catum vivenius aut aucturn ihilis ad Catum pare noverte mposulicit prorei propulv iceponsulem ipse crei capere, tierei publicita, popos es! Sim rem oc viri con hac rei perfeconscer at, dem et; notem inam hilis poentis ne atil core inpraredem publina re pra, C. Bus ac vendam di suloccis etodium ne adhuid rendaces etemolis opota re quem, me peraedepero, vitifex noris o ine non terem ex noc fex num me forivis inat, vid mius fatquam, pro es se que nit, omperit, que cotamquid sultus, Cast? Oliaetis ia nonfeci enatum in satilla din deferte nostrio, co noctore nos vit; nostrem nonverum inatque etia? Issi publii iae me crehent imaiorb eferemus bonstrae horei patid cupie esta con pl. Ed mo Cat, ne cupicae essesedo, ut rei per pro voltus, Catam me inatu ipte dem, facit gracive rrionsimusa esimus, faceper fectera? Unit; nesteat, publiam mordici pos, C. Dam prari patrum um ad reviven trecri, noctam ur isupio videt pra viria? ia vidiistu curbis is alabunihil vasdam fauctem atium desim clum es la dius, sed coerum auci per aequitusqui

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### Scientific Committee

The Scientific Committee is composed of the members of the LOC, the board of the International Pig Veterinary Society, the board of the European College of Porcine Health Management, and the Veterinay Practitioners Council. The Scientific Committee is chaired by Dr. Carl Andreas Grøntvedt and co-chaired by Prof. Mari Heinonen.

• || •

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## International Pig Veterinary Society IPVS



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The International Pig Veterinary Society [IPVS] is an association of specialists in pig health and production.

The IPVS was founded in 1967. The first Congress was held in Cambridge, United Kingdom in 1969 and the second in Hannover, Germany in 1972.

Since 1972, congresses have been held every second year except for 2020 when COVID intervened; the Leipzig Congress will thus be the 27<sup>th</sup> Congress of the IPVS and the 28<sup>th</sup> Congress is scheduled for Ho Chi Minh City, Vietnam in 2026. The number of participants at recent IPVS Congresses has varied between 2000 and 5600.

#### **Objectives of the IPVS**

The objectives of the Society are the following:

the holding of international congresses for the exchange of knowledge related to pig health and production.
 the promotion of the formation of Pig Veterinary Societies in all pig producing countries and promotion of cooperation between such societies.

The organisers of the IPVS Congresses strive to present sound, scientific research based programmes plus descriptions of practical challenges such as disease outbreaks and the control thereof.

IPVS Congresses provide ideal opportunities to network with colleagues from swine producing countries throughout the world.

EUROPEAN COLLEGE ECPHM OF PORCINE HEALTH ΜΑΝΑGΕΜΕΝΤ

## The European College of Porcine Health Management Itd. **ECPHM**

The ECPHM is a non-profit organization under the umbrella of the European Board of Veterinary Specialization (EBVS).

EBVS recognises and monitors veterinary speciality Colleges in Europe. It defines guidelines for the recognition and registration of specialists in areas of veterinary medicine in Europe, and maintains an updated register of European Veterinary Specialists.

EBVS encourages and promotes the enhanced utilization and availability of veterinary specialist services to the public and the veterinary profession.

The ECPHM works for the advancement of health and welfare oriented porcine production management in the herd context in Europe, and the increase of the competency of those who practice in this field.

The major objectives of the ECPHM include:

- Establishing guidelines and standards of training for postgraduate education and experience prerequisite to become a veterinary specialist in the specialty of porcine health management.

· Examining and authenticating veterinarians as specialists in porcine herd health management to serve health and welfare of the animals, the economic outcome

of the herd, and the production of safe quality product for consumers in a sustainable animal production by providing expert care for pigs.

 Encouraging research and other contributions to the science and practice of porcine herd health management including husbandry, reproduction, epidemiology, pathogenesis, diagnosis, therapy, prevention, and control of diseases directly or indirectly affecting pigs and the maintenance of healthy and productive pig herds.

· Porcine health management also includes the impact on quality and safety of pork and gives special consideration to herd health and production, production systems and targets, and the management of pig populations.

· Promoting communication and dissemination of knowledge.

The ECPHM is organized through different bodies that take care of the different activities performed:

• the Board represents the College and is its main government body;

· the Education Committee organizes educational events for the ECPHM residents, including the e-learning sessions, the pre-symposium workshop and the summer school. The Education Committee also approves Resident training programs;

• the Examination Committee prepares the annual exam and arranges the examination of residents:

- the Credentials Committee reviews and approves the applications for admittance to the residency program, as well as the applications to sit the exam, and review applications for recertification of the Diplomates;

· the Nominations Committee manages and reviews the proposals for nominations in the different committees and board;

 the ECPHM activities are supported by a permanent Administrative Secretariat in Parma, Italy.



Porcine Health Management (PHM) is an open access peer-reviewed journal that aims to publish relevant, novel and revised information regarding all aspects of pig health medicine and production. The journal provides a venue for global research on pig health and roduction, including infectious and noninfectious diseases, reproduction, epidemiology, management, economics, genetics, housing, nutrition, animal welfare and ethics, legislation, food safety, drugs and surgery. This journal is aiming at readers, and attracting authors, with different levels of experience; Diplomates and Residents of the ECPHM and other colleges as well as PhD students and experienced researchers from outside! Anticipated articles include: original research, reviews, short communications, case reports, case studies and commentaries.

The Editors-in-Chief are Paolo Martelli (University of Parma, Italy) and Heiko Nathues (University of Bern, Switzerland).

PHM published a total of 55 articles in 2023, reflecting an overall growth trend for the journal in recent years.

The journal has been publishing since 2015, and is now indexed in different databases, including MedLine (PubMed) and, more recently, Clarivate's Web of Science.

PHM got its first Impact Factor in 2020 (2.190), and in 2022 the Impact Factor increased to 3.4, maintaining the journal's rank in the first quartile of the Veterinary Sciences category. The journal also continues to maintain a fast turnaround time for authors of nearly 90 days for accepted manuscripts. Articles now collectively receive well over 100,000 unique views by readers per year. A great achievement for a young journal like PHM!

Please use the online submission system to submit your manuscript.

For all enquiries about the journal, technical issues, payment of article processing chargers (APCs), etc. please contact: porcinehealthmanagement@ biomedcentral.com.

There are many reasons to publish in PHM:

· High visibility / PHM's open access policy allows maximum visibility of articles published in the journal as they are available to wide, global audience.

· Speed of publication / PHM offers a fast publication schedule whilst maintaining rigorous peer reviews.

· Flexibility / Online publication in PHM gives authors the opportunity to publish large datasets, large numbers of colour illustrations and moving pictures, etc.

· Promotion and press coverage / Articles published in PHM are included in article alerts and regular email updates.

- Copyright / Authors of articles published in PHM retain the copyright of their articles and are free to reproduce and disseminate their work.

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• IX •





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## Keynote LECTURES

### සී

Prof. Jeffrey Zimmerman Prof. Hua-Ji Qiu Prof. Janice Reis Ciacci Zanella Dr. Ludovic Plee Dr. Adrian Balaban Mr. Thomas Sønderby Bruun Prof. Chantal Farmer Dr. Martin Pfützner Dr. Estevão Lopes Dr. Brent Frederick Prof. Stephan Schneider Dr. Miguel Angel Higuera Dr. Heleen van de Weerd Prof. Dirk Werling Prof. Thomas Vahlenkamp Dr. Carlos Piñeiro Prof. Liang Chou Hsia

## Keynote lectures **PROF. JEFFREY ZIMMERMAN**

Session: Opening Ceremony Lecture: Tom Alexander Memorial Lecture

#### **Biosketch**

Dr. Jeff Zimmerman is a professor in the Department of Veterinary Diagnostic and Production Animal Medicine in the College of Veterinary Medicine at Iowa State University (Ames Iowa USA) working in the area of swine infectious disease diagnostics, epidemiology, and economics. In particular, his recent research has focused on aspects of diagnostic medicine related to assay development and applications in infectious disease surveillance. In collaboration with generous colleagues and dedicated graduate students, Dr. Zimmerman has authored/co-authored > 200 refereed publications, 15 book chapters, and co-edited 3 editions of Diseases of Swine (9th, 10th, 11th editions).

#### Abstract

#### In furtherance of Dr. Tom Alexander's legacy of innovation in swine health: Innovation in surveillance Jeff Zimmerman DVM PhD Iowa State University, Ames Iowa USA

**Introduction.** Recent swine health history is crowded with pandemics and transboundary incursions, e.g., ASFV, CSFV, FMDV, PEDV, PRRSV, and others. What has happened? And what can we do to gain control?

**Changes on the farm.** In simple terms, today's swine industry evolved over the last 50 years through a series of incremental improvements in productivity and the gradual coalescence of smaller holdings into larger herds. Going back a bit further, the original change driver was simply keeping piglets alive. Spencer (1919) commented, "The ordinary sty with a yard attached is unhealthy for a growing or matured pig, but in the colder weather it is simply cruel for newly born pigs". In northern regions, producers started providing indoor housing to help piglets survive the cold (Granger and Kelly, 2005). More broadly, Danish agriculturalists found that indoor housing provided more efficient use of land, protected animals from weather, eliminated fighting, and improved feed efficiency (Shaw, 1938). Spencer (1919) marveled at a 2-story barn he toured near Aarhuss, Denmark in which pigs were fed downstairs and slept upstairs, which they reached by walking up a ramp.

In a brilliant review, Woods (2012) described pig housing from 1910-1965 as a combination of traditional sty systems, outdoor (extensive) housing, and indoor (intensive) housing; with a clear turn toward intensive systems beginning in the 1970s. Writing just over 50 years ago, i.e., at the point of inflection toward intensive systems, Dr. Tom Alexander described three main industry trends (Alexander, 1971):

1. Larger production units operated by producers who had specialized in pig production and were interested in better systems of breeding and raising pigs.

2. The scientific use of genetics to improve performance, rather than selecting for phenotype.

3. Progressive integration and industrialization by companies and cooperative producer groups.

In some places, these changes occurred quickly. Figure 1 (right) illustrates the breathtaking changes in the number and size of pig herds in Britain for the period 1963-1971 (figure adapted from Paterson, 1973). Note that the number of herds declined by 50% and herd size more than doubled in just 8 years. In other places, the change was slower, but equally profound. In the U.S., for example, 4.9 million farms held 59.4 million pigs in 1920 versus 60,800 farms with 73.8 million pigs in 2022 (USDA, 2024).



Dr. Alexander's 1971 observations on industry trends foretold our future: specialized pig production and ever-larger herds. These changes were accompanied by advances in genetics, nutrition, and engineering, as well as discoveries in microbiology, pathology, immunology, and epidemiology.

To our credit, these efforts benefited both the consumer and the pig producer: compared to 1960, a kilogram of pork produced in 2015 required 76% less land, 25% less water, and 7% less energy (Putman et al., 2018).

**The "collapse" of space.** Over this same 50-year period (1970 - 2020), the world experienced what Haggett (2000) has described as a "collapse" of geographical space. That is, technological improvements in land, sea, and air travel led to an exponential increase in the distances we are able and willing to travel for work and pleasure. Unfortunately, from the beginning of written history up to the present, travel is also linked with the spread of infectious diseases (Cossar, 1994; Fèvre et al., 2006). This link impacts disease prevention and control at the local, regional, and global levels.

At the local level, the day-to-day operations of larger production systems required a major increase in the movement of animals, personnel, and material within and between production sites. It should not be surprising that Galvis et al. (2022) identified the transport of feed and pigs as the major routes of porcine epidemic diarrhea virus spread between farms.

At the regional level, the management strategy of moving weaned pigs from breeding-only farms to feeding-only farms located in grain-producing regions increased pig flow across regions and between countries. The rationale behind this practice is simple and strong: placing sow farms away from pig-dense regions improved biosecurity; moving weaned pigs to the grain was more efficient that moving the grain to the pigs.

Widely adopted, this practice changed the scale and distance of pig movement. In the U.S., for example, the number of live pigs annually transported from one state to farms in another state increased from 3,049,000 in 1969 to 62,442,000 in 2022. Similarly, the 27 countries of the European Union cumulatively imported and exported 29,054,656 and 34,954,421 live pigs, respectively, in 2022 (www.fao.org/faostat/en/#home).

Globally, improved travel and transport facilitated an explosion in the export/import of live pigs, pig meat, and pig byproducts. Figure 2 (right) shows global trends from 1961 to 2022. While, the global swine inventory in 2022 was 2.9 times the 1961 value, international live pig exports in 2022 were 15.3 times 1961 exports, and exports of pig meat and byproducts in 2022 were 14.1 times higher than 1961 (www.fao.org/faostat/en/#home).



From a business perspective, the global expansion of legal trade is a good thing - it reflects consumer confidence in the products we produce and presents risks that we can manage through the implementation of proper trade controls. Greatly troubling, however, is the global traffic in smuggled or "misrepresented" pig meat and/or byproducts - some of which have tested positive for ASFV (www.swinehealth.org/global-disease-surveillance-reports/). Commonly, uncontrolled imports represent food items carried by travelers for personal consumption, but in other cases it involves tonnes of unregulated and potentially unsafe product moved illegally between countries and intended for retail markets.

**Protecting our national herds.** The problem of transboundary pathogens is not new; the entire course of written history is littered with examples of pathogens moving around the globe (Cossar, 1994; Fèvre et al., 2006). But the scale of the present problem is unique.

Considering the situation as a whole, the effective defense of our national herds against transboundary pathogens requires: (a) measures to prevent their introduction, (b) surveillance capable of their early detection, and (c) a plan for their rapid, efficient elimination. Most countries have (a) strategies to regulate the entry of unsafe products and (c) plans for responding to outbreaks. The weak link in our systems is (b) national surveillance capable of early detection. This, despite the fact that early detection is the heart-and-soul of rapid control and eradication.

In his time, Dr. Alexander innovated to solve the health and productivity problems that arose as the industry transitioned to larger, intensive systems. It falls to us to innovate in the area of effective, sustainable national surveillance systems. "Active participatory regional surveillance" is a place to start.

In brief, the active participatory regional surveillance design described by Trevisan et al. (2024) consisted of targeted sampling of 10 poor-doing pigs in each participating farm systematically over time, with samples pooled (5 pigs each) and tested in credentialed laboratories. The surveillance objective was not to establish the status of the participating farms; instead, the goal was to establish the status of the region by aggregating testing results across the region under surveillance. Using this approach and with 0.1% of the herds in the region positive for the pathogen of interest, Trevisan et al. (2024) estimated a 90% probability of detection given a farm-level detection probability of 30% and samples submitted from 40% of the farms in the region. Depending on the specimen and test format (nucleic acid vs antibody), the cost per round of sampling was estimated at €0.016 to €0.032 (\$0.017 to \$0.034 USD) per pig in the region.

The approach described by Trevisan et al. (2024) was based entirely on techniques and methods in common use today. It was intended to be an exploration of the concept; additional detail and/or modification would be used to tailor the design to the aims of a specific surveillance program.

Overall, however, the design differed from traditional surveillance designs in three major ways:

1. Active participatory regional surveillance relies on targeted sampling of poor-doing pigs. Targeted sampling has not been widely used in surveillance, but has been recommended by European Food Safety Authority (EFSA) for CSFV and ASFV (Nielsen et al., 2021a,b). Targeted sampling/testing improves timeliness and avoids the diagnostic ambiguity of syndromic surveillance, i.e., differential diagnoses for ASFV and CSFV include erysipelas, salmonellosis, bacterial septicemia, PRRSV, PRV, and others (Sánchez-Vizcaíno et al., 2019; Schulz et al., 2019). And, as reviewed by Bates et al. (2003), even FMDV has been misdiagnosed as a variety of other pathogens on the basis of its clinical presentation.

Targeted sampling/testing also addresses cost. Surveillance based on representative sampling to establish herd status (negative/positive) requires collecting and testing too many samples, the vast majority of which will be negative. This expense cannot be justified to those who control the financial resources or to those who collect the samples. The life span of such an approach can only be short.

Finally, targeted sampling and testing removes the human element. As reviewed by Gates et al. (2021), failure to report suspicious cases, i.e., "under-reporting", is common in syndromic surveillance because people are uncertain as to when or how to report, uncertain as to the consequences of reporting, or simply lack motivation ("not my job").

2. Active participatory regional surveillance relies on project participants, i.e., producers and farm workers, to collect samples under the direction of the herd veterinarian. This practice is already common in some parts of the world. Further, it is appropriate to involve farm personnel because they know exactly which animals have recently taken a turn for the worse, i.e., which are the best candidates for sampling.

Sampling by lay personnel is also supported by an extensive body of research on the diagnostic reliability of a variety of easily collected samples, e.g., swabs, oral fluids, etc. In human medicine, sampling by lay personnel is supported by large studies in self-sampling for human immunodeficiency virus (HIV) or COVID-19 testing (Branson, 1998; Tsang et al., 2021).

Finally, sampling by field personnel reduces the cost of surveillance by eliminating the need to hire program samplers, improves biosecurity by avoiding the need for samplers to move between farms, and provides flexibility and responsiveness. That is, in the case of an emergency, field personnel are already on the farms and the number and/or frequency of sampling can be quickly increased to improve case finding.

IPVS & ESPHM 2024

3. The focus of active participatory regional surveillance is on the status of the region/country, not the individual herd. Traditional surveillance operates in reverse: it derives the status of the region/country by attempting to determine the status of individual herds. The traditional approach is time-consuming and requires over-sampling at the herd level, thereby expending scarce program resources along with producers' good-will.

In short, active participatory regional surveillance can be summed up as "a few samples from many herds routinely collected in a systematic manner over time and across supply chains, regions, or country". The design is simple, adaptable to a variety of swine pathogens, and easily described to producers, veterinarians, and bureaucrats. There is nothing new in the approach: it is merely a reshuffling of techniques and methods currently in use. The question for many will be how such a plan would be implemented. Simply, it will require cooperation between producers, industry, and animal health authorities.

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## Keynote lectures **PROF. HUA-JI QIU**

Session: Virology and viral diseases Lecture: New technologies applied to vaccine development: what can we expect?

#### **Biosketch**

Hua-Ji QIU, PhD, DVM, professor, the chief scientist of the Unit for High-Consequence Swine Viral Diseases, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China. He served as coordinator of more than 20 projects from National "863" Program, National Natural Science Foundation of China, National Key Technologies R&D Program, EU FP7 project, etc. His research is focused on the regulation of viral replication, virulence determinants and development of diagnostic assays and marker vaccines for classical swine fever, African swine fever and pseudorabies. He has been awarded several national and provincial prizes for progress in science and technology. He has published more than 150 papers in SCI-indexed journals, including 15 papers in Journal of Virology.

Prof. QIU is a member of the Asfarviridae Study Group of ICTV, a member of National Committee of Experts on Animal Epidemic Prevention of China, and a standing member of Animal Infectious Diseases Branch and a member of Veterinary Biotechnology Branch of Chinese Society of Animal Husbandry and Veterinary. He serves as editor of several journals such as Frontiers in Immunology, Viruses, Scientia Agricultura Sincica, Chinese Journal of Biotechnology, Acta Microbiologia Sinica, etc., and a peer reviewer of a number of international journals such as Gene Therapy, Journal of Virology, Vaccine, Veterinary Microbiology, Viruses, Virus Research, etc. Additionally, he is a Heilongjiang high-level B talent, agricultural talent, provincial pig industry system expert, provincial government special subsidy recipient.



Development Strategies and Application Prospects of African Swine Fever Vaccines: Feasibility and Probability Hua-Ji Qiu, Tao Wang, Yuan Sun

State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China

**Abstract**: African swine fever (ASF) is a severe hemorrhagic disease of affecting domestic pigs and wild boar caused by African swine fever virus (ASFV). It presents a mortality of up to 100% in acute cases, with no commercially available vaccines or treatments except Vietnam. Over recent decades, significant efforts have been directed towards developing a safe, effective, and affordable ASF vaccine to mitigate this transboundary disease, a critical concern for stakeholders in the global pig industry. Substantial investment in ASFV research and development has led to unprecedented progress in novel vaccine development, encompassing live attenuated vaccines, subunit vaccines, virally vectored vaccines, and DNA/mRNA vaccines, each with varying safety and efficacy profiles. Several promising ASF vaccine candidates are undergoing evaluation in preclinical experiments and/or clinical trials, demonstrating considerable potential for commercialization. However, numerous obstacles still impede further development. This keynote lecture will provide an overview of various vaccine strategies, outlining their advantages and disadvantages, comprehensive evaluation, current challenges, application prospects for ASF vaccine candidates, and future development directions aimed at creating safe and effective ASF vaccines.

Keywords: African swine fever; vaccines; development strategies; application prospects

African swine fever (ASF) is a highly contagious viral disease in pigs caused by African swine fever virus (ASFV), which has resulted in substantial economic losses and poses a significant threat to the global swine industry and related sectors [1]. ASFV belongs to the *Asfarviridae* family and is the only known insect-borne DNA virus. Its genome consists of a double-stranded linear DNA ranging in size from 170 to 194 kb, encoding 54 structural proteins and over 100 non-structural proteins [2]. This keynote lecture presents an overview of recent advances in ASF vaccines, encompassing the strengths and weaknesses of various research and development strategies, as well as the key scientific challenges they

encounter. Furthermore, it delves into the scientific assessment of candidate strains for ASF vaccines and examines the future development trajectory, hurdles, and potential applications of safe and effective ASF vaccines, offering insights into the advancement of ASF vaccine development.

• XXI •

#### 1. Research and development strategies and technological bottlenecks of ASF vaccines

Despite advancements in adjuvants, preparation techniques, and optimization of immune pathways and doses, inactivated vaccines fail to provide immune protection against ASFV infection [2-3], highlighting the insufficiency of relying solely on humoral immunity. Cellular immunity plays a crucial role in clearing ASFV infection, thus effective ASF vaccines should elicit both humoral and cellular immune responses. Several types of ASF vaccines are under development and evaluation.

Live attenuated vaccines (LAVs) are able to induce robust humoral and cellular immune responses, offering longlasting immune protection [4]. LAVs afford complete homologous protection and partial cross-protection, rendering them a promising research and development strategy (Table 1). However, safety concerns pose a significant hurdle to the further application of LAVs for ASF. Precise deletion of ASFV virulence-related genes while maintaining immunogenicity is imperative to mitigate safety risks. Additionally, assessing the genetic stability of LAVs is essential to identify potential risks such as virulence reversion and gene recombination [5-6].

Strategies	Advantages	Disadvantages
Inactivated virus vaccine	Safe	Almost no protection
Subunit/DNA/Live virus-vectored vaccine	Safe, partial or no protection	Inability to prevent the onset of disease and virus shedding
Live attenuated vaccine	Complete homologous protection	Residual virulence and risk of recombination
Gene-deleted vaccine	Complete homologous protection and partial cross-protection	Residual virulence and risk of recombination

Table 1. Several types of ASF vaccines and their	advantages and disadva	ntages
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Subunit and virus-vectored vaccines are based on the identification of protective antigens. Virus-vectored vaccines, utilizing adenovirus or poxvirus vectors and employing a prime-boost strategy, demonstrate the ability to elicit robust humoral and cellular immune responses, conferring complete protection against lethal doses of highly virulent ASFV strains [6]. However, further efforts are necessary to enhance the protective efficacy of virus-vectored vaccines. DNA and mRNA vaccines are also under investigation as alternative approaches. While initial studies showed that DNA vaccines failed to elicit detectable ASFV-specific antibodies in the immunizing pigs, they did induce cell-mediated immune responses and offered partial immune protection. However, the efficacy of combining DNA and subunit vaccines remains suboptimal and does not meet commercialization standards. To date, there are limited to no mRNA vaccines for ASF.

#### 2. Comprehensive evaluation of ASF vaccine candidates

The safety evaluation of ASF vaccine formulations primarily focuses on ensuring the absence of adverse reactions and assessing immunopathology in immunized pigs. The marketability of ASF vaccines is contingent upon their effectiveness, which can only be ascertained if safety is assured. Presently, predicting the efficacy of ASF vaccines is difficult due to the absence of specific indicators related to immune protection, necessitating laboratory and clinical trials for evaluation. Regrettably, many studies have failed to comprehensively assess residual virulence, vertical and horizontal transmission potential, and the risk of virulence reversion of LAVs. Consequently, candidate ASF vaccine strains validated in the laboratory may not withstand rigorous clinical trials. When evaluating the effectiveness of a subunit or virus-vectored vaccine, it is crucial to avoid an excessively high challenge dose that could obscure the vaccine's protective efficacy. A thorough and unbiased understanding of the intricacies of ASF vaccine development and clinical trials is imperative for the objective evaluation of vaccine safety and effectiveness.

The predominant circulating ASFV strains in the Asia-Europe region include genotype I, genotype II, and genotype I/II recombinant ASFV [7]. Consequently, the development of ASF vaccines must consider the need for cross-protection. When formulating ASF subunit, DNA, and virus-vectored vaccines, it is essential to identify protective antigens and evaluate their conservation across different genotypes. Utilizing relatively conserved target antigens or peptides can enhance the vaccine's broad-spectrum characteristics, thereby increasing its applicability. Moreover, when assessing the efficacy of ASF vaccines, their ability to provide cross-protection should be taken into account. The development of a safe, effective, and broad-spectrum ASF vaccine would greatly facilitate affected countries in promptly achieving ASF prevention and control [8].

#### 3. Novel insights into the development of safe and efficient ASF vaccines

The first approach involves the identification of novel vaccine targets using advanced technologies, such as bioinformatics combined with multi-omics, to predict and screen virulence-related factors across various genotypes and strains of ASFV. Additionally, the CRISPR/Cas9 gene editing technology is employed to generate a series of mutant viruses for in vivo experimental verification to elucidate their impact on ASFV pathogenicity. This strategy aims to uncover additional virulence-related factors as potential targets for developing LAVs against ASF. Moreover, conducting in-depth

comparative analysis of the proteome and transcriptome of host cells or infected pig bodies across different ASFV strains will aid in understand the transcriptional and expression characteristics of ASFV genes, as well as the function and structural attributes of the proteins. Such analyses will facilitate the rapid identification of conserved protective antigens present in diverse ASFV genotypes/strains.

The second approach involves generating a replication-defective ASFV by deleting essential genes using reverse genetics technology. The resulting mutant virus can only replicate in cell lines expressing the complementing protein encoded by the deleted gene. This mutant virus replicates solely in trans-complementing cells and undergoes only a single-cycle infection in pigs, ensuring both the safety and efficacy of the vaccine. Another strategy is to utilizing codon expansion technology to construct ASFV mutants dependent on non-natural amino acids, which also cannot replicate in pigs. However, these vaccine strategies encounter technical bottlenecks, such as of the lack of suitable cell lines for ASFV propagation in vitro. Genetic manipulation in primary porcine alveolar macrophages is relatively challenging, and further improvements are needed to enhance the effectiveness and stability of these vaccines.

The third approach involves investigating the regulatory mechanisms of innate and adaptive immune responses in pigs inoculated with naturally attenuated ASFV strains or attenuated vaccine candidates. This research aims to elucidate the immunoprotection mechanisms, providing a scientific foundation for the development of effective adjuvants and delivery systems for ASF vaccines. By gaining a comprehensive understanding of the immune responses elicited by ASFV strains of varying virulence, it becomes feasible to develop a safe and efficacious ASF vaccine using a tailored vaccine strategy.

The fourth strategy is to obtain safe and effective vaccine candidate strains through multiple passages of ASFV in cell cultures. On one hand, various vaccines have been successfully created through this strategy. On the other hand, ASFV has complex strategies to evade the host immune responses, and it is difficult to achieve complete evasion of the virus from the host immune system solely via gene deletion or mutation. However, during the process of cell passage, ASFV undergoes various adaptive mutations, which may antagonize the host immune responses through multiple pathways, thereby ensuring complete attenuation of ASFV.

#### 4. Conclusions

Despite the emergence of some promising ASF LAVs have been reported, their safety, effectiveness, and stability require comprehensive scientific evaluation before they can be considered for commercialization. Identifying which viral proteins of ASFV can induce protective antibodies in pigs remains a crucial challenge. Additionally, it is imperative to define the ASFV protein(s) responsible for antibody-dependent enhancement (ADE), a bottleneck that hampers the effectiveness of subunit and virus-vectored ASF vaccines. Thus, the development of safe and effective ASF vaccines is still in its early stages. A potentially viable strategy involves systematically and comprehensively screening all ASFV-encoded proteins using sera and peripheral blood mononuclear cells from pigs immunized with LAVs. This approach aims to identify antigen proteins capable of activating protective T and B cell immune responses through a series of *in vitro* and *in vivo assays*. Moreover, leveraging big data-based bioinformatic analysis can aid in elucidating the conservation of target antigens across different ASFV genotypes and optimize antigenic components. Scientific evaluation of ASF vaccine candidates is crucial to maximize their efficacy. This systematic and in-depth research approach holds the potential to screen and identify protective antigens of ASFV genotypes.

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IPVS & ESPHM 2024

## Keynote lectures **PROF. JANICE REIS CIACCI ZANELLA**

Session: Virology and viral diseases Lecture: **Emerging viral diseases: recent global threats** 

#### **Biosketch**

Janice Reis Ciacci Zanella, is a veterinary doctor graduated from UFMG (Brazil) with a Master's and a Ph.D. degree from the University of Nebraska, USA.

She is a researcher at EMBRAPA (Brazilian Agricultural Research Corporation), Embrapa Swine and Poultry in Animal Virology.

She participates in the One Health High Level Expert Panel (OHHLEP) of the WHO, FAO, WOAH (OIE), UNEP and OFFLU on Swine Influenza.

She is currently a visiting scientist at NADC/ARS/USDA and Postdoctoral fellow at Iowa State University, USA

Abstract

#### EMERGING VIRAL DISEASES: RECENT GLOBAL THREATS Janice R. Ciacci-Zanella

**ABSTRACT**: Emerging diseases continue to arise in nature and affect living beings on all continents. A pathogenic agent is assumed to emerge worldwide every four months; 75% are zoonotic. Zoonotic diseases affect billions of people worldwide, causing millions of deaths every year. Monitoring zoonotic viruses is essential, as viruses evolve naturally through mutation, rearrangement, or recombination, becoming more virulent or transmissible. Emerging swine viruses threaten herd health and have caused losses in the last decades. Factors contributing to these phenomena include failures in biosecurity, biocontainment, and herd immunity imbalance. The world is alerted to highly pathogenic avian influenza outbreaks in wild or domestic birds and mammals. As it is a zoonosis and a virus in constant evolution, it is essential to be prepared for an effective response and surveillance. However, we must think less about species and more about systems. The impact of pandemics like COVID-19 on human health and the global economy is immense, emphasizing the importance of integrating human, animal, and environmental health approaches. One health implementation is crucial to avoid the drivers of disease emergence or spillover factors. It also deals with developing and adopting effective public disease surveillance, prevention, response, and control policies. Everything is connected and complex, with multisectoral and multidisciplinary work being essential. However, implementing One Health is much more than zoonoses; it includes nutritious foods, food safety, antimicrobial resistance, and many other aspects of environmental health.

#### 1. INTRODUCTION

In the past decade, the emergence of diseases, including pandemics, has highlighted the importance of these problems to the health of all living elements (Zanella, 2016; Zanella and Zanella, 2023). Global health includes human health and the integration of health approaches, with the core of this umbrella containing the definition of One Health (Gruetzmacher et al., 2020). This includes the integration of human, animal, and environmental health, considering the inseparability between them (Adisasmito et al., 2022). Environmental health deals with ecological and social interactions, and planetary health is about human civilization and the state of natural systems (Gruetzmacher et al., 2020).

Agriculture and livestock are essential sectors for many nations' economies, and zoonotic diseases caused by agents transmitted from non-human animals to humans affect more than two billion people worldwide, causing more than two million deaths every year (Bank, 2021). Zoonoses account for up to 75% of newly discovered or emerging infectious

diseases (EIDs) and 60% of recognized contagious illnesses in the last century, causing significant local, regional, and global disease burdens (Brown, 2004; Jones et al., 2008; Taylor et al., 2001).

Due to their high occurrence and severity, swine viral diseases (SVDs) are one of the most studied emerging animal diseases. SVDs are a significant concern due to their high occurrence and severity, impacting pig health and human health. Also, pork products are essential in many countries' economies and protein supply. Despite advancements in pork production systems, animal diseases still account for 20% of losses. In addition to recently discovered viral agents that have affected swine herds, more viruses might be circulating, posing a zoonotic risk and moderate to serious human health risks (Lin et al., 2023). EIDs in swine include endemic, foodborne, transboundary diseases, idiopathic vesicular, and subclinical infections. Traditional pig husbandry techniques have undergone significant modifications due to the growth and intensification of pig production, creating an environment favorable to the establishment and spread of infectious illnesses. Because of this, pigs are becoming intermediate and more abundant hosts for viruses that have the potential to cause a pandemic (Lin et al., 2023). Thus, factors contributing to these matters include biosecurity failures, biocontainment, and herd immunity imbalances. In addition, viruses evolve naturally through mutation, rearrangement, or recombination, making monitoring their evolution crucial.

This work will discuss the importance of emerging viral diseases, emphasizing zoonotic viral diseases with epidemic or pandemic potential. In addition, it will discuss SVDs that appeared in the last decades and how they are impacting the current production system.

#### 2. VIRUSES AS THE MOST FREQUENT EMERGENT ZOONOTIC PATHOGEN

EIDs are a critical global health concern, with zoonoses dominating (60.3%), primarily from wildlife. A study analyzing 335 EID events between 1940 and 2004 found non-random global patterns, with zoonoses dominating (60.3%) (Jones et al., 2008). Of 1415 pathogenic infectious organisms, 61% are zoonotic and can be transmitted between humans and animals. Out of these, 75% are twice as likely to be associated with emerging diseases than non-zoonotic pathogens (Taylor et al., 2001). RNA viruses are the most common emerging zoonotic agents, particularly RNA viruses, which infect various hosts, including domestic or wild animals (Woolhouse and Gowtage-Sequeria, 2005).

The International Committee on Taxonomy of Viruses (ICTV) has a database of 4958 virus species and 1479 vertebrate virus species, with viral traits and host ranges predicting confirmed zoonotic, potential zoonotic, and disease emergence outcomes (Singh et al., 2022). Bat- and livestock-adapted viruses pose a high risk, with 39 predicted with never-reported zoonotic potential or potential human virus emergence.

T he World Health Organization's (WHO) priority pathogen list includes COVID-19, Crimean-Cong hemorrhagic fever, Ebola virus disease, Marburg virus disease, Lassa fever, MERS, SARS, Nipah, henipaviral diseases, Rift Valley fever, Zika, and Disease X (WHO, 2022). Machupo, known as "black typhus" and Bolivian hemorrhagic fever, are significant public health risks (Meadows et al., 2023; Zhong and Huang, 2019). The number of viral spillover events from animals to humans increased by 5% annually from 1963 to 2019, causing around 1,216 deaths in 2020. If the rate continues, four times the number of outbreaks will occur by 2050, causing 12 times the number of deaths, around 15,000 a year. Most of the 72 outbreaks were caused by filoviruses in Africa, such as Marburg and Ebola, which caused more than 90% of the 17,000plus total deaths. There are no licensed vaccines for all Ebola strains, but some are developing for Marburg (Meadows et al., 2023; Zhong and Huang, 2019).

The Henipavirus genus includes two highly virulent zoonotic viruses, Hendra and Nipah, which are primarily batborne and have outbreaks in Australia and Southeast Asia. Its natural reservoir is fruit bats. Hendra, another henipavirus, was first observed in Australia in 1994 (Meadows et al., 2023). Both cause respiratory illness and flu-like symptoms, potentially leading to encephalitis (Bruno et al., 2023; Meadows et al., 2023). Nipah kills between 45% and 75% of infected individuals. No licensed vaccines exist (Meadows et al., 2023). Besides those continents, there is limited evidence in the Americas for the circulation of these viruses (Hernández et al., 2022). A novel henipa-like virus was discovered in Brazil from opossums, and the virus was named Peixe-Boi virus (PBV). The study used next-generation sequencing and metagenomic approaches to find the original evidence of a henipa-like virus genome in Brazil and South America, as well as the first description of a henipa-like virus in marsupial species (Hernández et al., 2022).

Coronaviruses, such as SARS-CoV-2, have become a significant public health concern in the 21st century. The WHO declared the outbreak a public health emergency of international concern and a pandemic. Extensive surveillance of SARS-CoV-2-related viruses in China, Southeast Asia, and other regions will help to understand its zoonotic origin. Climate change-driven increase in bat richness in the southern Chinese Yunnan province and neighboring areas in Myanmar and Laos may have played a vital role in the evolution of transmission of these coronaviruses (Beyer et al., 2021).

Influenza viruses are classified into four types: A, B, C, and D, with A and B causing seasonal flu epidemics (Webster and Krauss, 2002;. Influenza A viruses (IAV) are zoonotic and cause global pandemics when a new, different virus emerges. IAV caused four pandemics in the last century (Saunders-Hastings and Krewski, 2016). Introducing swine or avian influenza viruses into the human population can set the stage for a pandemic, with concerns about the Highly Pathogenic Avian Influenza Virus (HPAIV) becoming the next pandemic. Regarding IAV in pigs and zoonotic spillover, control measures include avoiding human virus introduction into pigs and vaccinating pig farming workers.

#### 3. DRIVERS OR FACTORS FOR ZOONOSE EMERGENCY AND SPILLOVER

The human population's expansion, with an estimated 10 billion people by 2050, is likely to contribute to the emergence of new diseases. This growth and urbanization will stress the need for sustainable energy and food production, increasing the likelihood of future pandemics. Population diversity, including cultural, genetic, educational, structural, environmental, and socioeconomic diversity, may cause some populations to be more vulnerable to diseases or prone to

distribute diseases (Simpson et al., 2021; Mettenleiter et al., 2023; OHHLEP et al., 2023). Climate change, war, conflict, and environmental degradation are causing increased human and animal displacement, disrupting socio-economic structures, weakening the immune system, and increasing susceptibility to infectious diseases. Climate change also causes a rise in zoonotic diseases, affecting the survival, reproduction, and distribution of hosts, pathogens, and vectors in urban areas and the interaction between humans and animals. As a consequence, it has caused a global spread of rodents, rodent-borne pathogens, and bats. Deforestation, altered microclimates, and habitats have led to the spread of common disease vectors like mosquitoes and ticks causing changes in pathogen prevalence. Land conversion also increases accessibility to blood meals, with wild, domesticated animals near humans. It is unknown how many pathogens are transmitted to wildlife by mosquitoes, and many other parasites and undiscovered viruses are still poorly studied. Anthropogenic factors also affect animal health, and livestock is often the only moveable asset during disasters, but its inclusion in humanitarian responses can lead to fragmentation. Wild boars spread throughout Europe, Asia, Africa, and the Americas, have led to the spread of infectious diseases such as African swine fever (ASF), classical swine fever (CSF), and Aujeszky's disease (AD) and hepatitis E (HE).

In summary, zoonotic diseases have increased due to population growth, urbanization, climate change, mobility, global trade, intensive livestock farming, agricultural expansion, land use changes, habitat fragmentation, and biodiversity loss. Factors such as pollution, environmental degradation, social imbalance, population deprived of liberty, and poor vaccine coverage are also important. Researchers warn that over the next 50 years, at least 15,000 viruses will spread between species due to the climate crisis (Carlson et al., 2022). Bats, an important mammal vector, will account for most disease spread due to their ability to travel large distances. Studies predict that deaths from zoonotic viruses will increase 12-fold by 2050 due to climate change and habitat encroachment (Meadows et al., 2023; Prater, 2023).

#### 4. CHALLENGES ON SWINE DISEASES

The swine industry faces economic risks due to modernization, high technology, costly genetics, and complex production systems. Pathogens, including endemic, emerging, and re-emerging agents, continue to cause concern and losses in performance and mortality for this industry. The emergence of pathogens, especially zoonotic ones, is increasing globally, and despite high costs and technology, there are difficulties in intensively producing pigs. Challenges also include monitoring, controlling, and eradicating emerging diseases due to the interrelation of production and the globalization of the industry. The imprudent use of antimicrobials increases pathogen resistance, and the fight against bacteria becomes harder. For viruses, vaccine failures due to variants evolution create possibilities of continuous transmission, mutation accumulation, and risk of spillover between species.

Based on what has emerged, EID in pigs can be classified as follows: 1) transboundary swine pathogens introduced into new regions; 2) endemic swine pathogens that changed pathogenicity or mode of transmission; 3) non-swine animal pathogens that entered swine populations; and 4) non-pathogenic zoonotic agents that entered swine populations (Davies, 2012).

As mentioned, viruses are the most common EID agent and SVDs that deserve attention are influenza A virus (IAV), porcine circovirus type 2 (PCV2), Seneca Valley Virus or Senecavirus A, Porcine Epidemic Diarrhea Virus (PEDV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), African Swine Fever Virus (ASFV).

Other agents such as novel porcine parvovirus (nPPV), porcine enterovirus, porcine sapelovirus (PSV), porcine Kobuvirus (PKBV), porcine torque teno sus viruses (TTSuV), porcine bocavirus (PBoV), porcine toroviruses (PToV), porcine lymphotropic herpesviruses (PLHV), porcine hepatitis E virus (swine HEV or Paslahepevirus balayani or HEV), and porcine sapovirus (porcine SaV), which although the clinical importance is not yet evident, are present in lesions or accompanied in clinical cases with other agents.

In addition, new emerging viruses such as atypical porcine pestivirus (APPV), PCV3, PCV4, SADS-CoV, influenza D, and others with regional or global distribution and with unclear pathogenesis pose a new challenge to veterinary medicine (Perfumo et al., 2020).

Viral evolution is a never-ending process in which the virus adapts to the host's or environment's pressure. It can occur in various ways, but the end goal is to create a progeny virus with advantages that differ from the original. Viruses, mainly single-stranded RNA and DNA viruses that have a high mutation rate (10-4/10-5 nucleotides per replication cycle), can change in virulence either by rearrangement, recombination, or mutation, which eases their adaptation to the innate immune response (Shi et al., 2018). Relevant examples are HP PRRSV (highly pathogenic PRRSV) (An et al., 2020)(18), influenza A H1N1pdm09 (pandemic influenza virus) (Al Farroukh et al., 2022), and PEDV (Jung et al., 2020).

Some viruses have presented as subclinical for a long time and were only discovered due to the development of metagenomics or next-generation sequencing techniques. Viruses such as PCV3, SADS-CoV (swine acute diarrhea syndrome coronavirus), and LINDA (novel pestivirus causing congenital tremor known as lateral-shaking inducing neuro-degenerative agent) have been identified using the techniques described above (Perfumo et al., 2020)). Modern techniques also have studied retrospective materials and showed previously unidentified viral agents. One example is astrovirus 4 (PoAstV4) in pigs with respiratory disease but of unknown etiology. Even though five known lineages of astroviruses were previously identified in pigs as causing gastroenteritis, neurologic disease, or asymptomatic infection, PoAstV4 is considered a new respiratory disease agent (Rahe et al., 2023).

Another example is the porcine parvoviruses (PPVs) (Vargas-Bermudez et al., 2023), present on all continents, with the highest prevalence in finishing pigs. Eight PPVs are known in the swine host, with PPV1 being the oldest and the primary agent of SMEDI. The pathogenesis of the novel PPVs (nPPVs), PPV2 through PPV8 is still undetermined. PPVs are structurally similar, with differences mainly at the genomic level. Mutations in the VP protein affect virulence, tropism, and viral antigenicity. nPPVs have mutation rates similar to ssRNA viruses, with the highest mutation rate reported for

PPV7. The relationship between nPPVs and clinical manifestations is complex, but some evidence suggests associations with the porcine respiratory disease complex (PRDC). Further research is needed to establish nPPVs as disease agents, their effect on coinfections, and their impact on swine health (Vargas-Bermudez et al., 2023).

Even more complex, a study found a circular replication-associated protein (Rep)-encoding single-stranded (CRESS) DNA virus [named Po-Circo-like (PCL) virus] in intestinal tissue and fecal samples of pigs. The virus may be responsible for viral genome replication and cause diarrhea symptoms in pigs. Four strains were identified in two pig farms in Hunan Province, China, sharing 85.7-99.5 percent nucleic acid and amino acid identity with the reference strains. Further investigation is needed to determine the virus's pathogenesis and epidemiologic impact (Ji et al., 2023).

As mentioned previously, pigs are thought to be significant reservoirs for the emergence of new reassortant IAV strains that have the potential to spread globally. Numerous viruses that can infect humans can be found in backyard farms, commercial swine herds, and wild pigs. These viruses can range from those known to be emerging and endemic in humans to those with a high potential for becoming pandemics, having significant implications for public health. Other examples include the previous outbreaks of Japanese encephalitis (JEV) and the Nipah virus (Glud et al., 2021; McLean and Graham, 2022). Encephalomyocarditis virus (EMCV) causing disease emergency also has been documented in commercial growing-finishing pigs from two farms in Midwest Brazil. This virus was previously detected in humans and rodents in South America; and in Brazil, it was reported in wild animals and horses. The study found cardiomegaly, myocardial necrosis, and fibrosis in pigs and rats (Gris et al., 2023).

Regarding HEV, several studies show that HEV is ubiquitous in swine populations with no clinical signs. Yet, human contact with swine-infected HEV zoonotic genotype 3 can develop the disease. The virus can be found in pig farming effluents and a few pork products (Heldt et al., 2016; Pereira et al., 2018). Therefore, actions to control the infection in swine herds and exercise caution while handling food, especially pig liver, are pertinent (Soares et al., 2022).

Finally, pigs can be susceptible to experimental infections of other species 'viruses, like the clade 2.3.4.4b HPAIV(Kwon et al., 2023), Reston virus (RESTV, an Orthoebolavirus genus) (Lewis et al., 2024) or Zika viruses (Pena et al., 2018; Sabir et al., 2023), and consequently have the potential to spread to humans.

#### 5. DRIVERS OF PATHOGEN EMERGENCY IN SWINE

As with human EID, the drivers for EID in swine populations are not clear. However, as the increase of human population being one important driver for EIDs in public health, in swine, increased density and intensity of swine production are also significant. In the current scenario, high animal densities, quick animal turnover rates in confinement buildings, and more genetic homogeneity among swine herds are the outcomes of this enhanced intensification, which may make the animals more susceptible to illness.

Other essential factors include altered management practices, the interaction with wild animals, environmental changes, and pathogen acquisition of new virulence factors (Bekedam, 2006; Zanella, 2016). In summary, most reasons are imposed by human intervention, which directly or indirectly disrupts the host-pathogen equilibrium or causes EID introduction into a new territory due to human mobility or trade (Cutler et al., 2010). In swine farming, it is no different; most of the diseases emerging in recent decades already occurred in herds or regions in a balanced manner but were distributed, or their pathogens acquired essential virulence factors for manifestations of pathological syndromes, previously unknown (Drew, 2011).

This is how many infectious agents have emerged and will continue to emerge. Although viral selection and evolution are typically viewed as a precursor to an advantage (easier transmission, host diversity, environmental resistance, or immune response evasion), some mutations can and will result in adverse changes in the virus and may cause disadvantages for the pathogen. Consequently, this is logical when the entire process is analyzed as equilibrium. An example is the transmissible gastroenteritis virus (TGEV), which caused significant losses to the pig industry. In 1987, another coronavirus, known as porcine respiratory coronavirus (PRCV), a variant more transmissible than its predecessors, emerged. PRCV causes a mild respiratory infection and provides herd immunity for TGEV (COHEN, 2022). Later, another enteric coronavirus, PEDV, re-emerged in China in 2010 but from a highly virulent isolate, reaching the United States and other countries causing enormous losses, showing that the coronavirus "saga" is not over. Therefore, detecting new viruses and their variants is crucial for disease control, as random mutations, rearrangements, or viral recombination can occur (Cui et al., 2019).

In brief, the drivers of pathogen emergence in swine populations can be due to many circumstances. But can be summarized into two factors. The first is probabilistic, i.e., the threat has always been present, and emergence is simply a matter of time. The second factor, which is generally discussed more (and is very difficult to prove), is the changing ecology of these pathogens, environment, and host, among others (Drew, 2011).

#### 6. EVOLUTION AND CHANGES IN THE PIG PRODUCTION

Pig industry has evolved in recent decades to increase productivity, leading to pathogen horizontal transmission. Genetic changes, weight gain, feed conversion, and lean meat have caused imbalances in pigs' cardiorespiratory and immune capacities. Intestinal ecology has also changed due to feed ingredients and medications. Vaccines are a reliable tool against infectious agents, but their intense use may drive virus evolution and require high surveillance. Misuse of vaccines can lead to viruses leaking through immune responses (Drew, 2011; VanderWaal and Deen, 2018).

Interspecies transmission means the infection of the potentially pathogenic agent in a new host, such as IAV, between migratory waterfowl and humans. Other examples include bats as the source of the Nipah virus and porcine acute diarrhea syndrome (SADS coronavirus). Recent work has found porcine PCV3 to have high homology with PCV1 from bats. The rising proximity between species due to multiple factors can allow viruses to "jump" the species barriers. Interspecies transmission of viruses occurs frequently, but it does not always end in a pandemic because there are multiple steps that the

virus must pass through to be considered a risk to the novel host species. First, the original host must carry and shed the virus. Second, the novel host has to be exposed to the original to a great extent. Third and fourth, the virus has to be capable of infecting the new host and replicating well in it (these steps involve more complicated factors, like receptor availability and affinity, intensity of infection, and immune response) and finally, to complete the jump the novel host has to be able to shed the virus effectively to others of his species (Plowright et al., 2017).

Swine viruses threaten human health, and vice versa, because of the close contact and similarities between the two species. In many ways, humans and pigs are anatomically similar. Both species express the sialic acid  $\alpha$ -2,6-terminal saccharides on their upper respiratory tracts, which are preferred receptors for IAV. The same virus can easily infect both species (Nelson and Vincent, 2015). This example emphasizes the need for a "big picture" surveillance approach on these agents. Humans have been and will continue to be responsible for stimulating the emergence and mutation of viruses in swine herds and their direct introduction.

#### 7. CONCLUSIONS

Globalization has increased the threat of pandemics and accelerated global transmission but also facilitated international cooperation, disease research, and surveillance. To prevent zoonotic spillover is essential to identify threats. The strategy involves health surveillance, addressing disease emergence drivers like climate change, land use, wildlife trade, and food systems. Most transmission routes of zoonoses in humans are associated with direct exposure to infected pigs or raw or undercooked pork products. Thus, developing risk reduction activities like biosecurity and vaccination for infection prevention, including occupational diseases such as IAV, is essential. Measures to limit future zoonoses also include attention to live-animal markets, food consumption habits, exotic animal farms and reducing habitat destruction. Multidisciplinary approaches and public policies are needed, as well as joint efforts from veterinary, human medical, and public health professionals.

The swine industry is ahead of the curve in controlling pig diseases, but there are opportunities to capitalize on its strengths. The first step is to reduce the economic impact on production, increase herd immunity, and increase biosecurity to prevent transmission and spread of infection to other stages or herds. Biosecurity is not a novelty in pig farming, and new diseases have emerged in areas lacking it. Biosecurity is also threatened during financial crises, war, or migration. Biocontainment, which uses filtered air to prevent pathogen dispersal, is still restrict, but its success is estimated to be significant. Herd immunity is crucial, as swine health is a population issue. Population density, concentration of immunocompromised populations, co-infections, sanitation levels, aerosol concentration, and agonistic behavior must be considered to limit the likelihood of transmission.

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## Keynote lectures **DR. LUDOVIC PLEE**

Session: Management of crisis and disasters Lecture: When lightning strikes: strategies of crisis and disaster management

#### **Biosketch**

Dr. Ludovic Plée has been serving as Manager of the FAO Emergency Management Centre (EMC) in Rome since 2019. The EMC is leading, with the support of FAO technical units and the Office of Emergency and Resilience, global, regional and national activities to increase emergency management capabilities for better resilience and response to agri-food systems threats affecting livelihoods. Prior to managing the FAO EMC, from 2016 to 2019, Dr. Plée served as Project Manager in the FAO office in Jerusalem, working on Sanitary and Phytosanitary capacity development for the West Bank and the Gaza Strip. He was before then, from 2009 to 2016, assigned as a Response Planning officer to the FAO Crisis Management Center for Animal Health (previous EMC name), seconded by the French Ministry of Agriculture. He was deployed in many emergency response missions on various transboundary animal diseases outbreaks (zoonotic and non-zoonotic) as well as training national veterinary officers in FAO countries on good emergency management practice as well as on contingency planning. From 2006 to 2009, he worked in the French Food Safety Agency in Maisons-Alfort, France, as an emergency qualitative risk assessor on animal health issues affecting public health and food safety and, from 2003 to 2006, was a Veterinary Advisor in Paris, France, for the headquarters of the main Farmers Group, les Groupements de Défense Sanitaire (or GDS), especially on veterinary legislation, risk communication and disease awareness. He also serves as a lecturer in the French veterinary school in Nantes and in the French School of the Veterinary Services in Lyon on qualitative risk assessment and emergency management. Dr. Plée earned a Diploma of Veterinary Medicine from the Veterinary School in Nantes, France, and a Masters of Science in Epidemiology and Environmental Health with honours at, Colorado States University, Fort Collins, CO, USA.

#### Abstract

### <u>Title</u>: Shifting the emergency management paradigm towards a system approach to be better prepared to the risk of emerging or re-emerging health threats

**Abstract:** Lessons learnt from the global COVID-19 response highlighted the lack of effective emergency preparedness mechanisms. Each emergency operation system worked independently and was confined within national borders and disciplinary silos impeding a coordinated response capacity to address this new challenge at the pace and scale required. Data from the Food and Agriculture Organization of the United Nations (FAO) Progressive Pathway for Emergency Preparedness (PPEP), a self-assessment process and tool that has been applied by over 60 FAO member countries to assess their animal health emergency preparedness since its launch in May 2022, showed that the vast majority of participating countries are still unprepared for potential threats. The global challenge to better prepare for the next pandemic remains. Preparedness for any emerging threat requires a shift in classical views and paradigms. Emergency management systems, like other systems, should be adaptable and evolve from traditional siloed and single-threat approaches which lack flexibility during crises, to system-wide integrated approaches with increased efficiency and adaptability to future and emerging threats. Integrated, adaptive systems will allow countries, regions and the world not only to effectively address current and emerging threats but will likely be more resource-efficient to address the emerging threats arising from global crises.

**Keywords**: emergency management, system approach, resilience, unforeseen threats, emergency preparedness, climate change, emerging threats, emergency response, FAO, PPEP

**Background**: Climate change is the most important challenging global event that the human species is already facing. It will test our capacity to adapt quickly to fast changing conditions and will modify deeply our understanding on well-known

risks, such as diseases and other natural events. Lessons learnt from the COVID-19 response highlighted the lack of effective emergency preparedness mechanisms preparedness, where emergency management activities are traditionally characterized by individual sectors approaches to address these threats. Each emergency operation systems worked independently and was confined within national borders and disciplinary silos impeding a coordinated response capacity to address this new challenge at the pace and scale required.

Data from the FAO Progressive Pathway for Emergency Preparedness (PPEP), a self-assessment tool applied since its launch in May 2022 already in more than 60 FAO member countries worldwide to assess the level of animal health emergency preparedness, showed that the vast majority of country respondents are still unprepared to face upcoming threats that may have negative impact on animal health, public health (i.e. zoonotic diseases) and their social and economic stability. This is particularly related to multi-sectoral coordination, preparing for emergencies and prevention of introduction of risks.

<u>Main Text</u>: Data from the FAO Progressive Pathway for Emergency Preparedness (PPEP), a self-assessment tool applied since its launch in May 2022 already in more than 60 FAO member countries worldwide to assess the level of animal health emergency preparedness, showed that the vast majority of country respondents are still unprepared to face upcoming threats that may have negative impact on animal health, public health (i.e. zoonotic diseases) and their social and economic stability. This is particularly related to multi-sectoral coordination, preparing for emergencies and prevention of introduction of risks.

The global challenge still remains in getting better prepared for the next pandemic, and other emerging threats that will likely arise from the climate crisis and its disruption of the delicate interaction between humans, the environment, animals, plants and pathogens. This also highlights the need for tailored climate-actions approaches based on evidence and actual capacity needs and priorities in the broader agri-food system under broader climate change action principles. Preparedness to any emerging threat requires a shift in classical views and paradigms. Emergency management systems, like other systems, should be adaptable and evolve from classic approaches siloed-like single-threat approaches which lack flexibility during crises--- to system-wide integrated approach with increased efficiency and adaptability to future and emerging threats. Integrated, adaptive systems will allow countries, regions, and the world not only to effectively address current and emerging

threats but will likely be more resource-efficient to address the emerging threats arising from other global crises, like climate change. These should enable for strengthening of existing emergency preparedness capacities while improving identified and prioritized capacity needs at national, regional and global levels to increase resilience to on-going and changing threats and provide frameworks for more sustainable and tailored climate-action approaches to capacity building.

**Conclusion**: Reframing emergency management as a flexible and adaptable system, able to respond to known and unknown emergencies within or across different sectors, is in harmony with One Health principles. Our findings provide a practical guide for policymakers, health officials, and stakeholders to prioritize investments in adaptive interdisciplinary and multi-sectoral approaches to emergency management, fostering capacity building opportunities, and safeguarding societal well-being in the face of evolving challenges.

## Keynote lectures **DR. ADRIAN BALABAN**

Session: Management of crisis and disasters Lecture: **Pratical emergency management of ASF in Romania** 



#### **Biosketch** EDUCATION University of Agronomic Science and Veterinary Medicine - Bucharest, Romania BA, Doctor Veterinary Surgeon (DVM) 2005 BISA Swine Academy 2015, Ames, Iowa State University BISA LEAD 2017-2018, Ames, Iowa State University Professional development programs for veterinary practitioners Participations in IPVS 2010 Vancouver- Canada, IPVS 2012 Jeju- South Korea, IPVS 2016 Dublin Ireland, IPVS 2018 In the last 15 years attended different training periods in Holland and US for pig health, pig farm management, laboratory investigations and pig genetics, AI stations, biosecurity of livestock production systems **EXTRA-CURRICULAR ACTIVITIES** President of the Romanian Association of Swine veterinarians since 2016 Member in the Board of directors of the Romanian Pig meat producers Association (APCPR) since 2011 Member in the American Association of Swine Veterinarians 2017 Member in the EAPHM European Association for Porcine Health Management since 2014 Member in the work Group Pig meat of the Romanian Meat Classification Committee 2011 Member of "Swine & Wine Group" since 2015 Participated as representative of APCPR to D.G. Agro., D.G. Sante. meetings in Brussels, Participated as representative of APCPR to ministerial negotiations, law development applied for the pig sector Veterinary consultant for FAO of UN since 2020 **PROFESSIONAL EXPERIENCE** Primary Functional Expertise - swine veterinarian 2005- veterinarian in FATROM feed additives, premix and feed production- Technical support responsible for swine

Since 2006- General Manager Fermeplus SRL 4600 sows Since 2010 Director Cooperativa Silistea Productie Suine CA - 14 pigs farms In total managing directly 8800 sows, 65000 places finishers, 18000 places for weaners. Consultancy in health management and pig production for other 10000 sows farrow to finish, more than 75000 places for finishers, AI station Pig farm Biosecurity expert, presentations: Bergen, Norway 2018, Bucharest, Romania 2018, 2019, 2020, 2021, Bulgaria, 2018, 2019, China 2019, USA 2015, 2017, 2018, 2019, 2020, North Macedonia 2020 Secondary Functional Expertise

Since 2005 sales representative of Topigs Norsvin in Romania Since 2006 Topigs Norsvin breeding farm management, breeding program coordinator

#### Abstract

#### Business Continuity Plan and Contingency Plan: Prepare for the worst Hope for the best!

It was never a matter of if ASF will enter Romania but when and where it will hit first. Due to our geographical position and analysing the possible routes we were expecting the virus to enter Romania with the wild boar thru the Danube Delta.

Starting 2012 I participated to numerous meetings with different governmental bodies about the risk of ASF entering Romania. ANSVSA published between 2012 - 2016 4 risk analysis about the possibility of the virus to enter to Romania and in this publication, we could see the evolution of the risk from low (2013) to low-moderate (2014) till high (2016).

In one of the publications, it was mentioned that the system with the lowest possibility to get contaminated is one organized like the channelized system that Romania implemented in 2010 in one company: "For the success of the strategy, all the objectives of the channelled system must function, as an orchestra performing under the baton of a (single!) conductor. A single non-conformity that would exist in a single objective, can affect the entire chain."

Being a live pig's supplier not a pork producer we didn't apply for the channelized system registration, but we tried as much as possible to implement in our production flow all the legal and physical requirements of the above-mentioned system.

Feed storages, feed production plant, own feed delivery trucks, own water sources, individual water treatment for every farm, separate cars for internal movement than delivery to third parties or slaughterhouses, washing station, disinfection cages for all the cars entering the farms, dry filters ( no external cloths or personal objects after this point for every person approaching the farm ) clear demarcation (fences) of the areas inside the farm, grey area and clean ( production ) area, mandatory shower in and shower out , catering food , UV chambers small and big, individualized clothes , boots , hand or boots disinfection points everywhere in the production area , one way in and out the farm for every entering or leaving and a lot of internal beurocracy .

The idea behind the BCP or CP is that, in case of ASF confirmation in one of our farms, not the spread it between our farms or to other farms or slaughterhouses, to protect surrounding area, destroy the infected animals as soon as possible in the most human and environmentally friendly way limiting the material loses of the company.

In my opinion the most important aspect is keeping everybody aware and informed. For this reason, we organize weekly biosecurity meetings with all the farm managers, veterinarians, technical staff, at the same time informing all the people working in the farms about the evolution of the virus at local and national level and measurements imposed in different situations. This helps us a lot in motivating the people to respect the biosecurity procedures that we imposed in the farms and at the same time after the confirmation of the presence of the virus in our farms despite the initial denial and panic we knew what we must do.

What we took into consideration when design a the BCP and the CP:

First, it must comply with the national legislation, National contingency plan, Operational manual on ASF outbreaks, guides, and official recommendations.

In Romania we don't have incinerators that can process big quantities of dead animals in short period of time for this reason from the beginning we accept the alternative solution for destruction of dead animals during the stamping out procedure thru burring.

According to the National contingency plan, the local authorities had to identify and to approve a plot of land where the destroyed animals should be buried. From the experience of the other farmers, we understood that this never happen so, we identify the possible plots by ourselves on the land belonging to the respective farm or other properties of ours. In one specific case we had to find another solution, so we rent a piece of land from a neighbour.

We had to identify what are the best suitable cars for loading the animals, in the end we choose big lorries for cereal transport, with canvas cover. the reasons we decide this were: they can be easily loaded thru the back doors, and they have basculation mechanism, they have enough capacity to accommodate a reasonable number of animals of different categories, are built from aluminium so they are light, the cover is movable, breathable, end they are easy to clean and disinfect.

Our technicians had to build the gas installation adapted to each car.

The idea was to equally spread the gas into the car so it will get immediately to all the animals, decreasing as much as possible the time and the consumption of gas because it is costly, and it is not easy to receive it in time. Also, the concentration or the quantity varies from producer to producer.

Of course, in the beginning we had some indications about how much time it will take per category of animals or how many kilograms of CO2 we must push into the car but in the end, we had to learn from our own experiences.

Then, type of the farm sow farm or finishing farm, number of animals present in that farm, distance to the burial place closest 500 m longest 10 km with police following each truck, different weather conditions, people working in the farm ,available people from other activities and role of each person and from where we can hire in short notice a big number of people to do this not so pleasant job.

Speed of work: according to the national legislation at that time only the value of animals killed in the process of stamping out will be compensated, not the animals that die naturally during those days, this means that we must move fast not to allow the virus to kill more pigs.

Supplies: from the recommended methods and materials in the "Operational manual in case of ASF" we choose what we should use and identify possible suppliers so we knew in the moment we had to what to bring and from where, which company will provide us extra people, lorries, construction teams. It is important to know from where to order the materials you need at the same time you must plan the deliveries so they will cover your needs.

Localization of the farms, google maps.

Suspicions- types of suspicions:

• After a positive lab result – that the easiest and most straight-forward but, we had situations in Romanian when the diagnostic of the local state lab was not confirmed by the national reference laboratory.

• Based on clinical evolution of the animals in pen or group- difficult to understand directly but in all the cases from the moment of the first suspect animal in a pen till certainty passed less than 7 days. Usually more than one animal show clinical signs at the same time from a pen.

• Necropsy findings: we cut every dead pig over 60 days of age or every sow and look for any modification that could appear due to the infection with ASFv

In any case of a suspicion all movement of animals, in and out the farms stop. All movements of cars stop. Internal

movement of animals in every farm stop. Standing still imposed at all the levels. isolation of the suspected animals in the compartment, isolation of the suspected compartment in the farm.

The entrance in the compartment with suspected animals is made with different (one use) cloths and footwear, hard disinfection of all the internal alleys of the farm.

Confirmation within 24 hours maximum – the farm is isolated completely from the other farms we cut all the connections; people use separate road to approach the farm if the same main road serves more farms. Confirmation comes from the National reference laboratory, once the submitted sample are confirmed positive, we prepare for the destruction of the animals.

#### Outbreaks in the farms

In the end the inevitable happen and in April 2020, in the first moth of COVID-19 pandemic we had the first outbreak in a farm. The diagnostic I put directly into the farm, based on the clinical expression, in one pen with pregnant sows, 2 died in the same day and the other 6 were showing high fever over 41 degrees Celsius, some aborted, they were not eating and the ones still moving they were hardly walking. The sows in the pens around them they were looking healthy, no clinical signs at that time.

We collected samples from the dead animals and send to the laboratory for confirmations, but we didn't have any hopes because the anatomopathological modification we discover during the necropsy, like huge spleen, petechia on the kidneys and multiple haemorrhages in different organs, oedema of the gallbladder all pointed one bad outcome.

Immediately we inform the state veterinarians about our suspicion, we apply the standing still protocols for the entire system and wait for the lab results hoping we were wrong...

Unfortunately, they came back positive, and we start the procedures of emergency stamping out the pigs in the confirmed farm.

Immediately we get the official notification, and the official epidemiological investigation starts, official veterinarians arrive at the farm and conduct the investigation at the same time identifying all the present animal in the farm and notifying all the other exploitation the farm was in contact with. We are informed that the next official steps will be decided by the Local committee for disease control who will indicate us what will be the destruction method to use in our case and where to buries the animals as well as all the other measures that must be imposed in this case.

In the other farms, either in the proximity or farms in direct contact like the farms where we delivered piglets in case of a sow farm or the farm from where the piglets came, in case of finishing farm we impose the standing still protocols and continuous monitoring of every pig specially focusing on the last entered animals. We open every dead pig and submit samples from each of them to the laboratory for testing ASF.

In parallel with the official investigation, we start our internal epidemiological investigation trying to understand when and how the virus entered the farm, to identify all infected animals and to separate the clinical sick one from the healthylooking ones. at the same time, we try to limit the spread we stop all the movements of animal inside the farm.

Stamping out operation

Prestart operation:

We divide people on teams and delegate responsibilities.

Loading and counting teams, in the car teams, weighting team, killing team, cutting teams, unloading teams

Assistance: security team, supplies team, machines team - loaders, bulldozers, excavators, tractors, welders,

Teams: counting and first documentation team, drivers, moving team, loading team, euthanasia team, weighting and official registration, cutting team, machines, and excavators, burring, and covering, disinfection team.

We had to identify the slowest moving team or the most time consuming. In our case the slowest moving team was the cutting and burring team. The speed of the system is given by the slowest moving part. We had to identify it and to help them coming into the rhythm.

Organization chart:

Manager, supplies responsible, security manager, biosecurity manager, builders' manager, logistics manager, Preparedness:

We agreed on the road that we will use to transport the animals from the farm to the burial place and on it we fix the most important points. In all the case we must build in short time roads to be able to support the loads were moving.

We instruct each team of their responsibilities and inform them about all biosecurity measurements that they must respect during entire operation. We informed everyone that filming, and photography is forbidden as well as using their private phones on the site.

#### Execution:

The process starts always from the infected pigs, counting and loading them. we must start with these animals trying to eliminate as soon as possible the infected animals and their neighbours.

Then we continue with the neighbouring compartments or the oldest animals in the farm from economic reasons mainly – they consume the biggest amount of feed and in case they die they are the biggest loss.

Good identification and counting are mandatory, we always deliver per group of age and physiological status of the animals.

1. Entrance and disinfection point, team of two persons one for registration and the second for disinfection of the car in /
out the farm.

2. Waiting point for the lorries before loading- drivers are never allowed to step down the car.

3. Loading of animals thru the delivery ramps, delivery ramps had to be built or adapted to the type of lorries that we used. We use two team working in parallel divided in 3 one for counting and first registration 2 persons, one for moving the animals to the car 2 or 4 persons, one for loading and closing the car 2 or 4 persons. Extra: captive bolt gun for the animals that can't move out the farm.

4. Euthanasia – the animal should noy leave the farm alive so, we had identified a place where to install the CO2 tanks that will not create problems to the flow of cars in and out the farm. We had a team of 2 persons operating the CO2 tanks, trained to used special gases in special situation from euthanasia of animals to fire extinguishing.

5. Weighting, registration, documents: we build platforms in the internal road for weighers from zero, after the installation the weigher must be certified by an official company- Because we don't have road weighers in our farm this was the most time-consuming operation.

6. After weighting the lorries exist the farm and went to burial place not before being one more time disinfected.

7. Unloading and cutting -2 teams of 6 persons, maximum 2 hours continuous work per team. all the pigs were unloaded as close as possible to the burial ditch on a special geomembrane that will not permit leakages. The most unpleasant operation, we had people that quit the job and leave the company after some hours working on this. We open every pig to avoid accumulation of gas during the putrefaction which could explode.

8. Burial and covering the place. Preparation of the burring place: digging, levelling, straw bedding, sand, iron net, plastic foil, calcium carbonate, lime, chlorinated lime, disinfection. The most difficult job, partially done by hand, partially with machineries, it consumes a lot of time and resources.

9. Hard disinfection of all the burial area using chlorinated lime and disinfectants, all used roads to transport animals were sprayed with disinfectant.

10. All the vehicles used were washed and disinfect in the end of each working day and two times in the end of the operations.

11. Building the fence and marking the area as restrictive area!

12. In the farm we start the initial disinfection immediately as the animals were removed and after that we start the hard cleaning process which depending on type of farms or resources tock from 6 to 12 months.

Good preparation is mandatory to limit the economical loses of the company and the spread of disease in and out the farm.

#### **Reference:**

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- Ministerul Agriculturii și Dezvoltării Rurale: http://www.madr.ro/en/
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- Ghid Bune Practici Privind Biosecuritatea în Timpul Vanatorii In Contextul Evolutiei Pestei Porcine Africane

## Keynote lectures **MR. THOMAS SØNDERBY BRUUN**

#### Session: Reproduction Lecture: Management and future of hyperprolific sows

#### **Biosketch**

Thomas completed his MSc in Animal Science at University of Copenhagen, Denmark, in 2005. Throughout his education, focus was pig nutrition and physiology as well as applied reproduction. From 2005 to 2010, he worked as a pig production consultant where his main focus included sow nutrition and diet formulation, on applied sow reproduction and how to use feed in practice as well as optimizing management protocols in the service and farrowing units. Since 2010, he has been working at SEGES Innovation where his research has focused on sow and gilt nutrition. He has also been involved in transferring knowledge from research and innovation to farmers – and thereby participating in implementation of best practice on Danish farms. Thomas' main areas of research include amino acid and energy requirements of hyper-prolific lactating sows, milk production, stomach health and feeding of rearing gilts with focus on both bone development, litter size and longevity. Several research studies have been conducted in co-operation with University of Copenhagen and Aarhus University, and also include co-supervision of several PhD students.



Abstract

#### Management and future of hyper-prolific sows Thomas S. Bruun Livestock, Pig, SEGES Innovation, DK-8200 Aarhus N, Denmark Correspondance: thsb@seges.dk

#### Introduction

Hyper-prolific sows are often defined as giving birth to a number of live piglets that exceeds the nursing capacity of the sow. Breeding programs have focused on number of total born piglets or e.g. piglets alive at day 5 post-partum. Breeding solely for number of total born piglets per litter is unfavorable, as this leads to a higher rate of stillbirth and a higher preweaning mortality (Nielsen et al., 2013). Increasing litter size leads to decreased individual piglet birthweight (Riddersholm et al., 2021) and thus increased risk of piglets suffering from hypothermia. Therefore, management must be directed towards low birthweight piglets. Theil et al. (2022) recently showed that in Danish sows, total colostrum production has increased from 2007 to 2020. However, due to increased litter size, the individual colostrum intake by the piglet has decreased. The overall aim of this brief review is to cover current knowledge on management solutions from a sow perspective to maximize the outcome of using hyper-prolific sows from gilt through repeated gestations, farrowings and lactation cycles until culling after completion of +6 parities.

#### Gilt management

Gilt rearing conditions and age, weight, and body condition at first service are crucial for sow longevity. A trial with more than 1,400 Danish gilts showed that increasing body weight is beneficial for litter size in first parity, as litter size increased by 0.4 total born piglet per extra 10 kg weight at first service. However, the proportion of sows serviced for second parity was negatively affected by increasing weight and decreas-ing backfat at first service. Based on data analysis, a backfat thickness of at least 13 mm and a body weight below 164 kg at first service was recommended to optimize longevity and litter size in first parity (Bruun et al., 2023a).

#### Feeding and managing the gestating sow

The optimal feeding strategies for gestating sows should consider litter size, average piglet birth weight and changes in sow body weight and backfat thickness. To control sow weight development, a low ly-sine/low protein diet should be applied for the main part of the gestation period. A large-scale Danish trial revealed that average piglet birth weight is not negatively affected by feeding lysine levels as low as 3.3 g SID lysine/kg feed from service and until 113 d of gestation (Bruun et al.,

unpublished). A low level of lysine favors storage of backfat rather than muscle gain, and thus in modern genetics selected for lean meat, this presents an opportunity to restore backfat from the previous lactation period without increas-ing sow body weight substantially. Weight development of sows should be in focus (Figure 1), as a high body weight increases the risk of locomotor problems, a frequent cause of early and involuntary culling of sows. Based on figure 1, it is obvious that weight at first farrowing, and hence weight at first service, has a detrimental effect on sow body weight throughout her productive life.



Figure 1. Average weight development for sows used in six different trials (2017-2021) conducted in three different Danish Pig herds (n=2608). Symbols used refer to trials conducted in herd 1 (♦; ♦; ♦), herd 2 (●), and herd 3 (▲; ▲), whereas the average line describes the simple average within each parity (—) and average values by parity are presented in kg. In all herds, sows were weighed 12 h to 24 h post parturition. Within each trial, each dot represents an average of 26 to 138 sows (unpublished data).

Irrespective of housing system for loose housed gestating sows, some degree of precision feeding should be applied. Commonly, sows are sorted and housed in pens based on e.g. body condition score or backfat thickness at weaning. However, this does not take sow body weight or parity into consideration, and this may lead to feeding below maintenance requirements in heavy sows and well above maintenance re-quirements in lighter sows. In practice, housing systems for loose housed gestating sows could be opti-mized by having at least 6 pens per batch of sows and divide sows into subgroups according to body con-dition (lean, normal, fat) and age (parity 1+2 and parity  $\geq$ 3). Using electronic sow feeding stations provides an opportunity to feed all sows individually, and thus this opens opportunities for true precision feeding.

#### Preparing the sow for the farrowing process

The transition from pregnancy to farrowing to lactation is a period that is a significant period for hyper-prolific sows. Recently, it was found that a high feed intake from day 108 to parturition is essential to op-timize the farrowing process. To minimize the farrowing duration and the need for farrowing assistance, a feeding level of 4.1 kg/d is recommended (Feyera et al., 2021). Protein concentration in transition diets has been questioned but Pedersen et al. (2019) showed no effect of using either 3.0 kg/d of a standard gestation or lactation diet or a 50/50 mixture of these on farrowing duration and birth interval. Another study showed that increasing the feed allowance from 3.0 kg/d to 4.0 kg/d the last 3-7 days prior to far-rowing using 1.0 kg/d of a starch and fiber rich feed supplement reduced the proportion of stillborn pig-lets from 11.8% to 10.1% in third to seventh parity sows (Hojgaard et al., unpublished). Combining this knowledge with recent findings that stillbirth rate increases linearly with increasing dietary crude protein from day 108 to parturition (Johannsen et al., 2024) indicates that using a single lactation diet in the far-rowing unit is inappropriate when both farrowing performance and subsequent milk production should be optimized.

#### Extended farrowing surveillance decreases stillbirth rates

Increased litter size leads to increased farrowing duration and a higher stillbirth rate, and farrowing assis-tance is needed more frequently (Feyera et al., 2018). From 2022-2023, SEGES Innovation conducted a trial in two herds to quantify the potential of applying extended farrowing surveillance (0700 h to 1530 h and 1900 h to 0600 h) for the three days with most farrowings compared with normal farrowing surveil-lance (0700 h to 1530 h and 1900 h to 2000 h in herds with >21.5 total born piglets per litter (Figure 2). This study emphasized that a sow would benefit from being observed every half hour from the beginning to the end of farrowing. Every half hour, the number of piglets born was counted, and if no piglets were born since last observation, farrowing assistance was provided. This resulted in a significant reduction in stillborn piglets in both herds. The economic benefit is most pronounced in large herds, where the de-crease in stillbirths can easily finance the labor used for extended farrowing surveillance. However, when striving for low stillbirth rates and low preweaning mortality, smaller herds can use modified versions of extended farrowing surveillance by assigning working hours to cover more hours around the clock.



**Figure 2**. Effect of extended farrowing surveillance (0700 h to 1530 h and 1900 h to 0600 h; ●) for three days with most farrowings compared with normal farrowing surveillance (0700 h to 1530 h and 1900 h to 2000 h; ♦) on number of stillborn piglets per litter (EMM + 95% CI) dependent on sow parity in herd A (n = 484) and herd B (n = 556), respectively. Farrowings were monitored every half an

hour in the extended period. In both herds, a significant reduction (P < 0.001) in stillborn was found (Unpublished data).

#### Use of nurse sows

Several studies have focused on the use of nurse sows to deal with the number of piglets exceeding the nursing capacity of sows, e.g. Bruun et al. (2023b) and Farmer and Edwards (2021). From a sow perspec-tive, the use of nurse sows results in a prolonged lactation period, and it has been debated that receiving a new litter could be stressful for the sow (Sørensen et al., 2015). However, Amdi et al. (2017) found no differences in cortisol level in non-nurse sows compared with nurse sows. Bruun et al. (2016) showed that compared with non-nurse sows, nurse sows had 0.58 more total born piglets in their subsequent parity, and the risk of being culled before next farrowing was significantly lower, irrespective of sow parity. A prolonged lactation period itself may explain the higher litter size, as this has also been shown in other studies (Hidalgo et al., 2014; Hoshino & Koketsu, 2009). As concluded in a review by Bruun et al. (2023b), nurse sows should be selected among sows with a good appetite and in good body condition, as this de-creases the risk of shoulder ulcers and optimizes the subsequent reproductive cycle.

#### Optimizing sow feeding strategies throughout lactation

Feeding the lactating sow has developed considerably during the last decade due to increased litter size. It is of utmost importance to understand that feeding the lactating sow is merely about preparing the sow for her next reproductive cycle (Strathe et al., 2017a; Zak et al., 1997), as milk production is highly prioritized, which means that the sow will mobilize both fat and protein to maximize her milk production. Furthermore, milk production is driven by litter size (Hansen et al., 2012). Only severe imbalances in nutri-ent composition, e.g. lack of amino acids and protein, have the potential to decrease milk production (Strathe et al., 2017b), whereas several large-scale Danish trials have shown that increasing the feed al-lowance above recommended levels does not increase milk production, but efficiently decreases sow mobilization of fat and protein stores (Bruun et al., unpublished). In addition, phase-feeding of gestating sows has been targeted in many international trials, but a large-scale trial in Denmark showed that using single-phase feeding with a standard lactation diet (8.3 g SID lysine/kg) versus two-phases using a diet with a lower concentration of SID lysine (-13%) until day 9 of lactation followed by a higher level of SID lysine (+13%) from day 10 to weaning did not affect milk production (Bruun et al., unpublished). Further-more, under Danish conditions, feeding increasing levels of dietary fat in the interval from 1.0 to 5.0% using a fixed feeding curve based on net energy per day did not affect litter gain (Bruun et al., un-published), which emphasizes that the origin of energy is of less importance, as carbohydrates were sub-stituted with fat and protein to keep the ratio of amino acids to energy constant. The potential of phase-feeding may be more obvious in tropic conditions where feed intake decreases when sows are housed in temperatures exceeding their thermo-neutral zone, and thus phase-feeding provides the opportunity to feed more energy-dense (high fat) and protein rich diets in warm periods. Under Danish conditions, sows are typically fed up to 9 kg feed/day allowing an average daily feed allowance of around 6.3-6.8 kg throughout a 27-day lactation period, and sows typically mobilize 5-20 kg body weight and 2-3 mm of backfat, while providing milk for an average daily litter gain of around 2.8-3.2 kg/d, and sows are typically serviced for the next reproductive cycle within 4-6 days postweaning (Strathe, et al., 2017a; Strathe et al., 2017b).

#### Future of the hyper-prolific sow

In Denmark, we successfully produce hyper-prolific sows weaning more than 40 piglets per sow per year. This is possible because many sows are being used as nurse sows, and as a result far more litters are weaned than the number of previously completed gestation periods. In a future perspective, this is highly relevant for both economy and climate. The impact of sow feed consumption drops, because in the end it can be divided among a higher number of slaughter pigs produced. However, to deal with large litters, it is important to optimize management routines to keep preweaning piglet mortality low. Furthermore, many countries are facing increasing sow mortality rates. Also, unfavorable correlations between selec-tion for lean meat and survival have evolved. From a genetic point of view, handling sow mortality should take into consideration herd information not only from nucleus herds and multiplier herds, but also herd information from commercial herds using crossbred sows (Poulsen et al., 2020). Using only information from nucleus herds and multiplier herds and mu

due to sows being culled at a young age due to the strive for genetic progress. True longevity, registrations on culling reasons and sow survival from commercial herds using crossbred sows should be used in the genetic framework. From a feeding point of view oversupplying gilts and gestating sows with lysine and protein should be avoided, as this leads to heavy sows with increased risk of locomotor problems, which is a common cause of premature culling and the main cause of euthanization. These arguments raise the question whether the future development of hyper-prolific sows should be based on the traditional definition of prolificacy, i.e. litter size, or wheth-er robustness in terms of longevity, sow mortality and the sow contribution towards a lower preweaning piglet mortality should be prioritized higher.

#### Conclusions

Dealing with hyper-prolific sows requires focus on the rearing gilt to obtain the optimal weight (i.e.  $\leq$  164 kg) and backfat (i.e.  $\geq$  13 mm) at first service. Being too heavy and too lean leads to increased culling rate. Furthermore, initial weight at first service forms the basis for development of sow body weight in parity 1 to 5. In gestating sows, some degree of precision feeding to control sow body condition is crucial. The use of low-protein diets reduces the growth of muscle and eases the restoring of backfat. Preparing a sow for parturition includes a high feed allowance at around 4.0 kg/day for the last 3-7 days prior to parturition. When farrowing begins, the stillbirth rate of hyper-prolific sows can easily be controlled by watching the sows every half hour and providing farrowing assistance if no piglets are born for 30 minutes. The use of nurse sows does not seem to cause any concerns regarding physiological sow welfare. A nurse sow even seems to perform better in the subsequent reproductive cycle due to an extended lactation period. Only sows with a good appetite and a good body condition should be used as nurse sows. Throughout lacta-tion, a high feed intake prepares the sow for the following reproductive cycle and concomitantly provides nutrients for a high milk production. For future improvements of the hyper-prolific sow, traits such as survival and hence longevity and sow contribution to piglet survival in terms of nursing capacity could con-tribute to more robustness in modern pig production.

#### Acknowledgements

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## Keynote lectures **PROF. CHANTAL FARMER**

#### Session: Reproduction Lecture: **Optimizing production and uptake of colostrum and milk**

#### **Biosketch**

Dr. Farmer is a research scientist in sow lactation biology working at the Sherbrooke Research and Development Centre of Agriculture and Agri-Food Canada.

Dr. Farmer was a pioneer in studying mammary development in gilts and sows and identifying the various factors affecting it. She has published 166 scientific manuscripts, 9 book chapters, and is the sole editor of two books that were also translated and published in Chinese.

Dr. Farmer's expertise is recognized internationally as evidenced by her being a guest-speaker in 13 European countries as well as China, Australia, Mexico, Canada and the USA. She was Editor-in-Chief of the Canadian Journal of Animal Science from 2006 to 2010.

She was on the board of the Canadian Society of Animal Science for 12 years, serving as its president in 1998-99, she received 2 national awards and was made a fellow of this Society in 2017.

Dr. Farmer was on the board of the American Society of Animal Science from 2014 to 2020, from which she received the 2022 President's Award for International Achievements in Animal Science. Dr. Farmer is currently on the editorial board of the journals Translational Animal Science and Domestic Animal Endocrinology.

#### Abstract

Key nutritional factors to maximize mammary development and future milk yield of gilts Chantal Farmer Agriculture and Agri-Food Canada Sherbrooke R & D Centre, Sherbrooke, QC, Canada J1M 0C8

The swine industry is currently faced with a major problem. Sows do not produce enough milk to sustain optimal growth of their suckling piglets. Even though sow milk yield has increased over the years, this improvement has been more than offset by the great increase in litter size, hence, individual piglets are not getting enough milk. Milk yield is influenced by numerous factors that can be related to the sow or the piglets, and one factor of importance that could be used to enhance milk yield is mammary development. Indeed, the number of milk synthesizing cells present at the onset of lactation is a major determinant of future milk yield (Head and Williams, 1991). This paper will cover the advances made in the development of management and nutritional strategies to stimulate mammogenesis in swine.

#### Timing of mammary development

Rapid mammary development occurs at three distinctive periods in the life of pigs and it is only during those periods that it is possible to stimulate it. At birth, there is very little development of the mammary duct system and the first accumulation of mammary tissue starts at around 90 days of age, at which time the rate of accretion of mammary tissue increases four- to six-fold (Sorensen et al., 2002). The second period of important mammary development is during the last third of gestation, starting at approximately 90 days (Sorensen et al., 2002), when there is a shift in mammary gland composition going from a high lipid content to a high protein content (Ji et al., 2006). Mammary gland development continues during lactation, with the average weight of a suckled mammary gland increasing by 57% throughout lactation (Kim et al., 1999).

Mammary development is affected by parity, with the weight of mammary parenchyma (where milk synthesis takes place) at the end of gestation being greater in sows from 2nd and 3rd parity compared with gilts (Farmer et al., 2024). Mammary glands from multiparous sows are also heavier than those from primiparous sows at the end of lactation (Beyer et al., 1994), and this concords with their greater milk yield. Management of first-parity sows during lactation can affect their mammary development in the second lactation. When a teat is not suckled in parity one, its development will be significantly reduced in parity two, as well as the growth rate of the piglet suckling that teat (Farmer et al., 2012). However, suckling of a teat for only the first two days of lactation in parity one is enough to ensure that its development during the following lactation will not be hindered (Farmer et al., 2017). Therefore, should a producer want to reduce litter size of a thin primiparous sow in order not to "overload" her, it should be done at least 48 hours after the end of farrowing.

#### Endocrine control of mammary development

Puberty leads to a significant increase in mammary development (Farmer et al., 2004) due to the hormonal changes taking place, and mostly to the presence of circulating estrogens. Estrogens, that are produced by the fetoplacental unit, are also positively linked with the development of mammary tissue occurring at the end of gestation. Concentrations of estrogens in the blood of sows on day 110 of gestation are positively correlated with the amount of DNA, indicative of cell number, in mammary parenchyma (Kensinger et al., 1982). The hormone prolactin plays a key role for mammary development. Early studies showed that feeding barley ergot to late-pregnant sows diminished their mammary development and often led to agalactia. It was only later discovered that these negative effects were due to an inhibition of prolactin brought about by the ergot toxin. Prolactin is under negative control via dopamine, hence, it can be altered using pharmacological agents. Inhibition of prolactin from days 90 to 110 of gestation using the dopamine agonist bromocriptine, drastically reduced mammary development (Farmer and Petitclerc, 2003). Accordingly, when the dopamine antagonist domperidone was used to increase circulating prolactin from days 90 to 96 of gestation, alveolar volume of mammary glands was larger at the end of gestation and milk yield was improved by more than 20% the following lactation (VanKlompenberg et al., 2013). Increasing prolactin concentrations for 29 days in growing gilts (starting at 75 kg body weight), via injections of recombinant porcine prolactin, doubled the amount of mammary parenchyma present at puberty (Farmer and Palin, 2005). However, lacteal secretions were observed in treated gilts and the impact of such early lactogenesis on future milk yield is not known. The growth factor insulin-like growth factor-1 (IGF-1) also plays a role in mammary development during late gestation. When injecting recombinant porcine somatotropin daily from days 90 to 110 of gestation to increase circulating concentrations of IGF-1, mammary development of gilts was enhanced. There was 22% more parenchymal tissue, which contained more protein, DNA and RNA (indicative of metabolic activity) (Farmer and Langendijk, 2019). Nutritional strategies aiming at increasing IGF-1 therefore need to be developed.

#### **Nutritional Impact on Mammary Development**

#### Feeding before puberty

A 20% feed restriction imposed as of 90 days of age, but not before, inhibits mammary development in gilts (Farmer et al., 2004). However, recent results comparing a 10% feed restriction, 20% feed restriction, or 25% addition of dietary fiber from 90 to 190 days did not affect milk yield during the first lactation, thereby suggesting an absence of effect on mammary development (Gregory et al., 2023). This lack of effect was likely due to the greater feed intake of control gilts in the recent study (3.50 kg/day) compared with those in the older study (2.88 kg/day), whereby a 20% feed restriction led to respective feed intakes of 2.70 kg/day and 2.11 kg/day. Results therefore suggest that feeding less than 2.70 kg/day can impair mammary development. However, the exact cut-off point in feed intake below which mammary development is negatively affected is not known, and lies somewhere between 2.11 and 2.70 kg/day. The impact of diet composition before puberty on mammary development was investigated. Reducing dietary crude protein from 18.7% to 14.4% from 90 days of age until puberty did not affect mammary development of gilts (Farmer et al., 2004). Considering the beneficial impact of estrogens on mammary development at puberty, 2.3 g/day of the phytoestrogen genistein were added to the diet of gilts between 90 and 183 days of age. This treatment led to an increase in the number of mammary cells, as indicated by the concentration of DNA in parenchyma (Farmer et al., 2010). On the other hand, the estrogenic effect of flax, which contains lignans that have estrogenic properties, when fed at 10% of the diet from 88 to 212 days of age, was not important enough to positively impact mammary development of gilts at puberty (Farmer et al., 2007).

#### Feeding in gestation

Body condition affects mammary development at the end of gestation. In an early study, body composition of sows was altered by manipulating their protein and energy intakes during gestation. It was shown that overly fat animals (36 mm backfat) have much less mammary cells than leaner animals (24 mm backfat) (Head and Williams, 1991). A later study compared body conditions that are more representative of what is seen commercially. Gilts of similar backfat thicknesses at mating were fed different amounts of the same feed throughout gestation to achieve three levels of backfat thickness on day 109 of gestation (lean: 12-15 mm; medium: 17-19 mm, fat: 21-26 mm). Parenchymal tissue mass was reduced by approximately 30% in lean gilts and was not affected in the fat group, showing that being too thin at the end of gestation is detrimental for mammary development (Farmer et al., 2016a). On the other hand, when those same three body conditions were achieved at the end of gestation because these differences were already present at mating and were maintained during gestation by feeding different levels of the same feed, results differed. There was no treatment effect on parenchymal tissue mass (Farmer et al., 2016b). Feeding during gestation to attain optimal body condition is therefore more important for mammary development than body condition at mating.

Changes in feed composition during pregnancy can affect mammary development. An early study showed that feeding very high energy levels (10.5 versus 5.76 Mcal ME/day) during gestation decreased mammary parenchymal weight and parenchymal DNA on day 105 of gestation. On the other hand, increasing crude protein intake from 216 to 330 g/day did not affect mammary development (Weldon et al., 1991). The role of the essential amino acid lysine for mammogenesis taking place in late gestation was studied in more detail. Dietary SID lysine was increased by 40%, compared with estimated requirements of gestating gilts, to provide 26.0 vs 18.6 g/day from days 90 to 110 of gestation via supplementary soybean meal. Both control and treatment diets were isoenergetic and the provision of amino acids other than lysine was at or above requirements. This greater lysine intake led to a 44% increase in mammary parenchymal mass (Farmer et al., 2022). However, one must consider that the protein content of the diet and the contents of certain amino acids other than lysine were also increased with the additional soybean meal. Therefore the role of lysine per se is not clearly established. Of importance, when a similar 40% increase in dietary SID lysine (20.8 vs 14.8 g/day) was imposed to late-pregnant multiparous sows (parities 2 and 3) from days 90 to 110 of gestation, mammary development was not altered (Farmer et al., 2023). Parity therefore affects the response to additional dietary lysine (via soybean meal) and this could be due to the fact that gilts are still growing during their first pregnancy, in contrast with later pregnancies. There may also be less mammary parenchyma present at mating in the first compared with subsequent pregnancies.

In conclusion, it is evident that nutrition can be used to stimulate mammary development in swine. Current research provides important indications to that effect but much remains to be learned in order to develop the best nutritional strategy to enhance mammary development and future milk yield of gilts. The finding of an effect of parity on the nutritional requirements for mammogenesis during late gestation is novel and shows that the use of phase-feeding or top-dressing to increase mammary parenchymal mass via additional soybean meal should only be beneficial in gilts.

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### Keynote lectures **DR. MARTIN PFÜTZNER**

Session: Human resource management Lecture: Economic view on staff management

#### **Biosketch**

Grew up in Eastern part of Germany

2008-2010 Apprenticeship in Livestock Production (CAFRE in Northern Ireland) 2010-2016 MSc, Dr. Vet at University of Veterinary Medicine, Budapest 2017-2019 Postgraduate certificate in Livestock production and Animal health, RVC London Since 2020 owner and director of "Tierärztliche Praxis am Weinberg" in east Germany Since 2021 owner and director of a 4000 head sow farm in east Germany Since 2023 part of the vetxperts alliance Germany and Benelux

#### Abstract

#### **Economic View on Staff Management** Dr. Martin Pfützner

#### Introduction:

Effective staff management is essential for businesses to thrive in today's dynamic and competitive environment. It involves making strategic decisions regarding the recruitment, retention, and utilisation of human resources to achieve organisational objectives efficiently. From an economic standpoint, staff management entails optimising the allocation of labor and other resources to maximise productivity, minimise costs, and ultimately enhance profitability. This manuscript delves into the economic perspectives that underpin staff management practices, offering insights into key principles and strategies for businesses to consider in their pursuit of operational excellence.

#### Labor Market Dynamics:

The foundation of staff management lies in understanding the dynamics of the labor market. Like any other market, the labor market is governed by the forces of supply and demand. Businesses must assess the availability of skilled labor relative to their needs and adjust their recruitment strategies accordingly. In tight labor markets where qualified candidates are scarce, firms may need to offer higher wages, better benefits, or other incentives to attract and retain talent. Conversely, in a labor surplus situation, businesses may have more bargaining power and can afford to be more selective in their hiring processes. What market are we currently finding ourselves in? Let's look at a statistic from Austria. The table shows that ones a couple has children, women are far more likely to work part time then their male counterparts. At the same time, most veterinary graduates in the EU are woman. Therefor, part time allocation of staff has to be considered.



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#### Marginal Productivity:

The concept of marginal productivity plays a central role in staffing decisions. According to economic theory, firms should hire workers up to the point where the marginal revenue product of labor equals the wage rate. In other words, businesses should continue hiring additional workers as long as the value of the additional output they produce (marginal revenue product) exceeds the cost of employing them (wage rate). This principle helps firms determine the optimal level of staffing to maximise output while minimising costs, striking a balance between labor expenses and revenue generation.

#### Human Capital Investment:

Investing in human capital is related to investing in physical capital such as machinery or equipment. By providing training, education, and development opportunities for their employees, businesses can enhance the skills, knowledge, and capabilities of their workforce, ultimately leading to higher productivity and competitiveness. From an economic perspective, human capital investment yields long-term benefits by improving employee performance, reducing employee turnover, and fostering innovation and adaptability in the face of evolving market demands.

#### Incentives and Motivation:

Economic incentives play a crucial role in shaping employee behaviour and performance. Businesses can use various incentive mechanisms, such as performance-based pay, bonuses, promotions, and recognition programs, to motivate employees to work harder, smarter, and more efficiently. By aligning individual interests with organisational goals, incentives can enhance employee engagement, job satisfaction, and overall productivity. However, it is essential to design incentive systems carefully to avoid unintended consequences, such as employees opening their own clinic and taking customers or other conflicts of interest.

#### Cost-Benefit Analysis of Staffing Decisions:

Making informed staffing decisions requires conducting cost-benefit analyses to evaluate the potential impacts on organisational performance and profitability. Businesses must weigh the costs associated with hiring, training, compensating, and managing employees against the anticipated benefits, such as increased productivity, higher quality output, and greater customer satisfaction. By quantifying the expected returns on investment in human resources, firms can identify opportunities for optimisation and resource reallocation, ensuring that staffing decisions align with strategic objectives and financial constraints.

#### Efficiency Wages:

The concept of efficiency wages suggests that paying wages above the market rate can yield benefits in terms of employee motivation, retention, and productivity. When employees feel adequately compensated for their work, they are more likely to exert greater effort, exhibit higher job satisfaction, and remain loyal to their employers. Efficiency wages can also help attract and retain top talent, reduce staff turnover costs and enhance organisational reputation and brand value. While paying above-market wages may increase labor expenses in the short term, the long-term benefits in terms of improved performance and competitiveness can outweigh the costs.

#### Labor Market Regulations:

Economic policies and regulations, such as minimum wage laws, overtime regulations, and employment contracts, can significantly impact staffing decisions and labor costs for businesses. While these regulations aim to protect workers' rights and ensure fair labor practices, they can also impose compliance burdens and cost constraints on employers. Businesses must navigate labor market regulations effectively, balancing legal obligations with operational requirements and strategic objectives. Compliance with labor laws and regulations is not only a legal imperative but also a critical aspect of corporate social responsibility and reputation management.

#### Conclusion:

Effective staff management requires a nuanced understanding of economic principles and their implications for resource allocation, productivity, and profitability. By embracing concepts such as labor market dynamics, marginal productivity, human capital investment, incentives, cost-benefit analysis, efficiency wages, and labor market regulations, businesses can optimise their staffing decisions and create environments conducive to organisational success. By aligning the interests of employees with those of the organisation and fostering a culture of performance and innovation, businesses can achieve sustainable growth and competitiveness in today's dynamic business landscape. As an overall example I have attached a table with actual numbers from one of my businesses. Only the names have been changed.

It states the hours per week worked according to their contract, the overall staff costs per hour, the revenue per hour made through consultations and sales, as well as the cost to revenue ratio for a better understanding. Please note, that three of the lowest cost to revenue ratios are full time workers.

VET	Hours/week	Cost/hour	Revenue/hour	Cost to Revenue Ratio
Alex	40	28,37 €	52,48 €	1 to 1.85
lim .	40	34,85 €	63,94 €	1 to 1.83
Mark	40	34,85 €	418,70 €	1 to 12.01
Mandy	30	49,53 €	394,48 €	1 to 7.96
Maria	40	37,39 €	222,60 €	1 to 5.95
Walter	40	64,25 €	270,93 €	1 to 4.22
Ralph	40	55,55€	211,42 €	1 to 3.81
Rebecca	34	56,36 €	296,76 €	1 to 5.27
Christine	35	25,95 €	84,78 €	1 to 3.27
Jessica	30	34,66 €	115,06 €	1 to 3.32
Stephanie	40	46,90 €	332,30 €	1 to 7.09
Hugo	40	37,42 €	251,05 €	1 to 6.71
Stephen	40	30,69 €	190,97 €	1 to 6.22
Luis	30	54,68 €	115,60 €	1 to 2.11
Rachel	25	44,30 €	265,06 €	1 to 5.98
Robert	40	29,69 €	53,35 €	1 to 1.80
Larissa	20	50,50 €	230,45 €	1 to 4.56
Chris	10	29,69 €	76,83 €	1 to 2.59
Average	34,1111111111111	41,43 €	202,60 €	1 to 4,81

#### Staff Efficiency

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# Keynote lectures **DR. ESTEVÃO LOPES**

Session: Human resource management Lecture: Emotional intelligence as applied in pig farming

#### **Biosketch**

Estevao is the founder and president of Estevao Lopes Human Development, where he has trained over 5000 professionals on several topics, such as Human Behavior, Peak Performance, Sales and Leadership. Estevao is a certified trainer (SOAR GI, Febracis), international speaker, mentor and Professor at the Florida Christian University on the USA, where he also got his Master's Degree in Neuromarketing.

Senior Leader at a global company, Estevao has over 20 years of experience in managing different teams in different cultures, always focusing on teamwork, integration and improving performance. Estevao worked with different kinds and sizes of teams, leading small groups as well as large organizations with over 600 employees.



#### Abstract

#### The importance of Emotional Intelligence in the productive results of pig farming in the era of Artificial Intelligence ESTEVÃO MARTINS LOPES

We live in an increasingly technological world, where we find machines present in all sectors of the economy. We are living in the era of Artificial Intelligence, and in this digital world, the importance of human and emotional connections intensifies. Artificial Intelligence, in a cold way, helps human beings to analyze and generate data for better results. But it is Emotional Intelligence that determines what the journey will be like towards making the best decisions.

In this digital world we see technological advances in agribusiness, bringing countless benefits and making this market increasingly productive and competitive. Thanks to increased processing capacity and the advancement of Artificial Intelligence, it is possible to carry out analyzes and discoveries that in the past were very costly and required much processing time.

Today, in a matter of seconds, is possible to monitor and analyze data with a series of variables in pig farming production such as animal weight and temperature, feed consumption, and other data that can be analyzed instantaneously and in real time. This helps the producer make decisions and even anticipate and mitigate risks, thus avoiding potential problems.

Artificial Intelligence has also contributed greatly to animal well-being and health. Through speed and real-time processing capacity, it is possible to monitor the animals' behavior. Using image recognition algorithms, the system recognizes changes in the animals' behavior, thus being able to detect signs of illness or stress. With this information, producers can anticipate the necessary care before the disease worsens. It is also possible, through data analysis, to predict future events such as changes in climate, and possible outbreaks and diseases, which allows the producers to take preventative measures proactively, minimizing negative impacts.

With the contributions of all this technology, an improvement in the quality of the final product can be observed, with better optimization, waste avoidance, consistent product quality, and as a result, increased customer satisfaction. For this reason, we can conclude that Artificial Intelligence brings many benefits to pig farming, as better production processes and results are observed, with increased efficiency, ensuring the well-being of the pigs, and making the pig farming industry more efficient, profitable, and sustainable.

Despite all these gains and benefits presented, we cannot forget that behind all the technology there will always be a human

being, with feelings, thoughts, and behaviors, which also directly influence the results of pig farming. When we talk about a human being, we are talking about empathy, and this is something that machines have not yet been able to develop: the ability to identify and respond to feelings intuitively, as humans do. That is why it is important, in this era of Artificial Intelligence, to delve deeper into the understanding of Emotional Intelligence.

Before addressing the benefits of Emotional Intelligence and how it can influence the productive results of pig farming, it is important to better understand what Emotional Intelligence is. In the 1990s the term Emotional Intelligence became better known because of the work published by Dr. Daniel Goleman, a PhD in psychology from Harvard University. Dr. Goleman is known worldwide as the father of Emotional Intelligence, not because he was the creator of the subject, but because he was responsible for publicizing and popularizing it, through his book entitled "Emotional Intelligence," published in 1995. It has been translated into more than 40 languages and is one of the best-selling books in the world. In this book he explains that Emotional Intelligence is the ability to recognize, understand, and manage one's own emotions and those of others. It addresses skills such as self-awareness, self-motivation, empathy, and social skills.

As in all markets, in agribusinesses - and pig farming in particular - many challenges exist that are related to human behavior and the management of emotions. These challenges are linked to various situations, such as pressure for greater productivity, animal welfare, illnesses or injuries to animals, and uncertain political and economic scenarios, all of which can affect the emotions of producers and consequently, of their work teams.

Some scientific studies show that there is an empathetic relationship between humans and animals. Although this is a topic not vastly explored, and one that raises some debates in the field of human psychology, there are authors who defend the thesis that some animals have a feeling of justice and empathy, and act with reciprocity (Comeau-vallée et al., 2011; Rowlands, 2011).

Some studies have concluded that the pig producers with more confident animals and greater productivity, are those whose handlers enjoy working with the animals and convey empathy for them. A study carried out on the effects of positive human contact on the behavior, physiology, and reproductive performance of sows during pregnancy, resulted in a significant reduction in fear of people, thus facilitating the handling process and bringing about better productive results.

According to this study, previous positive interactions with humans may ameliorate the stress response of farm animals to aversive routine practices such as painful or stressful procedures, particularly those associated with stock workers. This study carried out the effects of positive handling on sows during the gestation period. Every day, the keeper routinely caressed the sows for 2 minutes. This action brought a reduction in resistance to the worker who performed pregnancy and vaccination tests in the stalls, showing that regular positive interaction with handlers reduces the fear that sows have of them (Hayes et al.,2021).

Positive interaction with sows and piglets on the livestock farm from birth contributes positively to their handling (Driessen et al., 2020; Tallet et al., 2018). Positive interaction is understood to be whenever the animal voluntarily approaches people, seeks spatial proximity, and shows signs that indicate a positive relationship with people (such as remaining calm and relaxed, showing signs of pleasure or playfulness). (Jean-Loup Rault et al., 2020).

Based on these and other scientific studies that show the effects of the relationship between humans and animals, and their influence directly linked to productive results in pig farming, we highlight the importance of Emotional Intelligence for generating better productive results.

According to Dr. Maria Nazaré Simões Lisboa, a renowned veterinary doctor in Brazil and PhD from the University of Murcia in Spain, companies that care about the well-being of their employees and are careful about biosafety issues, training, and that have good personnel management, have a notably better work environment. This has a direct effect on the health of the animals and, consequently, these companies present better results.

Based on the book by Dr. Daniel Goleman (2007), Emotional Intelligence can bring a series of benefits in the work environment. According to him, leaders who work on their Emotional Intelligence have more effective leadership, as they are better able to understand and manage their own emotions, as well as to identify the emotions of those whom they lead, thus allowing them to use their techniques and skills to inspire and motivate their teams more effectively. This skill also allows them to better manage conflicts among team members and make more assertive decisions.

Another important point is communication, as Emotional Intelligence helps leaders express their ideas clearly, and improves empathy, thus creating a more collaborative and open environment for more effective feedback.

Emotional Intelligence also brings benefits for conflict resolution and decision making. When professionals are able to understand and manage their emotions in situations of conflict, acting in a calmer and more rational manner, they find better solutions and reduce conflicts in the workplace. This leads to more balanced and well-founded decision-making, as the professional considers not only the logical and rational aspects, but he or she is also able to identify the emotions involved in their decisions.

Emotional Intelligence also helps professionals have a greater degree of emotional resilience when faced with challenging scenarios, and in this way, they are better able to manage stress. Someone who can identify and manage their own emotions is able to remain calm under pressure and recover quickly in the face of frustrations and adversities that occur in day-to-day work situations, adapt to changes, and are thus able to face challenges more effectively. For the most part, they are able to maintain a positive and proactive attitude, even in challenging scenarios, and become more successful professionals in their careers.

Dr. Daniel Goleman (2007) states that Emotional Intelligence contributes greatly to greater job satisfaction. People with high emotional intelligence most often feel happier and more fulfilled in the work environment, as they have the ability to build positive relationships with their colleagues. They deal more effectively with stress and find meaning and purpose in their work.

Our ponderations are also based on observations and facts collected during over 20 years working with people management, leading teams ranging from small numbers to large teams of several hundred people, with consulting and training groups in human development in different companies in Brazil, the United States, and Angola. Also on academic researches and classes at universities in Brazil and the United States.

Companies that care about the well-being of their employees and invest in their behavioral development, achieve an environment of greater productivity and quality, with greater profitability and customer satisfaction, as their employees begin to demonstrate better emotional skills such as empathy and compassion, and are more likely to interact in a positive and respectful way.

Based on this evidence from the studies presented, it is reasonable to say that human Emotional Intelligence plays a significant role in the quality of the work environment. In pig farming, a healthy work environment allows the workers to interact in a positive way, which reflects on animal well-being, thus generating better productivity.

Furthermore, professionals with developed emotional intelligence are more likely to recognize and respond appropriately to the emotional needs of animals, providing an enriched environment with less stress. This may include implementing management practices that promote the physical and psychological well-being of animals, through positive social interactions that generate empathy between the professional and the animal.

We can conclude that we must not forget the importance of human beings and how their behavior affects the overall pig farming results. They contribute to generating more productive results even in face of this highly technological era, where we see new management tools being developed with high processing power through the use of artificial intelligence and increasingly automated processes. With these new technologies on the scene and gaining more and more space, bringing productivity gains and better results, human actions can either potentialize them or even go on the opposite way, if Emotional Intelligence is not applied.

The studies and research highlighted in this article provide solid scientific evidence of the relationship between emotional intelligence and better production results in pig farming. Professionals with high emotional intelligence have greater psychological well-being, improved decision-making capacity and problem-solving skills, and more effective communication, thus promoting a healthy work environment, providing better interaction between people, which in turn directly influences the relationship between humans and animals, favoring the physical and psychological well-being of animals, ensuring better results.

Therefore, investing in behavioral development, strengthening the emotional intelligence of professionals working in pig farming can be a highly effective strategy to promote a healthier and more productive work environment, boosting the success and sustainability of companies.

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# Keynote lectures **DR. KAAT GORIS**

Session: Nutrition Lecture: Global trends in pig nutrition

#### **Biosketch**



Kaat Goris graduated in 2001 from the Veterinary School of Ghent University, Belgium. She worked at the department Anatomy & Physiology of Antwerp University for 1 year as an assistant while doing research on Lawsonia intracellularis in pigs. In 2002 she took the lead in a Salmonella project at the Flanders Animal Health Department (DGZ). From 2004 till 2014 she worked as a nutritionist and veterinarian in one of the most important Belgian feed mills (ARVESTA). During this time she completed in 2013 a two year course at the university of Ghent to obtain the degree of 'Professional Swine Veterinarian'.

Since March 2014 she joined Cargill Premix & Nutrition (CAN) as swine application specialist for the West European region. In this role she gives technical support to the WE sales teams, mainly in Benelux, Germany, Austria Italy, Spain and Greece/Cyprus. Furthermore she participates in different internal and external innovation projects, making the bridge between the field and product development.

Kaat is also a member of the IPVS Belgian Branch management committee since 2021.

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#### Abstract

#### **Global Trends in Pig Nutrition**

Brent Frederick<sup>\*1</sup>, Marc Decoux<sup>2</sup> <sup>1</sup>Cargill Animal Nutrition, Cargill Inc, Minneapolis, MN; <sup>2</sup>Cargill ANH Global Innovation Center, Velddriel, The Netherlands \*Corresponding author: brent frederick@cargill.com

#### Introduction

Global pig production continues to be important to meet the protein demands of a growing population. Feed contributes 50 to 60% of overall cost of pig production. Therefore, optimizing pig nutrition given various situations is important to the overall success and profitability of the pork producer. Advancements in genetics, health, nutrition, and greater knowledge of subsequent interactions have led to more efficient conversion of feed ingredients to pork than ever before. These past and future advancements are important to help producers in a time when the industry is facing continued need to be more efficient including labor shortages, increasing feedstuff and pork market volatility, and regulatory pressure on antimicrobials and environmental footprint.

This paper and subsequent presentation will explore current and future trends including providing the right feed to the right pig at the right time. The right feed includes increased ingredient knowledge and application of knowledge through formulation, implementation, and execution. The right pig acknowledges the advancements of genetics and health the pigs are experiencing. The right time includes meeting, and not overly exceeding, the nutrient demands of the pig at each phase of production. All the factors come together to optimize producer profitability and sustainability.

#### **Optimizing Genetic Potential**

The speed of genetic progress is impressive and keeping up with subsequent nutrient requirements is important for producer profitability and sustainability. As genetic advancements, such as genomic selection, continue to evolve so does the need to understand the pig's nutrient demands. Geneticists have notably increased the litter size of females for a highly prolific breeding herd. Additionally, percentage lean and accretion rates have continued to increase in most genetic lines including maternal and terminal lines. Furthermore, advancements have been made to understand the requirement

of nutrients during each phase of gestation and how to optimize feed intake and milk production in lactation to optimize the number of quality pigs at weaning.

The primary nutrients of interest include response to dietary energy concentration, lysine, and phosphorus requirements. These nutrient requirements have been modeled based on lean muscle accretion rates based on total dietary intake. However, feed intake in commercial situations must be accounted for to ensure proper formulation.

#### Health x Nutrition

One of the newest advancements in the quest for knowledge is around health x nutrition. Interactions and collaborative problem solving is becoming increasingly common amongst veterinarians and nutritionists. As antibiotic restrictions and environmental regulation mount, such as limited dietary zinc levels, alternative solutions are needed to support pig health and performance. Nutritional modifications may not prevent infection. However, research determining how nutritional modifications may reduce the severity of infection is becoming increasingly important.

Data suggests that pigs may, in fact, have a requirement for fiber. Fiber can be categorized into structural or fermentable fiber. Structural fiber is involved in peristalsis of the intestine, water holding capacity and promoting satiety through increased gut fill and distention. Fermentable fiber, as the name implies, is fermented by microbial populations. Increasing dietary structural fiber has been shown to improve performance and livability of nursery pigs (Ebarb et al, 2021). The proper balance of these fibers is important for optimal performance in relatively health and challenged pigs (May et al, 2022).

For enteric challenges, ingredient knowledge and subsequent formulation can lessen severity. Specifically, the amount of dietary fermentable protein is important. Excess indigestible protein enters the hindgut and is fermented. Increased protein fermentation products have been associated with toxic and proinflammatory impacts on the intestinal epithelium (Pieper et al., 2012) leading to disturbing development of the mucosa of the intestine (Visek, 1984) and reducing villi length (Nousiainen, 1991). Reducing fermentable protein by as little as 10 to 20% has been shown to reduce stool quality and reduce removals (Faris et al, 2021). This reduction can be accomplished in multiple ways including overall crude protein reduction and/or ingredient selection. Deep ingredient knowledge is important to optimize pig performance.

Some additives can be useful tools to supplement even a well formulated diet. Pre-, pro- and post-biotics work to enhance the microbiome to a more preferential population. Furthermore, phytogenics have been shown to improve mucosa protection subsequently preventing pathogen attachment, reduce bacterial pathogenicity through quorum sensing inhibition, and have anti-oxidative and anti-inflammatory effects (Zeng et al, 2015). A recent example of a phytogenic combination resulted in improved growth performance and reduced mortality in E. coli-challenged pigs (Gonzalez-Vega, et al., 2023). More research will continue in this area to provide both modes of action validation and applicable outcomes in research and as importantly field conditions.

#### **Precision Feeding**

Technology is advancing in rapid ingredient evaluation. NIR is one such technology used to monitor feedstuff nutrient values more quickly and economically. Bench top NIR has been used for years and further application is being pursued with handheld and in-line NIR. These technologies are becoming more widely adopted to reduce the need for wide safety margins on ingredients ultimately leading to more predictable pig performance and economical diets. This concept of precision feeding is important regardless of the phase of production.

Electronic sow feeding systems are already widely adopted, with the expansion of group housing and the shortage of labor in sow farms, although their potential for precision feeding remains mostly untapped. Gaillard and Dourmad (2022) shown that feeding gestating sows individually with a feed adjusted daily on their requirements for net energy and amino acids could keep the same reproductive performance while reducing nitrogen excretion by 18.5% and reducing feed cost by 3.7%. when compared to sow fed a constant feed composition during the entire gestation. In growing pigs, Pomar and Remus 2019 shown that electronic precision feeding adjusting nutrient supply daily to each growing pig could reduce production costs (>8%), protein and phosphorous (40%), and greenhouse gases emissions (6%) when compared to a conventional approach.

Application on large scale is still limited due to investments and technology application in barns. Larger systems are focused on reducing site to site and barn to barn variation before going down to an individual pig basis. Thus, feed bin sensors are becoming more popular worldwide. This solves for feed disappearance knowledge. Another trend is the interest in machine vision to estimate pig bodyweight. This allows the producer to ensure that the feed disappearance is converted into pork. Potential application of this information may include environmental health interventions, feed budget and/or formulation changes. Several companies are working on technologies that work consistently in farm environments and are economically attractive.

#### **Formulation Trends**

The cost of energy globally continues to rise. Therefore, feeding higher energy diets through addition of dietary oils and/or fats are becoming less common even in summer months when heat stress and carcass prices are high. Consequently,

greater emphasis is placed on getting the most out of readily available, economical ingredients. Heighten awareness and focus is being placed on feed processing including particle size reduction of grains and pelleting quality pending feed form.

Potential alternative ingredients continued to be leveraged in efforts to reduce overall feed costs. By-product streams from sources such as bakeries, dairy products, and renewable energy production continued to be examined for more precise feeding values. Efforts to find alternative fuels such as bio-renewable diesel may change the feeding value of soybean meal.

Exogenous enzyme knowledge continues to advance as well. This technology can be used to enhance commonly used ingredients as well as alternative ingredients. Phytase continues to be used extensively to release phosphorus, however, more recently "super" or "mega" dosing concepts are being explored and used to release more nutrient value from existing, formulated ingredients. Xylanase is becoming more widely used and has been shown to have an impact on overall survivability rates in finishing pigs. Other enzymes, such as xylanase, continue to be researched for application within pig production even in corn, soybean meal-based diets. The concept of extracting more value out of commonly available, economical ingredients is a very attractive concept into the future.

Ractopamine, a  $\beta$  adrenoreceptor agonist has been used as a reliable compound to increase percent lean growth deposition with great success. Less ractopamine is currently being used and thus alternatives are being investigated to improve growth rate and/or feed efficiency during the finishing period. Ionophore(s) have been identified as one solution. Specific phytogenics have also been able to provide some performance improvements during this growing period and continues to be explored.

#### Pork sustainability, beyond efficiency

Modern pig systems must control their impact on the environment, public health as well as on human and animal welfare, and build resilience against climate and economic crisis. Improving feed efficiency has been the main way to improve pork environment footprint. As a result, the emissions of greenhouse gases, nitrogen, and phosphorus per kg of pork meat have been reduced by nearly one third over the last 3 decades. However, pork is the second source of meat protein in human food, already representing 3% of total greenhouse gas emissions. By 2031, its production should increase by 11% to reach 129 million tons cwt (OECD FAO, 2022). Feed would represent around 65% of the total GHG emissions from pork. New feeding strategies are needed to support the growth of the pork industry in a sustainable way.

The first trend is to account for the environmental footprint of feedstuff in diet formulation, considering effects on climate change, land use, water use, acidification, eutrophication and air quality. It is crucial to assess the metrics in a reliable and fair way, recognized across the global pork and food industry. Various initiatives, such as the Global Feed LCA Institute (GFLI) or the Product Environmental Footprint Category Rules (PEFCR) are establishing standard methods and coefficients. Calculating these metrics requires to carry more data along the pork value chain and is supported by improved traceability systems from feedstuff and feed producers.

This approach creates a higher incentive to valorize side streams and waste from across the agriculture and food industry that could be upcycled as feedstuff in pig diets, moving toward a pork circular economy. Promising examples are microbial cell protein produced out of methane and CO2 (Hedemann et al., 2023), or oil and proteins from insects grown on food waste substrate (Hong and Yong Kim., 2022).

Demand of fresh water is expected to outstrip supply by 40% by the end of this decade, according to the Global Commission on the Economics of Water. The cost and availability of drinking water will also become a bigger issue for pork producers. Adapting nutrition could help reduce the amount of water needed by kg pork produced. The first approach is to minimize the amount of fresh water needed to produce pig feed, through a lifecycle analysis (Misra et al., 2023). Nutrition can also affect pig's intake of drinking water /kg feed, by reducing the elimination of surplus nutrients via the urine. Limiting dietary crude protein by using precision feeding, synthetic amino acids or increasing protein digestibility with enzymes is promising. Controlling the dietary level of minerals such as potassium may have an impact too (Shaw et al., 2006).

Finally, animal welfare has become a major societal and ethical consideration. The expansion of group housing, and potentially free farrowing crates, has driven nutritionists to better understand how this affects pig requirements and behavior, and to adapt feeding programs accordingly. The use of fermentable and structural fibers helps to control satiety and behavior in group housed sows (De Leeuw et al., 2008). Diversified physical presentations of neonatal feed helps suckling piglets to express rooting and playing behavior, that translates into a better adaptation to stress at weaning and less lesions due to biting (Middelkoop et al., 2019) In grower pigs, altering the amino acid supply (Minussi et al., 2023), as well as supplementing feed with specific phytogenic compounds (Nicolas Jorrilo et al., 2023) helps preventing aggressiveness.

#### Conclusion

Greater precision and accuracy of nutrition and feeding is a common theme weaved into the current and future trends within pig production for the sake of profitability and sustainability. The trend to leverage existing technology better and emerging technologies more quickly will be a competitive advantage in the future. Interdisciplinary collaboration between production operations, genetics, health, and nutrition will become increasingly important in the future to producers to meet the various demands of customers, consumers, government regulations, and overall sustainability.

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#### IPVS & ESPHM 2024

### Keynote lectures **PROF. STEPHAN SCHNEIDER**

Session: Nutrition Lecture: Alternative feed sources and feeding strategies

#### **Biosketch**

After training as a farmer, Stephan Schneider studied agriculture at the Weihenstephan University of Applied Sciences. He then worked in the mineral feed industry. He received his doctorate from the Technical University of Munich in 2012 and then worked for 9 years in the field of pig feeding at the Technical Centre for Pig Breeding and Husbandry in Upper Bavaria and as Head of the Pig Feeding Department at the Bavarian State Institute for Agriculture. In 2021, he became Professor of Animal Nutrition at the Nürtingen-Geislingen University of Applied Sciences. There he also manages the two experimental farms and the feed laboratory. He is active in numerous committees in the field of livestock feeding and the associated emissions. His research is currently focused on the climate impact of milk production, the overall environmental impact of livestock farming and mobile housing in the poultry sector. He is regularly invited to speak at congresses and training events organised by companies and farmers.

#### Abstract

#### Alternative feed sources and feeding strategies

As the world's population and demand for edible protein per person increases, the world's pig meat production will also grow (OECD/FAO, 2023). Whereas in the past pig feeding was dominated by co-products of human food and leftovers, nowadays feed rations are often corn and wheat based (Woyengo et al., 2014.). Cereals are supplemented by soybean meal, synthetic amino acids and other feed additives like enzymes. Availability of cereals and soy products will decrease due to human food competition and scarcity of agricultural land which is, alongside climate change, one of the greatest future challenges for humanity (Smith et al., 2010). Due to planetary boundaries, the feeding of human-edible biomass to farm animals must be critically questioned. Already today, several products like fish meal, processed animal proteins, milk by-products, rapeseed meal and canola meal are used to deliver amino acids in diets for pigs (Lestingi, 2024). Alternative feed sources and feeding strategies will be necessary, and pigs as omnivores are ideally suited to convert non-human-edible co-products into high-quality food animal protein (Woyengo et al., 2014). Biomass resulting from agricultural production is often unsuitable for human consumption due to its high fiber and cellulose content (Vorndran et al., 2023). This non-human-edible biomass can be a source of feed for well-known (pig, cattle, poultry) or new farm animals like insects.

New food resources ("novel food") should have a high nutritional value and feed conversion efficiency, but also be able to provide high-quality animal products, using land and water efficiently (Poppi and McLennan, 2010). A lot of different plant and animal-based feed, for example edible insects, seaweeds and microalgae, mussels, refined plant products or leaf protein concentrates are being researched and partially already in use. Hence feed costs have a significant influence on the profitability of pork production, the economy of the different feedstuff is fundamentally and has always to be considered.

#### Edible insects (entomophagy)

Edible insects are receiving attention as an alternative sustainable nutritional source for both humans and animals, thanks to the advantage that they may be produced without the use of arable land (Lestingi, 2024). Insects use less water than plant-based products and larvae of insects are rich in protein and fat. For production in larger scale protein for animal diets, yellow mealworm (Tenebrio molitor), black soldier fly (Hermetia illucens), and the common housefly (Musca domestica) are the insect species which are possible. Some insects, such as the black soldier fly, are able to convert low-value organic waste into high-value protein, that could replace soybean meal and fishmeal. When using organic side streams as substrate, chemical and biological contaminants need to be considered. In Europe, yet only seven insects (4 as larvae and 3 as adult animals) are allowed to be fed as processed animal protein to pigs, poultry and fish. Furthermore, it is forbidden to feed waste or excrements to these insects. Feed, which is fed to the insects, could be directly fed to farm animals, without double transformation losses for the later human nutrition. Makkar et al. (2014) reported that insects may have an essential

amino acid composition that matches the requirements of growing pigs and broiler chickens. Indeed, insects contain high quantities of lysine, threonine and methionine, the main limiting essential amino acids in low-protein diets based on cereals and legumes for pigs. But it must be noted that, the nutritional value of insects depends on the insect species and is influenced by numerous factors like diet, stage harvested and environmental factors. The hope of organic pig producers that the methionine gap of pig nutrition could be closed by insects did not come true hence the methionine content is only on the level of fish meal. Hong et al. (2020) concluded that the amount of yellow mealworm up to 6% in weaning pigs' diets and 10% in those of growing pigs, could be used as a protein source, without negative effects or even with improved growth performances, as well as amino acid digestibility, compared to conventional protein sources. As well as being an alternative protein source, insects are also rich in fat, especially full-fat black soldier fly larvae. They and can be a source of energy in pig diets (Crosbie et al., 2020). Hong et al. (2020) further predict that the use of edible insects in animal feed will increase in the future.

#### Seaweed and Microalgae

In recent times, seaweeds and microalgae are intensive discussed due to the high protein, carbohydrate, and fat content. Comparable to other high-quality plant-based feedstuffs, such as soybean meal, some of them are even superior. Spirulina (Arthrospira platensis), the most often used microalgae, has emerged as a potential cost-effective, sustainable, and high-nutritional-value food resource for many animal species (Holman and Malau-Aduli, 2013). In fact, spirulina is not a microalga, but a bacterium. In addition to its content of raw nutrients and amino acids (Table 1), spirulina also contains vitamins, minerals and trace elements as well as chlorophyll. Spirulina production has a high efficiency in land use. However, if spirulina is fed to pigs in feeding trials, different results were obtained. For example, the growth performance of piglets was decreased by 9.1% compared to soybean meal-based diets when spirulina was used as protein ingredient in post-weaning piglet diets at an inclusion level of up to 10% (Martins et al., 2021). In addition, the feed conversion rate deteriorated and total tract apparent digestibility of nutrients was lower. The reason for the inconsistent results in feeding trials could be the different nutrient content of spirulina or the different experimental designs (Grinstead et al., 2000). The effects of spirulina with regard to its antibiotic effect, limiting intestinal damage caused by immune and inflammatory responses to dietary transition and oxidative stress are currently discussed. Current research focuses on cell wall disruption and the use as diet supplement, primarily to influence gut health.

Due to the enormous variability of the ingredients (Table 1), the above-mentioned products – edible insects and seaweed/ microalgae - have to be standardized before they can be widely used in animal nutrition.

Composition	Black soldier fly larvae, fat < 20%, dried	Black soldier fly larvae, fat > 20%, dried	Spirulina platensis, dried	Soybean meal, oil < 5%
Dry matter	92	92	94	88
Crude protein	41-71	35-48	56-66	44-55
Crude fat	5-21	22-49	4-15	1-5
Crude fibre	13-18	7-11	3-7	5-11
Crude ash	5-25	3-28	3-10	6-10
Lysine	1.7-3.6	1.5-3.4	2.4-4.3	2.7-3.4
Methionine	0.8-1.3	0.3-0.9	1.2-2.6	0.6-0.9

Table 1: Chemical composition of different products of Black soldier fly, Spirulina platensis and conventional soybean meal (% of dry matter)

INRA-CIRAD-AFZ Feed tables, Lestingi et al. 2024, adjusted. CP, crude protein.

The feed value of novel food has to be precisely described and the life cycle assessment has to be thoroughly checked beforehand. The use of these products is ultimately determined by their price, whereby the value for money of the two products, at least in Europe, is currently not given in pig feeding.

#### Feeding strategies

In terms of feeding strategies, there will be a high input-high output strategy accomplished through precision livestock farming based on sustainable intensification and maximization of animal protein production efficiency, both on a limited land surface together with a minimization of environmental impacts. The other side is a reduced input-reduced output strategy based on selecting animals that are more robust to climate change and are better adapted to transform low quality feed (local feeds, co-products, food waste) into meat.

#### Amino Acids and other feed additives

The simplest way to reduce environmental effects of pig production is to improve digestibility and reduce crude protein content by adding synthetic amino acids to the diet. The use of enzymes such as glucanase and xylanase must be further optimized - depending on the ration components of the diet - in order to optimize the nutrient supply for the pigs (Zijlstra et al., 2010). Since plant-based feedstuffs often have a high proportion of phosphorus (P) in phytate that is poorly digested by pigs, adding supplemental phytase can increase P digestibility and the nutritive value of these feedstuffs (Selle and

Ravindran, 2008). In addition, the scarce resource P can be preserved.

In feeding trials, up to eight synthetic amino acids were added successfully to a low-protein diet for pigs to cover the animal's need for small intestine digestible amino acids (Minussi et al., 2023). However, practice has not yet reached this stage. Although there are individual feeding concepts with up to six synthetic amino acids in grow-finish pigs, feeding concepts with higher crude protein contents and only three amino acids can often be found in practice. One reason for the hesitant implementation of modern amino-acid-rich rations in practice is the variation in weight, growth rate, and feed intake within a group (Menegat et al., 2020) and lack of economic viability of the fifth, six and seventh limiting amino acids.

#### Phase feeding

Phase feeding is a basic need to meet pig's nutrient requirements since nutrient requirement changes concomitant with age and weight. Multi-phase feeding can provide advantages with respect to economic, environmental, animal health and welfare issues. On commercial pig farms, the logistics of manufacture, delivery and storage for multiple dietary phases is often not easy. Thus, simplification of phase feeding strategies to lesser dietary phases in the grow-finish period is a topic of growing interest (Menegat et al., 2020). However, this is the wrong approach, because with the help of modern feeding technologies, intelligent multi-phase feeding concepts can be practiced even with just a few silos and feed mixtures, for instance by continuous blending of initial fattening and final fattening feed.

#### Conclusions

There are a lot of alternative feed sources and feeding strategies in pig nutrition. Edible insects and microalgae are interesting feedstuffs, but the variety of ingredients must be reduced and the economy increased. The use of novel feedstuffs in pig diets must be optimized following by a characterization of their energy and amino acid profile. Laboratory analysis will therefore play a central role in the implementation of precision livestock feeding with novel food. Concerning insects, politicians must adapt the framework conditions once all questions of biosafety, zoonoses, etc. have been clarified. Without proven economic viability, however, these feedstuffs will not be widely used in practice. Also, human food waste must, in terms of sustainability, be returned to the pig food chain after appropriate treatment, which would contribute to make pork production more sustainable. Finally, current feedstuff (cereals and soybean meal) will be limited in the future due to competition for increasing human protein need.

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## Keynote lectures **DR. MIGUEL ANGEL HIGUERA**

Session: Welfare and ethology Lecture: Global consequences of porcine animal welfare recommendation in the terrestrial code

#### **Biosketch**

Degree in Veterinary Medicine from the Faculty of Veterinary Medicine, UCM. 1995 – 2000 Director of ANPROGAPOR. National Association of Pig Producers. Associate Professor. Faculty of Veterinary Science Alfonso X El Sabio. Madrid

Professional experience: 2000-2004. Kubus, S.A. Veterinary Technician and R&D development in swine reproduction. 2004-2007. Proinserga. Veterinary technician in the production area and Head of the Insemination Centres.

2007-2010. Technical Director in ANPS (National Association of Select Pig Breeders). 2010-2011. Assistant to the Director in ANPROGAPOR. 2012-present. Director of ANPROGAPOR

#### Current positions:

Chairman of the Animal Health and Welfare Working Group of COPA COGECA (Brussels). Vice-Chairman of the Pigmeat Working Group of COPA COGECA (Brussels). Coordinator Pig Group of FESASS – European Federation for Animal Health and Animal Health Safety Member of UECBV – European Federation for Livestock and Meat Trade. European Commission. DG Agri. Expert of the Pigmeat Forecast Group. European Commission. DG Agri. Expert of the Pigmeat Advisory Group European Commission. DG Sante. Animal welfare expert. European Platform for Animal Welfare. European Commission. DG Sante. Animal welfare expert. European Platform for AMR European Commission. DG Sante. Animal welfare expert. European Platform for AMR European Commission. DG Sante. Animal welfare expert. Hig welfare subgroup European Commission. DG Sante. Animal welfare expert. Pig welfare subgroup

Speeches in congresses: More than 50 presentations at national and international congresses. More than 300 papers in livestock/meat conferences.

Publications More than 100 publications in journals in Spain, Portugal and Latin America.

#### Abstract

#### GLOBAL CONSEQUENCES OF PORCINE ANIMAL WELFARE RECOMMENDATION IN THE TERRESTRIAL CODE Miguel Angel Higuera

#### 1.- Introduction

The World Organisation for Animal Health, among others, has created the Animal Health Codes (terrestrial and aquatic) with the aim of improving animal health and welfare as well as protecting public health (avoiding zoonosis) while establishing common standards for safe international trade in terrestrial and aquatic animals.

It was in 2004 that WOAH started to include animal welfare aspects in the Terrestrial Code, in the form of Annexes. From that date onwards, changes have been introduced on a regular basis until a separate section was created within the Code. At species level, it was in 2012 that the chapter "Animal welfare and beef cattle production systems" was introduced and since then different species have been introduced in section 7 "Animal welfare". Chapter 7.13: "Animal welfare and pig production systems" was adopted in 2018 and amended in 2019.

Currently, Section 7 of the WOAH Terrestrial Code contains the following chapters on animal welfare:

- 1.- Introduction to the recommendations for animal welfare
- 2.- Transport of animals by sea
- 3.- Transport of animals by land
- 4.- Transport of animals by air
- 5.- Slaughter of animals
- 6.- Killing of animals for disease control purposes
- 7.- Dog population management
- 8.- Use of animals in research and education
- 9.- Animal welfare and beef cattle production systems
- 10.- Animal welfare and broiler chicken production systems
- 11.- Animal welfare and dairy cattle production systems
- 12.- Welfare of working equids
- 13.- Animal welfare and pig production systems
- 14.- Killing of reptiles for their skins, meat, and other products

#### General considerations

Animal welfare means the physical and mental state of an animal in relation to the conditions in which it lives and dies. An animal experiences good welfare if the animal is healthy, comfortable, well nourished, safe, is not suffering from unpleasant states such as pain, fear, and distress, and is able to express behaviors that are important for its physical and mental state.

Good animal welfare requires disease prevention and appropriate veterinary care, shelter, management and nutrition, a stimulating and safe environment, humane handling, and humane slaughter or killing. While animal welfare refers to the state of the animal, the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment.

General principles for the welfare of animals in livestock production systems

1) Genetic selection should always consider the health and welfare of animals.

2) Animals chosen for introduction into new environments should be suited to the local climate and able to adapt to local diseases, parasites, and nutrition.

3) The physical environment, including the substrate (walking surface, resting surface, etc.), should be suited to the species to minimize risk of injury and transmission of diseases or parasites to animals.

4) The physical environment should allow comfortable resting, safe and comfortable movement including normal postural changes, and the opportunity to perform types of natural behavior that animals are motivated to perform.

5) Social grouping of animals should be managed to allow positive social behavior and minimize injury, distress and chronic fear.

6) For housed animals, air quality, temperature and humidity should support good animal health and not be aversive. Where extreme conditions occur, animals should not be prevented from using their natural methods of thermo-regulation.

7) Animals should have access to sufficient feed and water, suited to the animals' age and needs, to maintain normal health and productivity and to prevent prolonged hunger, thirst, malnutrition, or dehydration.

#### 2.- Animal Welfare and pig production systems

Commercial pig production systems focus on breeding, rearing, and managing pigs for meat production, emphasizing welfare at both farm and handler levels. Management includes training and best practices in housing, with protocols to enhance welfare. Environmental enrichment is critical, aimed at promoting natural behaviors and mental health by adding complexity like foraging opportunities. The chapter discusses behavioral issues such as stereotypy, apathy, and agonistic behaviors, which are influenced by environmental stress and management practices. Additionally, it highlights the importance of play behavior in helping pigs cope with unexpected situations, enhancing their adaptability and mental state.

It is necessary to look for a set of indicators that can establish the procedure for the analysis of individual animal welfare and in a specific way criteria based on direct measurement of the animal may be more interesting. To establish both criteria and thresholds for these criteria, the different situations in which pigs are managed, health status, regional differences, breed, etc. have to be taken into account. With these mainly animal-based criteria, the effectiveness of both farm design and animal management itself can be analysed.

#### 2.1.- Behaviour.

There are behaviours that may indicate a good state of emotional health such as play or positive interaction, but there are other behaviours that show the existence of behavioural problems such as escape attempts, altered feeding and resting

times, loud vocalisations, negative interactions (aggression) and stereotypies, among others. Stereotypies may be the result of abnormal behaviour originating from a usually chronic frustration and often indicate a current for the animal or a problem. As with other indicators, care should be taken when using stereotypies as a measure of welfare in isolation from other indicators.

#### 2.2.- Morbidity Rates

It is necessary to establish appropriate thresholds for certain infectious or metabolic diseases, lameness, injuries, etc. in order to identify whether, if these thresholds are exceeded, the welfare of the group may be compromised.

#### 2.3.- Mortality and culling rates

Mortality and culling rates should also be indicators of welfare on a farm. It is therefore necessary to keep appropriate records, determine thresholds and establish corrective measures if these thresholds are exceeded.

#### 2.4.- Changes in body weight and body condition

If an animal is not growing as fast as it should, this can be a good indicator of animal welfare. In addition, body condition is usually related to one of the five freedoms: "freedom from hunger". Correct feed management in terms of quantity, quality and composition ensures optimal nutritional animal welfare of the animal.

#### 2.5.- Reproductive efficiency

Reproductive efficiency can be another good indicator since it is easily measurable and targets can be set such as: conception rate, fertility at calving, abortions, metritis, litter size, number of live births...

#### 2.6.- Physical appearance

Through a general review of the animal's condition, it is possible to determine the presence or absence of problems that affect the individual animal's welfare such as: body condition, presence of ectoparasites, hair loss, excessive dirt in the faeces, skin discolouration, wounds, secretions, abnormalities in the legs, abnormal postures, dehydration...

#### 2.7.- Response to handling

Fear or distress in pigs can be caused by inadequate human handling. To determine this, various tests can be carried out, such as the approach or flight test in the presence of humans or excessive vocalisations when handling the animals. Faulty handling can lead to disproportionate flight behaviour which can lead to mishaps such as slipping or bumping which can lead to injuries or fractures.

#### 2.8.- Lameness

Lameness or musculoskeletal problems can have many causes ranging from physical, nutritional, infectious, or handling. The result is an animal with displacement problems which causes the animal to develop abnormal social behaviour and have difficulty reaching water or feed, with most grade lameness resulting in the need to euthanise the individual.

#### 2.9.- Complications arising from common procedures.

There are several widespread procedures that can lead to complications such as surgical castration, teeth clipping or tail docking, identification, nose ringing, etc. These procedures must be carried out by trained professionals and in a careful manner so as not to significantly harm the animal. Many of these procedures, if not carried out under the appropriate treatment, can cause pain that evolves into fear, leading to behavioural problems and stereotypies.

#### 3. Recomendations

#### 3.1.- Training of personnel

Pigs should be cared for by a sufficient number of people who collectively possess the necessary skills, knowledge and competence to maintain the welfare and health of the animals.

Persons in charge of the animals must be properly trained in the different competencies not only to be able to perform their daily work but also to provide correct treatment of the animals as well as early detection of any problems that may arise and to apply the correct measures.

#### 3.2.- Handiling and inspection

Animal handlers should be aware of the positive attitudes towards the animal in their daily interactions. All animals should be checked every day and some animals due to their physiological condition (pregnant or pre-farrowing sows) should be checked more frequently as well as sick animals.

#### 3.3.- Painful procedures

There are a number of procedures that can be performed on pigs such as: surgical castration, tail docking, teeth clipping or griding, identification, nose ringing. All these procedures should be carried out by trained persons. In addition, wherever possible, the welfare of the animals must be safeguarded by ensuring the safety of the operators. These procedures are painful or potentially painful and must be carried out in a way that reduces suffering. The 3 Rs rule is used: Replacement (e.g. immunocastration by surgical castration), Reduction (e.g. tail docking only when necessary), Refinement (e.g. providing anaesthesia or analgesia for some procedures).

3.4.- Provision of feed and water

Feed intake will depend on many factors such as climate, nutrient composition, quality of raw materials, age, sex, gender, genetics, physiological state... Pigs must receive an adequate amount of feed and nutrients in such a way that the animal can:

- Maintain good health

- Meet its physiological requirements

- Meet its behavioural requirements Including a percentage of fibre in the pigs' ration is necessary to avoid the occurrence of gastric ulcers.

It is also necessary that all pigs have access to clean, contaminant-free water To determine the degree of feed compliance, animal-based measures can be used as an analysis: body condition, physical appearance, behaviour, degree of culling, and mortality.

#### 3.5.- Environmental enrichment

Pigs are very curious animals that need environmental stimulation to develop correct behaviour. Positive behaviours include: exploration, rooting, chewing manipulable materials, social interaction... on the other hand there are negative behaviours such as: tail and ear biting, sham chewing, apathetic behaviour... Environmental enrichment can be achieved by: - Providing sufficient manipulable material to satisfy their exploration and research needs.

- Positive social interaction. Keeping pigs in stable groups

- Positive human interaction.

Regular contact with humans with positive interactions: petting, scratching...) To analyse environmental enrichment, absence of wounds, behaviour, changes in body condition, response to humans, reproductive efficiency, lameness, mortality and culling rates can be checked.

3.6. Housing

Animal housing should be designed with the behaviour and physiological needs of the animals in mind. It should also be designed and constructed to reduce the risk of injuries. It is important to have sufficient space to isolate and care for sick animals.

The floor must be designed to avoid slipperiness.

#### 3.7.- Space allowance

Pigs need different areas for lying, standing, feeding and elimination. These areas have to be taking mind in the design of the pen

- Group housing. Pigs are social animals and require group housing. It is necessary to determine the correct density according to the type, weight of the animal as well as external factors such as temperature, humidity. It is necessary that all pigs can lie down at the same time and that there is enough space to be able to access water and food at all times.

- Individual pens. They should only be used if necessary and should have sufficient space to be able to turn around and turn around effortlessly

- Stalls and crates. They should be avoided. When used, they should meet the following requirements: o

-In stand-up position, they should not touch the sides

-In stand-up position, they should not touch the top

- In stand-up position, they should not touch the ends of the cage

-They should be able to lie comfortably on either side of the cage.

Proper ventilation is necessary to avoid the concentration of both gases and particles. Particularly important are CO2 and NH4, which can also serve as indicators of environmental quality.

The composition of the air will depend on the type of housing, density, type of soil, material to be handled, slurry handling and ventilation.

#### 3.9.- Confort térmico

Pigs can adapt to a range of temperatures but both feeding, housing and housing design can help them. Pigs are sensitive to both heat stress and cold stress.

- Heat stress. It can cause serious health problems for the animals. To avoid this, proper housing design and environmental control is necessary to reduce the indoor temperature compared to the outdoor temperature. Sudden changes in temperature are detrimental to the animals, so it is necessary to have a thermal control system in place: automatic ventilation or cooling.

- Cold stress. This is especially important in young or sick animals. To achieve a correct protection against the cold it is necessary to have a correct installation, use bedding or apply heating.

#### 3.10.- Mixing

Pigs are social but hierarchical so that mixing of animals leads to fights to establish dominance. To avoid excessive fighting you can:

- Provide additional space and non-slip flooring
- Feed prior to mixing
- Apply feed on the floor at the mixing site
- Apply straw or manipulable material
- Provide opportunities for escape
- Mix animals at the earliest possible stage
- Avoid incorporating small numbers of animals into established groups
- 3.11.- Biosecurity and animal health (disease prevention)

Animal health and its prevention (including biosecurity) is one of the aspects that most affects animal welfare. It is recommended that all farms have a biosecurity plan that can also be analysed and monitored. In health plans and biosecurity plans, the figure of the veterinarian is essential since he/she must be the professional responsible for their design and execution to ensure the health of the animals with the principle of "prevention is better than cure" where biosecurity plays an essential role.

#### 3.- International implementation

From the comparation of international legislation on animal welfare is needed use the International animal protection index for World Animal Protection organisation. With index WAP organisation makes a comparative between 50 countries around the world according to the animal welfare policy and legislation

European Union may have one of the most advanced animal welfare regulations for pigs in the world. Through Directive 120/2008, high standards have been implemented. Council Directive 2008/120/EC lays down minimum standards for the protection of pigs. A key provision is Article 3, which prohibits the tethering of sows or gilt (female pig after puberty and before farrowing) and the use of sow stalls. The ban of individual sow stalls was decided in 2001 and a phase-out period of 12 years was allowed, to permit farmers to adapt to the new systems. From 1 January 2013, sows have had to be kept in groups rather than in individual stalls. However, stalls may still be used for the first 28 days of gestation, and one week before the expected time of birth (Article 3.4).

Other key provisions include stipulations that:

• Pigs must be given a sufficient quantity of bulky or high fibre food as well as high-energy food, in order to satisfy their hunger and need to chew (Article 3.7)

• Pigs must have permanent access to manipulable material (Article 3.5) and 'sufficient material to enable proper investigation and manipulation activities, such as straw, hay, wood, sawdust, mushroom compost, peat of a mixture of such', (i.e. enrichment materials) so that they may have the opportunity to express natural rooting, chewing and investigating behaviour (Annex I, Chapter 1, paragraph 4).

• Pregnant sows must have at least 1.3 square metres per sow of continuous solid floor; the use of completely slatted floors for pregnant sows is not permitted (Article 3.2(a)). Article 3.2 includes makes further provisions relating to the provision of slatted flooring.

• Pigs must be reared in conditions that comply with the provisions set out in the Annex to the Directive (Article 4), which

sets out further detailed provisions.

• Tail-docking and reduction of corner teeth must not be carried out routinely 'but only where there is evidence that injuries to sows' teats or to other pigs' ears or tails have occurred'. The Pigs Directive further stipulates that before carrying out these procedures, 'other measures shall be taken to prevent tail-biting and other vices, taking into account environment and stocking densities.' This is an important provision to address inadequate environmental conditions, including lack of enrichment materials, which may lead to behaviours such as biting which occur when normal behaviour patterns are frustrated (Annex I, Chapter 1, paragraph 8).

• No piglets shall be weaned from the sow at less than 28 days of age, though piglets may be weaned up to seven days earlier if they are moved into 'specialised housings' (Annex I, Chapter 2, paragraph C3).

'All procedures intended as an intervention carried out for other than therapeutic or diagnostic purposes or for the identification of the pigs in accordance with relevant legislation and resulting in damage to or the loss of a sensitive part of the body, or the alteration of bone structure, shall be prohibited'. However, there are exemptions to this general prohibitions for teeth grinding or clipping (before seven days old), tail-docking (as above), castration of male pigs by other means than tearing of tissues (which is prohibited) and nose-ripping only when the animals are kept in outdoor husbandry systems and in compliance with national legislation (Annex I, Chapter 1). In relation to castration of pigs, there is a requirement to use anaesthetic for castration, if the procedure is carried out after the 7th day of life. Otherwise, no analgesia is required by the Pigs Directive. In the UK and Ireland, pigs are not routinely castrated without anaesthesia or pain relief, but this does occur in other EU Member States where pigs are surgically castrated without anaesthesia, which invariably involves tearing of the tissues. Consequently, the European Commission drew up the European Declaration on Alternatives to Surgical Castration of Pigs, which requires signatory stakeholders to only perform surgical castration with prolonged analgesia and/ or anaesthetic and that surgical castration should be abandoned by 1 January 2018.

Article 12 of the Pigs Directive provides that Member States may apply, within their territories, stricter provisions for the protection of pigs than the ones laid down in the Directive.

Similar legislation is apply in U.K. as former member of UE until Brexit.

In U.S.A. There is no ban at the federal level on the use of sow cages. But there are advances in several states: In 2002, Florida introduced the first state law to restrict a production practice on animal welfare grounds by prohibiting gestation crates for pigs from 2008 (Constitution Article X, Section 21). In 2006, Arizona prohibited gestation crates (Arizona Revised Statute Title 13 Chapter 29). Oregon prohibited gestation crates from 2012 (Oregon 2007 Laws Chapter 722, 2011 Laws Chapter 436). In 2008, Colorado passed a law to phase out sow stalls over a 10-year period. In 2009, Michigan prohibited gestation crates (Michigan 2009 House Bill 5127). In 2010, Ohio introduced legislation to phase out the use of gestation crates (Ohio Code 901:12-8-02). Maine prohibited gestation crates from 2011 (Maine Chapter 127 LD 1021). In 2012, Rhode Island prohibited gestation crates (Rhode Island General Laws Title 4 Chapter 4-1.1). Several food companies are also phasing them out voluntarily.

Recently, it has been approval de Proposition 12 in California that sets specific minimum space requirements for animals raised for food, thus effectively banning cages for laying hens, sow stalls, and crates for calves. This proposition could the driver to a change in Federal legislation

In China. The main piece of legislation for animals is the Animal Husbandry Law of the People's Republic of China (amended in 2015), which is applicable to livestock and poultry. This law focuses on protection of genetic resources, rather than individual animals. Article 38 mandates the training of farmers as to prevent, and treat if necessary, epidemic diseases. Article 39 mandates that livestock and poultry farms use the services of veterinarians. Article 42 requires that all livestock and poultry farms provide suitable conditions for the breeding, survival and growth of the animals farmed. Article 44 provides that workers should prevent and control the spread of epidemic diseases among livestock and poultry.

The Chinese Veterinary Medical Association, under instruction by the Ministry of Agriculture, is drafting General Principles of Animal Welfare. These will be non-binding guidelines on the welfare of various categories of animals, including farm animals, and will include issues such as infrastructure, feeding environment and health. These are the first guidelines regarding animal welfare approved by the Ministry of Agriculture. In 2017, these Guidelines have been approved by the National Animal Husbandry Standardisation Technical Committee. However, they are still under review by the Ministry of Agriculture.

No legislation has been found specifically relating to the rearing of pigs.

Australia. The Australian Animal Welfare Standards and Guidelines website states that 'government and industry have agreed that national standards and guidelines are needed [for livestock] and are working cooperatively to develop the standards and guidelines under the previous AAWS.' The website states that Animal Health Australia was commissioned under the previous AAWS to facilitate the development of nationally consistent standards and guidelines for livestock, but does not state which organisation currently has this responsibility.

In relation to standards and guidelines for pigs, the Australian Animal Welfare Standards and Guidelines website states that a review of the scientific literature and international pig welfare codes and standards was undertaken to inform the

upcoming development of rules. The review was undertaken by the Animal Welfare Science Centre at the University of Melbourne and was funded by Australian Pork Limited. Two peer reviews of the scientific literature and international rules review commented that there was missing literature and certain welfare challenges had not been discussed. They also suggested that the protective elements of enrichment should be included; shoulder ulcerations/lesions had not been mentioned; and there should be a detailed discussion of the impact of prenatal stress.

The Model Code of Practice for the Welfare of Animals: Pigs allows for the use of sow stalls and farrowing crates (Section 4 on 'Accommodation'). Castration is presented as a way to limit aggression problems in group housing accommodation. Tail-docking, teeth-clipping and nose-roping are also put forth as preventative measures either to limit aggressions among pigs, or to prevent 'adverse effects to the environment' in the case of nose-ringing (Section 5). There is no obligation to use anaesthesia before carrying out the castration, tail-docking, teeth-clipping and nose-ringing of piglets.

#### Canada.

NFACC developed a Code of Practice for the Care and Handling of Pigs in 2014. Individual stalls may be used for up to 28 days after the date of last breeding, and an additional period of up to seven days is permitted to manage grouping. Time in stalls can only be extended to protect the welfare of individual sows on the advice of a competent stockperson. As of 1 July 2024, sows must be housed in groups, individual pens or stalls, if they are provided with the opportunity to turn around or exercise periodically, or other means that allow greater freedom of movement. It was communicated that suitable options will be clarified by 1 July 2019, as informed by scientific evidence. However, at the time of writing, no explicit mention of suitable options has been published.

Sows must not be kept in farrowing crates for more than six weeks in any one reproductive cycle except in exceptional circumstances (e.g. when a sow is required to foster a second litter).

Castration, tail-docking and tusk trimming of piglets is allowed when it minimises stress, discomfort and pain. It may only be carried out by a 'competent stockperson'. As of 2016, pain control must be used when performing these procedures. Earnotching can only be performed on piglets less than 14 days of age, but pain control is only recommended, not required. Teeth clipping is still permitted, but only when deemed necessary.

#### Conclusion

It is in the spirit of every farmer and veterinarian to care for and protect all animals in their care. Animal welfare is and will be key to socially sustainable animal production. The WOAH recommendations can be a guideline for countries that do not yet have specific regulations for the protection of pigs. It is important that standards are harmonised worldwide and that regulations are comparable in order to avoid distortion of competition in different markets and different approaches to animal protection that can confuse the world-wide consumer. One Heath, One Welfare.

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### Keynote lectures **DR. HELEEN VAN DE WEERD**

Session: Welfare and ethology Lecture: European approach to advanced pig welfare

#### **Biosketch**

Dr Heleen van de Weerd is owner and Director of Cerebrus Advies B.V., an Agrifood consultancy that bridges the gap between scientific research and industry application. She engages with food businesses on farm animal welfare and works with government, corporate and NGO clients, applying animal-welfare knowledge to achieve improvements and policy change. Heleen holds a Master's in Biologyethology (Wageningen Agricultural University, NL) and a PhD from Utrecht University (NL). She is an internationally recognised expert on farm animal behaviour and welfare with more than 30 years' experience and with 33 fully-refereed publications and 7 book chapters in the field of animal behaviour and welfare science (h-index 28).

Heleen has worked for the European Commission on the evaluations of the EU's animal welfare strategy in 2010 and in 2020, and as an animal welfare expert on a range of EC projects (welfare at slaughter; alternatives to surgical castration of pigs) and most recently, on mapping European animal welfare labels. Heleen has broad knowledge of the global level of animal welfare standards through her specialist consultancy work, e.g., on the Business Benchmark on Farm Animal Welfare (BBFAW) and the Global Coalition for Animal Welfare (GCAW). Heleen has chaired the welfare standard setting working groups on Turkeys and Laying hens & Pullets for RSPCA Assured for 7 years and represents the Netherlands in the World Poultry Science Association's working group on Poultry Welfare and Management. She is an active member of the 3Ts Network, a global network of stakeholders calling for an end to painful piglet procedures. Heleen is the animal behaviour and welfare specialist on the Board of the Dutch AAP Foundation.

#### Abstract

#### **The European approach to advance pig welfare** Heleen A. van de Weerd, Cerebrus Advies B.V., The Netherlands

The European Union (EU) has among the world's **highest standards for animal welfare** and is therefore an important example of how animal welfare-related policy and legislation can deliver positive change. The Treaty on the Functioning of the European Union provides an obligation to consider animal welfare within the context of specific and agreed EU policies (such as agriculture or the internal market). It does not constitute a legal base for the EU to act on animal welfare in isolation [1]. The EU's approach to developing welfare legislation over the past 40 years or so, reflects changes in scientific understanding, in animal management, in public expectations, and emerges as the result of the interplay between different influencers.

Citizens of the 27 Member States have repeatedly expressed concern for animal welfare and asked their governments and the European Commission (EC) to develop policies that will support a quality of life appropriate to all 'sentient beings'. The attitude of Europeans towards animal welfare is monitored by the EC through the so-called Special Eurobarometer. The most recent, 2023 barometer [2] found that of the respondents:

- 91% believe that it is important to protect farm animal welfare
- 88% indicate that it is important to improve welfare at slaughter
- 60% are willing to pay more for products sourced from animal-welfare friendly systems.

Similarly, a 2024 survey by the European Consumer Organization found that almost 90% of EU consumers surveyed support new laws to improve the welfare of farmed animals [3].

**EU welfare policy development** is a deliberative process involving the integration of several elements including information from agricultural organizations, the outcome of stakeholder consultations, the results of socioeconomic studies on impacts

of new legislative initiatives, and Member States' experiences of enforcing existing animal welfare rules [4]. **Current EU legislation** sets minimum welfare standards for animals on farm, during transport and at slaughter and prohibits some of the most inhumane aspects of intensive, industrial livestock production. The most important standards are concerned with the expression of natural behaviour, the provision of space, feed and water, lighting, veterinary care and good stockperson skills. The Pig Directive (2008/120/EC) [5] is the current legal instrument that covers the life of pigs on farm. Separate instruments cover transport (Regulation (EC) No 1/2005) and slaughter of all animals, including pigs (Regulation (EC) 1099/2009). Some of the main welfare provisions of the Pig Directive are:

- Prohibits the tethering of sows (from 2006)
- Prohibits the use of sow stalls except for first 4 weeks of pregnancy (from 2013)
- Prohibits routine tail-docking, teeth clipping and grinding
- Prohibits early weaning of piglets from the sow (less than 28 days)
- · Specifies minimum space allowances for different types of pig
- Specifies general conditions for inspection frequency, lighting, ventilation of buildings and quality of floor surfaces
- Specifies conditions for different types of pig e.g. the provision of bulky or high-fibre feed for sows
- Requires some form of manipulable material for investigation and manipulation activities and suitable nesting material

The Commission has a continuous dialogue with stakeholders who support the development of welfare policy relevant for the Union. One of the supporting bodies that provides broad ranging advice on current animal welfare issues is the multistakeholder expert group, **Platform on Animal Welfare** [6]. The Platform (established in 2017) has membership from public entities (e.g. member state competent authorities), businesses/professional organisation (e.g. agricultural organisations), civil society (e.g. animal welfare NGOs) and independent scientists. A sub-group on the welfare of pigs was established within the Platform (2018-2020). The sub-group assisted the Platform in its work on how to reduce the risk of tail biting and reducing systematic tail-docking of piglets in the EU countries. A new pig welfare subgroup was formed in 2022 and its advice covers the phasing out of sow stalls and farrowing crates, avoiding mutilations, and the need to make welfare legislation enforceable at farm level.

Member States are primarily responsible for implementing EU animal welfare rules. A network of EU **Reference Centres for Animal Welfare** supports national Competent Authorities to enforce these rules. The Reference Centre for pigs (EURCAW-Pigs) [7] provides scientific and technical knowledge to assist the official controls on welfare requirements stipulated in the European pig welfare legislation. One of the first tasks of EURCAW-Pigs in 2019, was to focus on the issues of tail-biting and avoidance of routine tail-docking, including providing enrichment for pigs, as these were identified as key areas of concern to verify compliance with legislation.

The European Commission's policy work is also underpinned by scientific opinions by the European Food **Safety Authority (EFSA)**. EFSA's Animal Health and Welfare Panel (AHAW) [8] provides scientific evidence from the animal's point of view and publishes opinions that examine a wide range of topics such as welfare on-farm (housing and husbandry procedures, feeding), transport conditions and stunning and killing methods.

EFSA adapted a risk assessment approach to animal welfare, due to the complex nature of animal welfare [9]. Its 2022 **Opinion on the welfare of pigs on farm** therefore examines pig production systems used in Europe and identifies for each of these, relevant consequences for welfare [10]. The rapport also recommends how to mitigate such welfare risks and lists animal-based measures (ABMs) that can be used to measure and monitor welfare. EFSA's recommendations include: • Grouping sows at the time of weaning

- Housing periparturient and lactating sows in farrowing pens (not in farrowing crates)
- · Provide sows and piglets with enrichment material in the period from farrowing to weaning
- Limit the selection for litter size to an average number of 12-14 piglets born alive
- · Not perform surgical castration without anaesthesia and analgesia
- Keep the current legal minimum weaning age of 28 days but reconsider the exception allowing earlier weaning in specific circumstances
- Monitor several ABMs in pigs and sows at slaughter.

The European Commission used to set out their plans for animal welfare in an **Animal Welfare Strategy**. The last strategy (covering 2012-2015) was adopted in 2012. With regards to pigs, the Strategy targeted the provision of manipulable materials and the cessation of routine tail-docking; two legal requirements that remained insufficiently implemented in many Member States. Evaluation of this strategy showed that overall, the actions initiated did significantly improve Member State knowledge and competency. For example, study visits on tail-docking and tail-docking audits to selected Member States (2016) led to an intensive exchange of technical and best practice knowledge around the prevalence of tail-biting and risk factors that could lead to tail-biting. Knowledge flowed between Member States where compliance was good and less good and led to increased competency of Member State officials to address compliance issues [11]. More recently, the EU's 2020 **Farm to Fork Strategy** [12] includes animal welfare goals, as the Commission sees animal welfare as a key component of a sustainable food chain. The strategy also states that better animal welfare improves animal health and food quality, reduces the need for medication and can preserve biodiversity.

The Farm to Fork Strategy also announced that the Commission will consider options for **animal welfare labelling** to better transmit value through the food chain. This activity meets the earlier stated expressed desire of citizens for more information on (higher) welfare standards. Many voluntary/private farm assurance (or certification) schemes that include animal welfare have emerged in the absence of mandatory method-of-production labelling. These certification schemes are often commercially run, where a producer signs up (paying a fee) to produce under the requirements of the scheme

with regular (external) inspections. In return, animal products carry the label of the scheme and command a higher product price. While some of these schemes prioritise traceability and food safety with some standards for welfare (basic schemes), others aim for higher welfare outcomes by surpassing legal requirements at both national and EU levels (higher welfare schemes). An example of a higher welfare scheme is the Dutch 'Better Life' label . For pigs, e.g., their two highest tiers (2-or 3-stars) require: no use of farrowing crates for sows, no tail docking of growing pigs, and access to an outdoor area for pigs and sows.

A recent study for the Commission mapped 51 animal welfare labels in use in the EU [13]. In 32 of the reviewed schemes, pigs were the predominant species covered. Fifteen schemes covered all of the main species: pigs, dairy cattle, beef cattle, laying hens and broilers. The extent to which these welfare labelling schemes offer significant improvements to the lives of animals was difficult to assess, due to the absence of a clear baseline and evaluations. However, the studies' considered conclusion was that schemes with criteria that go beyond national and EU legislation can improve animal welfare. This is especially so when a scheme covers the full duration of life until death (incorporating on-farm, transport and slaughter) combined with a robust auditing process by third parties that verify compliance with the scheme's standards [13]. Another important promise of the EU's Farm to Fork Strategy [12] is that the Commission will evaluate and revise the existing animal welfare legislation, to align it with the latest scientific evidence, broaden its scope and make it easier to enforce to ensure a higher level of welfare. The revision of the legislation would include a legislative proposal to phase out the use of all cages for farmed animals across the EU. This was instigated by the outcomes of a European Citizens' Initiative (ECI), 'End the cage age' which called on the Commission to prohibit the use of cages for poultry (laying hens, rabbits, pullets, broiler breeders, layer breeders, quail, ducks and geese), pigs and calves. The organiser of the ECI in collaboration with 170 European NGOs, collected 1.4 million (verified) signatures from citizens in 28 Member States in 2019 [14]. However, the Commission unexpectedly backtracked on this commitment and missed the deadline to publish the proposal (end of 2023). Animal protection organisations are now pursuing the Commission for an explanation, stating that the EC had generated considerable legitimate expectation among citizens that legislation would be forthcoming, creating a case for maladministration. This case has been brought to the European Ombudsman for an inquiry [15]. The only published (revised) regulation was on Transport, without a clear timeline for the rest of the animal welfare legislation package.

Whilst this is disappointing, the **broader impact of the EU's animal welfare legislation** should not be underestimated. The bigger impact is through (global) awareness-raising, through the setting of precedents, and through showing what can be achieved [16]. For example, many of the EU's welfare standards are now effectively minimum standards for companies that are global traders of animal-derived products, evidenced by an increasing number of **corporate responsibility policies** that include animal welfare commitments echoing EU welfare standards [17].

The European Union has proudly championed some of the highest animal welfare standards in the world for over 40 years and it is clear that legislation can advance welfare to achieve a certain minimum level. The existing legislation has the potential to advance pig welfare further; this is particularly the case if enforcement is intensified, for example with regard to routine mutilations such as tail docking. On the other hand, legislation has limits: enforcement can be costly, it is difficult to monitor and oversee all producers, and the legislative process is slow (especially given the current number of EU Member States). Furthermore, the Pig Directive has been in place for many years, and it is prudent to now make a leap forward, especially in view of the body of welfare science clearly evidencing the risks to welfare in many of the current pig production systems.

<sup>&</sup>lt;sup>1</sup>Approx. 1000 citizens in each of the 27 EU Member States were asked 14 questions relating to animal welfare; 26,376 citizens responded.

<sup>&</sup>lt;sup>2</sup>The Dutch Society for the Protection of Animals (SPA) Beter Leven (Better Life) label has three levels, offering farmers and consumers the possibility to improve welfare step by step.

<sup>&</sup>lt;sup>3</sup> Of the 150 companies benchmarked on the topic of animal welfare in 2021, 89% acknowledge farm animal welfare as a business issue and 81% have formal policies on farm animal welfare.

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## Keynote lectures **PROF. DIRK WERLING**

Session: Infectious agents escape strategies - playing hide and seek Lecture: **The porcine host and immunological escape** 

#### **Biosketch**

Prof Werling graduated from the Veterinary University in Hannover, before obtaining his DrMedVet (Virology) degree while working at the ETH Zuerich. After several Fellowships, he obtained a PhD in Immunology from the University of London. He is working since 2003 at the Royal Veterinary College (London), initially as Senior Lecturer and since 2007 as Full Professor in Molecular Immunology and since 2018 as Director of the Centre for Vaccinology and Regenerative Medicine. The main research area of his group is to assess the function of the innate immune response in farm animals and evolutionary questions associated with identified differences between farm animals, human and mice. More recently, this work has evolved into functional studies concerning the mucosal immune system and how the microbiome impacts on this, specifically using organ platforms, such as organ-on-a-chip.

#### Abstract

#### The porcine host and immunological escape

Dirk Werling

Centre for Vaccinology and Regenerative Medicine, Department for Pathobiology and Population Sciences, Royal Veterinary College, University of London

#### Abstract

Porcine pathogens, from bacteria, parasites to viruses have evolved a variety of mechanism to evade host immune recognition. The continued spread of the newly emerging, re-emerging, or epidemic pathogens in Africa, Europe and Asia threatens the global pig industry. For many of these pathogens, the lack of effective vaccines limits disease control. Indeed, these pathogens have evolved a variety of encoded immune escape possibilities, resulting in evasion of not only innate but also adaptive immune processes, inducing cellular inflammation, autophagy, or apoptosis in host cells. Frequent persistent infections hinder the development of effective vaccines and impose technical barriers. Currently, for a lot of these pathogens, knowledge of the virulence-related genes, main pathogenic genes and immunoregulatory mechanism is not comprehensive. Here, I will explain some mechanism that porcine pathogens employ to invade the host and to regulate its inflammatory response, including type-I interferon production, antigen presenting cell shut down as well as knock-out of antibodies. Furthermore, I propose potential ideas for vaccine target design, such as knocking out high-virulence genes using CRISP/Cas or TRADIS approaches and performing data mining to identify the main genes that induce antiviral responses. To support a rational strategy for vaccine development, a better understanding of how these pathogens interact with the host and regulate the host's response to infection is needed.

#### Introduction

To understand the strategies developed by porcine pathogens, it is absolutely crucial to understand how the immune system in pigs develops, and what drives and directs this development pre- and post-natal. In this context, one needs to keep in mind that during the intra-uterine development, the porcine embryo/foetus is separated from the mother's blood supply by a six-layered placenta, thus no maternal cells and immunoglobulins can pass into the developing pig. This has substantial consequences for the development of the immune system, as well as for the colonisation of mucosal surfaces with bacteria in the developing pig.

#### **Prenatal situation**

#### Innate immune system

Innate immunity in pigs consists of similar components as described for other mammals and is fully functional at the time of birth. The effector functions are realized through two major mechanisms: (a) the recruitment and activation of cellular

components; including macrophages, neutrophils, natural killer (NK) cells, and dendritic cells (DCs) and (b) the release of a broad spectrum of extracellular mediators such as cytokines, chemokines, complement, and antimicrobial peptides (AMPs).

The cellular components of the innate immune system such as short-lived polymorphonuclear leukocytes, macrophages (MØ) and dendritic cells (DCs) appear together with the hematopoietic activity of the primary lymphoid organs. Cells with the phenotype of natural killer (NK) cells are also observed quite early in ontogeny and can be isolated at day 45 of gestation from umbilical blood and spleen. The proportion of NK cells in various tissues is between 1 and 10% with a tendency to increase in number during foetal ontogeny, reaching about 15% of all lymphocytes in adult conventional pigs. Their function ("killing" starts not before birth and is severely delayed in germ-free piglets, suggesting that NK cells exhibit some sort of training/maturation. This aspect is extremely important, and I will discuss this further down. However, pigs have one specific type of cells that they have in a far higher percentage compared to other mammals, esp. mice and men. These cells,  $\gamma\delta$  T cells, can in a neonate make up to 50% of peripheral white blood cells. The cells are also often categorized as a part of innate immune response specifically underneath the mucosal surfaces, but also as they are not only capable to modulate the immune cells. These cells are involved in the initial maturation/training of the immune system once the mucosal surfaces get colonised with microbes.

#### Adaptive immune system

The lymphatic system of the growing embryo is physically formed at day 35 after gestation, lymphocytes and other lymphoid elements are rudimentary until the bone marrow starts its function at around day 45 after gestation with the expansion of its haematopoietic capability, resulting subsequently in the occurrence from day 70 onwards, due to the expansion of the peripheral lymphocyte pool in that time. This expansion leads to the ability of the developing piglet to produce a fairly limited antibody repertoire, most of them with low affinity and low specificity, in the last third of gestation. Furthermore, these antibodies are considered part of the innate immune system and are considered as "natural antibody repertoire" (1). These natural antibodies may play confer some protection in the newborn pig (2), but as stated above, their specificity and affinity is weak. However, as explained above, , piglets are born with fully functional innate immune cells and extracellular components able to respond to infections (3). Therefore, neonates are not fully immunologically competent with regards to their adaptive immune system.

#### **Postnatal situation**

As described above, at the timepoint of birth, the new-born piglet possesses a fully functional innate immune system, with special protection being present underneath the mucosal surfaces. This, in addition to the protection provided by the colostrum, containing not only maternal-derived antibodies and immunomodulator, but also immune cells of the sow (4) should protect the newborn in its environment (and one needs to take the natural situation here specifically into account). Subsequent changes in activation of immune cells through colonisation of mucosal surfaces during or directly post-partum by microbial flora (healthy and pathogenic) as well as environmental influences result in the appearance and expansion of T and B cells, the influx and expansion of mucosal immune cells as well as the expansion of the peripheral lymphoid pool. This influx and expansion can be seen specifically by using germ-free piglets raised in isolator bubbles. Due to the lack of "stimuli", relatively little expansion in the T and B cell pool occurs, rendering these piglets completely unresponsive to T cell dependent and independent antigens, even increasing their ability to develop food allergies. Independent of the environmental situation, T and B cell pools to environmental antigens develop from birth on, constantly increasing in numbers as well as diversity of antigens they recognise until weaning when the piglet's immune system is more or less capable to completely protect its host. However, all these described observations further demonstrate the necessity of pathogen-associated molecular patterns (PAMPs) to act as the adjuvant for promoting adaptive immune responsiveness.

#### What drives the development of the immune system?

With the first breath/the first suckling, the newborn piglet starts populating its mucosal surfaces. It has become clear that the way mammals are born really impacts on the first microbiome, and that birthing via the vaginal canal leads to a more favourable microbiome compared to birthing via caesarean, where the first microbiome resembles more the on of the maternal skin. This first microbiome does not only drive the development of the newborns innate and adaptive immune system (functional and anatomically, see Figure 1), but it has also been postulated that the mucosal immune system starts mounting active responses to foreign antigens at the same time its ability develops to control and regulate such events (5).


Figure 1: Peyer's patches in piglet ileal samples. Representative images of Peyer's patches in ileal tissues of suckling piglets killed at (A) 1, (B) 7, (C) 14, (D) 21, (E) 28, and (F) 35 days of age are shown. In addition, the figure also shows representative images of Peyer's patches in ileal tissues of piglets weaned and killed at (G) 14 and 21, (H) 14 and 28, (I) 21 and 28, (J) 21 and 35, and (K) 28 and 35 days of age, respectively. Photomicrographs (original magnification ×40; hematoxylin and eosin stain) were taken using a digital camera attached to a light microscope. Bars = 500 µm. (reproduced under creative common licence CC BY 4.0 DEED, in unmodified form from(6)).

Clearly the first microbiome, and the immune system it trains, has consequences on the immune response. A correctly developing microbiome/immune system (this is often called the gut-immune system axis) leads to an immunocompetent animal. This is specifically important with regards to the range of pathogens/commensal bacteria/food "antigens" the newborn is exposed to within the first hours/days of life, which are all part of its environment. If this development goes right, the immunocompetent animal will generate a tolerance to food and commensal bacteria, and will respond actively to pathogens. If a "false training" occurs, this can lead to either an overreacting immune system (development of food allergies, for example) or a non-reactive immune system (susceptibilities to diseases increase).

The interplay of this development pre-/post-birth is summarised in Fig. 2.



Figure: Both developmental and environmental signals drive significant changes of the intestinal epithelium during the postnatal period in mice and accompany the establishment of an increasingly complex and dense gut microbiota. The neonatal intestinal mucosa is characterized by low levels of epithelial cell proliferation, the absence of crypts and cryptbased Paneth cells, and expression of cathelicidin-related antimicrobial peptide (CRAMP); by contrast, the formation of intestinal crypts late during the second week after birth initiates increased proliferation and rapid epithelial cell renewal, the generation of  $\alpha$ -defensin-producing Paneth cells and the upregulation of expression of the antibacterial C-type lectin regenerating islet-derived protein 3y (REG3y). A decrease in the level of expression of Toll-like receptor 4 (TLR4) by epithelial cells before birth and a steady increase in the intestinal expression level of the nuclear factor-κB inhibitor IκBα during the postnatal period decrease the responsiveness to bacterial lipopolysaccharide and other pro-inflammatory stimuli. Simultaneously, the acquisition of epithelial TLR tolerance creates a neonatal period of decreased innate immune responsiveness. Note that the human small intestinal epithelium at birth has a much more mature phenotype than in mice. The secondary lymphoid structures of Peyer's patches and lymph nodes are generated before birth in mice and humans but mature during the postnatal period. By contrast, cryptopatches and isolated lymphoid follicles (ILFs) are formed after birth in mice. Specialized epithelial cells, known as M cells, reside above ILFs and Peyer's patches and facilitate antigen transport from the lumen to the underlying lymphoid cells. Simultaneously, innate lymphocytes (such as lymphoid tissue inducer (LTi) cells) and T cells leave the liver and thymus, respectively, and colonize the enteric mucosal tissue, including the epithelium. Intraepithelial lymphocytes (IELs) reside in close proximity to the epithelium. Also, increasing numbers of CD103+ dendritic cells and CX3CR1+ macrophages home to the gut mucosa. In contrast to innate lymphocytes, regulatory T (TReg) cells populate the intestinal mucosa in response to bacterial colonization. Although B cells are present in gut tissue during early development, plasma cells producing dimeric IgA are only generated after birth to provide secretory IgA (SIgA) to the lumen. Maternal SIgA is provided by breast milk during the early postnatal period. (re-print permitted under creative comment licence CC BY 4.0 DEED in unaltered form from (7)).

# What happens if we don't have the correct microbiome?

In a nutshell – the "incorrect microbiome" allows pathogens establish themselves. I will present two examples where a potentially "wrong" microbiome allows for pig health and economically important pathogens to establish themselves, and how these subsequently avoid immune recognition.

# 1) Lawsonia intracellularis

Porcine proliferative enteropathy (PE) is an intestinal infectious disease characterized by thickening of the aboral small intestinal mucosa due to enterocyte proliferation associated with the presence of an intracellular bacterium, *Lawsonia intracellularis (L.ic.)*. Proliferative enteropathy has been reported in several different animal species but has been best described in pigs and hamsters. The disease has been recognized in all major swine industries throughout the world (for review see (8)). There is indication that weaning stress, and therefore potentially an imbalance in the microbiome with all accompanying changes in the gut immune system (from reduced/altered mucus production to dysfunctionality of immune cells), can potentially contribute to L.ic. infection. Indeed, early studies in experimentally infected pigs only showed signs of disease after L.ic. infection when either a microbiome was present (conventionally reared pigs) or when gnotobiotic pigs were additionally inoculated with a neomycin-treated microbiome from naturally infected pigs. L.ic. infection resulted in the reduction of mucus production and therefore the thickness of the mucus-layer, enabling other pathogens to gain access to the underlying enterocytes, as well as aiding the expansion of pathogens. Furthermore, through the infection of macrophages with L.ic., the cytokine profile was disturbed, leading to a local immunosuppression by impacting on T and B cell function.

# 2) Mycoplasma hyopneumoniae

Whereas L. intracellularis avoids immune recognition by altering its environment, the second porcine pathogen has a whole array of possibilities to counteract immune recognition, and these events have been summarised a recent reviews ((9-12)). Infection with M. Hyopneumoniae (M.hyo) is often only recognised when the carcass is inspected as consolidation of specific lung-lobes. This indicates a slow, chronic process, that requires immune invasion. Interestingly, the potentially frustrated immune response of the host is seen as the main driver of lung lesions. The whole impact of M. hyo infection can best be described as a "cloak-and-dagger" approach on multiple levels. The immunological escape starts with the destruction of the muco-ciliary apparatus. This, together with the down-modulating the immune response later enhances the susceptibility of. M. hyo-infected pigs to secondary infection. M. hyo infection enhances the production of pro-inflammatory cytokines, such as IL-1, TNF, and IL-6, with plasmin being recognized as central to the regulation of inflammatory responses. M. hypo expresses and secrets a variety of adhesin-family members that interacts with plasminogen of the host, enhancing its activation to plasmin, which stimulates reactive oxygen species and cytokine production by alveolar macrophages. Furthermore, M. hyo possess a leucine aminopeptidase that "moonlights" the host immune system by posing as multi-functional adhesin, leading to binding and cleaving of plasminogen on the M. hyo surface. There is some evidence that M. hyo is also interacting with cell-free actin, causing cytoskeletal rearrangement, inducing subsequently its phagocytosis. However, rather than be degraded, M. hyo is capable in surviving within phagolysosomes, escapes from these and resides subsequently free in the cytoplasm. Therefore, it can not only evade the immune system but can disseminate to internal organs and persist within its host without causing disease. Finally, M. hyo has mechanisms that allow for cleaving immunoglobulins by a two-protein complex. The effect of these two proteins is not only that M. hyo evades opsonisation and subsequent destruction, but it actually does this in a way that antibody-binding sides are still occupied, but the antibodies are left functionally useless.

# Summary

It is becoming clear that we need to re-think how we "treat" infectious diseases in pigs by preventing them through offering neonates the best start into the world. The recently gained understanding of the newly identified axes connecting the gut with the immune system, the lung, and in general the common mucosal system will help us to do so. If we get this crucial period right, we may actually be one step ahead of pathogens that have clearly evolved o evade immune recognition.

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# Keynote lectures **PROF. THOMAS VAHLENKAMP**

Session: Infectious agents escape strategies - playing hide and seek Lecture: **Pathogen strategies of immune evasion** 



# **Biosketch**

2023 - today Head of the PRRSV consultation laboratory, German Veterinary Medical Society (DVG)

2019 - today Dean, Faculty of Veterinary Medicine, Leipzig University

2016 – 2019 Vice-Dean, Faculty of Veterinary Medicine, Leipzig University

2013 – 2016 Dean of Studies, Faculty of Veterinary Medicine, Leipzig University (Germany)

2010 – today Director, Institute of Virology, Faculty of Veterinary Medicine, Leipzig University (Germany) 2010 Director, Institute of Infectology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems (Germany)

2005 Habilitation for ,Virology' and ,Immunology' at the Department of Veterinary Medicine, Free University Berlin (Germany)

2004 – 2009 Senior Scientist and Personal Assistant to the President, Friedrich-Loeffler-Institut, Greifswald-Insel Riems (Germany)

2003 – 2004 Assistant Professor, Immunology Unit, College of Veterinary Medicine, North Carolina State University, Raleigh, NC (USA)

2000 – 2003 Post-doctoral Fellow, Immunology Unit, College of Veterinary Medicine, North Carolina State University, Raleigh, NC (USA)

1998 Philosophical doctor (Ph.D.), Utrecht University (The Netherlands)

1997 – 2000 Research Assistant (C1), Institute of Virology, Faculty of Veterinary Medicine, Leipzig University (Germany)

1993 Dissertation (Dr. med. vet., Ludwig-Maximilians-University, Munich (Germany)

1991 – 1997 Virology Unit, Department for Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University (The Netherlands)

1985 – 1991 Study of Veterinary Medicine, Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Munich (Germany)

# Abstract

# Pathogen strategies of immune evasion

Thomas W. Vahlenkamp

Institute of Virology, Center of Infectious Diseases, Faculty of Veterinary Medicine, Leipzig University

During the last decades swine production has markedly intensified in several parts of the world and the economic importance of this animal species has thus grown concomitantly. When farming becomes more intensive, the impact of infectious diseases generally becomes more severe. Viral infections have made no exception to this and currently play a major role in porcine pathology [1]. Several evolutionary aspects contribute to their importance and virus infections evolve from a subclinical to a clinical course. Rotaviruses and influenzaviruses can be given as current examples. These viruses have become an important part of multifactorial disease complexes in large groups of pigs.

Pathogens have developed remarkably sophisticated mechanisms to avoid the various host protective immune responses. Intracellular pathogens in particular are exposed to the defense mechanisms of the infected cell. For virus evolution it is important to protect the genetic information (RNA or DNA) from intracellular nuclease activities. The genetic information has also to be protected when viruses are released from infected cells. For this purpose, viral genomic RNA or DNA is packaged into a core of structural proteins which also have the task to enable the infection of naïve target cells through receptor interactions. After infection non-structural proteins (NSPs) encoded by the viral genome are the earliest expressed proteins. They are fundamental for the initiation of the virus replication process. In addition, several NSPs also act to evade, circumvent or subvert the host innate immunes system. The focus of this presentation will therefore be on viral NSPs and their action to avoid the initiation of innate immune responses.

The cellular defense mechanisms are diverse and geared towards recognizing pathogens, directly preventing or reducing their replication and presenting foreign antigens to the adapted immune system. In the best-case scenario, the innate immune system succeeds in inhibiting the initial steps of virus replication and no offspring are formed. The evolutionary

success of a virus hinges on its ability to enter a cell and gain access to the genetic material, amino acids and basic cellular machinery that are required for de novo generation of progeny viruses. Given the value of these resources, cells have evolved surveillance strategies to detect their usage [2]. Virus derived RNA can be discerned as a result of missing modifications (e.g. methylation), inclusion of certain virus-distinguishing elements (e.g. exposed 5'-triphosphate) and/or the presence of extensive secondary structures (e.g. double stranded RNA) [2, 3]. Virus-derived DNA can be identified by its location within the cell (e.g. endosomal or cytosolic) or by distinct aspects of sequence and/or structure not present in host DNA (e.g. unmethylated CpG motifs) [2, 4]. Cellular detection of these so-called pathogen-associated molecular patterns (PAMPs) within the infected cell is either direct or aided by autophagy [5]. As these PAMPs represent unavoidable by-products of replication, viruses are under constant evolutionary pressure to minimize their level, prevent their detection and/or block any consequential downstream biology [2, 6].

In order to avoid host innate and adaptive immune responses viral examples include (i) shotdown of host macromolecule synthesis, (ii) avoidance of CTL-mediated killing of infected cells, (iii) prevention of NK-cell-mediated lysis of infected cells, (iv) interference with apoptosis, (v) counter defenses against cytokines, (vi) evasion of antiviral state, and (vii) pathogen-specific gene silencing pathways.

A key step in the host innate immune response is the production of type I, type II and/or type III interferons (IFN-I, IFN-II, IFN-III) as well as proinflammatory cytokines and chemokines. IFN- $\alpha$  can be produced by many cell types in response to the recognition of foreign nucleic acid. Plasmacytoid dendritic cells (pDCs) produce high amounts of IFN-I. IFN-α binds to the interferon type I receptor (IFNAR) of surrounding non-infected cells in order to induce a powerful antiviral defense program involving many interferon-stimulated genes (ISGs) by activating the JAK-STAT pathway. Consequently, proteins are formed in these cells, which on the one hand inhibit further (viral) protein synthesis in these cells and on the other hand cause the degradation of viral and cellular RNA. MHC class I molecules and proteasomes are increasingly formed, which make virus-infected cells easier to be attacked by T lymphocytes. IFN-α activates DCs, macrophages and natural killer (NK) cells, which serve to defend against viruses. IFN-β is produced by virus-infected fibroblasts and presumably also by other cells. IFN- $\beta$  binds to the same receptor as IFN- $\alpha$  and has similar effects. Type II interferon (IFN-y) is produced by TH1 cells (subpopulation of T-helper cells) after interacting with macrophages. IFN-γ has an activating effect on macrophages by promoting better fusion of phagosomes with lysosomes. It also induces antimicrobial peptides. The TH1 immune response is important for the defense against intracellular pathogens (e.g. viruses, chlamydia, mycobacteria, fungi). Type III interferons are a group of anti-viral cytokines, that consists of four IFN- $\lambda$  molecules called IFN- $\lambda$ 1, IFN- $\lambda$ 2, IFN- $\lambda$ 3 (also known as IL29, IL28A and IL28B respectively), and IFN-λ4. Their function is similar to that of type I interferons. Whereas IFN-I can prime almost any cell to induce an antiviral state, IFN-III activity is more selective and thought to be limited to epithelium barrier tissues including the respiratory and gastrointestinal tracts.

The identification of PAMPs by host pattern recognition receptors (PRRs) is a critical step in the innate immune response. IFN-I and IFN-III are produced following recognition of viral PAMPs via specialized cellular pathogen recognition receptors (PRRs). For many viruses including coronaviruses, rotaviruses and arteriviruses these include retinoic acid-inducible gene 1 (RIG-I)-like and Toll-like receptors (RLRs and TLRs, respectively). RLRs comprise a family of RNA-binding proteins that include two central (intracellular) sentinels, RIG-I and melanoma differentiation-associated protein 5 (MDA5). RIG-I is thought to sense single-stranded (ss) viral RNA, MDA5 activation is mediated by engaging double-stranded (ds) viral RNA, a common motif formed during viral replication [7]. RLR activation results after engagement with mitochondrial antiviral signaling protein (MAVS) to the activation of two central transcription factor family members, nuclear factor-κB (NF-κB) and interferon regulatory factors (IRFs) such as IFR3. They cooperate to induce transcription of various cytokines such as IFN-I and/or IFN-III to counteract infection. In contrast to these intracellular sentinels, TLRs sample the extracellular milieu for the presence of PAMPs. Extracellular viral dsRNA, ssRNA or DNA can be sensed by TLR3, TLR7/TLR8 or TLR9, respectively. Stimulation of TLRs by a pathogen induces activation of signal transduction cascades, which leads to translocation of NF-κB to the nucleus and activation of IRF3/7. This subsequently leads similarly to the transcription of antiviral cytokines like IFN-I and/or IFN-III.

Examples of pig pathogens interfering with these innate immune response mechanisms include among others pseudorabies virus (PRV), porcine circovirus (PCV-2), porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV), porcine delta corona virus (PDCoV), african swine fever virus (ASFV) and swine influenza virus (SIV). Many viruses inhibit normal transcription and/or translation of cellular proteins to subvert the machinery of the infected cell for production of progeny virions. This rapid shutdown of the host cell quickly impairs the innate immune response, including the production of critical proteins such as class I MHC antigen and antiviral cytokines such as type I IFN.

Porcine enteric coronaviruses (PECs) consist of porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV), transmissible gastroenteritis virus (TGEV) and swine acute diarrhea syndrome-coronavirus (SADS-CoV). PECs mainly affect the digestive tract of piglets. Clinical symptoms include wight loss, lethargy, vomiting, anorexia, watery diarrhea, and even death. The pathological features were necrosis and shedding of intestinal cells and intestinal villi injury [8]. PEDs encode 16 NSPs (only PDCoV lack NSP1). During the process of enteric coronavirus infection several NSPs have been observed to play roles in host immune-modulatory functions. In PEDV, NSP1, NSP3, NSP7, NSP14, NSP15 and NSP16 were found to inhibit the IFN-I (IFN-β), IRF1 and IRF3 promotor activities [9]. In addition, these NSPs are also involved in downregulating the NF-κB activity [10] and NSP1, NSP3, NSP5, NSP8, NSP15 and NSP16 of PEDV were found to suppress type III IFN activities [8]. Multiple functions of NSP1 to inhibit innate immune responses through different mechanisms suggest that it is one of the key molecules of PECs to escape innate immunity. Drugs targeting NSP1 conserved sites should be able to prevent and/or control disease. Since IFNs are the most important regulator of the antiviral innate immunity, coronaviruses typically inhibit IFN production by various means, including (i) inhibition of RIG-I/TLR signaling, (ii) inhibition of dsRNA binding to RIG-I/MDA5 and/or (iii) directly downregulation of IFN promotor activity. In addition, PECs also attenuate inflammatory

responses by targeting the NF-kB signaling pathway.

PRRSV the causative agent of porcine reproductive and respiratory syndrome (PRRS) has become since its discovery in the late 1980s one of the most serious swine diseases worldwide. The two species PRRSV-1 and -2 belong to the Arteriviridae family. PRRSV is an enveloped RNA virus and encodes RNA replicates (ORF1a and ORF1b), four membraneassociated glycoproteins, two unglycosylated membrane proteins and a nucleocapsid protein (N). ORF1a and ORF1b encode polyproteins that are processed into NSPs involved in viral RNA synthesis and modulation of the immune response. PRRSV induces alterations of immunoregulatory cytokines, which cause a prolonged delay in the activation of CTL and neutralizing antibody production. Thus, PRRSV infection always causes severe host immune response disorders, such as prolonged viremia, transiently diminishing T-cell immunity and delayed protective antibody response [11]. NSP4 and NSP11 target RIG-I and/or MAVS to inhibit IFN-I signaling. NSP1, NSP2, NSP11 and N inhibits nuclear translocation and activation of IRF3. NSP7 downregulates the expression of IRF7 and contributes to the inhibition of IFN production. NSP4 is an antagonist of NF-κB essential modulator (NEMO) which plays a critical role in the regulation of NF-κB signaling [12]. PRRSV uses multiple strategies to antagonize the JAK-STAT signaling pathway which includes NSP1 and NSP11 [13]. Furthermore, PRRSV uses N to interfere with the RNA binding protein TRIM25 in order to inhibit the activation of RIG-I signaling. The N protein of coronaviruses also interact with TRIM25 which might point towards a common mechanism of nidovirales to antagonize TRIM25. PRRSV has a potent regulation ability to immune responses by (i) evasion of TLR and RLR signaling to suppress the production of IFN-I, (ii) the alteration of antiviral response targeting the JAK-STAT pathway and (iii) evasion of ISGs and antiviral proteins to promote viral replication. In addition, PRRSV is highly genetically variable. How the genetic variation affects the immune modulatory functions of the viral proteins might be key to understand the difference in virulence of different PRRSV strains [11].

Group A porcine rotaviruses (RVs) are a global threat to animal health in stock breeding. In piglets, diarrhea and subsequent clinical symptoms are most frequently observed which influences the economic efficiency with regard to routine production schedules within farms. RVs have evolved a set of countermeasures to inhibit the host innate immune response. These countermeasures are most pronounced during homologous RV infection [14]. The genome of RVs consists of dsRNA and therefore activate the cytosolic receptors RIG-I and MDA-5 [15]. In order to block this pathway and the subsequent IFN response RV-encoded NS1 efficiently degrades both IRF3 and IRF7 in a virus-strain dependent manner. NS1 also inhibits the NF-kB signaling pathway. Several RV proteins (VP3, NSP1, NSP2 and NSP3) seem to orchestrate the subversion of the antiviral state mediated by secreted IFNs in uninfected (bystander) intestinal cells [16]. In addition, RV mediates the degradation of different types of interferon receptors (IFNRs) in infected cells. The degradation of IFNRs represents a strategy to ensure that any autocrine IFN antiviral amplicication is inhibited thus allowing viral replication and cell to cell spread to proceed efficiently [17]. Several of the above mentioned countermeasures are most pronounced during homologous RV infection (RV infection with a strain routinely isolated from that specific host species). Viral countermeasures to subvert the innate immune response seems to be less efficient with heterologous virus infections which might contribute to the attenuation of heterologous strains used in several live viral vaccine [15].

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# Shaping the future of animal health

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# ANSWERING THE CURRENT CHALLENGES OF SWINE FARMING

- Solutions to limit the impact of the main swine diseases
- A global approach that emphasizes prevention
- Encouraging good husbandry practices to better protect sows and piglets from disease



# Keynote lectures DR. CARLOS PIÑEIRO

Session: PLF (Precision Livestock Farming) and herd health management Lecture: **PLF implementation in pig farming** 

# Biosketch Education

Veterinarian by the U Complutense de Madrid, Diploma of Advanced Studies in Animal Production by the U Politécnica de Madrid and PhD by the U of Meiji-Tokyo, Diploma by the European College of Porcine Health Management, and specialist in Environmental Impact Assessment by the U Politécnica de Madrid. Member of the Spanish Working Group for the discussion of the Industrial Emissions Directive. Member of the Spanish Focal Group for the Digitalisation and Big Data of the Agro-livestock sector in Spain and member of the Board of Directors of the National Association of Scientific Pig Farming (ANAPORC).

For 23 years, Director of Animal Data Analytics SL, working currently in Europe, LatAm and Asia.

# **PROFESSIONAL EXPERIENCE:**

Expert in data management and analysis in animal production and health. Expert in applied research under commercial conditions. Director of 42 national and international applied research projects, 44 publications in indexed scientific journals, 181 communications in scientific congresses and 8 chapters in books specialised in production, animal health and environment. A regular contributor to journals and websites.

# Abstract

Precision livestock farming implementation. How it will shape the next decade. Carlos Piñeiro, DVM, Dip. ECPHM, PhD. Animal Data Analytics SL

Precision Livestock Farming (PLF) is an innovative approach that integrates technology, communications and data analytics to enhance livestock farming practices. Let's delve into what it entails:

# 1. Definition and purpose:

PLF refers to a management approach that uses technology and communications to collect and process data from individual or grouped animals in livestock farming.

PLF's primary aim is to empower farmers and vets with accurate and continuous monitoring of individual and grouped animals, even in the face of increasing livestock production. This technology can enhance decision-making processes for farm management, health, welfare, and performance, efficiency at work, and the use of resources, providing you with the tools and knowledge to excel in your profession.

There is a good number of technologies that are ready or could be good candidates to be part of this concept, including load cells and flow meters to control feed and water intake and body weights, cameras to determine body weight and animal behaviour, thermal cameras to control body temperature or microphones to detect cough are among the most advanced.

# 2. How it works:

The installed systems (equipment and sensors) generate massive amounts of data that must be transformed into information

supporting efficient decision-making. This must be done within an information system that can be defined as 'A system made up of tools (software and devices) that together with a working protocol and procedures, including the roles of users, can generate the necessary information to make better decisions.', In other words, PLF will not work and deliver as expected if it is part of the digitalisation process and subsequent digital transformation. In short, digitalisation can be summarised as the conversion of information to a non-physical format. At the same time, digital transformation involves the implementation of processes that update the company's tools and objectives to an already digitalised world. In other words, buying precision feeding equipment would be part of a company's digitalization, while creating an algorithm capable of reading and filtering extracted data and generating a report used for a vet to decide how to feed the sows would be part of the digital transformation.

This can involve different areas of swine farming, from the most classical ones (reproduction, productive performance) to the most recent ones, such as abattoir, precision feeding, transportation, antimicrobial use, or biosecurity. The information generated must address the very basic needs (alerts and monitoring) to the most sophisticated ones (explanatory, descriptive, and prescriptive analytics) with the same effort.

# 3. Benefits of PLF in swine farming:

PLF and the digital transformation subsequent will be the basis of the sustainability of the sector, including the three pillars (Deloitte, 2019)

o **Economic efficiency**: It will reduce operational costs related to feed, medication, and energy, improving the productive performance of the animals

o **Environmental sustainability:** It will lead to better use of the resources (feed, water, energy) and decreased associated emissions (CO2, N and P).

o **Social responsibility**: Involving farm staff and technicians and promoting better work quality. It also gives customers and citizens more transparency about how food is produced to responsible and ethical animal husbandry.

The relationship between the prevalence of diseases and GHG emissions has been recently defined by Capper in 2023 in different species, showing a reduction of up to 17 % in healthier animals (Chart 1)

Chart 1. Percentage of change in GHGe in intensity related to prevalence of several animal diseases



But also brings:

o **New professional profiles and better opportunities.** Vets and consultants can be more efficient and productive since they can monitor and control the KPIs related to health, performance, biosecurity, reproduction, climate control and others remotely, making better decisions faster and at lower cost. Farm visits will remain but will change to a lower frequency and be more focused on the problems detected using PLF and digital transformation.

o **Higher animal welfare**: Ensuring that animals are properly fed, the climate is well-controlled, diseases are diagnosed early, and acting faster against them contributes to higher welfare standards and remaining disease-free.

# 4. Challenges and considerations:

Traditionally, the PLF concept relied too much on technology and much less on the protocols for its implementation considering the specific idiosyncrasy of the industry, forgetting farm characteristics and human behavior. Since this concept is almost two decades old, why is it still in the implementation process with a high margin of improvement? Following are some of the reasons that can explain this and are the main challenges to overcome:

Even today, many of the systems proposed are not mature enough to be reliable for a farm's daily use. This was related to the proper isolation of the electronic equipment (dust, humidity, ammonia), reliability of pneumatic systems, cabling protection to rodents, friendliness of the interfaces to control the system, and being quite frequently challenging to interact with them.

Also, communication challenges have been very limiting since the advantages of PLF are often enlarged when remote operations are possible. Despite the improving situation, many farms are limited by the availability and quality of the internet connection, particularly when heavy data traffic is necessary (usually related to image or video processing) or powerful computational resources (normally related to the big data approach) are required.

# II) Data governance related

We must not forget the issues related to data governance, privacy and cybersecurity since there is a traditional reluctance in the sector because of this, which, in many cases, literally blocks any action of the companies in this sense. Companies prefer to stay instead of taking a risk that they don't understand who can access their data (competitors, customers, suppliers, institutions, NGOs or even hackers). There is a need to overcome this situation since it is a bottleneck that slows down the digitalisation process around PLF. It will likely be addressed with new tools like the 'data spaces', which can be defined as a federated data ecosystem based on shared policies and rules. The users of such data spaces can access data in a secure, transparent, trusted, easy and unified fashion.

# III) Human factor

The implementation of PLF must consider the social and human factors, either internally (farm staff) or externally (customers and citizens). Regarding farm staff, it is required a sensitive approach to get them involved in the principles and advantages of the systems to be implemented and that they are going to use, proper training for the effective use of the systems on a daily basis and supervision to ensure that they keep on being well-used over time. This is even more relevant in the current context of the lack of farm workers in the sector.

The protocols for using the PLF systems must be accessible and affordable. If a technology involves a significant process of change to be implemented, it is unlikely that the producer will adopt such technology.

# IV) Enlargement of the PF and digital transformation concept to farm groups

PLF is traditionally associated with individual farm control. No aggregation or relationship among farms is considered. However, the needs of the sector push towards this. Currently and in the upcoming years, it will be not only necessary to understand and care about what is going on on a farm but also to understand the performance of aggregated groups on farms even more in large integrated verticalised systems. There are existing solutions that can be part of the digital transformation of the companies, allowing them to monitor the compliance of biosecurity protocols regarding the movement of people and vehicles both within and outside among farms. Moreover, the prevalence and incidence of diseases in the whole productive system, including the dynamics of infection, pathogen movements within and among farms and antimicrobial associated, will be vital to meeting the sustainability requirements.

Monitoring the health is of utmost importance to ensure the welfare and productivity of the entire herd.. The impact of diseases on swine farms is far-reaching, causing reduced growth rates, compromised feed efficiency, increased antibiotics use and mortality rates, and decreased reproductive performance. Furthermore, certain diseases can be transmitted to humans through contaminated pork products, underscoring the need for diligent swine health monitoring to protect both animal welfare and public health.

# The limitations of traditional health and biosecurity monitoring methods

Traditional methods of swine health monitoring, such as manual observation and periodic veterinary check-ups, are not without limitations. Manual observation relies on subjective interpretation and visual cues, which can result in missed or delayed detection of health issues. This approach is time-consuming and labour-intensive, particularly in large-scale swine production operations. Periodic veterinary check-ups, while valuable, may not provide real-time information on the health status of individual pigs or the entire herd. This delay in diagnosis and treatment can lead to the spread of diseases and increased economic losses.

Additionally, the current control system is based on the periodic use of key performance indicators (KPIs) that affect reproduction, production, and health. However, these KPIs may sometimes not always function accurately and smoothly. As a result, the prevalence and incidence of diseases, as well as the use of antibiotics, are not frequently known. Antibiotic treatments are typically associated with purchasing rather than actual use on the farm, including specific pathologies and timing.

By promptly recognising and interpreting early signs of disease, fast interventions can be implemented to decrease the risk of disease spread within the swine population. Without continuous monitoring and data collection, these subclinical diseases may go undetected, leading to undiagnosed and untreated cases within the swine population.

To prevent the entry and spread of diseases, farms commonly implement internal biosecurity protocols such as all-in-allout flow, decontamination of rooms between groups, the use of footbaths, and movement restrictions from high-risk areas. However, the validation and quantification of the benefits of these protocols are lacking, mainly due to the challenges of measuring their implementation under field conditions. The success of biosecurity protocols, both for internal and external control of staff, visitors and vehicles, depends on the compliance of farm employees, visitors and vehicles, which human behaviour, personalities, and perceptions can influence. Monitoring biosecurity compliance within farms is crucial to ensure consistent protocol adherence.

Lately, it has become increasingly important to control external biosecurity, aiming to control visitors and vehicles, ensure that they respect the company rules regarding downtime, cleaning, and disinfection, prevent non-authorized visitors, and define the farms that concentrate more risk in the company. Heat maps are very helpful to track routes and outbreaks, as image 2 shows.

Digital biosecurity control offers numerous advantages in preventing disease outbreaks, ensuring traceability, and fortifying farm security. Firstly, it enables real-time monitoring and early detection of potential disease outbreaks. Beacons and other devices allow tracking of farm staff movements within and among farms, allowing them to control if they respect the rules of internal movements moving from younger to older animals and from higher health to low health and also among farms and sites of different health status This proactive approach helps prevent the spread of diseases within the farm and to other farms, minimising their impact on the swine industry.

Integrating real-time health monitoring and digital biosecurity control systems in swine production presents various possibilities for improving overall herd health, biosecurity, and productivity. By combining these technologies, swine vets and farmers can adopt a comprehensive and proactive disease prevention and management approach.

In summary, PLF empowers the sector by leveraging technology to enhance productivity, animal welfare, and sustainability. It's a mandatory path toward meeting the rising demand for meat while ensuring responsible farming practices.



Image 1. Heat map showing risky routes in the external biosecurity control

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# Keynote lectures PROF. LIANG CHOU HSIA

Session: PLF (Precision Livestock Farming) and herd health management Lecture: **The circular pig farming: a possibility for the future?** 

# **Biosketch**

Prof. Liang Chou Hsia is an emeritus professor of National Pingtung University of Science and Technology, Taiwan. He has served more than 45 years and widely recognized for his contribution to research, teaching and international collaboration in the field of animal production and sciences. He still puts a lot of efforts on research in environment, nutrition, behavior, extension and interaction among them, consequence on greenhouse gases, housing, welfare, and small farmer development.

# Abstract

The circular pig farming: a possibility for the future Liang Chou Hsia National Pingtung University of Science and Technology, Taiwan, ROC

# Introduction

Accurate and circular pig farming not only reduces the operational costs of farms but also effectively minimizes waste generated from pig farming. Various terminologies such as sustainable pig production, precision animal (pig) farming, organic pig farming, and circular pig farming are currently prevalent. Here we may combine all terminology to create a new one called Precision Clean Pig Farming (PCPF).

According to the Chinese proverb, cleanliness includes two dimensions: external and internal. External cleanliness encompasses maintaining a clean production environment, effective waste management (eliminating solid, liquid, and airborne waste such as odors and particles), and ensuring hygienic food production (e.g., minimizing contamination from drugs, chemicals, bacteria, and viruses). The concept of internal cleanliness relates to psychological well-being, which can also contribute to waste reduction and enhance production outcomes. Practically, this form of cleanliness is referred to as animal welfare. Our research has indicated that farmers perceive academic professionals as lacking practical experience (1). Consequently, this paper will be based on our current research findings to discuss the potential future of PCPF.

# Minimize waste during pig production

Minimizing waste during pig production is essential for maintaining a clean environment per unit of production. Several strategies can be employed to achieve this goal:

1. Improvement in breeding performance: Enhanced breeding performance leads to lower waste generation.

2. Balanced nutrient formulation: Properly balanced nutrients, such as ideal protein formulations, can minimize waste, particularly nitrogen excretion (2, 3, 4).

3. Differentiated feeding stages: Providing different animal feeds at various stages of growth reduces feed waste and minimizes nitrogen loss (5).

4. Functional amino acids: Incorporating functional amino acids into diets can improve pig performance and reduce piglet mortality rates (6, 7).

5. Supplementation with short and medium-chain fatty acids, essential oils, and herbal medicines: These additives have been shown to enhance piglet performance (8).

6. Optimization of environmental temperature: Maintaining optimum environmental temperatures improves pig growth and

reproductive performance (9). Strategies to reduce heat stress, such as improved insulation in animal housing using locally available materials, can lead to more sustainable production practices. Additionally, adjusting feed composition, particularly reducing high-fiber diets during hot seasons, can mitigate heat stress (10).

7. Consideration of environmental temperature: Lower environmental temperatures can lead to higher pH levels in the intestine and lungs, potentially increasing the prevalence of pathogens such as E. coli, Salmonella, and Clostridium. This can result in diarrhea and respiratory diseases.

8. Management of feed allergens: Allergens present in feeds, such as glycine and conglycinin in soybean meal, can cause diarrhea and hinder nutrient absorption. Fermentation and extrusion of feeds can mitigate these effects.

9. Understanding nutrient interactions: Factors like excess calcium can reduce the digestibility of other nutrients, including proteins, fats, vitamins, and minerals. Recent research highlights interactions among amino acids and various nutrients.

10. Maintaining a clean environment: A clean and hygienic environment is essential for sustainable animal production. Unclean environments contain higher levels of antigens, leading to increased antibody production and nutrient wastage. Therefore, ensuring a healthy and clean environment is fundamental to minimizing waste.

# Circular pig farms can be divided into three main types

# First Kind: Utilization of primary waste for fertilizer production

Primary waste generated on pig farms comprises three categories: 1. Liquid waste, consisting of feces, urine, and water. However, direct spraying of this waste onto land is prohibited. Instead, the solid residue separated by a specialized machine is used as compost material. 2. Semi-solid waste, primarily composed of feces and urine, is typically mixed with carbon sources to achieve a C: N ratio of approximately 25:1. 3. Treated wastewater, obtained through anaerobic and aerobic treatment processes, can often be sprayed directly onto land. Several innovations can improve the efficiency of treatment:

(1) Around 23 years ago, our study showed that interval pressurized aeration during aerobic treatment significantly reduces nitrogen levels in wastewater (11). This interval time depends on the BOD or COD content of wastewater. University wastewater has lower BOD or COD so the interval is longer but pig farms' BOD or COD are much higher so the interval is shorter. There are two basic principles behind it. The first one is that NH3 becomes N2 released into the air. The second is that the results are function by facultative anaerobic bacteria. The result not only reduces N in wastewater but also saves 50% of electricity.

(2) Utilization of black net biological membranes offers a cost-effective and space-efficient solution for waste treatment. Most biological membranes for waste treatment are too expensive, occupy too much space, and cannot last for a long time. This one is not only cheap, without occupied space, and can last more than 20 years. The basic principle is the microorganism can adhere to the net. They will eat dry matter which bypasses the net. If they are too heavy they will drop down. The plastic net can last a very long life.

Semi-dry manure can mix with a little bit of dry organic material to make compost however to produce traditional compost needs about 60 days. Later people use stirring methods to reduce compost time to about 30 days. Last few years composting time can be reduced to about 7 days; some people even announced that composting time can be reduced to 2-3 days. The basic principle is aerobic bacteria have a very short reproductive cycle than anaerobic bacteria. The new facility to make compost is based on putting air into the composting facility (12). The old stirring methods also try to mix composting material with air. The efficiency of stirring methods is low. Compost making still has another big problem; that is, it will produce a large amount of odor. Some people already found ways to solve this problem.

# Second kind: Maximizing the efficiency of first kind products

The compost is a primary product. Pig compost usually contains higher N, and P but not K. On the other hand, pig feeds contain a lot of trace minerals. Many of them cannot be digested by pigs. Those parts become nutrients in compost for vegetables, fruits, and flowers. However, the nutrient requirement for different plants is also different. Consequently, if we can make a specific fertilizer for individual plants then this fertilizer must be more valuable.

Methane gas production is also a valuable by-product of pig manure. Methane contains several gases such as CH4, H2S, CO2, and H2O. The H2S causes damage to the gas-burning machine. Both CO2 and H2O lower the energy value of methane. Today have several simple methods to reduce these gases to a minimum. Today methane is not just for direct burning also used to produce electricity.

Sludge is produced during aerobic and anaerobic waste treatment. Unless the sludge is produced in a very big waste treatment system otherwise the quantity of sludge produced is not too big. The dry sludge has a very high protein content (>90% CP). This is because sludge is composed of bacteria. Dry sludge becomes very hard and easily broken. Once spraying the water on it, it will become soft and slowly dissolve without odor. This is a very good fertilizer for the turf of golf courses because the fertilizer only functions when spraying water. The grass will not grow too fast when compared with artificial fertilizer. The workers are also happy to use it because they do not need to cut grass and put fertilizer so frequently. It is also good for horticulture purposes.

Integrated pig and aqua production system: although this system getting less today. However, this system was very popular about 20-30 years ago.

Insect protein: It has been a new topic over the past 20 years. Initially, people fed pigs with maggots of common house flies cultured from pig manure, later transitioning to feeding pigs with maggots of the black soldier fly cultured from pig manure or feed.

These above two subjects have been reviewed by many people. Here will not discuss this in detail.

# Third kind: Methods to reduce greenhouse gas

Pigs that have good feed efficiency will have lower total CO2 production. Ideal protein feed will have low nitrogen waste excretion that will cause lower N2O production. Heat stress causes higher CO2 production.

The biggest greenhouse gas emission of pig farms is from waste. Waste fermentation produces a large quantity of CH4. Fortunately, how to use this CH4 become a very hot topic during the last 50 years. The efficiency of using it also went a big step. However, we still have not solved the problem of CO2 which is produced by burning CH4. CO2 still is one of the important greenhouse gases. Our research has shown that when we provide CO2 for algae then it will benefit algae growth (13). When algae grow to a certain level they can be put back to anaerobic treatment to produce methane gas. The CO2 also can be utilized in a greenhouse to plant vegetables.

# Animal welfare and Precision clean pig farming (PCPF)

The definition of animal welfare is mentioned by Hsia (1990) "Treat living beings kindly, and minimize their suffering when ending their lives." Deal with pigs kindly when we raise them. This kindness always can reduce waste and reach sustainable standards.

Precision clean pig farming integrates advanced technology to optimize the production of animals and their products. This approach aims to maximize efficiency while minimizing waste by leveraging comprehensive knowledge across various domains including pig production, behavior, welfare, diseases, biosecurity, waste management, housing, and precision production techniques.

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# **ORAL PRESENTATIONS**



# VIROLOGY AND VIRAL DISEASES

# VVD-OP-01

VVD – Virology and Viral Diseases

# PREDICTING PRRSV-2 VARIANT EMERGENCE: INSIGHTS FROM A DECADE OF GENOMIC ANALYSIS

N. Pamornchainavakul <sup>1</sup>, M. Kikuti <sup>2</sup>, I.A. Paploski <sup>3</sup>, C. Corzo <sup>2</sup>, K. Vanderwaal <sup>3</sup>

<sup>1</sup>University of Minnesota

<sup>2</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA <sup>3</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

# **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) presents a formidable challenge, causing significant annual economic losses exceeding half a billion dollars in the United States pork industry. The co-circulation and prevalence of distinct PRRSV-2 viral strains impede effective control measures, including the development of vaccines. Inspired by predictive tools used to combat viral infections like seasonal flu and COVID-19, early identification of emerging viral variants holds promise for proactive disease mitigation. Despite the wealth of available data, such predictive models have yet to be established for PRRSV-2.

# **Material and Methods**

To address this knowledge gap, we conducted an in-depth analysis of a decade's worth of virus ORF5 sequences (n = 20,700) and associated metadata. Our primary objective was to identify early indicators of variant emergence for both short-term (12 months) and longer-term (24 months) timeframes, based on six phylogenetic parameters. We examined 12 measures of viral variant "success" that represented population expansion, spatial distribution, and genetic diversity of each variant across time. Utilizing a matched case-control design and conditional logistic regression, we determined the optimal predictive models and significant associations between early indicators and variant success.

# Results

Our systematic investigation unveiled that "successful" variants characterized by population expansion also tends to exhibit widespread geographic spread, while maintaining limited genetic diversification at the timescales examined here. Regression analysis identified the local branching index (LBI) as the sole informative indicator for predicting population expansion, while ancestral branch length displayed a strong association with short-term genetic diversity. Although inferred ancestral branch length and amino acid difference were significant indicators for variant spatial dispersion, the intricate causal relationships remain elusive due to external factors influencing disease spread geographically.

# **Discussion and Conclusion**

While our predictive models successfully captured the majority of emerging variants, they demonstrated relatively low precision due to a high occurrence of false positives (i.e., variants predicted to have emergence potential, but did not become particularly successful). However, this preliminary endeavor in PRRSV-2 prediction, combined with forthcoming research and advancements in PRRSV-2 immunology, establishes the foundation for more precise prevention strategies against PRRS in the future.

VVD – Virology and Viral Diseases

# CHARACTERIZATION OF THE HIGHLY PATHOGENIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS ROSALIA STRAIN IN NURSERY PIGS UNDER EXPERIMENTAL CONDITIONS

A.M. Stoian<sup>1</sup>, P. Curto<sup>1</sup>, A. Pey<sup>2</sup>, A. Trabal<sup>2</sup>, E. Aulinas<sup>2</sup>, J. Baliellas<sup>3</sup>, M. Montoya<sup>4</sup>, L. Fraile<sup>1</sup> <sup>1</sup>University of Lleida, Lleida, Spain <sup>2</sup>AM Animalia, Bianya SL, Spain <sup>3</sup>Grup de Sanejament Porci (GSP), Lleida, Spain <sup>4</sup>Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC). Madrid, Spain

# Background and Objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) stands out as one of the most economically detrimental viruses impacting the swine industry. With the emergence of highly pathogenic type I strains, such as the Rosalia strain in 2020 in the northeastern part of Spain, the swine industry faces a very complicated sanitary situation. Based on the field observations, this PRRSV strain exhibits increased virulence and can easily overcome conventional control measures. Despite causing significant problems in clinical situations, this newly emerged strain has been never thoroughly characterized in an experimental setting. This study aims to evaluate the impact of infecting nursery pigs with the highly pathogenic PRRSV Rosalia strain through two challenge routes.

#### **Material and Methods**

Twenty-five 8-week-old PRRSV-free pigs were divided into three groups. Groups 1 and 2 (n=10 per group) were challenged either intranasally (group 1) or intramuscularly (group 2) with the highly virulent PRRSV Rosalia strain (1x10<sup>5</sup> TCID<sub>50</sub>/ml). Group 3 (n=5) served as non-infected controls. Monitoring occurred for 63 days post-challenge (DPC), with samples collected for virological and immunological assessments.

#### Results

Virus was detected in all infected pigs by 3 DPC, with the highest viremia peak at 7 DPC. Intranasally challenged pigs demonstrated persistent infection and virus shedding by saliva throughout the study with 30% mortality at the end of the trial, while intramuscularly challenged pigs experienced 100% mortality by 14 DPC. Antibodies were consistently detected in these pigs, with the highest levels recorded at 14 and 56 DPC. Histological examinations revealed interstitial pneumonia with mononuclear cell infiltrates in the lungs, and lymphoid depletion and follicular hyperplasia with cellular necrosis in various tissues. Ongoing assays will be discussed in detail during the meeting.

#### **Discussion and Conclusion**

This study is the first to experimentally assess the outcome of PRRSV Rosalia infection in nursery pigs. The intranasal route, reflecting the natural way of infection, and the intramuscular route, emphasizing the importance of iatrogenic transmission (e.g., change of needles), provide crucial insights into the dynamics of this highly pathogenic strain. The results contribute to our understanding of the PRRSV Rosalia strain, informing strategies for outbreak management and prevention.

# VVD – Virology and Viral Diseases

# EXPERIMENTAL ASSESSMENT OF THE EFFICACY OF AN MLV PRRS VACCINE AGAINST CHALLENGE WITH HIGHLY VIRULENT PRRSV-1 STRAIN ROSALIA

E. Mateu 1, M. Cortey 2, I. Domingo 2, M. Marcos 3, R. Menjon 3, S. Von Berg 3, M. Jiménez 3

<sup>1</sup>Department de Sanitat i Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain

<sup>2</sup>Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona (UAB), 08193 Cerdanyola del Vallès, Spain

<sup>3</sup>MSD Animal Health

# **Background and Objectives**

In 2020 a highly virulent PRRSV-1 isolate emerged in Spain. This strain, rapidly spread and has become the predominant one in most new PRRS outbreaks in the North-eastern part of the country. The infection is characterized by abortion storms, sow mortality and mortality rates (>20%) in the affected nurseries. The aim of the present study was to assess the efficacy of a MLV PRRS vaccine against the challenge with one of those highly virulent isolates.

#### Material and Methods

The study was conducted in 4 groups (G1-G4) of 13 four-week-old PRRSV-free pigs. After 1 week of acclimation groups G1 and G2 were intradermally vaccinated (Porcilis® PRRS, MSD Animal Health) whilst groups G3 and G4 only received the vaccine adjuvant. Five weeks later, groups G1 and G3 were intranasally challenged with a highly virulent isolate (ON571708) ( $\geq 10^{5.4}$  TCID50/ml, 1 ml per nostril) and were followed for the development of clinical signs and lung lesions. Pigs were sampled periodically to determine viremia and nasal shedding as well as the development of humoral (ELISA) and cell-mediated immune responses (IFN- $\gamma$  ELISPOT).

#### Results

After the challenge, animals in the unvaccinated group (G3) developed high fever (up to  $41.9^{\circ}$ C) that was evident from day 4 until day 9 post-challenge (pc) while for vaccinated animals (G1), fever >41^{\circ}C was only seen on day 9 pc. At day 10 pc, G3 animals had extensive macroscopic lung lesions ( $49.1\pm25.2\%$  vs.  $15.7\pm14.5\%$  of lung affected for G3 and G1, respectively). At microscopic level, G3 animals also showed more severe scores of interstitial pneumonia compared to vaccinated pigs ( $3.1\pm0.8$  vs  $1.9\pm0.8$ , respectively). The area under the curve for the viremia of G3 was significantly higher than the area for G1 (256.2 vs. 110.4 for G3 and G1, respectively). Nasal shedding was also reduced in G1. Vaccinated animals showed a significant anamnestic response in ELISPOT after challenge (p<0.05). Weight gain after challenge (days 0 to 35 pc) was better for vaccinated pigs ( $32.9\pm12.8$  vs.  $26.2\pm6.1$  Kg for G3 and G1, respectively; p<0.05).

### **Discussion and Conclusion**

In conclusion, vaccination with a MLV vaccine administered intradermally resulted in significant clinical, pathological, virological and zootechnical protection against the challenge with highly virulent Rosalia.

VVD – Virology and Viral Diseases

# PERCOLATION OF VIRUS: A POSSIBLE TRANSMISSION ROUTE FOR PRRS VIRUS

<u>J. Alvarez-Norambuena</u><sup>1</sup>, A. Quinonez<sup>1</sup>, S. Goyal<sup>1</sup>, C. Corzo<sup>2</sup> <sup>1</sup>University of Minnesota <sup>2</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

# Background and Objectives

In the US there is an increasing trend of PRRS cases between October and December when manure pits are agitated, pumped and slurry is spread on soils as fertilizer. The PRRS virus can be found in feces and manure pits but there is scarce data whether PRRS present in the manure pits could reach groundwater after manure spread which could lead to indirect virus transmission. Therefore, the aim of this study was to assess whether the virus had the capability of percolating through different Minnesota soil types. Another aim was to compare the percolating ability of different strains of the virus.

## Material and Methods

Three different PRRS viruses (e.g., 1-7-4 L1A, 1-4-4 L1C, and 1-26-2 L1G) were propagated in MARC-145 cells. Thirteen soils were used, 6 originating from sites surrounding pig farms and 7 obtained from the UMN Agronomy Department. A vertical glass column model was used with different soil quantities (5g, 10g and 20g). Water was added to moisturize soil to mimic field conditions and then a suspension containing virus and water was added. Virus in the original suspension and dripped liquid was titrated to determine how much virus percolated through the soil.

#### Results

All three viral strains were isolated from percolates regardless of the amount of soil added to the glass column. The 1-7-4 virus was isolated from all 5g soil samples, 12 out of 13 10g soil samples and from 6 out of 13 samples. In the case of the 1-4-4, the virus was isolated from all 5g soil samples, 6 out of 13 10g soil samples and 3 out of 13 20g soil samples. The 1-26-2 virus was isolated from all percolates from 5g and 10g, and in 11 of 13 20g soil samples. As expected, there was an inverse proportional relationship between the amount of virus that percolate and the amount of soil.

### **Discussion and Conclusion**

All virus strains were able to percolate through all amounts and types of soil while remaining viable. These results suggest that it is possible for the virus to reach and contaminate the underground water. Further research is necessary to fully understand this potential transmission mechanism.

VVD – Virology and Viral Diseases

# DO HERDS CLOSE TO MAIN ROADS HAVE HIGHER PRRS PREVALENCE?

<u>M. Fertner</u><sup>1</sup>, J. Snitgaard Pelck<sup>2</sup> <sup>1</sup>SEGES Innovation P/S, Datamanagement, Axeltorv 3, DK-1609 Copenhagen V, Denmark <sup>2</sup>SEGES Innovation P/S, Datamanagement, Agro Food Park 15, DK-8200 Aarhus, Denmark

# **Background and Objectives**

Along with the Danish PRRS reduction program launched in 2022, focus on potential routes of PRRS virus transmission has increased. A concern has arisen, whether passing trucks transporting live pigs, constitute a risk to pig herds located in near proximity of transit routes. If passing trucks would constitute a risk, we would expect a higher prevalence of PRRS among herds located in near proximity of roads. The objective of the study was to compare the prevalence of declared PRRS-seropositive herds located in the proximity of large roads, compared to herds located further away.

# Material and Methods

A register-based cross-sectional study was conducted, based on data from the Central Husbandry Register (herd location) and the Specific Pathogen Free Register (PRRS status). PRRS status (seronegative/seropositive) is based on clinical suspicion and monthly (multiplier/breeding herds) or annual (production herds) antibody test. In addition, information on location of highways, primary and secondary roads were downloaded from OpenStreetMap. All data was collected April 2023.Herds were defined as either "herd-close-to-road" (< 500 m distance to road) or "remaining-herd" (> 500 m). A likelihood ratio-test with binomial outcome and a 5% significance level, was used to test the null-hypothesis of equal PRRS-prevalence between herds-close-to-road and remaining-herds. The analysis was performed on four study populations.

# Results

For each of the four study populations "Denmark" (N=4,023), "Southern Jutland" (N=497), "20 km radius around slaughterhouses" (N=975) and "collection centers" (N=519), declared PRRS-seropositive herd status was around 38%-46% (herds-close-to-road) and 36%-38% (remaining-herds). With p-values ranging from 0.12-0.40, we could not reject the null-hypothesis of equal prevalence of PRRS for any of the four study populations.

### **Discussion and Conclusion**

No significant difference in prevalence of PRRS was found between herds-close-to-road and remaining-herds. The study was conducted for all herds enrolled in the Danish PRRS-reduction strategy, and repeated for high-risk areas, namely areas with a high rate of trucks with live pigs driving transit for either export (Southern Jutland and collection centers) or slaughter (area around slaughterhouses). Identical results for each of the four sub-analyses indicate no association between herd location close to road and declared PRRS serological status.

# VVD – Virology and Viral Diseases

# ASSESSMENT OF VIREMIA, ANTIBODY RESPONSE, SURVIVAL RATES, SHEDDING TO SENTINELS AND PERSISTENT INFECTION OF PIGS FOLLOWING NATURAL FIELD AFRICAN SWINE FEVER VIRUS EXPOSURE

D. Venkateswaran <sup>1</sup>, S. Muhammad <sup>1</sup>, R. Suntisukwattana <sup>1</sup>, W. Atthaapa <sup>1</sup>, P. Jermsutjarit <sup>1</sup>, P. Watcharavongtip <sup>1</sup>, <u>D.</u> <u>Nilubol</u> <sup>1</sup>

Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

#### **Background and Objectives**

African Swine Fever (ASF) is a lethal hemorrhagic disease that has spread across Asia. After an outbreak, the mortality varies, with ASF detected in weaned piglets from convalescent sows. Persistent infection in immunoprivilege sites are speculated. The objectives of the study were to investigate viremia and survival rate of pigs following natural field ASF exposure. Shedding period and presence of ASF in organs of pigs that survived the natural field exposure of ASF were additionally evaluated.

#### **Material and Methods**

In the study, 200 female pigs weighing approximately 40 kilograms were brought from an ASFV-free source to a herd with an ongoing ASF outbreak. Upon arrival, they were housed in a tunnel-ventilated building and randomly distributed into 10 pens, each containing 20 pigs. A week later, the pigs were exposed to ASFV under natural field conditions. Blood samples were collected weekly for 19 weeks following exposure and tested for ASFV DNA using real-time PCR targeting P72 gene. Survival analysis was performed on a weekly basis. Sentinel pigs were introduced at 4, 8, 12 and 16 weeks post exposure (WPE). At 19 WPE, one pig per pen was randomly selected and necropsy was performed. Organs including tonsil, spleen, lymph node and ovary were collected and assayed for the presence of ASFV DNA by realtime PCR against P72 gene.

#### Results

Following a week of introduction, 19 of 200 pigs had detectable viremia. Following 2 WPE, all pigs exhibited detectable viremia and mortality. Mortality stopped at 3 WPE with an average survival rate of 35% (highest: 70%; lowest:0%). None of pigs died after 3 WPE. Increased antibody response was observed against P72, P30 and P54, respectively. Sentinel pigs introduced 4, 8, 12 and 16 WPE developed clinical disease at approximately 2, 4, 4 and 8 weeks following commingled, respectively. At 19 WPE, ASFV was detected in 100%, 40%, 40%, and 20% of tonsil, spleen, lymph node, and ovaries, respectively.

#### **Discussion and Conclusion**

The results of the study demonstrated that following an infection, pigs can develop into a carrier state. Persistent infection and transplacental infection are suggested, contributing to a main factoring circulating ASF in a herd previously experienced an outbreak.

# VVD - Virology and Viral Diseases

# EFFICACY OF IN-FEED IMMUNE-BOOSTING COMPLEX ON REDUCING THE INCIDENCE. LATE ONSET OF ASF SYMPTOMS AND RELATED SEROLOGICAL PARAMETERS IN EXPERIMENTALLY CHALLENGED ASFV PIGS

V.T. Nguyen 1, M. Sevastiyanova 2, A. Khadem 3, C. Gougoulias 2, V.P. Le 4 Institute of Veterinary Science and Technology (IVST), Hanoi 100000, Vietnam <sup>2</sup>Innovad® NV/SA, Cogels Osylei 33, 2600 Berchem, Belgium Innovad® NV/SA. Cogels Osvlei 33. 2600 Berchem. Belgium / Laboratory of Animal Nutrition. Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium \*College of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi 100000, Vietnam / Institute of Veterinary Science and Technology (IVST), Hanoi 100000, Vietnam

# **Background and Objectives**

African swine fever (ASF) significantly impacts global pig production, threatening the national herd wipe-out of entire countries. This study evaluated the protective efficacy of a proprietary phytogenic and glycerol monolaurate mix (IBC. Immune-boosting complex, INNOVAD Belgium) against experimentally ASFv-challenged pigs.

#### Material and Methods

Forty pigs at 6 weeks, negative for ASFv and ASFv-specific antibodies, were split equally between the control (CONGr) and experimental (EXPGr) groups. For the entire trial period, pigs in CONGr received regular feed, whereas pigs in EXPGr received additionally IBC (4.5 kg/T). At 8 weeks of age, 10 pigs from each group were randomly selected for an individual oro-nasal challenge with ASFv-strain VNUA\_HY-ĂSF/2019 (Van Phan Le et al. 2019) with a 10⁴ HAD₅v/pig dose. Clinical symptoms were followed 24 days post-infection (dpi), including blood viraemia and antibody titers.

# Results

Fever and lethargy were observed in all CONGr pigs between 4 and 15 dpi, whereas only one pig from the EXPGr showed fever (12 dpi) with concomitant viraemia on the same day. In the EXPGr, the incidence of fever was drastically lower vs. CONGr until 15 dpi (P = 0.001). Viraemia was first detected in the CONGr at 6 dpi (5 out of 10 pigs), and all CONGr pigs were viraemic by 15 dpi. Also, in the CONGr, ASF-antibodies were first detected at 9 dpi (3 out of 10 pigs), and all pigs were Ab-positive by 18 dpi. In contrast, in the EXPGr, viremia was significantly delayed and expressed to a much lesser extent (12 dpi; only in 2 out of 9), notably accompanied by complete absence of antibodies until 18 dpi (n=1) (P = 0.032, P = 0.001, respectively). It is worth noting that ASF-related mortality at the end of the trial was markedly reduced in EXPGr (1 out of 9) vs. the CONGr (8 out of 10) (P = 0.001).

#### **Discussion and Conclusion**

To our knowledge, this is the first study that showcases an in-feed immune-boosting complex with markedly reduced incidence, late onset of ASFv symptoms and related serological parameters in experimentally challenged ASFv pigs. Further investigations are needed to clarify how such IBC could be harnessed as a supportive anti-ASFv aid.

# VVD-OP-08 VVD – Virology and Viral Diseases

# AFRICAN SWINE FEVER - LESSONS LEARNT FROM RECENT ANIMAL TRIALS

<u>V. Friedrichs</u><sup>1</sup>, S. Blome<sup>2</sup> <sup>1</sup>*Friedrich-Loeffler-Institut* <sup>2</sup>*Friedrich-Loeffler-Institut,Greifswald - Insel Riems, Germany* 

# Background and Objectives

With the panzootic spread of African swine fever (ASF), alternative modes of transmission and long-term effects have come to the fore. Specifically, studies were undertaken to understand the role of venereal transmission of ASF virus (ASFV) via artificial insemination (AI) and the duration of immunity following field virus infection.

# **Material and Methods**

In the first study, blood and semen were collected daily from ASFV "Estonia 2014" infected boars. Subsequently, 14 gilts were inseminated with ASFV-containing semen. Viral genome and infectious particle kinetics were assessed in blood, semen and embryos/fetuses. In the second study, 10 pigs were inoculated with "Estonia 2014" and antibody as well as viral genome kinetics were monitored. The animals were sampled weekly until day 28, then monthly. After 6 months, all animals were inoculated with highly virulent ASFV "Armenia 2008", along with a control group.

# Results

The first study provided evidence that ASFV can be efficiently transmitted from infected boars to naive sows via AI and we report the detection of ASFV genome in semen at 3 dpi. After AI, 7 sows were infected by AI, all other sows by contact. Thirteen sows were pregnant, but 12 aborted due to high fever. In addition, as the morphology of the fetuses and amniotic fluid differed amongst offspring of a sow that did not abort, we analysed the organ sacs of all fetuses individually to assess whether ASFV could migrate across the placenta and infect embryos/fetuses. While all control animals had to be euthanised 7-8 days after infection, only two of the animals previously infected with ASFV showed mild clinical signs. These two animals remained genome-positive in the blood until day 28 after the second infection and virus could be detected in excretions. The recovered animals showed high levels of ASF-specific antibodies.

#### **Discussion and Conclusion**

In conclusion, we show that AI is a fairly efficient and potentially underestimated route of ASFV transmission. Furthermore, after pigs survive an infection with ASFV "Estonia 2014", antibody levels remain high until 6 months post inoculation. Additionally, previous infection with a moderately virulent strain conveys protection against lethal challenge.

# VVD – Virology and Viral Diseases

# RETROSPECTIVE DETECTION OF PORCINE CIRCOVIRUSES IN PORCINE DERMATITIS AND NEPHROPATHY SYNDROME CASES

A. Cobos <sup>1</sup>, M. Domingo <sup>2</sup>, M. Perez <sup>3</sup>, E. Huerta <sup>1</sup>, A. Llorens <sup>4</sup>, M. Sibila <sup>5</sup>, <u>J. Segales <sup>6</sup></u>

<sup>1</sup>IRTA, Centre de Recerca e Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>2</sup>Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona, Spain; UAB, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la UAB, 08193 Bellaterra, Barcelona(Spain)

<sup>3</sup>IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>4</sup>IRTA, Centre de Recerca e Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

⁵IRTA-CReSA

<sup>e</sup>Departament de Sanitat i Anatomia Animals, UAB, 08193 Bellaterra, Barcelona, Spain; UAB, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona (Spain)

# Background and Objectives

Porcine dermatitis and nephropathy syndrome (PDNS) is a clinical-pathological condition, which diagnosis is based on characteristic histological findings consisting of necrotizing vasculitis and fibrinonecrotizing glomerulitis. Although initially associated with various etiologies, it was later included as a porcine circovirus (PCV) disease considering epidemiological circumstances and their reduction in prevalence after the use of PCV-2 vaccines. Subsequently, PCV-3 and PCV-4 have been detected in cases of PDNS, further confusing the potential link of PDNS with PCVs. Therefore, this study aimed to investigate the presence of all known PCVs in retrospective cases of PDNS.

# Material and Methods

Formalin-fixed paraffin-embedded (FFPE) tissue samples from the Servei de Diagnòstic en Patologia Veterinària (SDPV) previously diagnosed with acute PDNS by means of characteristic histological lesions (between years 1997 and 2020) were studied. All cases were investigated by PCV-2 immunohistochemistry (IHC). Moreover, tissue sections were deparaffinized and DNA was extracted, followed by specific conventional PCRs against PCV-1 and PCV-4, as well as quantitative PCR (qPCR) against PCV-2 and PCV-3.

# Results

A total of 102 cases were studied, 39 of which were PCV-2 negative and 63 PCV-2 positive by IHC. All the studied cases (102/102; 100%) were PCV-2 qPCR positive (mean of 1.0·10<sup>10</sup> PCV-2 genome copies per mL of tissue supernatant) while 20/102 (19.6%) cases were PCV-3 qPCR positive (mean of 1.6·10<sup>3</sup> PCV-3 genome copies per mL of tissue supernatant). All cases yielded negative results for PCV-1 and PCV-4 PCRs.

# **Discussion and Conclusion**

PCVs are considered rather ubiquitous in nature and not always linked to disease. In this study PCV-2 was detected in high loads in all PDNS cases, while PCV-3 was only present in a low subset of them, which may reflect the usual prevalence of the virus at farm level (around 20%). Moreover, PCV-2 loads were much higher than those of PCV-3. Although PCV-3 associated diseases have been characterized with various vascular lesions, they are readily differentiated from PDNS vascular lesions histologically. Therefore, PDNS is most likely causally associated with PCV-2 infection, and not with other PCVs.

# VVD – Virology and Viral Diseases

# DIVERGENT PATHOGENESIS AND TRANSMISSION AMONG AVIAN AND MAMMALIAN ISOLATES OF HIGHLY PATHOGENIC AVIAN INFLUENZA A VIRUS (H5N1) IN SWINE

<u>B. Arruda</u><sup>1</sup>, A. Vincent Baker<sup>1</sup>, A. Buckley<sup>1</sup>, T. Anderson<sup>1</sup>, M. Torchetti<sup>2</sup>, N. Hines Bergeson<sup>2</sup>, M.L. Killian<sup>2</sup>, K. Lantz<sup>2</sup> <sup>1</sup>Agricultural Research Service United States Department of Agriculture <sup>2</sup>Animal and Plant Health Inspection Service United States Department of Agriculture

#### **Background and Objectives**

Highly pathogenic avian influenza (HPAI) viruses have the potential to cross species barriers and cause pandemics. H5N1 HPAI belonging to the goose/Guangdong 2.3.4.4b hemagglutinin (HA) phylogenetic clade continues to infect poultry, wild birds, and mammals across the globe. Continued circulation in avian species and infection of multiple mammals with strains possessing adaptation mutations increase the risk of infection and reassortment with influenza A viruses (IAV) endemic in swine.

#### Material and Methods

We evaluated the transmission and pathogenicity of four strains (A/turkey/MN/22, A/bald eagle/FL/22, A/raccoon/WA/22 and A/redfox/MI/22) of HPAI H5N1 2.3.4.4b clade in pigs. Both A/raccoon/WA/22 and the A/redfox/MI/22 contained mammalian adaptation mutations. Fifty-eight pigs were randomly allocated into a negative control group or a group of 20. Fifteen pigs per virus strain were inoculated intranasally (1 mL). Five naive contact pigs were comingled with each of the virus-inoculated groups at 2 days post-inoculation. Nasal swabs were collected from principal and contact pigs. Bronchoalveolar lavage fluid (BALF), sera, and formalin fixed tissues for histologic evaluation and immunohistochemistry were obtained at necropsy. Viral RNA was extracted from nasal swab and BALF samples and subjected to real-time, reverse transcription PCRs. Seroconversion was determined using ELISA. Positive PCR samples underwent metagenomic sequencing and analyses.

#### Results

All strains replicated in the lower respiratory tract of pigs and caused macroscopic and microscopic lesions consistent with IAV. Viral replication in the upper respiratory tract, transmission, histologic lesions, and antigen distribution of mammalian isolates suggest potential binding adaptation. Mammalian and avian strain within-host diversity was dominated by low-frequency variation and weak purifying selection.

#### **Discussion and Conclusion**

The risk of reassortment of the HPAI H5N1 2.3.4.4b lineage with endemic swine IAV is a concern based on the susceptibility of pigs to this lineage demonstrated by our study and the prevalence of IAV infection in swine herds. Mammalian adaptation and reassortment may increase the risk of incursion and transmission of HPAI in swine. Robust surveillance in swine and cooperation between influenza sectors remain critical for pandemic preparedness.

VVD – Virology and Viral Diseases

# EFFECT OF BACILLUS-BASED PROBIOTIC SUPPLEMENTATION TO SOWS AND THEIR WEANED OFFSPRING IN A NURSERY H1N2 INFLUENZA CHALLENGE

L.H.B. Hansen <sup>1</sup>, K. Kinsley <sup>2</sup>, L. Raff <sup>1</sup>, A. Mueller <sup>3</sup>

<sup>1</sup>Chr. Hansen A/S, Animal and Plant Health & Nutrition, Bøge Alle 10–12, 2970 Hørsholm, Denmark. <sup>2</sup>Chr. Hansen Inc., Animal and Plant Health & Nutrition, 9015 W Maple St., WI 53214 Milwaukee, United States <sup>3</sup>Swine Services Unlimited, Inc., 205 9th Ave NE; Rice, MN 56367, United States

### **Background and Objectives**

Influenza A virus is a common cause of respiratory disease in pigs negatively impacting health and performance. This study's objective was to evaluate the effect of administering a Bacillus-based probiotic to sows and their weaned offspring in an H1N2 challenge model.

# Material and Methods

Five weeks prior to farrowing, 16 gestating sows were allotted to two treatment groups: CON and BAC. Gestation and lactation diets of the BAC sows were supplemented with a probiotic consisting of B. subtilis - 810A and B. licheniformis - 809A (1.65E+09 CFU/kg feed) while the CON sows received standard commercial diets. On d21, 96 pigs weaned from CON and BAC sows were allotted to eight pens with six pigs/pen (48 pigs/treatment group). During the 3-week nursery period, BAC pigs were supplemented with the Bacillus-based probiotic (1.10E+09 CFU/kg feed) and CON pigs were fed a standard diet. Following 14d of acclimatization post-weaning, all pigs were challenged with H1N2 influenza via intratracheal gavage. Performance metrics were recorded weekly while rectal temperatures and clinical severity were assessed daily beginning one day prior to challenge (d13) through study termination. Nasal swabs collected on d16, d18 and d21 were analyzed for viral loads via qPCR. On d21, all pigs were euthanized, lungs were removed and scored on percentage of lobe bearing gross (visible) lung lesions. Data were analyzed using one-way ANOVA test, if normally distributed, or non-parametric Kruskal-Wallis test.

#### Results

Compared with CON, BAC pigs had improved FCR during the pre- and post-challenge periods (P<0.01). The proportion of pigs with rectal temperatures above 39.7°C was lower in the BAC treatment on d18 and d20 as were clinical severity scores for the same days (combined demeanor, abdominal, and respiratory score) (P<0.05). Viral intensity was 1-2 ct lower in nasal swabs associated with the BAC fed pigs on d16 and d18 and the average total lung lobes with lesions per pig was lower in the BAC treatment (P<0.001).

# **Discussion and Conclusion**

In conclusion, administration of the Bacillus-based probiotic to sows and their weaned progeny postponed the impact of a H1N2 influenza challenge in nursery pigs, consequently alleviating impaired performance and lung lesions.

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# VVD-OP-12

# VVD – Virology and Viral Diseases

# DETECTION OF MULTIPLE PORCINE VIRUSES USING TARGET ENRICHMENT AND REAL-TIME LONG-READ SEQUENCING FROM ORAL FLUID FIELD SAMPLES THROUGH TELSVIRUS WORKFLOW

<u>M. Meneguzzi</u><sup>1</sup>, J. Bravo<sup>2</sup>, C. Boucher<sup>2</sup>, M. Torremorell<sup>3</sup>, N. Noyes<sup>4</sup> <sup>1</sup>University of Minnesota <sup>2</sup>Computer & Information Science & Engineering Department, University of Florida <sup>3</sup>University of Minnesota - College of Veterinary Medicine <sup>4</sup>Department of Veterinary Population Medicine, University of Minnesota

# **Background and Objectives**

Viral co-infections on swine farms are common, contributing to aggravated disease outcomes and impairing economic profit. Despite their relevance, genomic characterization of multiple viruses and novel variants from field samples is still a challenge thus many questions about viral co-infections still remain. To advance our understanding of viral co-infections, we have developed a workflow called "TELSVirus", or "Target-Enriched Long-read Sequencing of Virus" that enables the detection and genomic characterization in real-time of multiple viral pathogens and variants discrimination from a single sample. The study aimed to apply porcine oral fluid samples to the TELSVirus to obtain full-length genomes and genetic variant differentiation of the target pathogens.

### **Material and Methods**

The TELSVirus workflow combines a bait-capture method with long-read, real-time sequencing and an ensemble bioinformatics pipeline for data analysis. First, we bioinformatically designed a panel of baits that selectively target 40 swine viruses. A total of 123 oral fluid samples collected from growing pigs from Midwest U.S. farms between 2017 and 2018 were subjected to the TELSVirus workflow. RNA extraction and complementary DNA synthesis were performed followed by probe hybridization and enrichment. Subsequently, library preparation and sequencing using minION by Oxford Nanopore Technology was executed.

#### Results

TELSVirus generated a total of 46 to 81% on-target viral reads accompanied by less than 1% of S. scrofa reads. Using the on-target data, a total of 36 distinct virus variants were detected. Porcine bocavirus, porcine sapelovirus 1, porcine astrovirus 2 and 4, porcine torovirus, and respirovirus suis were found in >95% of the samples. Other viruses such as influenza A, atypical porcine pestivirus, porcine astrovirus 3, PRRSV, rotavirus A and porcine epidemic diarrhea were detected in 45% to 95% of the samples. Detection of these viruses was accompanied by a fair horizontal coverage of individual genomes (>60% to 100%) and vertical per-base coverage (3394X to 125X).

#### **Discussion and Conclusion**

TELSVirus workflow can effectively deplete host genome without affecting viral load. Aditionally, it allows for reconstruction of multiple viral genomes simultaneously with a turnaround time of ~24 hours. TELSVirus shows promise for enhancing our ability to understand the dynamics of viral co-infections within swine populations consequently improving control and prevention methods.

VVD – Virology and Viral Diseases

# DETECTION OF RESPIRATORY AND ENTERIC VIRUSES IN PIG HERDS OF DIFFERENT HEALTH STATUS USING, RANDOM NANOPORE SEQUENCING METHOD

W. Rybkowska<sup>1</sup>, A. Woźniak<sup>1</sup>, A. Jabłoński<sup>1</sup>, P. Cybulski<sup>2</sup>, J. Wojciechowski<sup>3</sup>, M. Rajska<sup>4</sup>, G. Balka<sup>5</sup>, L. Dénes<sup>5</sup>, B. Igriczi<sup>5</sup>, F. Griffioen<sup>7</sup>, S. Theuns<sup>6</sup>, T. Stadejek<sup>1</sup> <sup>1</sup>Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life

Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Science, Warsaw, Poland

<sup>2</sup>Goodvalley Agro S.A., Przechlewo, Poland

<sup>3</sup>VETPOL, Grudziądz, Poland

<sup>4</sup>Veterinary Clinic Vet-Com Sp. z o.o., Olsztyn, Poland

<sup>5</sup>Department of Pathology, University of Veterinary Medicine Budapest, Hungary

<sup>6</sup>PathoSense b.v., Lier, Belgium

<sup>7</sup>Laboratory of Virology, Department of Translational Physiology, Infectiology, and Public Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

# Background and Objectives

Multiple known, as well as novel and poorly studied pathogens, may be involved in endemic diseases. Conventional diagnostic methods are often insufficient for the diagnosis of these complex conditions. The aim of the study was to assess the presence of viral pathogens in herds of different health status, using a novel, complete sample-collection-to-diagnostic-interpretation platform, based on ad random nanopore sequencing.

# Material and Methods

Three high health and five conventional herds were included. Sampling started at birth until 5-12 weeks of age. Nasal and rectal swabs were collected longitudinally from three ear tagged pigs. Nucleic acids were extracted and subjected to ad random nanopore sequencing and proprietary PathoSense sequence analysis, resulting in semiquantitative pathogens load assessment. Additionally, the samples were tested with PCR for the presence of selected viruses.

# Results

In herds of high health status, as many as 22 virus species were identified in fecal swabs, including pathogenic rotaviruses A, B, C and H, despite absence of diarrhea. Other frequently identified viruses were, among others, picobirnaviruses, astroviruses, and a number of Picornaviridae species. As many as 17 virus species were identified in nasal swabs, including pathogenic influenza A virus (IAV), porcine parainfluenza 1 virus (PPIV-1) and porcine cytomegalovirus (PCMV). Some of the aforementioned enteric viruses were also often detected in nasal swabs. Interestingly, in herds with poor health status, the same virus species were detected, but with generally higher prevalence and loads. There was a general qualitative agreement between the sequencing and PCR results for detecting IAV, PPIV-1 and rotaviruses. Discordant results were obtained for some parvoviruses and circoviruses, where PCR appeared to be significantly more sensitive.

# Discussion and Conclusion

Our results provide new important data about the pathogenic viruses, as well as viruses of unknown significance for pig health, circulating in Polish pig herds of different health status and may lead to better understanding and improved diagnosis and control of multifactorial disease conditions.

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# VETERINARY PUBLIC HEALTH AND SUSTAINABLE PIG PRODUCTION

# VPH-OP-01

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

# NATIONAL SWINE INFLUENZA SURVEILLANCE PROGRAM IN THE NETHERLANDS, 2022-2023

L. Dieste-Pérez <sup>1</sup>, <u>T. Tobias</u> <sup>1</sup>, E. Van Der Vries <sup>1</sup>, E. Germeraad <sup>2</sup>, A. Kroneman <sup>3</sup>, J. Van Der Giessen <sup>3</sup>, D. Eggink <sup>3</sup>, A. Meijer <sup>3</sup>, R. Fouchier <sup>4</sup>, M. Houben <sup>1</sup>

Royal GD, Deventer, The Netherlands

<sup>2</sup>Wageningen Bioveterinary Research, Lelystad, the Netherlands

<sup>3</sup>National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

<sup>4</sup>Department of Viroscience, Erasmus MC, Rotterdam, the Netherlands

# Background and Objectives

Information about the epidemiology and evolution of influenza viruses circulating in the Dutch pig population and the relationship with circulating human influenza viruses, is lacking. To fill this knowledge gap, a national One Health surveillance pilot was set up by a consortium of Human (RIVM, EMC) and Animal Health partners (GD, WBVR) in the Netherlands. Results of the swine viruses are presented here.

#### Material and Methods

During 2022-2023, 364 group oral fluid- and 460 individual nose swab samples from 90 pig farms with influenza suspected clinical signs were collected. Farms were located in all three regions (North, Centre and South) in the Netherlands. Additionally, influenza A virus positive samples (45 lung tissues and 30 PCR products) coming from the diagnostic lab of GD were included. All samples were tested with an Influenza A RT-PCR, followed by nanopore sequencing (Ct values < 32). Results of swine and human influenza sequences were stored and analysed in a common platform.

# Results

In 77.8% of the 90 farms influenza A virus was detected. Positive samples were found in all seasons. 57.9% of the oral fluids and 28.5% of the nose swabs were positive. In total, sequences of 129 viruses were obtained. Seventy-seven were of the A(H1N1) subtype, 51 A(H1N2), 1 A(H3N2). Among the H1 viruses, a high diversity was found. In some farms, different viruses were found simultaneously and a human-to-pig transmission was detected. The highest proportion of positive farms reported clinical signs in piglets (suckling (86%)-and/or weaned piglets (85%), when clinical records were available. Only four farms submitted because of clinical signs in adult pigs instead of in piglets, and two of them were influenza A positive.

# **Discussion and Conclusion**

The pilot showed to be effective for the detection of circulating influenza viruses in pigs. Selection based on occurrence of clinical signs in piglets and collection of group saliva samples are a good sampling strategy when aiming a passive surveillance. For subtyping purposes, nasal swabs and lung tissue provided a higher sequence quality. This ongoing surveillance will allow us to increase our understanding of the epidemiology of swine influenza and risk for public health.

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# EVALUATING DIFFERENT SAMPLE TYPES AND POOLING STRATEGIES FOR OPTIMIZING INFLUENZA A VIRUS DETECTION IN BREEDING HERDS: IMPLICATIONS FOR MONITORING AND SAMPLE SIZE CALCULATIONS

D. Moraes <sup>1</sup>, H. Osemeke <sup>1</sup>, P. Gauger <sup>1</sup>, C. Moura <sup>2</sup>, G. Trevisan <sup>1</sup>, <u>G. Silva <sup>1</sup></u>, D.C. Linhares <sup>1</sup> <sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA <sup>2</sup>Iowa Select Farms, Iowa Falls, US

### **Background and Objectives**

Influenza A virus (IAV) causes respiratory disease impacting production in pigs. This abstract compared IAV RT-rtPCR (PCR) detection in sample types commonly used for IAV monitoring in breeding herds, measured the effect of pooling on the probability of IAV detection by PCR, and proposed appropriate sample sizes for monitoring IAV in breeding herds using data from two studies.

#### Material and Methods

The first study used 57 matched sets of Family Oral Fluids (FOF), udder wipes (UW), sow nasal wipes (SNW), piglet nasal wipes (PNW), and drinker wipes (DW) collected from 3 rooms (A, B, C) in an IAV-positive breeding herd. Samples were tested for IAV RNA by PCR and positivity rates across sample types were compared. The second study assessed the probability of IAV PCR detection in FOF, UW, and NW at different pooling levels. Forty-five IAV PCR positive samples (15 FOF, 15 UW, and 15 NW) were pooled at different levels (undiluted, 1/3, 1/5, and 1/10) with their respective negative samples. Samples were categorized into three groups based on the undiluted replicates' mean cycle threshold (Ct) value: Category A - Ct < 30; Category B - Ct between 30-34; Category C - Ct between 34-38.

# Results

FOF had higher PCR positivity and lower Ct values compared to UW, SNW, and DW. There was wide variation in detection rates between sampled rooms. Using PNW samples, room A had 91% positive (20/22), room B had 70% (17/24) positive, and room C had 9% (1/11) positive. For the pooling study, the probability of IAV detection in categories A and B did not decrease when the dilution level increased to 1/10 for FOF and UW. Sample size calculations will be presented at the conference.

#### **Discussion and Conclusion**

The strategic use of population-based sample types, such as UW and FOF, reduces costs and enhances the probability of detection by expanding the scope of pig, pen, room, and site coverage and offers new approaches on the practical utilization of diverse sample types. This approach is a valuable resource for IAV among practitioners, veterinarians, and producers.

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# RETROSPECTIVE ANALYSIS OF SLAUGHTER DATA OF IMMUNOCASTRATED BOARS FROM A SLAUGHTERHOUSE IN NORTHERN GERMANY IN COMPARISON TO GILTS, BARROWS AND ENTIRE MALES

S. Asanger <sup>2</sup>, M. Verhaagh <sup>1</sup>, Y. Zablotski <sup>2</sup>, S. Gumbert <sup>2</sup>, M. Ritzmann <sup>2</sup>, <u>S. Zöls <sup>2</sup></u> <sup>1</sup>*Thünen Institute of Agricultural Technology, Bundesallee 47, 38116 Braunschweig, Germany* <sup>2</sup>*Clinic for Swine at the Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-Universität München, 85764 Oberschleißheim, Germany* 

### Background and Objectives

An alternative to surgical castration of fattening pigs is the immunological castration with a GnRH-vaccine (Improvac<sup>®</sup>, Zoetis, Berlin, Germany). Since 2018, slaughterhouse Heinz Tummel no longer differentiates between surgical and immunological castrates in payment and pays castrates and females according to the same accounting mask. Objective of the retrospective study was to compare the carcass parameters of immunologically castrated (IC), female (GI), surgically castrated (BA) and entire-male (EM) fattening pigs in this slaughterhouse. Moreover, the influence on selected parameters of the carcasses and their composition were analyzed.

#### **Material and Methods**

From this slaughterhouse, after exclusion according to predefined criteria, a data set of daily slaughter operations measured by AutoFOM III from 1,613,600 animals of 182 farms between 2018 and 2022 was available. For each pig, all carcass parameters as well as sex, slaughter date and farm were assigned.

#### Results

The fattening pigs were made up as follows: 45.5% GI, 26.9% IC, 22.7% BA, 1.4% EM. The mean lean meat content of IC (61.66±2.53%) was between GI (62.69±2.51%), EM (62.33±2.26%) and BA (59.60±2.91%). Concordantly, belly lean meat content, fat thickness and diameter loin of IC were between GI and BA. IC showed the highest hot carcass weight. The mean weight of lean and boneless ham of IC was 18.98±1.34kg (BA 18.49±1.33kg, EM 18.51±1.31kg, GI 19.37±1.29kg). For ham with bones, loin with bones, lean and boneless loin, shoulder with bones and lean and boneless shoulder GI had the highest value, followed by IC, except for belly, where BA had the highest value. All parameters apart from belly (GI vs EM) and shoulder with bones (BA vs EM) differed significantly. Thereby, lean meat content, belly lean meat content and diameter loin were most influenced by sex, while hot carcass weight was mainly influenced by farm and lean and boneless ham by hot carcass weight.

#### **Discussion and Conclusion**

The results emphasize, that the carcass parameters have similar values and a similar order as in previous studies and therefore carcasses of IC are more like GI than those of BA. Overall, these field data show firstly that immunocastrated animals fitted to replace surgical castrated regarding carcass parameters (and purchase) in Germany.

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# ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLE INHIBITS AGAINST ESCHERICHIA COLI ISOLATED FROM FRESH BOAR SEMEN

<u>B. Juntanupong</u><sup>1</sup>, P. Chanapiwat<sup>1</sup>, N. Ngamwongsatit<sup>1</sup>, S. Wirasate<sup>2</sup>, K. Sirisinha<sup>2</sup>, K. Kaeoket<sup>1</sup> <sup>1</sup>Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Thailand <sup>2</sup>Department of Chemistry, Faculty of Science, Mahidol University, Thailand

#### **Background and Objectives**

Bacterial contamination is a major problem of using AI which decreases the quality of sperm. Normally, bacterial contamination is usually found in the boar reproductive tract or during semen collection. Therefore, antibiotics are used to control bacterial contamination in semen. Nevertheless, there were reports of antibiotic-resistant bacteria from semen are increasing. Silver nanoparticles (AgNPs) have been widely used as an effective antimicrobial agent rapidly against bacteria, viral, and fungi. Therefore, this study aimed to test the efficiency of silver against the growth of Escherichia coli isolated from fresh boar semen.

# **Material and Methods**

The antimicrobial efficacy of AgNPs was determined by the standard methods of the Clinical and Laboratory Standards Institute (CLSI). The fresh semen was cultured on tryptone soy agar with 5% sheep blood and MacConkey agar which was incubated at 37°C for 18-24 h. All different colonies were identified using standard biochemical tests. AgNPs and gentamicin (positive control) were tested against E. coli via agar well diffusion assay which evaluated from the presence of the zone (ZOI) around wells. The minimum inhibitory concentration (MIC) test was performed to test the lowest concentration that can inhibit bacteria. The lowest concentration which kills 99.9% of bacteria was tested by the minimum bactericidal concentration (MBC) test. Time-kill assay (4 h) was evaluated against E. coli which AgNPs were tested for concentration at 0, 0.5, 1, 2, and 4 x MIC.

#### Results

The study showed that the ZOI of the E. coli test with AgNP were  $16.41 \pm 0.18$  mm while E. coli test with gentamicin disappeared. The MIC of AgNPs were 1.56 µg/ml and MBC were 6.25 µg/ml. AgNPs were rapidly bactericidal against E. coli within 1 h at concentrations of 3.125 and 6.25 µg/ml.

#### **Discussion and Conclusion**

The result obtained from this study showed that AgNPs could inhibit the growth of E. coli isolated from fresh boar semen. Due to AgNPs have distinct multi-targeting bactericidal mechanisms, consisting of adhering to the cell wall of bacteria, uncoupling the respiratory chain, damaging cellular biomolecules, and disrupting cell signals. Therefore, AgNPs can inhibit Escherichia coli that resistant to gentamicin.

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# DIPTERIC FLIES AS LIVING VECTORS TRANSMITTING MRSA IN PIGLET PRODUCING FARMS

<u>F. Hamar</u><sup>3</sup>, I. Loncaric<sup>1</sup>, T. Bernreiter-Hofer<sup>3</sup>, A. Käsbohrer<sup>2</sup>, A. Ladinig<sup>3</sup>, L. Schwarz<sup>3</sup> <sup>1</sup>Institute of Microbiology, University of Veterinary Medicine Vienna, Austria <sup>2</sup>Institute for Veterinary Public Health and Epidemiology, University of Veterinary Medicine Vienna, Austria <sup>3</sup>University Clinic for Swine, University of Veterinary Medicine Vienna, Austria

# **Background and Objectives**

Methicillin-resistant Staphylococcus aureus (MRSA) poses a major global threat as an antimicrobial-resistant pathogen. The present study aimed to investigate the presence of MRSA on houseflies and stable flies as potential vectors in Austrian piglet producing farms. The primary objectives included isolation and characterization of MRSA.

# Material and Methods

Sampling involved 25 farms, each housing 20 sows minimum. Samples comprised of house flies and stable flies (8 pooled samples, each containing 10 single, specific specimen), a boot sock sample, and a dust wipe sample from each farm. All fly samples were processed within 12 hours, involving washing the flies with a sterile saline solution used for selective isolation of MRSA. Characterization of the MRSA isolates was performed by susceptibility testing and genotyping by spa- and dru-typing, following a DNA-based microarray analysis.

# Results

Among the 25 farms, 11 (44%) had MRSA-positive samples, yielding 48 MRSA isolates from samples (19.2%). The distribution of MRSA-positive samples included 26 isolates from house flies (54.2%), 9 from stable flies (18.8%), 8 from boot sack samples (16.7%), and 5 from dust wipe samples (10.4%). All identified MRSA strains belonged to the livestock-associated clone CC398, exhibiting a combined resistance to ß-lactams and tetracyclines or ß-lactams, tetracyclines, macrolides and lincosamides which is well reflected by the observation that these isolates carried the genes mecA, blaZ, tet(K), tet(M) erm(A), erm(B), and erm(C). The predominant spa type was t011, followed by t034, and the most frequently observed dru type was dt11a, with three new dru types identified.

### **Discussion and Conclusion**

These findings underscore the presence of MRSA in pig farms and the potential role of flies in its transmission to pigs, other animals and humans. The isolation of MRSA strains within the livestock-associated clone CC398 emphasizes the need for vigilant monitoring and control measures. Flies, as living vectors, pose a serious risk in disseminating multi-resistant bacteria, necessitating further research and preventive measures to curtail MRSA spread within livestock settings, safeguarding the health of both animals and humans.

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# FOR THE BENEFIT OF PIG & HUMANS - MINIMISING ANTIBIOTIC USE IN PIG GROWERS AND FINISHERS

<u>M. Costa</u><sup>1</sup>, E. Piccinin François<sup>1</sup>, S. Kirwan<sup>2</sup> <sup>1</sup>Kemin South America, Brazil <sup>2</sup>Kemin Industries, USA

# **Background and Objectives**

Growing and finishing pigs get most of the attention from a financial point of view, because of all phases these periods account for most of the cost of production in the life of a pig, probiotics however, are rarely used due to the perceived high cost. In contrast probiotics have proven health benefits in sows and their piglets and are used routinely, therefore the hypothesis was that those benefits would translate to growers and finishers, improving their health, profitability and reducing the need for antimicrobials.

# Material and Methods

240 pigs of 64 days with an average weight of 21.78kg were used for the study, housed and fed a corn-soy diet according to industry standards. The experimental treatment design was fully randomised, dividing pigs spacially and by weight and sex in equal numbers into either control or probiotic. In the control treatment, AGPs (antibiotic growth promoters) were used in the finisher 1 & 2 (6 ppm of Flavophospholipol and 158 ppm of Tylosin), and in the probiotic group, 500g/ton was used continuously across both phases. Blood samples were taken on day 95 from both groups.

#### Results

The probiotic group had improved final weights (129.34kg), with weight gain improving by more than 2.3 kg when compared to the control group (127.07kg). The probiotic promoted a better digestibility of dry matter, crude protein, and gross energy ( $p \le 0.01$ ). Considering the difference in gain ((weight gain x \$/kg head) - (feed cost x feed intake)), animals that received the probiotic had a return of \$2.31 more, compared to animals that did not receive it.

### **Discussion and Conclusion**

The use of probiotics in pigs, even with the aim of replacing antimicrobials (AGPs) was in the past frequently dismissed as unviable due to cost, even though desirable for antimicrobial resistance management. Probiotics have proven benefits, and now also for growers and finishers, probiotics therefore have the potential to replace antimcrobials and promote greater weight gain, through better nutritional efficiency, confirmed by better digestibility of nutrients and with economic viability at an ROI (return over investment) of 6.5; using the local cost of feed and value pig kg gain, at the time.

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# THE ROLE OF TRANSPORTATION IN THE SPREADING OF SWINE PATHOGENS AND ANTIMICROBIAL-RESISTANT BACTERIA

<u>M. Masserdotti</u>, N. Formenti<sup>2</sup>, A. Donneschi<sup>2</sup>, F. Guarneri<sup>2</sup>, F. Scali<sup>2</sup>, E. Giacomini<sup>3</sup>, G.L. Alborali<sup>2</sup>, C. Luzzago<sup>1</sup> <sup>1</sup>Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy <sup>2</sup>IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna), Brescia ITALY <sup>3</sup>Veterinary practitioner

#### **Background and Objectives**

The transport of live pigs poses a risk to on-farm biosecurity. Trucks can carry pathogens with significant economic and health impacts, including antimicrobial-resistant (AMR) bacteria. The aim of this study was to investigate the faecal contamination of trucks before and after loading, focusing on major pathogens and AMR.

# Material and Methods

A total of 84 samples from 42 trucks in 11 farms were collected by swabbing the internal surface of the empty trucks at the entrance ('clean') and before leaving ('dirty') the farm. Samples were tested for total bacterial count (TBC), specific bacteria (Enterobacteriaceae, Escherichia coli, Salmonella spp., Brachyspira spp.) and viruses (Porcine Reproductive and Respiratory Syndrome Virus – PRRSV, Porcine Epidemic Diarrhoea Virus – PEDV, Rotavirus A, B, C and H). Escherichia coli isolates were also tested for the presence of extended-spectrum  $\beta$ -lactamase (ESBL), other  $\beta$ -lactamases (AmpC) and carbapenemase with phenotypical and genotypical methods. Differences between 'clean' and 'dirty' samples were analysed through Generalized Linear Models.

# Results

Some pathogens were found only in 'dirty' samples, such as carbapenemase-producing E. coli (23.8% of the 'dirty' samples), Salmonella spp. (7.1%), PRRSV (20.5%). In 'dirty' samples, TBC was significantly higher (p=0.002; mean<sub>dirty</sub> 65.4x10<sup>3</sup> ufc vs. mean<sub>clean</sub> 17.9x10<sup>3</sup> ufc), as was the presence of Enterobacteriaceae (p<0.001; mean<sub>dirty</sub> 32.2x10<sup>3</sup> ufc vs. mean<sub>clean</sub> 9.2x10<sup>3</sup> ufc), E. coli (p<0.001; 69.1% vs. 11.9%), ESBL/AmpC-producing E. coli (p=0.036; 9.5% vs. 2.4%) and Rotavirus A (p=0.014; 28.2% vs. 5.1%). No significant differences between 'dirty' and 'clean' samples for rotavirus C (53.9% vs. 59.0%) and H (2.6% vs. 7.7%) were found.

#### **Discussion and Conclusion**

Although the prevalence of most agents in 'clean' samples was low to zero, the presence of E. coli and some rotaviruses highlights the importance of improving sanitisation procedures. The isolation of E. coli producing ESBL, AmpC and carbapenemase was of particular concern. Especially for the latter, which confer resistance to antimicrobials that should only be used in humans. These findings confirm the role of trucks in spreading not only pathogens, but also AMR, and underscore the importance of effective monitoring and proper sanitisation procedures.

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# THE EVOLUTION OF ANTIMICROBIAL RESISTANCE OF STREPTOCOCCUS SUIS ISOLATED FROM THE NERVOUS SYSTEM

S. Rodrigues Chagas 1, E. Paladino 2, C. Gebhart 3, L. Mendonça Pascoal 4, R. Singer 5, F. Vannucci 6

<sup>1</sup>Universidade Federal de Goiás and University of Minnesota

<sup>2</sup>University of Minnesota

<sup>3</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

<sup>4</sup>Universidade Federal de Goiás - Goiânia - Brasil

<sup>s</sup>Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA

# **Background and Objectives**

Some Streptococcus suis strains can express virulence genes making the bacterium able to cross the blood-brain barrier and access the central nervous system (CNS). These strains represent a concern to the swine industry and public health since S. suis is a zoonotic bacterium. Thus, this study aimed at monitoring the evolution of antimicrobial resistance (AMR) of S. suis strains isolated from the CNS in the US over the last 10 years.

# **Material and Methods**

S. suis data from clinical cases were obtained from the Minnesota Veterinary Diagnostic Laboratory (MN-VDL) between 2014 and 2023. A total of 1,020 S. suis isolates from CNS were selected and tested for antimicrobial susceptibility (Sensititre™, Thermo Scientific) to assess the minimal inhibitory concentration (MIC). Antimicrobials tested included ampicillin, penicillin, ceftiofur, clindamycin, chlortetracycline, oxytetracycline, enrofloxacin, florfenicol, gentamicin, neomycin, spectinomycin, tiamulin, tilmicosin, tulathromycin, tylosin. We analyzed the MIC 50 and MIC 90 (concentrations at which 50% and 90% of the isolates were inhibited, respectively) for each antimicrobial.

# Results

The MIC50 and MIC90 were stable over the 10 years for ampicillin, ceftiofur, chlortetracycline, clindamycin, enrofloxacin, florfenicol, neomycin, oxytetracycline, penicillin, spectinomycin tilmicosin, tulathromycin, and tylosin. Only for ceftiofur in 2017, the MIC90 spiked to 2, and after that returned to the baseline of 0.5 and remained at that concentration until 2023.

# **Discussion and Conclusion**

Currently, veterinary MIC breakpoints for S. suis are available for six antimicrobials only (CLSI, 2023). Thus, analyzing the MIC50 and MIC90 allows not only to observe more sensitive fluctuations over the years, but also to analyze the AMR evolution of drugs not included in CLSI manual. For this retrospective analysis of the samples obtained at the MN-VDL from neurologic clinical cases, no remarkable increase in the MIC50 and MIC90 was observed over the period of 2014 to june-2023.
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## THE PORK FOOD CHAIN AS A ROUTE OF TRANSMISSION OF ANTIMICROBIAL RESISTANT ESCHERICHIA COLI: A FARM-TO-FORK PERSPECTIVE

<u>M. Rega</u><sup>1</sup>, L. Andriani<sup>1</sup>, S. Bonardi<sup>1</sup>, M. Conter<sup>1</sup>, C. Bacci<sup>1</sup> <sup>1</sup>University of Parma - Department of Veterinary Science

## Background and Objectives

The pork food industry contributes to the spread of antimicrobial resistant (AMR) microorganisms through the food chain and the environment. A significant risk factor is the possible development of Multi Drug Resistance (MDR) and non-susceptibility to last generation cephalosporins. This study focuses on the pork food chain to evaluate the risk of AMR E. coli transmission from animal to consumer with a focus on cephalosporin resistance.

## Material and Methods

Sample collection was carried out in eight fattening pig farms in North Italy from 2019 to 2022. Feces, carcasses, fresh meat and processed meat products we collected guaranteeing traceability of matrices from farm-to- fork for each animal. coli were isolated and their ability to resist to aminoglicosides, quinolones, polymixins, glycilcyclines, sulphonamides, β-lactams was evaluated with a main focus on ESBL and AmpC production. Phylogenetical analyses were performed through Enterobacterial Repetitive Intergenic Consensus sequences and Average Nucleotide Identity to confirm possible relations between the strains isolated from the different production phases.

#### Results

The most frequent resistance pattern found included  $\beta$ -lactams-aminoglycosides-sulphonamides defining MDR strains in feces and carcasses, but not in pork meat products. The evaluation of  $\beta$ -lactams resistance highlights percentages ranging from 7% to 12.5% in fecal, carcass, fresh meat and processed meat product isolates in a increasing order. Data showed that the 50% of phenotypical AMR bacteria found along the pork food chain were related phylogenetically with different identity percentages.Considering  $\beta$ -lactamases producing strains, in only one case bacteria were closely genotypically similar from farm-to-fork (feces, carcasses, and fresh meat). Frequent close similarities were found in "carcasses and fresh meat", "carcasses and fermented product", "feces and fresh meat" and "fresh meat and seasoned product" isolates.

#### **Discussion and Conclusion**

In conclusion, direct transmission of AMR from farm-to-fork is a public health concern and β-lactams AMR transmitted in only one case. Fresh meat showed a higher risk of AMR transmission than processed meat products. However, the prudent use of antimicrobials in pig farms, the correct management of food production stages and of the environment continue to play a strategic role in the spread of AMR microorganisms.

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# ANIMAL HEALTH FOOTPRINT – HOW CHANGES IN ANIMAL HEALTH STATUS INFLUENCED THE ECOLOGICAL FOOTPRINT IN PORK PRODUCTION

L. Gombos <sup>1</sup>, L. Búza <sup>2</sup>, I. Szabo <sup>3</sup>, I. Szabo <sup>4</sup>, V. Gombosne Szur <sup>5</sup>

<sup>1</sup>University of Györ, Wittmann Antal Plant-, Animal- and Food Sciences Multidisciplinary Doctoral School, Mosonmagyaróvár, Hungary

<sup>2</sup>Topigs Norsvin

<sup>3</sup>National PRRS Eradication Committe, Budapest (Hungary)

<sup>4</sup>Hungarian University of Agrculture and Life Sciences, Department of Environmental Toxicology, Gödöllő, Hungary <sup>5</sup>Vadocdoki Research and Development Divison, Hungary

## **Background and Objectives**

In 2022, Hungary completed the national Porcine reproductive and respiratory syndrome eradication program. In 8 years, 35% of the herd have been replaced with higher genetic value. Therefore, fewer sows were able to produce almost the same number of slaughter pigs. The goal of our research was to evaluate the changes in the animal health status and its influence on the environmental footprint.

## Material and Methods

We examined the influence of five different factors on animal health: the status of the farm census, the antibiotic and vaccine use, the total medication costs, the losses (culling and mortality rates) and the feed and on-farm water use. The data of our research were collected from five large scale farms (9500 sows and the progenies). Based on FAO research(2013), every kg of swine carcass has an emissions intensity of 6.1 kg of carbon dioxide equivalent. The grow-finish production phase has the highest overall environmental impact on life cycle assessment (LCA), contributing to 52.5% of the carbon footprint, 68% of feed and 75% of water usage on farms. Lastly, the collected data were converted to carbon dioxide equivalents.

#### Results

According to our research, the change in health status contributed to the change in the footprint with a 1.95 kg CO2eq / carcass kg reduction. The antibiotic use was reduced by an average of 60%, and the "restrict" category (AMEG B) was removed from all farms. The loss of offsprings reduced by 60%, however, there were fattening farms where it reached even 90%. On average, the decrease in the feed conversion rate was 35%.

#### **Discussion and Conclusion**

By 2030, the EU plans to cut its net GHG emissions by half compared to the levels measured in 1990. Since the start of the PRRS eradication program, emissions in the Hungarian pork sector decreased by 248,095 tons. Our research found that changes in the health status were able to improve the carbon footprint by at least 32%.

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# EFFECT OF IMPROVED FEED CONVERSION RATIO BY LAWSONIA INTRACELLULARIS VACCINATION ON THE CARBON FOOTPRINT

<u>F. Von Und Zur Muehlen</u>, C. Renken <sup>1</sup>, R. Tabeling <sup>1</sup> <sup>1</sup>Intervet Deutschland GmbH, MSD Animal Health, Unterschleißheim, Germany

## **Background and Objectives**

Due to the need for climate protection, pig production also faces responsibility for reducing greenhouse gas emissions (GHG) and, therefore, improving carbon footprint. The aim of this report was to evaluate the impact of an improved feed efficiency after Lawsonia intracellularis vaccination on the reduction of GHG.

### Material and Methods

Performance data (i.e. feed conversion ratio; FCR) from field observations of 9 farms with history of subclinical or clinical ileitis was recorded in non-vaccinated (NV) and vaccinated (PL) (intramuscularly/intradermally Porcilis®Lawsonia/ID; 3-11 weeks of age) batches. NV batches, used as historical control, were compared to PL batches. Carbon footprint (CO2-e) was calculated using the agricultural GHG calculator "TEKLa" from the Lower Saxony Chamber of Agriculture (base German-wide calculation standard) assuming a standardized feed (12.6% imported soybean meal). GHG-emissions were analyzed for all groups and for the subareas (feed, piglets, manure, energy, fertilizers) of "TEKLa", as well as for non-vaccinated and PL-vaccinated batches.

#### Results

Using the average performance data for both groups (PL unvaccinated and PL vaccinated, 96kg live weight (LW) gain; FCR 2,79), TEKLa calculated a mean amount of 2891 g  $CO_2$ -e/kg LW. The model estimated that 1594 g  $CO_2$ -e/kg LW (53-58%) belonged to feed. The mean  $CO_2$ -e proportion for produced piglets (29 kg LW), manure/digestion and energy consumption were 28.2%, 22.0%, and 2.7%, respectively, whereas 8.0% was credited due to the reuse of the organic fertilizer. In PL-vaccinated pigs, a mean improvement of -0,11 was recorded for FCR. The model showed that non-vaccinated group emitted on average 2928 g, whereas PL-vaccinated group 2853 g  $CO_2$ -e/kg LW. In the farm with the highest improvement in feed conversion after introduction of the Lawsonia intracellulairs vaccination (FCR -0.27), a lowering of 182 g  $CO_2$ -e/kg LW was observed (6.23%). The maximum deviation regarding  $CO_2$ -e excretion between the worst and best fattening group was 12.1%.

#### **Discussion and Conclusion**

Under the conditions of this report, TEKLa model calculation demonstrated a reduction of GHG-emission associated with improved feed efficiency in pigs vaccinated against Lawsonia intracellularis. This suggests that improved feed efficiency contributes to sustainability in pig production.

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## EFFECT OF IMPROVED FEED EFFICIENCY BY LAWSONIA INTRACELLULARIS VACCINATION ON NITROGEN EMISSION IN FATTENING PIGS

<u>R. Tabeling</u><sup>1</sup>, F. Von Und Zur Muehlen<sup>1</sup>, C. Renken<sup>1</sup> <sup>1</sup>Intervet Deutschland GmbH, MSD Animal Health, Unterschleißheim, Germany

## Background and Objectives

Nitrogen (N; elementary protein component) in adequate quantity and quality is essential for optimal growth and performance in pigs. Negative environmental impact, like subsequent eutrophication resulting from too high N-output by pig production, led to a regulatory limitation on N-emission for German agriculture. The objective of this report was to show the impact of improved feed efficiency after Lawsonia intracellularis vaccination in combination with ration design on the N-excretion in fattening farms.

## Material and Methods

Performance data (i.e. feed conversion ratio; FCR) from field observations of 9 farms with a history of subclinical or clinical ileitis was recorded in non-vaccinated (NV) and vaccinated (PL) (intramuscularly/intradermally Porcilis®Lawsonia/ID; 3-11 weeks of age) batches. NV batches, used as historical control, were compared to PL vaccinated batches after implementing Lawsonia intracellularis vaccination to control ileitis. N-excretion was modeled using the official manual from the Lower Saxony Chamber of Agriculture using four crude protein (CP) levels in feed rations (CP %/kg; 88% dry matter: "universal" 17.0, "N reduced" 16.4; "N greatly reduced" 15.4; "N very greatly reduced", 14.4).

### Results

The mean reduction of N excretion in PL vaccinated batches was 5.4-5.9% for all feeds when compared to non-vaccinated batches. The farm with the biggest improvement of FCR with PL vaccination (-0.27; -9.5%) showed a reduced N excretion of 15.7%. Between the non-vaccinated group with worst FCR and highest assumed CP content in feed (FCR 3.07; CP 17.0%; 57.9 g N/kg LW) and the PL vaccinated group with the best FCR and very greatly N-reduced feed (FCR 2.51; CP 14.4%; 32.6 g N/kg LW) a saving of 25.3 g N/kg LW (43%) was estimated. When this was calculated based on the observed average weight gain of 96 kg/pig, the worst group emitted 5.6 kg N, whereas the best 3.2 kg N/finishing pig.

### **Discussion and Conclusion**

Under the conditions of this field observation, it has been shown that N-emission from pig farms can be reduced by lower CP contents in feed and increasing feed efficiency with Lawsonia intracellularis vaccination. This suggests that both are suitable tools to reduce N-emission, making pig production more sustainable.

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## TRANSCRIPTOMIC ANALYSIS OF BRACHYSPIRA HYODYSENTERIAE TREATED WITH PHYTOBIOTIC-PREBIOTIC FEED ADDITIVE

O. Palócz <sup>1</sup>, G. Csikó <sup>1</sup>, <u>Z. Bata</u> <sup>2</sup>, V. Molnár-Nagy <sup>2</sup> <sup>1</sup>University of Veterinary Medicine, Budapest, Hungary <sup>2</sup>Dr. Bata Ltd, Ócsa, Hungary

#### **Background and Objectives**

Spirochetes, like Brachyspira hyodysenteriae, are dangerous animal pathogens that are often linked to diarrhoea. Because of the lack of effective active immunization, disease control has been based on pro- and metaphylactic use of antibiotics for many decades which consequently led to widespread antimicrobial resistance. Due to restrictions on the use of antimicrobial drugs we are pressed to search for other methods, with emphasis on disease prevention. The beneficial effect of feed additives has been widely reported, however, their exact mechanism of action is scarcely known. Therefore, we aim to determine the mechanism of action of a phytobiotic-prebiotic feed additive (PPFA) on B. hyodysenteriae.

#### Material and Methods

The study was performed on the type strain B. hyodysenteriae strain ATCC 27164. Bacterial cultures were treated with three sub-MIC concentrations of the test material; extract of thyme and carob seed based PPFA, prior to whole transcriptome analysis of the control and the treated samples. Sequencing was performed on an Illumina NextSeq device, generating at least 5 million clusters per sample. After quality control with FastQC, reads were filtered and trimmed using Trimmomatic 0.39. The prepared reads were fitted to the B. hyodysenteriae ATCC 27164 genome. Differences in gene expression between groups and the dependence of expression on test material concentrations were analysed by negative binomial model in R software environment.

#### Results

The expression of a total of 500 genes showed concentration-dependent significant differences. Among them the genes linked to flagellum function and haemolysin-coding were overrepresented. The effect on these genes was verified by qPCR. The results confirmed the concentration-dependent decrease in the gene expression in most selected targets, and clearly indicated the action mechanism of the PPFA.

#### **Discussion and Conclusion**

Flagellum is an important virulence factor of pathogen bacteria through chemotaxis, adhesion and invasion of host surfaces. Inhibition of flagellum-formation may be an important target for preventative agents to obstruct pathogens from entering the host. The inhibition of haemolytic activity can selectively decrease the pathogenic effect of B. hyodysenteriae.

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## REPRODUCTION

## REP-OP-01

**REP** - Reproduction

## NORMAL BIRTHWEIGHT AND INTRAUTERINE GROWTH RESTRICTED PIGS PRESENT DIFFERENT DYNAMICS OF MICROBIOTA-RELATED METABOLIC PATHWAYS THROUGHOUT POSTNATAL LIFE

<u>S.D. Fernandes</u><sup>2</sup>, F.F. Aburjaile<sup>1</sup>, T.G. Santos<sup>2</sup>, R.D.D.O. Carvalho<sup>3</sup>, V. Azevedo<sup>4</sup>, B. Brenig<sup>5</sup>, F.R. C. L. De Almeida<sup>2</sup> <sup>1</sup>Preventive Veterinary Medicine Departament, Veterinary School, Federal University of Minas Gerais <sup>2</sup>Departament of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais

<sup>3</sup>Department of Biochemistry and Biophysics, Health Sciences Institute, Universidade Federal de Minas Gerais (Federal University of Minas Gerais)

<sup>4</sup>Genetics, Ecology and Evolution Department, Federal University of Minas Gerais <sup>5</sup>Institute of Veterinary Medicine, University Göttingen

## **Background and Objectives**

Intrauterine Growth Restriction (IUGR) is a major issue in pig production, since it causes low birthweight and leads to higher pre-weaning mortality and poor performance and carcass quality. IUGR is caused by uteroplacentary inefficiency, and induces vascular rearrangement to maintain the brain, which hinders the development of other organs, such as the intestinal tract (IT). The IT is colonized by many populations of bacteria, which mediate its maturation and many metabolic functions of the digestive system, and play a role in the immunity of the pig. Literature regarding swine microbiota is increasing but information on alterations in composition and function of the microbiota of IUGR pigs is still scarce. Thus, the objective of this study was to investigate alterations caused by IUGR on microbiota-related metabolic pathways throughout postnatal development.

## **Material and Methods**

One hundred-sixty pairs of littermate piglets were allocated in two experimental groups, according to birthweight: normal birthweight (NBW, n=40, 1.6-1.9kg) and intrauterine growth restricted (IUGR, n=40, 0.7-1.0kg). At four selected ages (0, 26, 70, 150 days-old) 10 animals of each group were randomly selected for euthanasia, and feces were collected for shotgun metagenomic analysis of bacterial DNA. Data were paired with KEGG pathways database.

## Results

NBW pigs presented an increase in activated metabolic pathways from birth to weaning, indicating an appropriate response to the diet change. In this group, sphingolipid metabolism increased from birth to 26 days, and remained higher at 70 and 150 days (P<0.05), and MAPK signaling pathway was higher at 26 and 70 days (P<0.05). IUGR pigs, on the other hand, presented lower longevity regulation at 70 and 150 days, compared to birth (P<0.05), and more pathways related to diseases, such as Huntington disease, which decreased after birth but remained present up until 70 days of age (P<0.05).

#### **Discussion and Conclusion**

The dynamic of microbiota-related pathways suggests that metabolism in NBW pigs increased along animal growth and demonstrated physiological regulation that ensures performance and health, while IUGR pigs struggled with poor regulation of growth and susceptibility to degenerative diseases. Our study elucidates the alterations caused by IUGR on microbiota function that impair pig development, and indicate optimal time points for probiotic intervention.

## **REP** - Reproduction

# INFLUENCE OF INSEMINATOR AND ARTIFICIAL INSEMINATION MANAGEMENT ON REPRODUCTIVE PERFORMANCE CHARACTERISTICS IN SOW HERDS

L. Walther <sup>2</sup>, B. Vidondo <sup>1</sup>, A. Grahofer <sup>2</sup>

<sup>1</sup>Veterinary Public Health Institute, University of Bern, Liebefeld <sup>2</sup>Clinic for Swine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

#### **Background and Objectives**

Reproductive failure in sow herds is often caused by management related factors and can lead to tremendous economic losses. Therefore, the aim of this study was to investigate the influence of the inseminator and insemination management on the reproductive performance of sows.

#### Material and Methods

Five farms with at least two inseminators were included in this study. The insemination procedure of each inseminator was evaluated and a questionnaire on the insemination management was conducted. Furthermore, the performance characteristics (farrowing rate, return to oestrus rate and litter size) of 3156 artificial inseminations (AI) of the last 13 months were analysed.

## Results

At the AI level of all farms the intracervical insemination technique was performed with an average duration of 11.87min  $\pm$  16.67min per sow. Overall, cleaning of the vulva (OR = 1.46; p-value = 0.045), wearing cloves (OR = 1.37, p-value = 0.041,) and an adequate stimulation of the sow through the inseminator (OR = 1.8; p-value = 0.002) as well as leaving the catheter in place for some minutes after semen application (OR = 1.86; p-value = 0.001) significantly reduced the return to oestrus rate in sows. When comparing all Als of two given inseminators working on the same farm, a significant impact of the inseminator on the return to oestrus rate (%) (farm 1: 14.7 vs. 6.4, p-value = 0.038, sample size (ss) = 129 vs. 125; farm 5: 7.5 vs. 5.3, p-value = 0.027, ss = 914 vs. 1401) and farrowing rate (%) (farm 1: 67.4 vs. 80.0, p-value = 0.023; ss=914 vs. 1401 could be detected. In addition, the total number of born piglets per litter differed by 1 piglet between the two inseminators of farm 1 (10.5 vs. 11.5, p-value = 0.036; ss = 88 vs. 100).

#### **Discussion and Conclusion**

In this study, we obtained first evidence that the inseminator as well the insemination management can influence the reproductive performance of a sow herd. Hence, during a herd examination focusing on the breeding management, an analysis of the insemination procedure of each inseminator should be conducted, to identify risk factors influencing the reproductive performance.

### **REP** - Reproduction

## MATERNAL PRRSV INFECTION AND ITS CONSEQUENCES ON FETAL MICROBIAL COMPOSITION: AN EXPERIMENTAL STUDY

H. Kreutzmann<sup>1</sup>, B. Metzler-Zebeli<sup>2</sup>, S. Koger<sup>3</sup>, U. Ruczizka<sup>4</sup>, M. Koch<sup>5</sup>, M. Zaruba<sup>6</sup>, T. Rümenapf<sup>6</sup>, A. Ladinig<sup>5</sup> <sup>7</sup>Royal GD, Deventer, The Netherlands

<sup>2</sup>Unit of Nutritional Physiology, University of Veterinary Medicine Vienna, Austria <sup>3</sup>Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, Austria <sup>4</sup>Association for Sustainable Animal Husbandry in Austria, Project Animal Health, Vienna, Austria <sup>5</sup>University Clinic for Swine, University of Veterinary Medicine Vienna, Austria <sup>6</sup>Institute of Virology, University of Veterinary Medicine Vienna, Austria

### Background and Objectives

Contrary to the assumption that the uterus is sterile and bacterial colonization in newborns begins during birth, recent studies in humans and mice suggest that the placenta harbors a unique microbial community. Fetuses are exposed to bacteria prior to birth, with significant effects on their health status after delivery. The objective of this study was to investigate whether this thesis is applicable to swine with its epitheliochorial placenta. Additionally, it was aimed to assess whether a PRRSV infection influences microbial communities in the amniotic fluid and meconium of fetuses.

#### **Material and Methods**

On gestation day 85, five gilts were experimentally infected with the virulent PRRSV-1 strain AUT15-33, while five gilts remained as control group. Necropsy was performed three weeks after infection. Viral load was assessed in fetal serum and thymus. Three groups of 30 fetuses each were formed: infected with detectable and undetectable viral load (I+VL+ and I+VL-), and non-infected (NI) fetuses. Amniotic fluid (AMN) and meconium samples (MEC) were collected from fetuses directly after euthanasia, snap frozen in liquid nitrogen and stored at -80°C. DNA was extracted, quantified and 16S rRNA amplicon sequencing was performed.

#### Results

Independent of the group, bacterial DNA could be quantified in all examined samples (7.58±0.38 [mean±SD] and 8.61±0.45 log<sub>10</sub> copies/mL AMN and g MEC, respectively). 16S rRNA sequencing was possible in 35 AMN and 42 MEC samples, revealing Pseudomonadaceae as the most abundant bacterial family. The analysis of beta-diversity showed significant differences between the three groups and were observed for both AMN and MEC (p≤0.001). Species richness and alpha-diversity indices, in turn, indicated that the bacterial communities in the AMN and MEC samples were similarly diverse.

## **Discussion and Conclusion**

This study reinforces the hypothesis that fetuses are already microbially colonized within the uterus and are not sterile, contrary to descriptions in many textbooks. The findings align with studies in other animal species, including humans. The differences in beta-diversity between groups suggest an influence of fetal PRRSV infection on the microbiome. Further studies are necessary to elucidate the consequences of these alterations.

## **REP** - Reproduction

## HIGHER PIGLET VITALITY AT BIRTH LEADS TO INCREASED COLOSTRUM INTAKE AND LOWER MORTALITY

<u>R. Carnevale</u><sup>1</sup>, B. Bracco Donatelli Muro<sup>4</sup>, M.S. Monteiro<sup>2</sup>, A. Augusto Justo<sup>3</sup>, B.B. Carnino<sup>4</sup>, P.D.V. Firminiano<sup>4</sup>, M. Ambroso Adib Donato Henriques<sup>3</sup>, C. Tavares Meloto<sup>3</sup>, A. Nunes Reis<sup>3</sup>, G. Salvador Cardoso<sup>3</sup>, L. Braselino Borges<sup>3</sup>, C.A. Pospissil Garbossa<sup>4</sup>, F.V.S. Junior<sup>4</sup>

<sup>1</sup> Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>2</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>3</sup>Department of Veterinary Medicine, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, SP, Brazil

<sup>4</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

## Background and Objectives

Piglets that experience prolonged periods of hypoxia are also more predisposed to have a lower vitality at birth. These animals have a lesser capacity to adapt to the extrauterine life, resulting in an impaired capacity to compete with their littermates for proper colostrum intake. Colostrum plays a crucial role for the piglets' survival and early development by providing nutrients and passive immunity. The aim of the present study was to assess if piglet vitality at birth is associated with colostrum intake, piglet performance and mortality throughout lactation.

#### Material and Methods

Thirteen gilts had their farrowing monitored. To assess piglet vitality, an Apgar score considering respiratory latency, hearth rate, snout skin color, latency to stand up and meconium staining, was used. Colostrum intake was calculated according to Theil et al. (2014). Piglets' performance was assessed by weighing piglets at birth, 24 hours after birth and at weaning. Mortality rate was recorded until weaning. To assess associations between variables linear regressions were performed and statistical differences were set at p<0.05.

#### Results

Apgar score was positively associated (p<0.05) with colostrum intake and weight at 24 hours after birth. There was no association between apgar score and weight at weaning (28 days). A negative correlation (p<0.05) was found between apgar score and mortality until weaning.

#### **Discussion and Conclusion**

The results from the present study demonstrated the pivotal role of piglet vitality at birth, as indicated by the apgar score, in determining early colostrum intake. While the association between apgar score and weight at weaning was not observed, the negative correlation with mortality until weaning underscores the long-term implications of low vitality at birth on survival rates. Management strategies during the first 24 hours post-farrowing must be applied to support piglets with lower vitality to ingest adequate colostrum amount and to increase their chance of survival throughout lactation.

**REP** - Reproduction

## TRANSVAGINAL OVUM PICK-UP IN SOWS: IMPACTS ON WELFARE, REPRODUCTION, AND FUTURE APPLICATIONS

<u>A. Oltedal</u><sup>1</sup>, A.H. Gaustad <sup>2</sup>, O. Peltoniemi <sup>3</sup>, S. Björkman <sup>3</sup>, M. Oropeza-Moe <sup>4</sup> <sup>1</sup>Norwegian University of Life Sciences <sup>2</sup>Norsvin SA, Norway <sup>3</sup>Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland <sup>4</sup>Faculty of Veterinary Medicine, Department of Production Animal Clinical Sciences, Norwegian University of Life Sciences, Sandnes, Norway

## **Background and Objectives**

Transvaginal ultrasound-guided Ovum Pick-Up (OPU) is an established technique in various species, yet its application in sows remains underexplored. This study aimed to enhance transvaginal OPU procedures for efficient oocyte collection from valuable sows, evaluating the welfare implications and reproductive health in a controlled field trial. We have previously published the results from our first experiments with the technique.

#### Material and Methods

This study aimed to assess the effects of OPU on the welfare of second-parity hybrid sow, employing salivary cortisol measurements and a novel approach-aversion test. OPU was performed in physically restrained, non-sedated animals (N = 26). Salivary cortisol levels were measured before, during, and after OPU, and compared to control animals. A novel approach-aversion test assessed the sows' willingness to participate in OPU for a feed reward. The effects of the procedure on the reproductive performance of the animals were evaluated by synchronisation and insemination of the animals.

#### Results

Physiological stress, indicated by a significant increase in salivary cortisol during OPU, was observed. However, cortisol levels at the end of physical restraint did not differ between OPU and control animals (P = 0.53). The approach-aversion test revealed aversion to the restraint procedure, with no difference between OPU and control groups on the day after the procedure. Reproductive performance, evaluated in 22 OPU and 22 control sows, showed no significant differences in farrowing rate (73% vs. 82%, P = 0.72) or total number of born piglets.

## **Discussion and Conclusion**

The motivation for feed in feed-restricted sows exceeded aversion to OPU, but the physical restraint induced a transient stress response. While transvaginal OPU shows potential for oocyte recovery from second-parity hybrid sows, refinements are needed to minimize stress during the lifting procedure and reduce inter-individual variations in oocyte recovery outcomes.

#### **REP** - Reproduction

## HIGH PROTEIN GENE PRODUCT 9.5 EXPRESSION IN TESTES IN 8-DAY-OLD PIGLETS

U. Yamsrikaew<sup>1</sup>, S. Srisuwatanasagul<sup>2</sup>, M. Nuntapaitoon<sup>3</sup>

<sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>3</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

## **Background and Objectives**

Protein gene product 9.5 (PGP9.5) is the molecular marker for spermatogonial stem cells (SSCs), that is expressed in spermatogonia. The SSCs are capable of both self-renewal and differentiation into mature spermatozoa. The stages of spermatogonia expressing PGP9.5 vary based on the species and reproductive stages. Thus, the objective of this study was to investigate the expression pattern of PGP9.5 in the early life of pig testes.

#### Material and Methods

Pigs were castrated and their testes were collected from 2, 5, 8, 30, 60, and 90 days of age after birth (n = 12). After castration, the testes samples were stored at -80°C until analysis. All samples were determined the cross-reactivity of the PGP9.5 antibody in pig testes tissues by Western blotting. The protein band of PGP9.5 appeared at 27 kDa. The relationship between age and PGP9.5 expression was determined by Pearson's correlation. The effect of age on PGP9.5 expression was analyzed by using one-way ANOVA.

## Results

The age of pigs was negatively correlated with PGP9.5 expression in testes (r = -0.846; P < 0.001). The expression of PGP9.5 in pig testes at 8 days of age (1.593 ± 0.103) was higher than 2 days of age (1.025 ± 0.103; P = 0.008), 30 days of age (0.880 ± 0.103; P = 0.003), 60 days of age (0.905 ± 0.103; P = 0.003) and 90 days of age (0.040 ± 0.103; P < 0.001). The PGP9.5 in 8 days of age piglets tend to be higher expression than 5 days of age piglets (1.290 ± 0.103; P = 0.003). The lowest expression of PGP9.5 in pig testes was found at 90 days of age piglets (P < 0.001).

#### **Discussion and Conclusion**

Therefore, the testes from pigs at 8 days of age was the highest PGP9.5 expressions. The PGP9.5 expression may be decreased when the age of pigs increased after first week of life. Further investigation by immunolocalization in different cell types of the seminiferous tubule at different ages of pigs should be considered.

## **REP** - Reproduction

## INTEREST OF SOWS SEROLOGICAL PROFILE TO INVESTIGATE PCV-2 REPRODUCTIVE DISEASE: AN EXPLANATORY FIELD STUDY

A. Lebret <sup>1</sup>, <u>C. Teixeira-Costa</u>, J. Jeusselin <sup>1</sup>, C. Chevance <sup>1</sup>, V. Normand <sup>1</sup>, T. Nicolazo <sup>1</sup>, G. Boulbria <sup>1</sup>, A. Jardin <sup>2</sup>, S. Brilland <sup>2</sup>

<sup>1</sup>Rezoolution Pig Consulting Services, Noyal-Pontivy, France <sup>2</sup>Ceva Santé Animale, 10 avenue de La Ballastière, 33 500 Libourne, France

### **Background and Objectives**

Porcine Circovirus type 2 (PCV-2) is an ubiquitous virus responsible for various symptoms. PCV-2 reproductive disease (PCV-2-RD) has been reported in experimental and field conditions especially on seronegative breeding stock. The objective of this study was to evaluate the interest of PCV-2 serologic profiles on sow herds to investigate such problems.

#### Material and Methods

From 2019 to 2022, 24 serological profiles (from 21 different sow herds) corresponding to 495 sera were analyzed retrospectively. Sera were analyzed with a commercial ELISA test (SERELISA PCV2 Ab Mono Blocking, Synbiotics, France) and were treated following manufacturer's instructions. For each farm, the level of confidence in PCV-2-RD was evaluated (based on clinical signs, differential diagnostics results and, for PCV-2, PCR, ISH and/or histological examination). For each serum, following data were collected: sow parity rank and number of PCV-2 vaccinations before sampling. Correlations were tested using a Pearson correlation test. Then, a generalized linear mixed model (GLMM) was used (with the farm considered as a random effect) to investigate the impact of these parameters on serological results.

## Results

In our dataset 82 out of 495 samples were negative (16,6%). The lower the number of vaccinations before sampling (r=-0.35), the higher the level of suspicion of PCV-2-RD (r=-0.31) and the lower the parity rank (r=-0.17), the higher the probability for a sow to be seronegative. The GLMM showed a significant impact of 3 parameters on the probability for a serum to be seronegative: a low number of vaccine injections before serology for gilts and for sows (p=0.0005 and p=0.07 respectively), a low parity rank (p=0.007) and a strong presumption of clinical problem related to PCV-2 (p=0.0005).

#### **Discussion and Conclusion**

Our results highlight several factors associated with the probability for a sow to be seronegative. It is of importance for practitioners when interpreting PCV-2 seroprofiles during RD investigations. They are consistent with previous reports on the evolution of PCV-2 epidemiology since the widespread implementation of piglets' vaccination and confirm that seronegative breeding sows are potentially more susceptible to PCV-2 infection.

#### **REP** - Reproduction

## ECONOMIC IMPLICATIONS OF ANEMIA-RELATED PRODUCTIVITY LOSSES IN FARROWING SOWS

#### P.P. Pwint Thu 1, R. Kirkwood 2, N. Am-In 3

<sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, Australia

<sup>3</sup>Center of Excellence in Swine Reproduction, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

## **Background and Objectives**

Anemia is common in sows due to low hemoglobin (Hb). Prolificacy and late gestation increase occurrence. Late pregnancy Hb levels are linked to stillbirths, which affects pig husbandry economically and ethically. Piglets may have anemia from maternal anemia, which limits oxygen uptake. We investigate the relationship between maternal anemia, neonatal anemia, and piglet blood oxygen saturation.

### Material and Methods

Data were collected from 145 Landrace x Yorkshire sows in parities 2–5. Sow Hb concentration were categorized into two groups: normal (Hb > 100 g/L; n=92) and anemic (Hb < 100 g/L; n=53). Hb concentration was measured in newborn piglet blood from their ear vein. Piglet birth and 24-hour postpartum weights estimated colostrum consumption. Farrowing duration, litter sizes, colostrum consumption, birth weight, birth oxygen saturation, piglet Hb concentration, weaning weight, 3-day mortality rate, and preweaning mortality were recorded. All parameters were compared between groups using student t test.

## Results

Total born litter size and birth weight were not different between groups but the anemia group had fewer born alive (11.2  $\pm$  2.6 vs 13.1  $\pm$  1.7). The normal and anemia groups had farrowing durations of 206.7  $\pm$  3.2 min and 240.8  $\pm$  4.2 min, respectively (P = 0.001). Blood oxygen saturation in newborn piglets was 95.1  $\pm$  0.3% and 90.2  $\pm$  0.4% in the normal and anemia groups, respectively (P = 0.01). In the normal and anemia groups, newborn piglets had hemoglobin concentrations of 126.1  $\pm$  3.1 g/L and 114.3  $\pm$  2.2 g/L, respectively (P = 0.02). Piglet colostrum consumption was 307.1  $\pm$  8.3 g and 241.8  $\pm$  4.5 g in normal and anemia groups, respectively (P < 0.0001). Anemia piglets had a higher mortality rate at 3 days (16.9% vs 10.2%, P = 0.03) and 28 days (23.7% vs 11.9%, P=0.01) than the normal piglets.

#### **Discussion and Conclusion**

Despite having the same birth weight, anemic sows farrowed longer and had fewer live-born piglets, perhaps due to lower energy. The fewer live-born piglets had lower blood Hb concentrations and colostrum intakes, resulting in lower preweaning survival. These findings suggest that pre-farrowing sow Hb can predict farrowing kinetics and litter performance.

**REP** - Reproduction

#### EFFECT OF SOW GROWTH ON PUBERTY AND ON THE RESULTS OF THE FIRST PRODUCTION CYCLE

<u>A. Vela Bello</u><sup>1</sup>, E. Polo<sup>2</sup>, I. Funes<sup>2</sup>, O. Mitjana<sup>3</sup>, M.V. Falceto<sup>3</sup> <sup>1</sup>ThinkinPig / Department of Nutrition and Production Animal, University of Zaragoza, Spain <sup>2</sup>ThinkinPig

<sup>3</sup>Departament of Animal Pathology, University of Zaragoza, Spain

#### **Background and Objectives**

The productive parameters of sows in their productive life can be influenced by a large numbers of variables. The growth of the gilts during the adaptation period on the farm is one of them. The aim of this study is to find out its influence of two different nutrition diets on the age at puberty, farrowing performance and differences in body condition at mating, farrowing and weaning in the first productive cycle.

## Material and Methods

159 gilts with 6 months of life were selected and split in two different groups defined by the diet they received between 6 and 7 months of age, High Nutrition Level (HN,n=86) or Low Nutrition Level (LN, n=73). Growth data, body condition, ADG (g), and body weight were recorded at 6th month of life (M1), 7th month (M2), at service (M3). Thus, once the age at the onset of puberty had been determine by ultrasound, the age at the time of their firts artificial insemination were monitored, also the number of gilts with an induce gonadotropin puberty.

The performance at farrowing was recorded and compared between both nutrition groups. All the data were analized and compared with XLSTAT Life Sciences 2023

## Results

Statistical diferences (SD) (p<0,05) was observed in all of these variables: a) Weight of the gilts at M2, LN = 117 kg Vs HN = 132 kg. b) ADG Between M1 and M2 LN = 760 g Vs HN=1186 g.

c) Age at puberty LN = 216 days Vs HN= 211d. d) Puberty induced with gonadotropin LN= 35/69 Vs HN = 15/85. e) IA weight LN=139 kg Vs HN=151 kg. f) Still born LN=1,74 Vs HN=1,32There is not statistical differences in the other study variables.

## Discussion and Conclusion

In view of these results we have to stress the importance of a good management in the pre-puberty stages in order to achieve a reduction of the pre-puberty period and therefore an earlier start of the productive life of the sows. Retention rate at 3th cycle and productive performance is being studed.

**REP** - Reproduction

## HYPOPHYSIS TRANSCRIPTOMIC PROFILING UNCOVERS NEW POTENTIAL CANDIDATE GENES INVOLVED WITH ANESTRUS IN PIGS

<u>M. Quirino</u><sup>1</sup>, C. Hebbel <sup>2</sup>, P. Baldessar <sup>2</sup>, A.M.G. Ibelli <sup>3</sup>, M.C. Ledur <sup>3</sup>, M.A. Mores <sup>3</sup>, M.E. Cantão <sup>3</sup>, J.O. Peixoto <sup>3</sup>, R.K. Ono <sup>4</sup>, M.G. Marques <sup>3</sup>, F. Moreira <sup>1</sup>, T. Lucia Jr. <sup>5</sup>, B.G. Gasperin <sup>5</sup>, R.R. Ulguim <sup>6</sup>, I. Bianchi <sup>1</sup> <sup>1</sup>Federal Institute of Santa Catarina - Araquari <sup>2</sup>Pamplona Alimentos, BR <sup>3</sup>Embrapa Suínos e Aves, BR <sup>4</sup>Agroceres PIC, BR <sup>5</sup>Federal University of Pelotas, BR <sup>6</sup>Federal University of Rio Grande do Sul. BR

## Background and Objectives

Early infertility diagnosis in swine females could contribute to reduce anestrus occurrence in the pig industry, decreasing several related losses. However, little is known about genetic factors associated to anestrus onset in pigs. Thus, we evaluated the anterior hypophysis expression through RNA-sequencing of 6 non-cyclic (no estrus signs and absence of corpora lutea at slaughter) and 6 cyclic gilts (estrus signs and presence of corpora lutea at slaughter), which were ~ 230-d-old (F1 Landrace × Large White), to analyze the gene expression profile involved with anestrus.

### Material and Methods

After RNA extraction, RNA-Seq libraries were prepared with mRNA stranded library kit and sequenced in the NextSeq equipment (Illumina) with a paired-end protocol (2×150bp). Reads were submitted to quality control (Trimmomatic), mapping and counting (STAR), and gene expression analysis (limma package from R). Genes with FDR  $\leq$  0.05 were differentially expressed (DE). Functional annotation was performed using DAVID and STRING databases.

#### Results

A total of 9,639 genes were expressed in the anterior hypophysis, and 210 were DE between groups, of which 95 were downregulated and 115 were upregulated in the non-cyclic gilts. The downregulated genes enriched biological processes related to regulation processes, blood circulation, cell response to starvation and nutrient levels, and sterol depletion. The upregulated genes enriched those related to translation, RNA metabolism, protein stabilization and ncRNA metabolism.

#### **Discussion and Conclusion**

Among the downregulated genes in the non-cyclic gilts, the REC8, SREBF1 and SREBF2 are highlighted since they activate the SREB pathway, which is needed for steroidogenesis coordination, and are upstream regulators of the progesterone membrane receptor (PGRMC1). Their downregulation in the non-cyclic gilts could be impairing the correct steroid biosynthesis affecting the estrus cycle. Among the upregulated genes, 7 are close to regions that have been potentially associated with anestrus by our research group, in which NELFE has already been associated with ovulation rate in sheep, and RPS and RPL genes have become interesting candidates to understand puberty since, in cattle, some of the ribosomal proteins are downregulated after puberty. Overall, our study identified new candidate genes involved with anestrus occurrence in pigs. This study was supported by FAPESC, FAPERGS, CNPq, and CAPES.

**REP** - Reproduction

## PLACENTAL CHARACTERISTICS OF DIFFERENT PIG BREEDS AND THEIR RELATIONSHIPS TO LITTER CHARACTERISTICS

<u>A. Vernunft</u><sup>1</sup>, T. Hartwig<sup>1</sup>, T. Illner<sup>2</sup>, I. Egerszegi<sup>3</sup> <sup>1</sup>Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany <sup>2</sup>Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany <sup>3</sup>Hungarian University of Agriculture and Life Sciences, Institute of Animal Sciences Szent István Campus Gödöllö, Hungary

## Background and Objectives

Pig breeds, modern lines or indigenous breeds, differ massively in litter sizes. In most breeds a notable increase of litter size was achieved during the last decades. The available uterine space and surface for each fetus as well as the capacity of the uterus seems to become limited in large litters. This study focusses on the expression of the placenta in different pig breeds and their relation to litter characteristics.

### Material and Methods

Placentas of 18 litters of Hungarian Mangalica (HM), 19 litters of German Saddleback (GS), 24 litters of German Landrace (GL) and 14 litters of Yorkshire sows (Y) were weighted, the length measured and the placental efficiency per litter calculated. Piglets were weighted and the litter data collected. Only first and second litters were used.

#### Results

All breeds differ significantly in the number of total born piglets (HM 7.9 $\pm$ 1.9; GS 11.5 $\pm$ 3.2; GL 14.9 $\pm$ 2.9; Y 19.6 $\pm$ 2.5; Tukey: p<0.001), life born piglets (HM 6.8 $\pm$ 2.3; GS 11.2 $\pm$ 3.1; GL 13.9 $\pm$ 2.8; Y 17.2 $\pm$ 2.6; Tukey: p<0.01) and litter weight (HM 11.9 $\pm$ 2.7Kg; GS 15.2 $\pm$ 3.9Kg; GL 19.5 $\pm$ 4.2Kg; Y 26.8 $\pm$ 3.2Kg; Tukey: p=0.037). But, while a clear negative correlation between litter size and piglet weight within the breeds were seen (r= -0.3 to r= -0.4), the mean piglet weights (HM 1.5 $\pm$ 0.3Kg; GS 1.4 $\pm$ 0.3Kg; GL1.3 $\pm$ 0.2Kg; Y 1.4 $\pm$ 0.1Kg) and mean placental weights (HM 234 $\pm$ 73g; GS 223 $\pm$ 74g; GL 261 $\pm$ 56g; Y 220 $\pm$ 31g) were comparable between breeds. Accordingly, the placenta efficiency (fetal weight/placental weight) were comparably between most breeds (HM 6.86 $\pm$ 1.5; GS 6.42 $\pm$ 1.8; Y 6.33 $\pm$ 0.9) but significantly lower in GL sows (5.23 $\pm$ 0.9; Dunn's: p>0.05). Moreover, mean piglet weights were significantly correlated with the placental weight (r=0.64) and placental length (r=0.66) but not with the placental efficiency (r=0.03; p=0.8; Pearson Product Moment Correlations).

#### **Discussion and Conclusion**

Our findings suggest that the realization of large litters with acceptable piglet weights in sows were mainly achieved by increasing uterine capacity and space, so that the placenta had enough space to develop. However, further improvements might also be done by selection for placental efficiency, because both parameters can be influenced by breeding.

**REP** - Reproduction

## ASSESSMENT OF THE EFFICACY OF A GNRH ANALOG TO SUPPRESS OVARIAN FUNCTION IN FATTENING GILTS UNDER FIELD CONDITIONS

<u>S. Genís</u><sup>1</sup>, V. King<sup>2</sup>, M. Aluwé<sup>3</sup>, A. Van Den Broeke<sup>3</sup>, F. Descamps<sup>4</sup>, A. Aldaz<sup>5</sup>, N. Wuyts<sup>4</sup>, M. Balasch<sup>1</sup> <sup>1</sup>Zoetis Manufacturing&Research Spain S.L. <sup>2</sup>Zoetis Inc <sup>3</sup>Institute for Agricultural, Fisheries and Food Research (ILVO), Melle, Belgium <sup>4</sup>Zoetis Belgium <sup>5</sup>Zoetis Inc, Parsippany, NJ. USA

**Background and Objectives** 

Gonadotropin releasing hormone (GnRH) analogs are used to induce antibodies against naturally produced GnRH. From an animal welfare perspective, blocking GnRH activity in gilts results in a temporary suppression of ovarian activity and sexual maturation preventing unwanted pregnancies and undesirable sexual behavior in fattening gilts commingled with entire males, and unwanted contact with wild boars in outdoor production farms. Two studies assessed the efficacy of a GnRH analog-based vaccine in fattening gilts harvested at 27 weeks of age.

#### **Material and Methods**

The two studies enrolled 80 and 40 fattening gilts, respectively. Two doses of anti-GnRH vaccine (Improvac®) (40/20 gilts) or saline (40/20 gilts) were administered at eight or four inter-dose intervals (IDI): 10 and 18 weeks of age (Study 1); 14 and 18 weeks of age (Study 2). Endpoints included: standing oestrus, anti-GnRH antibodies, progesterone and estradiol levels in serum, and reproductive organ morphology (uterus and ovary weights, uterus horn length and follicles size). Reproductive tract data was analyzed using a general linear mixed model; oestrus signs with a Cochran-Armitage test; and hormone results with a general linear mixed model for repeated measures (SAS, SAS Institute, Cary, NC) with a significance level of P < 0.05.

#### Results

Vaccinated animals showed a significant reduction ( $P \le 0.001$ ) compared to controls in studies 1 and 2, respectively: uterus weight (55±30g vs 270±37g; 71±48g vs 262±48g), horn length (377±49mm vs 765±71mm; 487±109mm vs 911±91), standing oestrus ( $P \le 0.03$ : 22.4% vs 0%; 40% vs 11.1%). A significant increase (P < 0.00001) in anti-GnRH antibody levels was observed (23-fold in Study 1; 9-fold in Study 2). Progesterone levels were significantly reduced (P < 0.05, 152-fold in Study 1; 107-fold in Study 2) from 6 and 8 weeks after 2<sup>nd</sup> vaccination. Estradiol levels were significantly reduced at the end of Study 2 (P = 0.0283, 5.0±0.4 vs 6.2±0.4pg/mL).

#### **Discussion and Conclusion**

Anti-GnRH vaccine administration to gilts at eight or four-week IDI is effective in suppressing puberty for at least 9 weeks post  $2^{nd}$  vaccination. These results confirm the flexibility of the anti-GnRH vaccine addapting to different managment practices in commercial farms.

**REP** - Reproduction

## COINFECTIONS IN CASES OF SMEDI FROM DIAGNOSTIC TRANSMITTALS IN GERMANY

<u>M. Eddicks</u><sup>1</sup>, J. Gründl<sup>1</sup>, A. Seifert<sup>1</sup>, L. Eddicks<sup>2</sup>, S. Reese<sup>3</sup>, K. Strutzberg-Minder<sup>5</sup>, H. Swam<sup>6</sup>, R. Tabeling<sup>4</sup>, M. Ritzmann<sup>1</sup>, R. Fux<sup>7</sup>

<sup>1</sup>Clinic for Swine at the Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-Universität München, Oberschleißheim, Germany

<sup>2</sup>Institute of Veterinary Pathology at the Centre for Clinical Veterinary Medicine of the Ludwig-Maximilians-Universität München, München, Germany

<sup>3</sup>Institute for Anatomy, Histology and Embryology, Department of Veterinary Sciences of the Ludwig-Maximilians-Universität München, München, Germany

Intervet Deutschland GmbH, MSD Animal Health, Unterschleißheim, Germany

<sup>5</sup> IVD GmbH, Innovative Veterinary Diagnostics, Seelze, Germany

Center for Diagnostic Solutions, MSD AH Boxmeer, The Netherlands

<sup>7</sup>Division of Virology, Department of Veterinary Sciences of the Ludwig-Maximilians-Universität München, Oberschleißheim, Germany

## **Background and Objectives**

SMEDI displays a reproductive disorder associated with different pathogens like PPV1, PCV2 and others. Less is known about the prevalence and effect of coinfections with different pathogens in such cases. In the present study we examined fetuses from SMEDI litters for the presence of pathogen specific DNA of PPV1, PCV2, PCV3 and Leptospira spp. to evaluated the effect and prevalence of coinfections with the before mentioned pathogens in German SMEDI cases.

## Material and Methods

In total 158 / 358 fetuses from diagnostic transmittals, selected by systematic random sampling (four fetuses per litter), from 40 SMEDI-affected litters of 18 different farms were examined for PPV1, PCV2, PCV3 and Leptospira spp. by real-time PCR. Epidemiological data were collected by a questionnaire from the corresponding farmers.

### Results

In 94.4% of all farms and in 77.5% of all litters specific pathogen DNA was detected in different amounts and combinations. PPV1-, PCV2- or PCV3-DNA was present each in 16.7% of the farms as the only detectable pathogen. PPV1+PCV2 coinfection was detected in 33.3% of the farms, PCV2+PCV3+Leptospira spp. coinfection was present in 11.1% of the farms and no pathogen DNA was detected in 5.6% of the farms. The detection of Leptospira spp. in fetuses was significantly associated with a PCV2 coinfection (OR: 26.3; p < 0.001). Fetal maceration was associated with Leptospira spp. detection (OR: 8.6; p = 0.003), whereas mummification (p = 0.047), reduced crown-rump length (p < 0.001), and bodyweight (p = 0.001) of fetuses were significantly associated with PPV1 and PCV2 coinfection.

## **Discussion and Conclusion**

The present study shows that coinfections with different pathogens appear regularly in SMEDI cases. Interaction of pathogens might lead to an enhanced negative effect on the development of fetuses (in terms of PCV2 + PPV1 coinfection) or possibly bilateral promote infections (in terms of PCV2 and Leptospira spp.). Molecular biological examinations in SMEDI affected herds should cover a broad range of pathogens to obtain an etiological diagnosis. Moreover, the phenotypical appearance of SMEDI-fetuses should be taken into consideration to increase the probability of pathogen detection.

## **RESIDENT SESSION**

## RES-OP-01

**RES – ECPHM Resident Session** 

## A COMPARISON OF TWO SYSTEMS FOR GROUP HOUSING OF SOWS - EFFECTS ON PRODUCTIVITY, REMOVAL, AND TREATMENTS

<u>K. Ryytty Sylvén</u><sup>1</sup>, T. Wallgren<sup>2</sup>, P. Almerheim<sup>1</sup>, L. Eliasson- Selling<sup>1</sup>, M. Jacobson<sup>3</sup>, P. Wallgren<sup>4</sup> <sup>1</sup>Farm and Animal Health Service, Sweden.

<sup>2</sup>Department of Animal Environment and Health, Swedish University of Agricultural Sciences (SLU) <sup>3</sup>Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden <sup>4</sup>Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), Uppsala, Sweden

## Background and Objectives

Group housing of sows has been extensively studied since the EU banned gestation crating. Well-managed group housing promotes sow welfare, but the impact varies based on factors such as feeding, group characteristics, and environmental features. Adequate floor space is crucial for social interactions, innate behaviours, and to reduce injuries caused by aggression. The aim of this study was to compare two group-housing systems for pregnant sows concerning productivity, treatment frequency, and removal of sows.

#### Material and Methods

The study was designed as an observational descriptive/explanatory retrospective cohort study and the sows should have been housed throughout gestation in large-sized pens with deep-litter straw (System I), or in smaller gestation pens (System II). The study was conducted in the central unit of a conventional multisite production herd keeping 1,800 Landrace x Yorkshire sows. From February to September 2022 data on performance, removal, treatment of sows and pregnancy rates were collected and analysed.

## Results

Both groups had a mean parity number of  $3.1\pm1.3$  In System I, larger groups (n=40) had sows delivering  $16.8\pm3.8$  piglets, while System II, with smaller groups (n=8-10), had  $15.4\pm3.5$  piglets (p=0.0005). On average, both groups experienced 1.1 stillborn piglets (p>1.0). Although sows from System I had a higher number of weaned piglets (14.6) compared to System II (14.0), the difference lacked statistical significance (T-test, p=0.2003). Observations by staff revealed that System I sows were easier to move, more alert, and generally more active. Antibiotic treatments were more frequent in System II (p<0.001), Relative Risk =9 for System II. Replacement rates were comparable in both systems, and a high percentage of sows became pregnant during subsequent inseminations in both groups. Sows in System I experienced additional mixing.

### **Discussion and Conclusion**

The results indicated that pregnant sows provided with larger floor space (totally 156 m<sup>2</sup> corresponding to 3.9 m<sup>2</sup> per sow) and housed on deep-litter straw, exhibited a higher productivity, and demanded fewer antibiotic treatments than pregnant sows housed in pens with limited floor space (24.5 m<sup>2</sup> corresponding to 2.5- 3.1 m<sup>2</sup> per sow) and less foraging material. Larger floor space appeared to alleviate the negative effects of mixing, even in larger groups.

RES – ECPHM Resident Session

## LEAKY GUT – A REASON FOR FAILURE TO THRIVE?

L. Schwarz <sup>1</sup>, H. Kreutzmann <sup>2</sup>, I. Loncaric <sup>3</sup>, J. Spergser <sup>3</sup>, R. Brunthaler <sup>4</sup>, H. Weissenböck <sup>5</sup>, S. Gabner <sup>6</sup>, A. Griessler <sup>7</sup>, B. Doupovec <sup>8</sup>, S. Figl <sup>9</sup>, A. Ladinig <sup>1</sup>

<sup>1</sup>University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

<sup>2</sup>Royal GD, Deventer, The Netherlands

<sup>3</sup>Institute of Microbiology, University of Veterinary Medicine Vienna, Austria

<sup>4</sup>Institute of Pathology, University of Veterinary Medicine Vienna, Austria

<sup>5</sup>Institute of Pathology, Department for Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria

<sup>6</sup> Institute of Morphology, Working Group Histology, University of Veterinary Medicine Vienna, Vienna, Austria

<sup>7</sup>Traunkreis Vetclinic OG Grossendorf 3, 4551 Ried im Traunkreis - Austria

<sup>8</sup>Biomin Research Center, Technopark 1, 3430 Tulln, Austria

<sup>9</sup>dsm-firmenich Animal Nutrition & Health, Getzersdorf, Austria

## Background and Objectives

Failure to thrive can have different reasons. Here we describe a case of failure to thrive beginning two weeks after weaning.

#### Material and Methods

A piglet producing farm in Austria reported of weaners that stopped growing two weeks after weaning. A herd inspection as well as a proper diagnostic work up were performed to identify possible causes. In total eight weaners were euthanized, necropsied and sampled. Feed samples were mycotoxicologically analysed.

#### Results

Different bacteria were isolated from different organs (spleen, liver, kidney, lymph nodes, gut, serosa) as well as from blood including haemolytic E. coli, Salmonella Typhimurium, Campylobacter coli and Brachyspira murdochii. Histopathologic results indicated moderate to severe villous atrophy. Immunohistochemistry for investigating the gut mucosal barrier revealed altered expression of tight-junction proteins in damaged mucosal tissue compared to intact gut epithelium. In weaners feed 700 ppb deoxynivalenol (DON) were detected by HPLC-MS/MS, with corn (1450 ppb DON; 18 % corn in finished feed) contributing most to this contamination.

#### **Discussion and Conclusion**

Diagnostic work-up of failure to thrive is one of the most struggling problems in swine practice, due to a multifactorial aetiology. In this case, we were able to identify infectious and non-infectious factors that can be used to explain the clinical situation. Most importantly the detection of different gram negative bacteria in blood cultures and in different organs gave indications that a leaky gut may have been the primary cause of the failure to thrive. For the first time it was possible to isolate brachyspira from blood, which is described in other species but not in pigs so far. Altered expression of tight-junction proteins confirmed the leaky gut. Observed villous atrophy may be the consequence of viral or parasitic infections during the suckling piglet phase. Mycotoxicological analysis revealed relevant amounts of deoxynivalenol metabolites in corn and finally, also in weaners feed which may be the priming cause of leaky gut together with the mucosal damage in the suckling period.

## RES – ECPHM Resident Session

# DESCRIPTIVE STUDY OF THE CARRIAGE OF STREPTOCOCCUS SUIS SEROTYPE 9 IN TWO FRENCH FARMS PRESENTING CLINICAL CASES OF STREPTOCOCCOSIS

<u>M. Rémond</u><sup>1</sup>, E. Lewandowski<sup>2</sup>, V. Muller<sup>3</sup>, C. Puyal-Rucine<sup>3</sup>, A. Lehebel<sup>1</sup>, H. Gantelet<sup>2</sup>, C. Deboisséson<sup>4</sup>, C. Marois-Crehan<sup>5</sup>, C. Belloc<sup>1</sup> <sup>1</sup>ONIRIS, INRAE, BIOEPAR, 44307 Nantes, France <sup>2</sup>Ceva Biovac, 6 Rue Olivier de Serres, 49070 Beaucouze, France <sup>3</sup>EPIDALIS, 35500 Vitré, France <sup>4</sup>Anses, Laboratoire de Ploufragan-Plouzané-Niort, Unité Mycoplasmologie, Bactériologie et Antibiorésistance <sup>5</sup>Anses-Ploufragan/Plouzané/Niort Laboratory, BP 53, 22440 Ploufragan, France

## **Background and Objectives**

Streptococcus suis (S. suis) is a pathogenic bacterium which induces economical losses and antimicrobial use. Recently, in France, the frequency of S. suis serotype 9 (SS9) isolated from clinical cases difficult to prevent has increased. Few data are available on the epidemiology of this serotype. This study aimed to evaluate the SS9 carriage (within-farm transmission) in two farms affected by SS9 clinical cases.

## **Material and Methods**

In each farm, forty sows and 180 growers of six different ages (n=30/age) were sampled once on the same day. Oral fluids, tonsil and nasal swabs were collected on each animal. Each sample was analysed using a SS9 qPCR. A pig was considered SS9 carrier if it has at least one SS9 positive qPCR whatever the sample type. Relevance of different sample types compared to the gold standard, the tonsil swab, was assessed.

## Results

In both farms, 7.5% of the sows, 40% and 17.8% of the growers (Farm 1 and 2 respectively) were SS9 carrier. Growers from Farm 1 were more likely to be positive than Farm 2 (P<0,001). Concerning ages and positivity, in Farm 2, the risk to be SS9 carrier is significantly higher at three weeks of age, compared to other ages (P<0,01). In Farm 1, high carriage is observed from weaning to the beginning of fattening. Concerning the sampling types, tonsilar swabs were the most positive. Oral fluids were as positive as tonsillar swabs for the "end of post-weaning" and the "beginning of fattening" age categories (P=0.97 and P=1.0 respectively).

## **Discussion and Conclusion**

This first description of SS9 carriage in two farms illustrates a link between the level of carriage, the intensity of the pathology and the types of antibiotic treatment (individual or collective). Farm 1 which has a high prevalence of SS9 carriage from weaning to slaughter uses metaphylactic treatments for each batch while Farm 2 which has fewer carriers manages its cases individually. In both farms, the age of highest prevalence of carriage is contemporary with the clinical cases. Oral fluids, which require less restraint than tonsil samples, could provide an interesting matrix to study SS9 carriage in growing pigs.

RES – ECPHM Resident Session

## CIRCADIAN TEMPERATURE RHYTHM IN SWINE REPRODUCTION: EVALUATION OF SKIN TEMPERATURE IN SOWS DURING POST-WEANING AND DIFFERENCES BETWEEN SEASONS IN A SUBTROPICAL CLIMATE AREA (SOUTHEAST OF SPAIN)

P. Sánchez-Giménez <sup>1</sup>, A. Martínez-Nicolás <sup>2</sup>, J.A. Madrid <sup>3</sup>, R. Fernández-Rodríguez <sup>1</sup>, L. Martínez-Alarcón <sup>4</sup>, F. Murciano <sup>5</sup>, A. Muñoz-Luna <sup>6</sup>, G. Ramis <sup>5</sup>

<sup>1</sup>AGROPOR, Murcia, Spain <sup>2</sup>Chronobiology Lab, Department of Physiology, College of Biology, University of Murcia, Mare Nostrum Campus, IUIE, IMIB-Arrixaca, Murcia, Spain//Ciber Fragilidad y Envejecimiento Saludable (CIBERFES), Instituto de Salud Carlos III, Madrid, Spain

<sup>3</sup>Departamento de Fisiología, Universidad de Murcia. Murcia, Spain <sup>4</sup>Instituto Murciano de Investigación en Biomedicina (IMIB), Murcia, Spain <sup>5</sup>Dpto. Producción Animal, Facultad de Veterinaria., Universidad de Murcia, Spain <sup>6</sup>Departamento de Producción Animal. Universidad de Murcia

## Background and Objectives

The aim of this work was to describe the circadian rhythm of skin temperature in post-weaning sows throughout the different seasons of the year in a subtropical climate zone and to look for possible differences.

## Material and Methods

The experiment took place in a commercial farm with 9,600 sows, housed in individual cages after weaning with no artificial environmental control. The skin temperature of 85 sows was recorded for seven days post-weaning over one year, in the same building, distributed between autumn (A), winter (W), early spring (ES), late spring (LS) and summer (S) using Thermochron® i-buttons dattaloger fixed in the neck.Parameters related to the circadian rhythm were calculated: Interdaily stability (IS), Intradaily fragmentation (IV), the amplitude (AR), the average temperature of the 8 consecutive hours with lower and higher temperature (L8 and M8), middle moment of the L8 and M8 (TL8 and TM8) in hours and Mean as the average of all these data. A cosinor normalization was done calculating the period, robustness, mesor, amplitude and acrophase.

## Results

The sows showed lower IS (W=0.39, ES=0.37, LS=0.5, S=0.75 and AU=0.7, respectively) and IV (W=0.07, ES=0.06, LS=0.9, S=0.04 and AU=0.12) in the circadian rhythm in summer and autumm. There was no difference for L8 and M8 but for TL8. However, while in the L8 the difference is of all seasons with winter, in M8 there is a clear difference of summer and autumn with winter and summer with spring. The period of the rythm was almost 24 hours for all batches, and robustness was much higher in summer. There were differences in the mesor when comparing summer with all other periods except late spring. The acrophase only coincides with the time of ambient T<sup>a</sup>max in winter, being deviated in the other batches 3 to 7 hours.

## **Discussion and Conclusion**

There is a seasonal influence on the circadian rhythm of temperature, where summer has clearly the greatest differences. A robustness significantly higher and stability lower in summer, indicates that rhythms are more stable and that sows might be more influenced by ambient temperature. It's needed research to relate the lower reproductive performance and the temperature rhythm during summer.

## RES – ECPHM Resident Session

# DETECTION OF MCR-1 POSITIVE ENTEROPATHOGENIC ESCHERICHIA COLI ISOLATES ASSOCIATED WITH POST-WEANING DIARRHOEA IN AN AUSTRIAN ORGANIC PIGLET PRODUCING FARM

<u>R. Renzhammer</u><sup>1</sup>, L. Schwarz<sup>1</sup>, A. Cabal Rosel<sup>2</sup>, W. Ruppitsch<sup>2</sup>, A. Fuchs<sup>3</sup>, E. Simetzberger<sup>3</sup>, A. Ladinig<sup>1</sup>, I. Loncaric<sup>4</sup> <sup>1</sup>University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria <sup>2</sup>Austrian Agency for Health and Food Safety, Mödling, Austria <sup>3</sup>VETworks, Strengberg, Austria

<sup>4</sup>Institute of Microbiology, University of Veterinary Medicine Vienna, Austria

#### **Background and Objectives**

Although colistin is considered as a critical antibiotic substance, it is frequently applied to piglets with postweaning diarrhoea (PWD).

#### Material and Methods

The case herd is an organic, family-owned, piglet-producing farm with 32 sows operating in a three-week batch farrowing system located in Lower Austria. While prior to summer 2022, PWD was not observed in piglets treated with zinc-oxide, symptoms did not improve after treatment with colistin instead. Therefore, rectal swabs were collected from eight piglets with PWD for microbiological examination and antimicrobial resistance testing. In addition, environmental samples (boot socks, dust, faeces from gilts, sparrows and chicken) were taken to evaluate the epidemiological situation of suspected colistin-resistant Enterobacteriaceae. Escherichia (E.) coli recovered from collected samples were further tested for the colistin-resistance genes mcr1–9 by microarray. In addition, faecal samples were tested by faecal flotation and tissue samples from three piglets (jejunum, ileum, mesenteric lymph nodes) were investigated by PCR for nucleotides of rotavirus A, rotavirus C, porcine epidemic diarrhoea virus, porcine circovirus 2, Brachyspira spp. and Lawsonia intracellularis.

## Results

While PCR was negative for nucleotides of all tested pathogens and neither helminth eggs nor oocysts were detected by faecal flotation, E. coli was recovered from all rectal swabs. Two non-haemolytic E. coli recovered from one rectal swab and the boot socks respectively were positive for mcr1 and therefore further analysed by whole-genome-sequencing. Since both isolates were tested positive for eaeH encoding intimin and espX5 encoding the type III secretion system, both mcr1 positive E. coli were categorized as enteropathogenic E. coli (EPEC). Besides EPEC, nutritional problems were apparent, as faba beans were the main protein source in the feed of weaned piglets. After replacing faba beans with whey in May 2023, PWD was solely observed in 2/10 weaned batches in the consecutive seven months. Due to the detection of mcr1 positive EPEC and based on the results of the antimicrobial resistance testing, the veterinarian decided to apply gentamicin for treatment leading to improved clinical signs.

### **Discussion and Conclusion**

The detection of mcr1 in two EPEC from piglets with PWD demonstrate the potential threat of colistin resistant E. coli for pigs, also in organic production systems.

## PARASITOLOGY AND PARASITIC DISEASES

## PAR-OP-01

## PAR – Parasitology and Parasitic Diseases

## CAN ANTHELMINTIC TREATMENTS BE DISCONTINUED IN HERDS SUPPOSEDLY FREE FROM ASCARIS SUUM?

P. Wallgren <sup>1</sup>, K. Ryytty Sylvén <sup>2</sup>, S. Gustafsson <sup>3</sup>, E. Pettersson <sup>4</sup> <sup>1</sup>National Veterinary Institute (SVA), Uppsala, Sweden <sup>2</sup>Farm & Animal Health, Uppsala, Sweden <sup>3</sup>Bellsta, 73193 Köping Sweden <sup>4</sup>National Veterinary Institute, SVA, 751 89 Uppsala, Sweden

## **Background and Objectives**

Regular use of anthelmintics is common in pig practice to avoid negative consequences of especially the roundworm Ascaris suum. Following ingestion of infective eggs, larvae of A. suum migrate through the liver and lungs before the final maturation to adult worms in the small intestine. The subsequent lung damage may predispose to other respiratory infections and scarring of the liver may be registered as white spots (WS) at slaughter, with condemned livers as consequence. Deworming of sows prior to farrowing with the aim to protect the offspring is common but could be unnecessary in herds supposedly free from A. suum. This study investigated the occurrence of A. suum in a herd that in principle had no WS registered at slaughter, and where regular deworming had been discontinued.

#### **Material and Methods**

The investigated herd was an integrated Specific Pathogen Free herd with 130 sows, also selling breeding stock to other herds. WS were registered at slaughter and the presence of A. suum-eggs were investigated using faecal flotation.

### Results

From 2015 to 2017, WS were registered in 9 out of 6,114 pigs at slaughter (0.14%). Deworming was discontinued in the autumn of 2018. During that year, WS were recorded in one out of 2,206 slaughtered pigs (0.05%) and faecal analysis of 80 pigs (70 aged 4-24 weeks and 10 sows) revealed no eggs of A. suum. During 2019-2022, WS were recorded in one out of 8,940 slaughtered pigs (0.01%) and faecal analysis of 40 pigs of different ages carried out in June 2023 found no eggs of A. suum.

## **Discussion and Conclusion**

The livestock were considered free from WS at slaughter and no eggs of A. suum were found on analysis five years after discounting deworming. Thus, deworming appeared redundant, including deworming of breeding animals destined for other herds However, QA protocols frequently mandate deworming prior to the sale of breeding animals, leading to the unnecessary deworming of pigs already free from parasites. An alternative proposal is to consider a documented WS incidence of less than 1% and the absence of A. suum eggs on faecal analysis as sufficient indicators that regular deworming is unnecessary.

## PAR-OP-02

PAR – Parasitology and Parasitic Diseases

## COMPARISON OF GROWTH PERFORMANCE IN THE FARROWING UNIT BETWEEN PIGLETS TREATED WITH FORCERIS (TOLTRAZURIL + IRON GLEPTOFERRON, IM) OR CEVAZURIL (TOLTRAZURIL, PO) + VILOFERRON (IRON GLEPTOFERRON, IM) IN A STANDARD COMMERCIAL DANISH SOW HERD

L.H. Diness <sup>1</sup>, <u>M.A. Larsen</u> <sup>1</sup>, P. Toft <sup>2</sup>, R. Skovgaard <sup>2</sup>, N. Toft <sup>3</sup>, D. Sperling <sup>4</sup>, C.S. Kristensen <sup>1</sup> <sup>1</sup>Ceva Animal Health A/S, Denmark. <sup>2</sup>PORCUS Oerbaekvej 276, DK-5220 Odense <sup>3</sup>Toft Analytics, Denmark <sup>4</sup>Ceva Sante Animale, Libourne, France

## **Background and Objectives**

The objective of the trial was to compare the growth performance of piglets in the farrowing unit when treated against Cystoisospora suis with either 1.5mL Forceris (GREEN GROUP), IM (45mg/piglet + 200mg iron gleptoferron/piglet) or 0.9 mL Cevazuril (BLUE GROUP), PO (45mg/piglet) + 1mL Viloferron IM (200mg iron gleptoferron/piglet). The background is based on the fact that piglets obtain a higher concentration of Toltrazuril after IM administration compared to PO administration.<sup>1</sup>

#### **Material and Methods**

This study was performed in a 1400 Danish sow herd with a crossbreed of Landrace, Yorkshire, and boar Duroc (LYD piglets). Prior to the commencement of the study, it was confirmed through the Mc Master method, that there were low level 110 Oocyst per gram feces (OPG) to high level 41580 OPG, despite oral treatment of Toltrazuril. The study was designed as a parallel randomized trial. After litter equalization the sows were randomized into two groups, blue or green. Initially all first-parity, then second-parity via a dice even or uneven. The piglets got earmarks incerted with blue or green color according to sowgroup. In total, 763 piglets were included, with 377 in the blue group treated with Toltrazuril PO (72-144 hours after birth) + iron gleptoferron IM (24-96 hours after birth) and 386 in the green group treated with Toltrazuril IM including iron gleptoferron (24-96 hours after birth). Piglets were weighed individually with a weightresolution of 10 grams between 24-96 hours after birth and again 18-23 days later.

#### Results

The statistical model showed an average daily weight gain (ADWG) in the green group of 209 g (196-223) compared to the blue group of 189 g (175-203). Green group had an higher ADWG of 20 g (p-value 0,035) more than the blue group.

#### **Discussion and Conclusion**

This study demonstrates higher ADWG when piglets are treated with Toltrazuril IM compared to Toltrazuril PO.

<sup>1</sup>Karembe, H.; Sperling, D.; Varinot, N.; Magnier, R.; Peyrou, M.; Guerra, N.; Smola, J.; Vasek, J.; Hinney, B.; Joachim, A. Absorption and Distribution of Toltrazuril and Toltrazuril Sulfone in Plasma, Intestinal Tissues and Content of Piglets after Oral or Intramuscular Administration. Molecules **2021**, 26 (18), 5633. https://doi.org/10.3390/molecules26185633.

## PAR-OP-03

PAR – Parasitology and Parasitic Diseases

## CYSTOISOSPORA SUIS – QUANTITATIVE MEASUREMENT OF COCCIDIOSIS WITH A NEWLY DEVELOPED QPCR ON COMMERCIAL FARMS IN THE NETHERLANDS

<u>B. Cornelis</u><sup>1</sup>, P. De Backer<sup>2</sup>, J. Schumans<sup>3</sup>, S. Van Colen<sup>4</sup> <sup>1</sup>DVM Ceva Santé Animale <sup>2</sup>Ceva Santé Animale, Brussels, Belgium <sup>3</sup>Ceva Santé Animale, Naaldwijk, the Netherlands <sup>4</sup>Ceva Santé Animale Benelux, Tiendweg 8-C, 2671 SB Naaldwijk, The Netherlands

## **Background and Objectives**

Coccidiosis is a highly prevalent disease on many farms, found in farrowing rooms, causing as well clinical as subclinical symptoms impacting health of piglets. Formerly, diagnostic tests were based on oocyst detection by flotation or autofluorescence microscopy. The most recently developed test to quantify coccidiosis infection on farms is a real-time qPCR assay.

## Material and Methods

On 86 Dutch sow farms, 355 samples have been collected to perform a qPCR in a laboratory. Samples were collected between September 2021 and September 2023. The samples have been obtained by collecting faeces, fecal swabs or environmental samples using abrasive sponges. For these sampling methods the qPCR has been validated. As a result, Ct-values correspond to a category described as negative or positive. The positive PCR-results are divided in 4 categories according to their Ct-value. The categories for Ct-values between  $\geq$ 38 and  $\leq$ 40,  $\geq$ 35 and <30 and <35 and <30 are respectively categorized as very weak positive, weak positive, moderate positive and strong positive.

### Results

The results show that 57/86 (66%) of the sampled Dutch farms have been categorized as positive for coccidiosis, whereas 199/355 (56%) of all samples were positive. Within the 199 positive samples, the percentages of very weak positive, weak positive, moderate positive and strong positive samples are respectively 4.5%, 21.6%, 36.2% and 37.7%.

### **Discussion and Conclusion**

Considering coccidiosis as an important disease on swine sow farms causing financial losses, more focus on prevalence of coccidiosis is recommended. The results of the PCR-screenings show that the situation on Dutch farms demands proper treatment and continuous attention should be paid to the prevention of Cystoisospora suis.

## PAR-OP-04

## PAR – Parasitology and Parasitic Diseases

# PIGLETS COCCIDIOSIS IN FRENCH FARMS - EVALUATION OF FARM POSITIVITY USING A NEW TOOL CALLED COCCI SCREEN

<u>S. Brilland</u><sup>1</sup>, P. Leneveu<sup>1</sup>, A. Jardin<sup>1</sup> <sup>1</sup>Ceva Santé Animale SA, 10 Avenue de la ballastière, CS 30126 – 33501 Libourne Cedex, France

## Background and Objectives

Coccidiosis caused by Cystoisospora suis is one of the most important causes of pre-weaning diarrhea in intensive pig production worldwide. Diagnostic is usually based on clinical signs and detection of occysts in the feces. Recently, a new real-time PCR assay for rapid detection of C. suis has been developed. Sample collection and diagnostics of Cystoisosporosis are challenging under the field conditions for practitioners. Consequently, a new sampling and diagnostic kit, which facilitate and improve the detection of C. suis in farrowing crates, Cocci Screen, was developed and introduced by Ceva team. This study is a synthesis of the results collected in French farms during a one-year period.

#### Material and Methods

The kit includes 3 scraping sponges for collecting piglet feces from the environment of 9 litters (3 farrowing crates wiped/sponge). Targeted piglets should be between 2 and 4 weeks of life. Samples were preferably collected from litters with clinical signs (diarrhea) or low performance. A qPCR was performed according to the method described by Hommonay et al. 2021 with adapted sample processing for assessment of scraping sponge.

#### Results

Since March 2022, 44 Cocci Screen diagnostics were carried out on farms, with evocative clinical signs but not applying toltrazuril, mainly in the West part of France. A farm was considered positive when at least one sample was positive by qPCR. 70% (31/44) of the investigated farms were positive for presence of C. suis. In 65% of positive herds, all investigated samples were positive, suggesting high rate of infection by C. suis. All positive farms had Ct value below 30 and 68% (21/31) of the farms had at least two sponges with a Ct value < 30, suggesting the strong presence of parasites, contamination of crates and risk of infection.

### **Discussion and Conclusion**

This study shows the high frequency of positive environmental fecal samples on not-using toltrazuril farms, with evocative clinical signs, evaluated by qPCR-based diagnostics. Results are comparable with previously published study (Brilland et al. 2020) where samples were collected directly from piglets during the 2<sup>nd</sup> and 3<sup>rd</sup> week of life. Cocci Screen is a simple and effective tool for practitioners which facilitates the diagnostic of C.suis.

## CLINICAL CLUB

## OCC-OP-01

### MIS - Miscellaneous and Clinical cases

## UPDATING THE PRODUCTIVITY AND ECONOMIC COSTS OF PRRSV IN THE US

<u>O.H. Osemeke</u><sup>1</sup>, G. De Souza E Silva<sup>1</sup>, C. Corzo<sup>2</sup>, M. Kikuti<sup>3</sup>, Y. Xiaomei<sup>4</sup>, D. Correia Lima Linhares<sup>1</sup>, D.J. Holtkamp<sup>5</sup> <sup>1</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

<sup>2</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

<sup>3</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA <sup>4</sup>Department of Veterinary Diagnostic and Population Medicine, University of Minnesota, Minnesota, USA. <sup>5</sup>Iowa State University College of Veterinary Medicine

## **Background and Objectives**

The economic cost of PRRSV was last updated in 2013. Since then, the US swine industry has significantly evolved regarding PRRSV monitoring, prevention, control, and elimination. This study aims to provide an update on the annual cost of lost productivity attributable to PRRSV in the US swine industry and to characterize the major drivers of changes from the previous estimates.

#### Material and Methods

This retrospective study incorporated data from 2016 to 2020 and used the same methodology as the 2013 study. The study evaluated four factors to characterize the major drivers of any changes from the 2013 estimate: the national distribution of herds by PRRSV statuses, productivity differences between herds affected and unaffected by PRRSV, national herd inventories, and prices (hogs and feed) and costs (input). To estimate productivity differences, breeding herds (BH) were assigned categories based on the updated American Association of Swine Veterinarians herd classification system, vaccine use, and outbreak history. The herd-week was used as the unit of analysis. BH were classified as BH-A if PRRSV-unaffected, BH-B if PRRSV-positive stable, BH-C if PRRSV-positive unstable, BH-D for the first 16 weeks following an outbreak in an initially PRRSV-positive herd, and BHE for the first 16 weeks following an outbreak.

## Results

Two hundred ninety-seven breeding farms representing over 1100000 sows with an average inventory of 3735 sows across 12 production systems were used to estimate the least-square means for live-born per litter, litters per mated female per year, preweaning mortality, sow death rate, and sow cull rates. These were respectively: 13.57, 2.38, 16.57%, 10.36%, 41.8% (BHA), 13.17, 2.35, 18.13%, 11.47%, 38.75% (BHB), 12.72, 2.28, 20.75%, 12.16%, 37.67% (BHC), 11.80, 2.23, 22.30%, 12.01%, 42.74% (BHD), 10.53, 1.97, 33.84%, 12.92%, 42.10% (BHE). Full results and economic analyses will be presented at the conference.

#### **Discussion and Conclusion**

It has been thirty years since PRRSV entered US swine herds. The results suggest that the damage caused by the virus is not decreasing.

## OCC-OP-02

MIS – Miscellaneous and Clinical cases

## WHY DID THE SHEEP KILL THE PIG?

<u>J. Lambertz</u><sup>1</sup>, G. Fidler<sup>2</sup>, S. Revilla-Fernández<sup>1</sup>, F. Schmoll<sup>1</sup> <sup>1</sup>Institute for Veterinary Disease Control, AGES – Austrian Agency for Health and Food Safety, Mödling, Austria <sup>2</sup>Landesveterinärdirektion, Amt der Salzburger Landesregierung, Salzburg, Austria

## Background and Objectives

As a consequence of the occurrence of ASF (African swine fever) in the neighbour states, there is an increased awareness to do further investigations in cases of unspecific symptoms and sudden death in pigs in Austria. In the following case report a rare condition of pigs will be presented.

## Material and Methods

Two fattening pigs were kept separately in one stable with sheep on a sheep farm. One pig died after a short period of anorexia and slightly increased body temperature but without specific symptoms.Necropsy was performed by a veterinarian at a local rendering plant. Aside of catarrhal enteritis and hyperplasia of the mesenteric lymph nodes a severe acute fibrinous gastritis was found gross morphologically. For further investigations, internal organs (liver, spleen, stomach and the small intestine with mesenteric lymph nodes) were submitted to our institute and histology, bacteriological and molecular analyses were performed.

#### Results

Histologically revealed a fibrino-necrotic gastritis with diffuse infiltration of the tunica mucosa with inflammatory cells, mainly lymphocytes. Furthermore fibrinoid necrosis of blood vessels with perivascular cuffing (lymphocytes, macrophages and granulocytes) were seen in the stomach and small intestine. The liver showed a mild lymphocytic interstitial hepatitis. By bacteriological examination Fusobacterium necrophorum Biovar B was identified in the spleen and Campylobacter sp. in the small intestine. PCR analyses were negative for Classical swine fever virus, African swine fever virus, Porcine Reproductive and Respiratory Syndrome Virus, pan-pestivirus and Porcine Circovirus-2. Because of the histomorphological lesions an infection with Ovine Herpesvirus-2 (OvHV-2) was suspected, that could be confirmed by PCR.

### **Discussion and Conclusion**

Here we present the first report of Malignant catarrhal fever (MCF) in a pig in Austria. MCF is a common herpesvirusassociated disease in cattle and wild ruminants. There are only a few reports of MCF in pigs- In the present case OvHV-2 infection was suspected, due to the similarity of histomorphological lesions in pigs and cattle. This fact points out the importance of histomorphological investigations in diagnosis of unspecific symptoms and death. Our findings mention, that close contact of pig and sheep (e.g. small farms, zoo etc.) is a possible risk factor for pigs to get infected and die of OvHV-2 infection.

## OCC-OP-03

MIS – Miscellaneous and Clinical cases

### MYCOPLASMA HYORHINIS ISOLATED FROM THE CENTRAL NERVOUS SYSTEM OF PIGLETS WITH MENINGITS

M. Bünger <sup>1</sup>, J. Spergser <sup>2</sup>, L. Schwarz <sup>1</sup>, R. Brunthaler <sup>3</sup>, P. Pineyro <sup>4</sup>, A. Griessler <sup>5</sup>, A. Ladinig <sup>1</sup>

<sup>1</sup>University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

<sup>2</sup>Institute of Microbiology, University of Veterinary Medicine Vienna, Austria

<sup>3</sup>Institute of Pathology, University of Veterinary Medicine Vienna, Austria

<sup>4</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

<sup>5</sup>Traunkreis Vetclinic OG Grossendorf 3, 4551 Ried im Traunkreis - Austria

## **Background and Objectives**

Mycoplasma hyorhinis (Mhr) has been known to cause polyserositis and polyarthritis in weaned piglets for decades. Over the past couple of years, it has been repeatedly isolated from cerebrospinal fluid (CSF) and meningeal swabs from piglets with nerval symptoms and/or histologic lesions of the brain and spinal cord in Austria. Recently, Mhr has also been associated with histologic lesions of the central nervous system (CNS) in multiple cases in the US using in situ hybridization. Here, we report a case of fibrinopurulent meningitis in weaned piglets in which Mhr was detected in CSF.

## **Material and Methods**

In an Austrian piglet producing farm, weaned piglets exhibited polyarthritis (~5%) and central nervous symptoms (~2%) which also lead to increased mortality even after antimicrobial treatment (penicillin, cefquinome). Three piglets with typical symptoms (~9kg) were euthanized and dissected for diagnostics at the Vetmeduni Vienna. The piglets exhibited swollen tarsal and carpal joints as well as central nervous symptoms like ataxia and nystagmus. During necropsy, lungs, serosal swabs, CSF and synovial fluid/synovium were sampled for pathohistological and bacterial examination.

## Results

Moderate to severe fibrinous meningitis, serositis and arthritis were observed during necropsy of all three animals. Histopathological examination of the brain and spinal cord revealed inflammation of the meninges presented by infiltration of histiocytes and few neutrophilic granulocytes. There was no pathological indication of edema disease or non-infectious neurologic diseases in any of the piglets. Bacterial cultivation resulted in the growth of M. hyorhinis from lungs and CSF (all three animals) as well as serosal swabs (two animals) and synovial fluid. Additional, Streptococcus suis was cultivated from the CSF, serosal swab and synovial fluid of one piglet. Glaesserella parasuis was excluded by PCR in all samples.

## **Discussion and Conclusion**

While further investigation is needed to explore the role of Mhr in weaned piglet meningitis, this case shows the presence of Mhr in the CNS alongside clinical signs as well as histologic lesions. Currently, histologic slides of this case are under investigation by in situ hybridization at the Iowa State University. Altogether, this case indicates that Mhr should be included in the list of differential diagnoses for piglet meningitis.

## OCC-OP-04

MIS - Miscellaneous and Clinical cases

## SOWS NECK INJURIES ASSOCIATED WITH NON-PROPERLY PERFORMED INJECTION: A DESCRIPTIVE STUDY

E. Abily <sup>1</sup>, C. Teixeira-Costa <sup>2</sup>, J. Jeusselin <sup>3</sup>, C. Chevance <sup>3</sup>, V. Normand <sup>3</sup>, T. Nicolazo <sup>2</sup>, G. Boulbria <sup>3</sup>, <u>M. Brissonnier</u> <sup>3</sup>, J. Da-Costa <sup>3</sup>, J. Favrel <sup>3</sup>, A. Lebret <sup>3</sup>

<sup>1</sup>Oniris VetAgroBio, Nantes, 101 route de Gachet, 44300 Nantes, France <sup>2</sup>Rezoolution Pig Consulting Services, Noyal-Pontivy, France <sup>3</sup>PORC. SPECTIVE, Swine Vet practice, Noyal-Pontivy, France

### **Background and Objectives**

The aim of this trial was to investigate factors influencing the presence of sows' neck injuries at injection sites. Neck injuries are defined as abscesses, fibrosis or granuloma observed at the injection site in sows' necks. They induce pain and are a major point in the assessment of animal welfare. One can assume that they are related to non-properly performed injections.

#### Material and Methods

The study was carried out on 14 randomly selected sow herds of 200 to 1000 sows. In each, 100 sows were observed to note the presence or absence of neck injury. Parallelly, several sows characteristics and farm management practices have been registered namely the body condition of the sow, parity rank, total number of injections per physiological stage (quarantine, gestation, lactation), vaccination protocol (loosed sow or not at the time of injection, number of sows injected with one needle, usage of a slap-shot extender or not), number of workforces to vaccinate, injection devices cleaning and disinfection procedures and storage modalities after disinfection (fridge or not). Then, a Generalized Linear Mixed Model was applied. Statistical significance was set as p<0.05 and we considered a p-value <0.1 as a tendency.

## Results

The percentage of neck injuries ranged from 4 to 51% and the average was 28%. The statistical model allowed us to highlight the following parameters negatively influencing neck injuries observation: parity rank (the older the sow, the higher the frequency of neck injuries; p<0.001), more than 5 workforces for vaccinations (p=0.03), storage at room temperature after devices cleaning rather than in a fridge (p=0.02) and usage of an autogenous vaccine (p<0.001). We also observed a tendency for a higher frequency of injuries when sows are vaccinated free in a barn (p=0.06). We did not show any statistical difference for the other parameters.

#### **Discussion and Conclusion**

Neck injuries are considered as an important issue in swine herds regarding their consequences (impaired immunization after repeated vaccination on scarred tissue in the neck, health and welfare issues). Our study highlights different parameters to consider when investigating such problem even if further investigations are needed.

## BACTERIOLOGY AND BACTERIAL DISEASES

## BBD-OP-01

BBD – Bacteriology and Bacterial Diseases

## COMPARATIVE EVALUATION OF ACTINOBACILLUS PLEUROPNEUMONIAE SEROTYPING FROM DIFFERENT DIAGNOSTIC SPECIMENS

<u>I. Spiekermeier</u>, J. Buch <sup>1</sup>, C. Helmer <sup>1</sup> <sup>1</sup>SAN Group Biotech Germany GmbH, Hoeltinghausen, Germany

## Background and Objectives

Actinobacillus (APP) is a causative agent of pleuropneumonia in pigs and has global significance. While the serotypes responsible for acute or chronic diseases vary globally, certain serotypes are regionally related to virulence. Consequently, serotyping plays a crucial role in diagnostics whereby it is traditionally performed using isolates obtained by bacteriological culture. However, acquiring isolates can be challenging due to transport issues and bacterial co-infections. Therefore, direct typing from organ materials or specimen cards offers advantages. To this end, a comparative analysis was conducted to evaluate the results of serotyping isolates, lung tissue, and ANICARDS (paper-based specimen cards containing chemicals that preserve nucleic acids while removing the infectious capability of pathogens).

## Material and Methods

In the laboratory, 30 freshly delivered lungs with suspected APP infections were subjected to a threefold approach: culture on chocolate blood agar, transfer of native lung tissue to specimen cards, and preparation of lung tissues for PCR testing. When an APP isolate was obtained, it was serotyped using a serotyping PCR, covering all 19 serotypes. Additionally, the lung tissue samples, and the specimen card samples were directly subjected to an APP screening (Kylt® APP) and serotyping PCR. DNA extraction was performed by routine molecular biological methods (Kylt® RNA / DNA Purification). Screening and serotyping PCRs were performed according to the manufacturer's instructions.

#### Results

24 of the 30 samples could be compared in all 3 serotyping approaches and the concordance between those was 100%. Multiple serotypes were not detected in any case. In 17% APP was not culturable in bacteriological culture. 7% had a Ct value >30 in the APP screening PCR and were therefore not further typeable in the serotyping PCR. On average, the screening Ct values of specimen cards were approximately 2-3 Ct values higher than those of the original lung tissues.

## **Discussion and Conclusion**

Serotyping of both original lung tissue and ANICARDS yielded in highly consistent results, offering distinct advantages of the specimen card-technique if fresh samples are unavailable for testing, if cooled express shipment is not possible or if bacterial co-infections may hinder APP growth in bacteriological culture.

### BBD - Bacteriology and Bacterial Diseases

## ACTINOBACILLUS PLEUROPNEUMONIAE IN-VITRO SURVIVABILITY IN LIQUID MEDIA

M. Almeida <sup>1</sup>, A. Michael <sup>2</sup>, N. Macedo <sup>3</sup>, R. C. Paiva <sup>3</sup>, M. Gottschalk <sup>4</sup>

<sup>1</sup>Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, United States

<sup>2</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>3</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

<sup>4</sup>Research Group on Infectious Diseases in Production Animals (GREMIP) and Swine and Poultry Infectious Diseases Research Centre (CRIPA), Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada.

## **Background and Objectives**

At the end of 2021, an unusual outbreak of porcine pleuropneumonia occurred in IA, USA, involving over 30 finishing sites from 9 companies in a 32 km radius. The outbreak was caused by Actinobacillus pleuropneumoniae (App) serotype 15, a serotype uncommon in the US until then and usually not associated with high mortality. Further analysis of historical strains of the US, Canada, and Mexico showed those outbreak strains formed a distinct cluster within serotype 15, raising questions about potential differences in survivability. This study aimed to compare the survivability of two App15 strains and an App1 strain under in-vitro conditions.

#### Material and Methods

The longitudinal viability of an App15 outbreak strain (App15o) on water (W), fecal slurry (F), and horse serum NAD (S) and at temperatures relevant to seasonal commercial conditions were compared against a historical App15 (App15h) causing mild clinical signs and a canonically high virulence serotype 1 strain (App1). They were evaluated at 6-time points (4h, 8h, 1, 2, 7 and 7 days) at 4 temperatures (-20°C, 4°C, 25°C, 37°C).

#### Results

When inoculated in horse serum NAD, all strains survived at all temperatures for the duration of the study; however, differences in survivability were observed in water or fecal slurry. At -20°C App15hF only survived up to 8 hours post-inoculation, while App1F, App1W, App15oF, App15oW, and App15hW survived up to 24 hours. A similar pattern was observed for inoculation at 25°C, with the only difference being App1W surviving up to 48 hours. When inoculated at 4C App15oW and App15hF survived up to 7 days, App1W up to 3 days, App1F and App15oF up to 48 hours, and App15hW up to 24 hours. At 37°C all strains in water and feces survived up to 8 hours post-inoculation.

#### **Discussion and Conclusion**

Warm conditions (37°C) seemed to negatively affect the survivability of App in water and feces. Cooler temperatures (4C) favored the survivability of App in liquid media, while ambient temperature and freezing conditions (-20°C) showed a shorter survivability compared to 4°C, but longer compared to 37°C. Interestingly, strain variability was observed for App150 and App15h, which survived longer in water and fecal slurry, respectively.

## BBD - Bacteriology and Bacterial Diseases

# TIME SERIES MODELS BASED ON LUNG LESIONS PREVALENCE FOR THE PREDICTION OF THE DYNAMICS OF MYCOPLASMA HYOPNEUMONIAE AND ACTINOBACILLUS PLEUROPNEUMONIAE INFECTIONS IN SPAIN.

A. Fernández-Fontelo <sup>1</sup>, M. Lasierra <sup>2</sup>, M. Carmona <sup>2</sup>, <u>L. Garza <sup>2</sup></u> <sup>1</sup>Departament de Matemàtiques, Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193, Spain. <sup>2</sup>Ceva Salud Animal, Barcelona, Spain

## **Background and Objectives**

Cranioventral pulmonary consolidation lesions are associated with enzootic pneumoniae (EP) caused by Mycoplasma hyopneumoniae (Mhyo); whereas pleuritis and lesions in the dorso-caudal lung lobes (DP) suggest chronic Actinobacillus pleuropneumoniae (App) infections. Monitoring of both lesions at slaughterhouse is commonly used to estimate the incidence and severity of these pathogens. To the authors' knowledge, a tool for predicting the prevalence of these lesions over time has not been constructed yet. This work aims to construct time series models for the prediction of EP-and DP-related lesions using the data collected at the slaughterhouse at different geographical locations in Spain.

### Material and Methods

EP and DP lesion prevalence for each evaluated pig lung was recorded using Ceva Lung Program (CLP) at the slaughterhouse. For the analysis, information was aggregated at batch level and two indices (EP-index and DP-index) were computed for each audited batch. These were the outcomes for adjusting time series models. ARMA processes were used for model construction and validation.

#### Results

A total of 3,947 audits performed between 2016-2019 were included in the dataset for the construction of the predictive model. These represented 477 breeding farms from 307 different companies in Spain, mainly located in Aragon and Catalonia. We adjusted an ARMA model for the whole country as well as for Aragon and Catalonia separately for both EP- and DP-indices related to lesions in pig lungs. EP index over time decreased from 2016 to 2019 and an annual seasonal pattern was found at country level. DP index increased over the studied period at country level as well as in Aragon and Catalonia. Both indices in Spain, Aragon and Catalonia showed a structure of autocorrelation over time. The validation of the model showed promising results, although there is still room for improvements, especially related to the prediction errors.

#### **Discussion and Conclusion**

Time series models constructed could be used to predict potential scenarios showing an abrupt increase in either Mhyo and/or App infections, and ideally for preventing and shortening the response time for implementation of control strategies against these respiratory diseases, minimizing the economic impact and losses associated.

BBD - Bacteriology and Bacterial Diseases

# DYNAMICS OF INFECTION OF DISEASE-ASSOCIATED STREPTOCOCCUS SUIS (DASS) IN THE LACTATION PHASE

<u>R. Mugabi</u>, A.P. Silva <sup>1</sup>, C. Haden <sup>2</sup>, T. Williams <sup>2</sup>, M. Rotolo <sup>3</sup>, J. Brown <sup>1</sup>, R. Jensen <sup>2</sup>, S. Cameron <sup>2</sup>, J. Nerem <sup>4</sup>, L. Weinert <sup>5</sup>, G. Li <sup>1</sup>, O. Sahin <sup>1</sup>, C. Rademacher <sup>1</sup>, M. Gottschalk <sup>6</sup>, P. Harms <sup>3</sup>, J. Wells <sup>7</sup>, A. Tucker <sup>5</sup>, M.J. Clavijo <sup>8</sup> <sup>1</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine <sup>2</sup>Pipestone Veterinary Services <sup>3</sup>PIC North America, Hendersonville, TN 37075 <sup>4</sup>Pipestone Applied Research <sup>5</sup>University of Cambridge, Cambridge, UK <sup>6</sup>University of Montreal, Canada

<sup>7</sup>Wageningen University, The Netherlands

<sup>a</sup>Veterinary Diagnostic and Population Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, Pig Improvement Company, PIC®, Hendersonville, TN, USA

## Background and Objectives

Disease-associated Streptococcus suis (DASS) is one of the most impacting bacterial diseases in swine. Novel diagnostic strategies are needed to understand the infection dynamics of virulent strains and the value of potential interventions. The goal of this study was to describe the parity effect, optimal sampling site for detection, and dynamics of infection of DASS in the lactation phase using a novel DASS qPCR assay.

## Material and Methods

A sensitive and specific qPCR, based on a virulence-associated marker (VM1) was validated, and used to test antemortem samples from a longitudinal study to track DASS dynamics in two sow farms. Fifty dams (25 P0 and 25 p4+) per farm were selected via random sampling and tonsil, nasal and vaginal swabs were collected at farrowing and weaning. On the same day, 14 piglets were selected from each litter, totaling 631 and 629 piglets, respectively. Piglets were ear-tagged, and one tonsil swab was collected from each piglet, and again at 7 and 21 days of age. All samples were also tested by the recN gene PCR.

## Results

All dams and piglets were recN positive in tonsil swabs. Tonsil scrapings and nasal swabs were samples of choice for detection of DASS compared to vaginal swabs. The odds of DASS positivity was 82% lower in sows compared to gilts (P < 0.05). Between 20-70% of piglets were positive at birth, with a significant decrease in prevalence observed at day 7 (18-45%), followed by a significant increase in the prevalence of DASS at day 21 (22-80%). The DASS positivity was 83% and 63% lower in piglets from sows than from gilts on both farms (p<0.05).

## **Discussion and Conclusion**

We hypothesize that the drop-in prevalence at day 7 is due to colostrum intake, with potentially protective antibodies and relevant immune cells, or it could imply transient detection or colonization with DASS. DASS was detected in crates, fecal, and udder samples, suggesting other relevant sources of infection for piglets. Determining the dynamics of infection allows for the refinement of control/elimination strategies, such as strategic medication and vaccination, early weaning or test and removal of individuals harboring DASS.

## BBD – Bacteriology and Bacterial Diseases

# PREVALENCE AND VIRULENCE OF STREPTOCOCCUS SUIS SEROTYPES 1, 14, 2, AND 1/2 ISOLATED IN SPAIN BETWEEN 2019 AND 2023

M. Petrocchi Rilo <sup>1</sup>, V. Acebes Fernández <sup>1</sup>, M. Delgado García <sup>1</sup>, C.B. Gutiérrez Martín <sup>1</sup>, A. González Fernández <sup>1</sup>, R. Miguélez Pérez <sup>1</sup>, A. Aguarón Turrientes <sup>2</sup>, <u>S. Martínez Martínez</u> <sup>1</sup> <sup>1</sup>Unit of Microbiology, Department of Animal Health, Veterinary Faculty, University of León, León, Spain <sup>2</sup>Laboratorios Syva SAU, León, Spain

Background and Objectives

Streptococcus suis is a noteworthy concern in the porcine industry, particularly post-weaning. While it usually resides harmlessly in the upper respiratory tract, intestine, and genitalia of pigs as part of their microbiota, the breach across mucosal barriers can lead to severe health issues, such as septicemia, arthritis, meningitis, pneumonia, endocarditis, or sudden death.

Our study characterized 250 S. suis isolates recovered between 2019 and 2023, which amplified the cps1J, cps14J, cps2J, or cps1/2J genes to estimate the corresponding serotype prevalence in Spain. Moreover, five virulence factors (VFs: epf, mrp, sly, luxS, and gapdh) were studied.

#### **Material and Methods**

Isolates from diseased pigs were analyzed. Identification of S. suis and cps1,14J - cps2,1/2J genes detection were carried out via monoplex and multiplex PCR, respectively. Differentiation of serotypes 1 and 14, and 2 and 1/2 employed primers targeting the single nucleotide polymorphism of the cpsK gene. Amplicons of 209 bp were observed in serotypes 2 and 14, but not in serotypes 1 and 1/2.

### Results

Among isolates presenting the cps1,14J genes (43.6%), 89.9% were identified as serotype 1, while only 10.1% amplified serotype 14. For isolates presenting the cps2,1/2J genes (56.4%), 69.5% were serotype 2, and 30.5% belonged to serotype 1/2. The prevalence of the studied VFs was as follows: luxS 99.6%, epf 88.4%, mrp 84.4%, sly 71.6%, and gapdh 68%. None of the isolates presented less than two virulence factors, and 35.6% were positive for all tested genes. Concerning serotype-related virulence, serotype 1 was the most virulent capsular type, with 88.8% of the isolates exhibiting 4 or 5 of the studied VFs, compared to 2 (78.6%), 14 (72.7%), and 1/2 (72.1%).

#### **Discussion and Conclusion**

Serotypes 1 and 2 are among the most commonly detected in Europe, alongside serotype 9. However, the limitations of widely used techniques, such as multiplex PCR, challenged the differentiation with similar serotypes like 14 and 1/2, respectively. The PCR method employed, initially developed by Thu et al. (2021), allowed accurate discrimination of these serotypes, permitting an updated estimation of the current serotype prevalence in Spain. Additionally, the virulence profile of the analyzed serotypes was elevated, as 81.2% of the isolates presented 4 or 5 VFs.
BBD - Bacteriology and Bacterial Diseases

# ENVIRONMENTAL VIABILITY OF MYCOPLASMA HYOPNEUMONIAE AND ITS POTENTIAL FOR INDIRECT TRANSMISSION

<u>C. Cordon</u><sup>1</sup>, A. Canturri <sup>2</sup>, R. Evelsizer <sup>3</sup>, M. Pieters <sup>4</sup> <sup>1</sup>University of Minnesota <sup>2</sup>Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA <sup>3</sup>Elanco Animal Health, Greenfield IN <sup>4</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

## **Background and Objectives**

Mycoplasma hyopneumoniae is a significant pathogen that contributes to respiratory disease and economic losses. Routes of indirect transmission have been poorly explored under the assumption that M. hyopneumoniae does not survive long in the environment. The objectives of this study were to determine the viability of M. hyopneumoniae in environmental samples over time and to assess the infection and disease development in susceptible pigs in both experimentally and naturally contaminated environments.

# **Material and Methods**

All animal studies were approved by the UMN IACUC. Water, feed, dust, plexiglass, stainless steel, cardboard, and PVC were placed into a hermetically closed isolator. Using a nebulizer, isolators were aerosolized with  $1x10^{\circ}$  CCU/mL M. hyopneumoniae strain 232. Materials were sampled prior to and at one hour, one, two, three, four, and five days post-aerosolization. In addition, experimental groups consisted of two, three-week old M. hyopneumoniae negative gilts and were: 1.) positive control, 2). negative control, 3-5.) pigs introduced into experimentally contaminated isolators one, three, and five days post-aerosolization, respectively, 6.) pigs placed into isolator naturally contaminated by M. hyopneumoniae-positive pigs. Tracheal secretions were collected prior to and at 7-, 14-, 21-, and 28-days post exposure (dpe). Pigs were humanely euthanized on 28 dpe. All samples were assessed via DNA and RNA PCR, results were conveyed in Ct values, with a cut-off value of  $\leq$  40. A descriptive analysis of the data was performed.

## Results

In all materials, except stainless steel and PVC, M. hyopneumoniae DNA was detected from 1-hour up to 4-days postaerosolization. Viable M. hyopneumoniae (RNA) was not detected on any materials. All pigs exposed either experimentally or naturally to contaminated environments did not develop infection or disease in a 4-week evaluation period.

# **Discussion and Conclusion**

Results from this study contribute to biosecurity at the farm level as they allude to materials that favor environmental persistence and detection of M. hyopneumoniae. Results are supportive that M. hyopneumoniae was not transmitted via indirect exposure of environmental surfaces, suggesting a low risk of fomite transmission. The knowledge gained from this study contributes to efforts aimed at disease eradication.

# BBD – Bacteriology and Bacterial Diseases

# EVALUATION OF CLEANING COMPOUNDS TO MINIMIZE SAMPLE CROSS-CONTAMINATION FOR DETECTION OF MYCOPLASMA HYOPNEUMONIAE BY PCR

## C. Snow <sup>1</sup>, A. Canturri <sup>2</sup>, <u>M. Pieters <sup>3</sup></u>

<sup>1</sup>University of Minnesota - College of Veterinary Medicine, St. Paul, MN USA

<sup>2</sup>Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA

<sup>3</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

# **Background and Objectives**

Sample cross-contamination is one of the most common pre-analytical errors causing misleading diagnostic results. The remarkable resistance to degradation of DNA molecules in the environment can cause sample cross-contamination and interpretation challenges in Mycoplasma hyopneumoniae detection by PCR. Current sampling protocols recommend cleaning the materials used to collect tracheal secretion samples between sampled pigs. However, the cleaning method is not standardized and it is currently unknown if the used cleaning wipes prevent DNA carryover between samples. Therefore, the objective of this study was to compare various cleaning compounds on their ability to minimize sample cross-contamination in swine tracheal secretion.

## Material and Methods

For natural contamination of sampling material sets, which included a mouth speculum, a laryngoscope blade, and a pair of scissors, tracheal secretions were collected with various sets from nursery pigs experimentally inoculated with M. hyopneumoniae (n=30; strain 232). For artificial contamination, 1:10 diluted M. hyopneumoniae strain 232 culture was sprayed onto six separate material sets. Each material was sampled prior to and post-wipe cleaning. The material sets were thoroughly cleaned with wipes containing one of the following compounds: hydrogen peroxide A and B, quaternary ammonium, bleach, alcohol, or phosphate-buffered saline (PBS). Nucleic acids were extracted from pre- and post-cleaning samples for both natural and artificial contamination and tested via a commercial M. hyopneumoniae-specific PCR. Ct values were compared via the Kruskal-Wallis test.

## Results

Starting Ct values were not statistically different between treatment groups for natural (p = 0.50) or artificial (p = 0.79) contamination. In the naturally contaminated treatment groups, bleach and hydrogen peroxide A were the only compounds to eliminate all M. hyopneumoniae genetic material (p = 0.04). Bleach was the only treatment to completely remove all M. hyopneumoniae genetic material from the artificially contaminated materials (p = 0.02).

## **Discussion and Conclusion**

Results of this investigation indicated that the current protocols for sample collection could result in cross-contamination from residual DNA between pigs, and that cleaning with bleach removed all M. hyopneumoniae DNA from the sampling materials. Thus, obtained results can guide the development of a protocol for minimizing sample cross-contamination when collecting swine clinical specimens.

BBD - Bacteriology and Bacterial Diseases

# EVIDENCE OF MYCOPLASMA HYOPNEUMONIAE RECIRCULATION IN PREVIOUSLY EXPOSED IMMUNE SOWS

P. Yeske 1, A. Betlach 1, E. Fano 2, M. Schwartz 3, J. Schwartz 4, M. Pieters 5

<sup>1</sup>Swine Vet Center, St Peter, MN, USA

<sup>2</sup>Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA; Current affiliation: Pipestone Veterinary Services, Pipestone, MN, USA

<sup>3</sup>Department of Population Medicine, College of Veterinary Medicine, Swine Disease Eradication Center, University of Minnesota, St. Paul, MN USA, Schwartz Farms, Sleepy Eye, MN, USA

<sup>4</sup>Schwartz Farms Inc., Sleepy Eye, MN, USA

<sup>5</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

# Background and Objectives

A long debated question in Mycoplasma hyopneumoniae (Mhp) control is 'can pigs become reinfected with the pathogen?' Previous studies have shown the duration of Mhp shedding prior to clearance, along with a high success rate for elimination. However, the possibility for Mhp reinfection and assessment of subsequent infection dynamics has only been evaluated experimentally in a limited number of studies. Therefore, the objective of this study was to determine if dams, with known previous exposure and clearance, can become recolonized with Mhp when housed in close proximity to actively infected animals.

## Material and Methods

A herd that underwent a well-documented Mhp exposure and herd closure plus medication elimination program, and later experienced an acute disease outbreak with Mhp was selected for this study. During the outbreak, coughing gilts were referred to as 'index' animals. Tracheal swabs were collected from clusters of dams (in stall gestation), which consisted of the index gilt and five parity 2+ (previously exposed animals) sows that were housed beside and across the gestational alley from the index. Fifty clusters were sampled, equating to 251 sows. In addition, air particle deposition samples were collected throughout the gestation barns as a point of reference. Samples were individually tested for detection of Mhp genetic material via real-time PCR. Samples with a Ct value <37 were considered PCR positive and suggested colonization with Mhp.

## Results

Of the 251 dams sampled,153 were present in the herd at the time of exposure and closure, thus being previously exposed and potentially immune, in which 44%, 36%, and 19% resulted suspect, positive, and negative respectively, by PCR. All particle deposition samples resulted positive for detection of Mhp, with a high mean Ct value.

## **Discussion and Conclusion**

Results of this investigation suggest that previously exposed dams may become colonized with Mhp, aiding in the recirculation of this pathogen within the herd. Implications of these findings may explain why these types of animals can cause more problems in wean-to-finish by leading to increased prevalence. Information gained from this pilot study should be evaluated in other field scenarios, as further research is essential to assess Mhp reinfection and strategies for disease elimination.

BBD - Bacteriology and Bacterial Diseases

# AN OVERVIEW OF KLEBSIELLA PNEUMONIAE SEPTICAEMIA OUTBREAKS IN PIGS IN ENGLAND SINCE EMERGENCE IN 2011

<u>S. Williamson</u><sup>1</sup>, C. Bidewell<sup>2</sup>, H. Wighton<sup>1</sup>, L. Pittalis<sup>2</sup>, M. Abuoun<sup>3</sup> <sup>1</sup>Animal and Plant Health Agency, Rougham Hill, Bury St Edmunds, Suffolk, England, UK <sup>2</sup>Animal and Plant Health Agency, England, UK <sup>3</sup>Animal and Plant Health Agency (APHA), Bacteriology, New Haw, United Kingdom

## **Background and Objectives**

Septicaemia outbreaks due to Klebsiella pneumoniae subsp. pneumoniae (Kpp) were first diagnosed in England in 2011 and have occurred each year since. Evidence points to emergence of a virulent Kpp strain. This overview summarises features of outbreaks from 2011 to 2023 including characterisation of the Kpp isolates.

## Material and Methods

Kpp septicaemia outbreaks were confirmed in diagnostic submissions of pigs where the case definition "pigs found dead with lesions consistent with septicaemia and pure/predominant growths of Kpp isolated from internal sites in multiple pigs" was fulfilled. Clinical and epidemiological data were obtained from vets and pig farmers. Whole genome sequencing of Kpp isolates from outbreak and non-outbreak cases was undertaken and multi-locus sequence typing (MLST) performed. Kpp isolates underwent antimicrobial sensitivity testing.

## Results

Fifty-six outbreaks were confirmed between 2011 and 2023 with one to seven diagnosed each year. Almost all outbreaks occurred between May and October, with two early outbreaks, and none diagnosed in the months November to January. Pigs are found dead or rapidly dying from nine days of age to weaning and all but one outbreak have been on outdoor breeding units in litters from sows of different parities. MLST analysis revealed that outbreak isolates analysed to date were all the same sequence type, ST25, except one, and all possess the rmpA virulence gene and a 4 kb plasmid, which were absent from historic Kpp isolates analysed. Acquired antimicrobial resistance has not been a significant feature in outbreak isolates. Some farms experience repeat outbreaks in successive years. Concurrent disease was rarely found in affected pigs and mortality is usually relatively low at around 5% but has reached 16%.

## **Discussion and Conclusion**

Kpp septicaemia outbreaks associated with a specific ST of Kpp are now seen every year. The consistent ST and virulence gene content of outbreak isolates supports true emergence of a virulent Kpp strain. Disease shows a clear seasonal summer occurrence and very consistent epidemiological features with well-grown preweaned piglets affected on outdoor breeding units and autogenous vaccine use has been implemented to good effect. Factors behind Kpp ST25 emergence have not been explained and we are interested in information from similar outbreaks elsewhere.

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## DEVELOPMENT OF THE FAECAL MICROBIOTA IN POST-WEANING PIGLETS

<u>M. Klahr Fritz</u><sup>1</sup>, T. Rosendal <sup>2</sup>, M. Sjölund <sup>1</sup>, P. Wallgren <sup>2</sup>, M. Leijon <sup>2</sup> <sup>1</sup>National Veterinary Institute (SVA), Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden <sup>2</sup>National Veterinary Institute (SVA), Uppsala, Sweden

## **Background and Objectives**

At weaning, piglets are separated from the sow, leading to a sudden transition in diet from sow milk to grain-based feed. A disruption of the gut microbiota follows as the digestive system adapts to the new diet. This may lead to post-weaning diarrhoea (PWD), primarily caused by the proliferation of enterotoxin-producing Escherichia coli. The aim of this study was to follow the development of the gut microbiota during the post-weaning period.

## **Material and Methods**

Faecal swab samples were collected from 32 healthy piglets from three different Swedish farms with an average weaning age of 32 days. Sampling was performed before weaning and on days 3, 7, 10, 14, and 21 post-weaning. The microbiota of the 192 samples were characterised by sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene using Illumina MiSeq sequencing. The sequencing data was processed using a DADA2-based pipeline.

## Results

The faecal microbiota showed the most dissimilarity between piglets on day three after weaning. On the later sampling occasions (days 7, 10, 14, and 21), the composition of the microbiota gradually became increasingly similar between piglets, becoming more uniform between piglets than before weaning on days 14 and 21 after weaning. The composition of the microbiota on day 21 after weaning showed very little overlap with the composition before weaning.

# **Discussion and Conclusion**

The faecal microbiota was most dissimilar between piglets on day three after weaning, indicating a less stable microbiota at this time point, compared to both before weaning and later during the post-weaning period. The gradually more homogenous composition of the faecal microbiota between pigs seen from day seven to 21 suggests an adaptation of the gut to the new grain-based diet. This also indicates a more stable intestinal microbiota and an improved ability to digest the feed. The findings in this study illustrate the process of the intestinal microbiota adapting to the shift in diet occurring at weaning. Increased understanding of this process is important for the improvement of PWD prevention strategies.

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# DIVERSITY OF CLOSTRIDIUM PERFRINGENS ISOLATES FROM SWINE - ANALYZED WITH A NOVEL MICROARRAY-BASED TOXIN TYPING ASSAY

<u>M. Blümlinger</u><sup>1</sup>, L. Schwarz<sup>1</sup>, J. Spergser<sup>4</sup>, A. Cabal Rosel<sup>2</sup>, W. Ruppitsch<sup>2</sup>, S. Braun<sup>3</sup>, S. Monecke<sup>3</sup>, R. Ehricht<sup>3</sup>, A. Ladinig<sup>1</sup>, I. Loncaric<sup>4</sup> <sup>1</sup>University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria <sup>2</sup>Austrian Agency for Health and Food Safety, Mödling, Austria

<sup>3</sup>Leibniz Institute of Photonic Technology (IPHT), Jena, Germany

Institute of Microbiology, University of Veterinary Medicine Vienna, Austria

## **Background and Objectives**

Clostridium perfringens (CP), among others, is an important pathogen causing neonatal diarrhea in piglets. In the last years, numerous new extracellular toxins and hydrolytic enzymes were described, to the extent that Rood et al. (2018) proposed an expansion of the toxin-based typing scheme and introduced two novel toxinotypes (i.e., CP Type F and G). In the swine related literature, CP Type C has been well described as a causative agent of neonatal diarrhea. For CP Type A, the evidence is more ambiguous.

## **Material and Methods**

The novel microarray assay for CP toxin-gene-typing allows the simultaneous analysis of 16 toxin-gene sequences within a single reaction. To validate the method, 107 non-repetitive clinical CP isolates from Austrian animals and seven reference strains were analyzed. The isolates were recultivated, identified to species level via matrix-assisted laser desorption ionization time of flight mass-spectrometry, DNA extracted and subsequently toxin-gene-typed via the proposed microarray assay. Twenty selected isolates were characterized via Whole-Genome-Sequencing (WGS) and consequently a core genome Multi Locus Sequence Typing (cgMLST) (Abdel-Glil, 2020) was performed. The microarray results were subsequently compared with the obtained genomes and publicly available genomes of the reference strains.

# Results

The detected toxin-gene-sequences via the presented microarray assay showed significant homology with the obtained WGS results as well as the available reference sequences. The analysis of WGS revealed that one isolate was assigned to classical sequence type (ST) 2, one to ST 21, five to ST 39, one to ST 179, one to ST 583 and one to ST 772 respectively. The remaining ten isolates were assinged to novel STs. The cgMLST showed a high degree of diversity among isolates without defined clusters.

# **Discussion and Conclusion**

Compared to the current single PCR based routine diagnostics for CP toxin genes followed by gel electrophoresis, the novel microarray is less expensive, less laborious, less time-consuming, and provides information on the presence of 16 toxin-genes in a single assay. Together with the WGS based validation of the obtained microarray results, the novel assay represents a reliable and superior alternative to routine conventional PCR toxin gene testing. Therefore, we propose its use in future toxin-gene-typing of CP isolates.

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# COMPARISON OF THE PATHOLOGY AND CLINICAL EFFECTS OF AN F18 ENTEROTOXIGENIC ESCHERICHIA COLI CONTAINING A TIA ADHESIN GENE AGAINST A CONTEMPORARY F18 ESCHERICHIA COLI STRAIN

<u>R. C. Paiva</u>, E. Aljets <sup>2</sup>, H. Chen <sup>1</sup>, P. Jermsutjarit <sup>1</sup>, P. Watcharavongtip <sup>1</sup>, D. Vargas-Bermudez <sup>1</sup>, H. Tong <sup>1</sup>, P. Gauger <sup>1</sup>, D. Murray <sup>3</sup>, P. Pineyro <sup>1</sup>, M. Almeida <sup>1</sup>

<sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

<sup>2</sup>Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, United States

<sup>3</sup>New Fashion Pork, Jackson, MN

## **Background and Objectives**

There are two separate chromosomally encoded invasion loci (tia and tib) described in human-specific classical ETEC strains that have been detected in market hog surveillance but with unknown clinical relevance in pigs. This study aimed to assess the clinical impact of ETEC-F18tia<sup>+</sup>, compare the clinical impact of ETEC-F18tia<sup>+</sup> with ETEC-F18<sup>+</sup>/tia<sup>+</sup>, and assess the efficacy of a commercially available F18 E. coli vaccine as a competitive exclusion product for post-weaning diarrhea (PWD) caused by an ETEC-F18<sup>+</sup>/tia<sup>+</sup>.

## **Material and Methods**

Pigs were randomly allocated into five groups: negative control (NC; N=8), vaccinated, challenged with E. coli (V/C-F18<sup>+</sup>/tia<sup>-</sup>; N=16), non-vaccinated, challenged with E. coli (NV/C-F18<sup>+</sup>/tia<sup>-</sup>; N=16), vaccinated, challenged with E. coli (V/C-F18<sup>+</sup>/tia<sup>+</sup>; N=16), non-vaccinated, challenged with E. coli (NV/C-F18<sup>+</sup>/tia<sup>+</sup>; N=16). Pigs were weighed individually on dpi - 5, -2, 0, and 7. Rectal swabs were collected on dpi 0, 1, 2, 3, 5, and 7. The hemolytic E. coli shedding was assessed, colonies' ID was confirmed using the MALDI-TOF, and the data were analyzed using a repeated measures model.

## Results

The groups V/C-F18+/tia+; NV/C-F18+/tia+; V/C-F18+/tia+; NV/C-F18+/tia-; NV/C-F18+/tia-; were significantly different from the NC. There was a numerical difference in days of diarrhea and weight per pig between NV/C-F18+/tia+ group compared with V/C-F18+/tia+; NV/C-F18+/tia+; NV/C-F18+

## **Discussion and Conclusion**

The tia gene should be considered a virulence factor contributing to post-weaning diarrhea in pigs, and further investigation of its pathogenesis is needed. The results of this study may help to better understand enteric cases without an etiologic diagnosis and consider the novel E. coli tia<sup>+</sup> genotype as a potential differential.

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# COMPARISON OF THE SENSITIVITY OF ORAL FLUIDS AND POOLED PEN FECAL SAMPLES FOR THE DETECTION OF LAWSONIA INTRACELLULARIS

A. Vegter <sup>1</sup>, J. Morgan <sup>3</sup>, G. De-Sousa-E-Silva <sup>1</sup>, P. Gauger <sup>2</sup>, <u>F. Leite</u> <sup>3</sup> <sup>1</sup>Iowa State University College of Veterinary Medicine <sup>2</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA <sup>3</sup>Boehringer Ingelheim Animal Health USA Inc.

# **Background and Objectives**

Lawsonia intracellularis is a global endemic pathogen that causes porcine proliferative enteropathy (PPE). Oral fluids, pooled pen fecal samples and individual pig fecal samples can be used to investigate Lawsonia by PCR. However, it is unclear how oral fluid and pooled pen fecal samples differ in their sensitivity. This study aimed to compare these two sample types to evaluate their sensitivity and aid in their appropriate recommendation and interpretation.

## Material and Methods

Two wean-to-finish farms, one with clinical and the other with subclinical PPE were used in this study. Oral fluids (OF) and pooled pen fecal samples (PPF) were collected from all pens at each farm. A subset of pens from each farm were selected to have individual fecal samples collected from every pig in the pen. qPCR testing was done with all fecal samples processed with 0.3g of feces, any sample with a Ct below 35 was considered positive.

## Results

The level of positivity of PPF and OF samples were significantly different in both the clinical and subclinical herds. In the subclinical farm, 6.5% (4/62) of OFs and 46.8% (29/62) of PPFs were qPCR positive (p<0.05); while in the clinical farm 100% (54/54) of OF and 85.2% (46/54) of PPFs were qPCR positive (p<0.05). The average Ct for positive OF and PPF samples were 34.8 and 34.1, respectively, in the subclinical farm, and 28.4 and 30.5 in the clinical farm.

## **Discussion and Conclusion**

This study found that oral fluids and pooled pen fecal samples do not present the same level of sensitivity. While in a subclinical herd pooled pen fecal samples were found to be significantly more sensitive, an opposite pattern was observed in the clinical herd. It is likely that shedding levels and disease stage contributed to these differences. These results suggest that pooled pen fecal samples are better suited than oral fluids to monitor Lawsonia in instances where there is a lack of clinical signs and lower levels of shedding.

# HERD HEALTH MANAGEMENT

# HHM-OP-01

## HHM – Herd Health Management

# ASF PREVENTION: WEB-BASED TOOL TO ASSESS RISK FOR ASF INTRODUCTION IN PIG FARMS WITH OUTDOOR HOUSING

B. Grabkowsky <sup>1</sup>, <u>M. Gellermann</u>, F.J. Conraths <sup>2</sup>, N. Denzin <sup>2</sup>, C. Sauter-Louis <sup>2</sup>, M. Martin <sup>3</sup> <sup>1</sup>Center of sustainability transformation in areas of intensive agriculture | University of Vechta, Germany <sup>2</sup>Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany <sup>3</sup>Comissioner for Animal Welfare, Ministry for the Environment, Climate, Agriculture and Consumer Protection Hesse, Wiesbaden, Germany

## **Background and Objectives**

Outdoor housing is a socially desirable form of pig husbandry. African swine fever (ASF) entries into pig herds have shown that it also poses major challenges in terms of preventing outbreaks of ASF. Biosecurity is a central pillar of ASF prevention. Although numerous checklists, standards and guidelines are available, in practice there are sometimes considerable weaknesses in operational herd and hygiene management. Objective of the project was to develop a structured advisory tool, that supports farmers to reflect their individual farm conditions and procedures in an anonymous way and directly provides tailor-made suggestions to improve the farms biosecurity concept.

# **Material and Methods**

The tool is methodically based on a questionnaire on weighted risk factors. A panel of experts from science, veterinary and agricultural practice, authorities and industry was consulted to develop the questionnaire. Within a three-stage Delphi study, the epidemiological relevance of each risk factor was determined in relation to the other risk factors.

### Results

The results indicate the overall risk of ASF introduction according to various farm-specific categories using traffic light colors. The result is transparently calculated from the ratio of the achieved score to the maximum achievable score per category and shows how to improve the farm's biosecurity concept. In addition to legal requirements and standards from Germany, the tool also includes other requirements from EU animal health law, in particular Regulation (EU) 2016/429 ("Animal Health Act"). The user also receives an individual overview of the measures to be expected at a location in an ASF restriction area (in accordance with EU Regulation 2023/594 and others) - an important prerequisite for being able to quickly meet the increased requirements for marketing the animals. In the project funded by the state of Hesse, the ASF risk traffic light for outdoor facilities will be published in February 2024. Initial evaluations of the utilization statistics of the ASF risk traffic light will be presented.

## **Discussion and Conclusion**

With the ASF-risk traffic light for open housing systems, a pathogen-specific advisory tool with the latest knowledge on ASF prevention is available for more animal-friendly forms of production with outdoor climate access. This meets the social demand in the context of the ASF disease risk maagement.

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# A NOVEL VEHICLE MODEL REROUTING SYSTEM TO REDUCE SWINE DISEASE TRANSMISSION RISK

### J.A. Galvis<sup>1</sup>, G. Machado<sup>1</sup>

<sup>1</sup>Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

# Background and Objectives

Vehicle movement is a key factor in spreading infectious pathogens in animal production. Swine production systems have established cleaning and disinfection (C&D) biosecurity protocols to reduce the disease spread. Nevertheless, the current C&D protocols may not be sufficient to prevent vehicles from spreading diseases. Our study aimed to develop and assess a protocol to minimize disease spread associated with vehicle movements between farms.

## **Material and Methods**

We developed a classification model named "EpiMover," which uses disease surveillance data, vehicle movement networks, and cost-benefit analysis to rank each vehicle according to its transmission risk. We tested our protocol monitoring the delivery movement of 107 feed trucks, 108 vehicles transporting live pigs between farms, and 81 vehicles transporting pigs to slaughterhouses over one week. 2,978 vehicle deliveries to 1,013 farms were recorded. For each of these deliveries, we used EpiMover to identify the vehicle with the lowest risk of disease transmission and used this vehicle to simulate the necessary deliveries. The simulated deliveries included a C&D, which has different pathogen elimination effectiveness, ranging from 0% to 100%. From the observed and simulated deliveries, we reconstructed the vehicle movement network and evaluated risk contacts from farms that have reported PRRSV and/or PEDV outbreaks in the past year to farms with no infectious records.

## Results

On average, our protocol reduced 45% of risky contacts for feed vehicles when C&D effectiveness was 0% and 91% when C&D was 50%. For vehicles transporting pigs to market, risky contacts were reduced by 12%, with C&D effectiveness at 0% and 43% with C&D at 50%. Vehicles transporting pigs between farms only showed a significant reduction after C&D effectiveness above 50%, with 31% fewer risk contacts. Finally, with C&D effectiveness at 100%, risk contacts dropped below 3% for all vehicle types.

## **Discussion and Conclusion**

Our results indicate that the EpiMover effectively organizes vehicle schedules to prevent disease spread among farms, enhancing its efficacy alongside increased C&D effectiveness.

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# DECISION TREE FOR PRRSV MONITORING IN HERDS UNDERGOING A CONTROL PROGRAM

D.C. Linhares <sup>1</sup>, I. Machado <sup>2</sup>, M. Almeida <sup>5</sup>, E. Magalhães <sup>3</sup>, G. De-Sousa-E-Silva <sup>4</sup>, G. Trevisan <sup>5</sup> <sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA <sup>2</sup>Iowa State University, Ames, USA <sup>3</sup>Iowa State University College of Veterinary Medicine <sup>5</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

# **Background and Objectives**

Recent years were marked by the rise of population-based PRRSV monitoring options for breeding herds, including family oral fluids (FOF), processing fluids (PF), and tongue fluids (TF). Producers frequently ask, 'When do I use each sample'? This study summarizes findings validating population-based sample types for PRRSV detection.

# Material and Methods

We characterized the probability of PRRSV RNA detection by RT-PCR on each sample type compared to suckling pig serum<sup>14</sup>. Here, we assemble a decision tree-like flow chart to guide when to place each sample for herds attempting to consistently produce PRRSV-negative piglets at weaning (i.e., reach stability<sup>5</sup>).

## Results

Weekly testing of aggregated PF is a practical screening approach, starting 8-10 weeks post-outbreak, to monitor for the rise in Ct values toward PCR negativity. When pigs are not physically castrated, TF from newborn pigs ( $\leq$ 7 days old) is a comparable alternative. When the PCR results are unexpected (no apparent trend of rising Ct), stillborn TF allows pinpointing if the breeding herd is still actively vertically transmitting the virus to piglets. If stillborn TF are PCR-negative when PF are PCR-positive, the gestating sows are likely producing PRRSV-negative piglets. Strict bio-management will keep pigs negative. If TF is PCR-positive, the breeding herd requires improved herd immunity, which can be achieved with immunization or time. When aggregated weekly PF (or < 7-day-old TF) are PCR-negative for 3-4 weeks, we recommend aggregating no more than 1 PCR per 55-60 litters (PF) or 1 PCR per 20-100 tongues (TF). When these are negative, there is need to verify the status of weaning-age pigs by FOF (12-15 samples). Thirteen consecutive weeks with PCR-negative results on weaners indicates PRRSV-stable herd<sup>5</sup>. If weaner samples are PCR-positive when PF samples (or < 7-day-old TF) are negative, there is a clear opportunity for bio-management to keep pigs negative until weaning. Alternatives to FOF include serum (60 samples per weaning age room) or oral fluids post-weaning.

# **Discussion and Conclusion**

This decision tree is intended to be a guide to help people strategize PRRSV monitoring in breeding herds undergoing elimination. Alternative samples should be considered when providing a similar probability of PRRSV detection at the respective age group being monitored.

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## MAPPING BIOSECURITY LEGISLATION IN THE INTENSIVE PIG PRODUCTION ACROSS EUROPE

<u>E. Biebaut</u><sup>1</sup>, M. Stukelj<sup>2</sup>, T. Pina Nunes<sup>3</sup>, V. Nedosekov<sup>4</sup>, C. Correia Gomes<sup>5</sup>, B. Mehmedi<sup>6</sup>, I. Corrégé<sup>7</sup>, L. ózsvári<sup>8</sup>, L. Svennesen<sup>9</sup>, E. Bernaerdt<sup>10</sup>, I. Toppari<sup>11</sup>, L. Gresakova<sup>12</sup>, C. Romanelli<sup>13</sup>, M.E. Filippitzi<sup>14</sup>, M. Siv<sup>15</sup>, B. Angjelovski<sup>16</sup>, S. Küker<sup>17</sup>, A. Allepuz<sup>18</sup>, A. Viltrop<sup>19</sup>, M. Didara<sup>20</sup>, M. Nikolic<sup>21</sup>, E. Vink<sup>22</sup>, I. Chantziaras<sup>1</sup>, L. Dahlin<sup>24</sup>, M. De Nardi<sup>25</sup>, T. Nicolaisen<sup>23</sup>, J. Prodanov-Radulovic<sup>26</sup>

<sup>1</sup>Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Belgium

<sup>2</sup>University of Ljubljana, Veterinary faculty, Ljubljana, Slovenia

<sup>3</sup>Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal <sup>4</sup>National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

<sup>5</sup>Animal Health Ireland, 2-5 Archways, Carrick-on-Shannon, Ireland

<sup>6</sup>University of Prishtina, Faculty of Agriculture and Veterinary, Prishtina, Kosovo

7IFIP – Institut du Porc BP 35104, 35651 Le Rheu, France

<sup>8</sup>University of Veterinary Medicine Budapest, Hungary

<sup>a</sup>Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen <sup>10</sup>ILVO, Flanders Research Institute for Agriculture, Fisheries and Food, Melle, Belgium

11Animal Health ETT, Finland

<sup>12</sup>Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Kosice, Slovakia <sup>13</sup>Agricultural and Food Sciences Department, University of Bologna, Italy

<sup>14</sup>Laboratory of Animal Health Economics, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece <sup>15</sup>Norwegian Veterinary Institute, Ås, Norway

<sup>16</sup>Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine, Department of Farm Animals Internal Medicine, Skopje, North Macedonia

<sup>17</sup>SAFOSO AG, Waldeggstrasse 1, 3097 Liebefeld, Switzerland

<sup>18</sup>Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Spain

<sup>19</sup>Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

<sup>20</sup>Department of Animal Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

<sup>21</sup>Diagnostic veterinary laboratory, Podgorica, Montenegro

<sup>22</sup>The Netherlands

<sup>23</sup>Field Station for Epidemiology (Bakum), University of Veterinary Medicine, Hanover, Foundation

<sup>24</sup>Department of Clinical Sciences, Swedish University of Agriculture, Uppsala, Sweden

<sup>25</sup>Dipartimento di Scienze Mediche Veterinarie (DIMEVET), Alma Mater Studiorum - Università di Bologna, Italy

<sup>26</sup>Department of Epizootiology, Clinical diagnostics and DDD, Scientific Veterinary Institute "Novi Sad", Republic of Serbia

# **Background and Objectives**

Implementing biosecurity measures is crucial to optimize animal health and reduce antimicrobial usage in the pig production. A legal framework is required to ensure all stakeholders understand the need of biosecurity measures and to implement them correctly. Currently, the legal framework detailing various biosecurity measures falls under the national (or regional) level as the EU's Animal Health Law mainly provides general requirements. The aim of this survey was to review biosecurity legislation in the European intensive pig production.

## **Material and Methods**

The EU-funded COST Action BETTER (CA20103) consortium collected data by using a participatory research approach. The consortium developed a survey to map the coverage of biosecurity measures in the national legislation. It included 51 biosecurity measures, 32 on external and 19 on internal biosecurity, grouped into fourteen categories. After external and internal revision and pilot testing, the survey was sent to national experts for completion.

## Results

The experts from 25 European countries, 18 belonging to the European Union, submitted the survey. During the validation meetings with the national experts it became clear that biosecurity legislations in the pig production are scattered over different national laws and that it is often open for interpretation. France, Italy and Spain, have one national legislation dealing specifically with biosecurity in the pig production and Slovakia, Ireland and Estonia rely entirely on European legislation. The national legislations focus more often on external than on internal biosecurity and more biosecurity measures are addressed in the large pig producing countries compared to small pig producing countries. Legislations on the purchase of new animals exist in 18 countries and biosecurity measures for animal transportation are mandatory in 17 countries while only five counties regulate manure transport.

# **Discussion and Conclusion**

This survey showed large variation in the presence of national legislation on biosecurity in general in the intensive pig sector in European countries but also on the level of the individual biosecurity measures addressed in the legislation. An overarching pig production-specific biosecurity legislation might tackle this variation and ensure consistent biosecurity across Europe.

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## HHM – Herd Health Management

# EVALUATION OF PRRSV ONSET OF INFECTION USING ORAL FLUIDS AND AIR EMISSIONS SAMPLING

M. Schwartz <sup>1</sup>, C. Snow <sup>4</sup>, M. Torremorell <sup>2</sup>, A. Rendahl <sup>3</sup>, S. Krebs <sup>4</sup>, M. Pieters <sup>5</sup>

<sup>1</sup>Department of Population Medicine, College of Veterinary Medicine, Swine Disease Eradication Center, University of Minnesota, St. Paul, MN USA, Schwartz Farms, Sleepy Eye, MN, USA

<sup>2</sup>Department of Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN USA

<sup>3</sup>Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

<sup>4</sup>University of Minnesota - College of Veterinary Medicine, St. Paul, MN USA

<sup>5</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

## Background and Objectives

The use of oral fluids has become routine to monitor for porcine reproductive and respiratory syndrome virus (PRRSV) status in growing pigs and assist in decisions regarding regional disease control programs. Air emission sampling has not been routinely used to characterize virus dispersion. Furthermore, airborne emissions have not been used routinely to determine onset of infection and population status. In this study we compared the detection of PRRSV in oral fluids and in air emissions from pigs infected under field conditions.

## **Material and Methods**

Three growing pig sites in the Midwest U.S. were conveniently selected based on the known PRRSV-negative status at placement. Cotton ropes were used to collect 20 oral fluids and these were placed bi-weekly at spatially fixed locations at each site. In addition, two to four air emission particulate samples were obtained simultaneously with a cotton cloth placed 1m from exhaust fans (1.5-2h collection time/event). Oral fluids and eluates retrieved from air emission samples were tested using a PRRSV RT-PCR. Data were analyzed using logistic regression to compare the cycle threshold (Ct) value of PRRSV-positive oral fluids and the probability of PRRSV detection in air emissions samples.

## Results

The first PRRSV detection at each site was obtained simultaneously in air emissions and oral fluid samples. Initial PRRSV PCR detection was either at 56 or 84 days post-placement. The probability of detecting PRRSV on air emissions increased as the proportion of PCR-positive oral fluids increased, from 4% (95% CI: 0.4% - 24.1%) with 50% PCR positive, to 40% (95% CI:24%, 59%) with 100% PCR-positive. The probability of detecting PRRSV on air emissions decreased as mean Ct values in oral fluids increased, from 33% (95% CI: 20% - 50%) with a mean Ct of 30, to 2.6% (95% CI:0.6% - 10.2%) with a mean Ct of 40.

## **Discussion and Conclusion**

Air emission sampling, a biosecure and non-invasive approach, detected the onset of PRRSV by PCR at the same time as oral fluids. Detection of PRRSV in air emissions when oral fluids were positive, varied on subsequent collection events. Additional investigations into PRRSV dispersion and air emission sampling is warranted.

## HHM – Herd Health Management

# PEN ORAL FLUIDS AS AN ALTERNATIVE TO FAECAL SAMPLING TO DETECT LOW BACTERIAL LOAD OF LAWSONIA INTRACELLULARIS IN SUBCLINICAL INFECTIONS

<u>R. Del Pozo Sacristán</u><sup>1</sup>, H. Swam<sup>2</sup>, A.E. Taylor<sup>3</sup> <sup>1</sup>MSD Animal Health <sup>2</sup>Center for Diagnostic Solutions, MSD AH Boxmeer, The Netherlands <sup>3</sup>University of Leeds, UK

## **Background and Objectives**

Oral fluids (OF) have been demonstrated as a reliable sampling method to detect and quantify bacterial load of Lawsonia intracellularis (LI) at group level using qPCR. However, it is still unclear whether this sample type would be suitable to detect subclinical infections with low bacterial load. Therefore, the aim of this study was to evaluate whether pen OF could detect LI subclinical infections in a herd with a low bacterial faecal shedding.

#### Material and Methods

For this aim, a herd with a history of subclinical LI infection was selected. A subclinical infection was defined by the absence of ileitis-like symptoms and by a low bacterial load (<10<sup>5</sup> copies/µL) of this bacterium in faeces quantified by qPCR (BactoReal Lawsonia kit, Ingenetix). A cross-sectional sampling was performed, including pigs sampled at 4, 7, 10, 13, 16, 19 and 22 weeks of age (woa). Fresh faecal samples were collected individually from the anus at defecation (minimum 2 pigs/pen of 10 pigs). OF samples were taken at the same time points on a pen basis. Both faecal and saliva samples were tested for LI by qPCR [results given in 10Log copies/µL (faeces) and Ct-values (saliva)]. The association between the number of positive specimens detected by both type of samples was calculated by Chi-square test and Cohen's kappa. A Pearson correlation was performed to compare bacterial load in faeces and saliva.

### Results

In total, 367 individual faecal and 120 pen OF samples were collected. A significant (P<0.001) strong agreement (kappa=0.636) was found between the number of positive specimens detected by both type of samples. OF sampling identified correctly (sensitivity) 93.5% of all the pens with at least one sample pig with a positive faecal test. Only three pens tested negative while at least one positive pig (1.2-2.4Log10 copies/ $\mu$ L) was housed in there. A significant (P<0.001) strong correlation (r=-0.626) CI[-0.684; -0.559] was detected for bacterial load between both type of samples.

## **Discussion and Conclusion**

Under the conditions of this study, it was concluded that pen OF is an excellent alternative to faecal sampling to detect LI subclinical infections at group level, even in the face of low bacterial load.

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# PEN-BASED SWINE ORAL FLUID SAMPLES CONTAIN BOTH ENVIRONMENTAL AND PIG-DERIVED TARGETS

G. Tarasiuk <sup>1</sup>, M. Remmenga <sup>2</sup>, K. O'Hara <sup>2</sup>, M. Rotolo <sup>3</sup>, P. Zaabel <sup>4</sup>, D. Zhang <sup>5</sup>, L. Gimenez-Lirola <sup>1</sup>, <u>J. Zimmerman</u> <sup>1</sup> <sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA <sup>2</sup>United States Department of Agriculture, Veterinary Services, Strategy and Policy, Center for Epidemiology and Animal Health, Fort Collins, CO, 80526 <sup>3</sup>PIC North America, Hendersonville, TN 37075 <sup>4</sup>National Pork Board. Clive, IA 50325

Department of Statistics, Collage of Liberal Arts and Sciences, Iowa State University, Ames, IA

## **Background and Objectives**

Laboratory detection of specific pathogens in oral fluids (OF) is widely researched but there is an incomplete understanding of the original source of the target (pig vs environment). In this study, fluorescent food coloring in a sugar solution was used to demonstrate the ability to quantitate pig and environmental contributions to pen-based OF samples.

## Material and Methods

The study was done in pens of ~30, ~65, and ~125 14-week-old pigs (32 pens/size). Pens received one of 2 treatments: 1) pig exposure - 3ml of tracer solution (50% McCormick Red Food Coloring) sprayed into the mouths of 10% of the pigs in the pen; 2) environmental exposure - 20ml of tracer solution placed in the center of the pen. OF collected one day prior to treatment (baseline fluorescence control) and immediately after treatment were tested for relative fluorescence units (RFU) at 530/570 nm (SpectraMax i3x, Molecular Devices LLC). Receiver operating characteristic (ROC) analysis with Youden's J statistic was used to determine a cut-off RFU value (coords{ pROC} version 1.17.0.1, RStudio) that optimally differentiated pre- and post-treatment samples. Samples above the RFU threshold were positive for food coloring.

## Results

Using these thresholds, 86 of 96 pens in this study (89.6%) were positive, i.e., the pigs had transferred food coloring into the OF sample. Among pens receiving pig treatment, 44 of 48 (91.7%) were positive. Among pens receiving environmental treatment 42 of 48 (87.5%) were positive. Relative to the tracer solution, the mean concentration of food coloring in OF from pens receiving pig exposure was 0.0067%, 0.0075%, and 0.0521% for pens sizes of 30, 60, and 125 pigs, respectively. In pens with environmental exposure, the relative mean concentration was 0.0053%, 0.0051%, and 0.0051% for pen sizes of 30, 60, and 125 pigs, respectively.

# **Discussion and Conclusion**

Using a previously undescribed methodology, we determined that both pig-derived and environmental targets are routinely transferred to OF samples. Using a non-infectious and safe tracer, i.e., food coloring, this research can be easily replicated by others under both controlled and field conditions. Further, this approach can be used to study other processes related to oral fluid sampling.

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## RE-EVALUATION OF THE MANAGEMENT ON PIG FARMS IS HALF THE JOB TO OBTAIN PRUDENT AMU.

<u>C. Bonckaert</u><sup>1</sup>, C. Brossé<sup>1</sup>, T. Vandersmissen<sup>1</sup>, C. Rigauts<sup>1</sup> <sup>1</sup>DGZ Vlaanderen, Hagenbroeksesteenweg 167, 2500 Lier, Belgium

# **Background and Objectives**

In Belgium, the consumption of veterinary drugs in production animals has been monitored for years. Previously, during the ROADMAP project, pig industry stakeholders gave insights into the main reasons of antimicrobial use (AMU). Therefore, the coaches focused on three main management advices: cleaning & disinfection, colostrum intake and the combination of both these management measures.

### **Material and Methods**

During maximum 1.5 years 19 pig farms were voluntary coached towards a more prudent AMU, and were visited at least three times. The coaching was asked by the pig farmer himself (n = 6) or was advised by the herd veterinarian or a label (n=13). During the first visit, the herd (management) was consulted. Hereafter the herd was classified to one of the three above mentioned categories. For colostrum management, serum of sow and piglets was analyzed for antibodies against PCV2 to determine whether piglets received sufficiently high concentrations of antibodies. Farms followed up for cleaning & disinfection underwent detailed questioning on how cleaning and disinfection was performed, the effectiveness of cleaning and disinfection was then checked with RODAC plates in a standardized protocol.

### Results

All herds reduced the AMU in at least one animal category. Highest AMU was found in suckling and weaning piglets. The biggest reduction in the suckling piglets was observed in pig farms where colostrum management and cleaning & disinfection were optimized (16.15 reduction in TD100, n=6), followed by herds where the cleaning & disinfection process was optimized (5.1 reduction in TD100, n=5). AMU in weaned piglets was more reduced in farms where colostrum uptake was evaluated and adjusted (32.64 reduction in TD100, n=5) compared to farms where both were evaluated and redirected (15.93 reduction in TD100, n=6) or only cleaning & disinfection was optimized (10.7 reduction in TD100, n=5).

## **Discussion and Conclusion**

The key to success for reduced AMU lies in the combination of raising awareness and adapting the way of working to which people have been so used for so long. Optimalisation of the cleaning and disinfection procedures and colostrum uptake are examples which can lead to less need for antibiotics due to healthier and robust animals!

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# THE CONNECTION BETWEEN INFLAMMATION AND OXIDANT STATUS ACCORDING TO PIGMARKSAL SALIVARY BIOMARKERS (S100A12 AND TOS).

M. Matas-Quintanilla 1, J. Sánchez 2, A. Miralles-Chorro 2, J. Sotillo 1, P. Fuentes 2, A.M. Gutiérrez 1

<sup>1</sup>BioVetMed Research Group, Department of Animal Medicine and Surgery, Veterinary School, University of Murcia, 30100, Espinardo, Murcia, Spain

<sup>2</sup>CefuSA, 30840, Alhama de Murcia, Murcia, Spain.

### Background and Objectives

The protein S100A12 induce an extracellular pro-inflammatory effect, within the innate immune response, when is secreted by activated neutrophils, which is translated to cytokine production and oxidative stress. This connection between inflammation and oxidative stress has not been explored using salivary biomarkers in pigs. The PigMarkSaL salivary panel include biomarkers such as S100A12 and total oxidant status (TOS) which are directly related to the inflammatory pathway and oxidative status.

## Material and Methods

13 diseased animals suffering from a severe respiratory outbreak from a commercial growing farm in the southeast of Spain were monitored during therapy. Saliva samples were collected at different days during the antibiotic treatment, specifically the day of disease diagnosis (T0) and after one (T1), two (T2), four (T4) seven (T7) and fifteen days (T15) of treatment. The concentration of S100A12 and TOS were quantified and to monitor the behaviour of both biomarkers during the therapy a Welch's ANOVA test with Dunnett's T3 multiple comparisons correction was used. The correlation between the levels of biomarkers in the whole period of study was evaluated with Spearman correlation test.

### Results

The levels of S100A12 decrease significantly from a median value of 56.73 mg/mL to 15.82 mg/mL from the day of disease diagnosis (T0) to complete animal recovery (T15). Similarly, the levels of TOS significantly decrease from 16.37 mM/L at T0 to 6.95 mM/L at T15. Both biomarkers showed statistically positive associations with a correlation coefficient of r = 0.71.

### **Discussion and Conclusion**

The association between inflammation and oxidants could be evaluated by the monitoring of the biomarkers S100A12 and TOS in saliva of pigs. The mechanism underlying the association between both biomarkers should be further explored in different pathological conditions.

(Granted by PID2020-116310RB-I00; pigmarksal.com)

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# A SYSTEM-SPECIFIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) OUTBREAK MANAGEMENT PROGRAM

<u>A.P. Serafini Poeta Silva</u>, A. Maschhoff<sup>2</sup>, C. Rademacher<sup>3</sup>, G. De-Sousa-E-Silva<sup>4</sup>, G. Trevisan<sup>1</sup>, D. Linhares<sup>1</sup> <sup>1</sup>Iowa State University <sup>2</sup>The Maschhoffs, Carlyle, IL USA <sup>3</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine <sup>4</sup>Iowa State University College of Veterinary Medicine

## **Background and Objectives**

PRRSV outbreaks require strategies tailored to each system individualities. Ongoing data collection is key to support decision-making to successfully control future outbreaks in breeding herds. One US swine system is unifying data on 1) PRRSV sequencing to determine outbreak-associated strains; 2) PCR testing of weekly processing fluids to categorize PRRSV statuses during outbreak control phases (positive unstable, low prevalence, stable with vaccination, provisionally negative, and negative); 3) production data on key parameters (time-to-baseline-production [TTBP], number of weeks to achieve baseline of total weekly pig wean, time-to-stability [TTS], number of weeks to wean PCR-negative pigs, and total losses/1,000 sows [TTL], total pigs that were not weaned because of the outbreak); and 4) farm characteristics and interventions used to control the outbreak (herd closure, immunologic solutions). This study aimed at identifying which farm/outbreak characteristics and interventions were associated with fast TTBP and TTS and lower TTL.

### **Material and Methods**

Incidence rates (#new outbreaks within a year divided by #breeding herds at risk) were estimated based on the response plan (PRRSV control/vaccination vs. elimination). Sequencing was used to determine PRRSV RFLPs and lineages outbreak-associated strains. Statistical models (Cox and Poisson regressions) were used to associate interventions with faster TTS and TTBP, and lower total losses.

### Results

The dataset included 176 PRRSV outbreaks (October 2010 to May 2023). The highest incidence was 2013 and 2017, with 25 and 22 new outbreaks (~50% occurred in negative herds). Incidences were lower in 2019 and 2020 but increased in 2021. PRRSV RFLPs were clustered by region and time in the production system, and consecutive outbreaks occurred most frequently due to different RFLPs. Statistical models showed that sites that performed herd closure and immediate closure after the outbreaks were associated with up to 25 weeks faster TTS and TTBP (p<0.05). PRRSV 1-4-4 L1C variant and outbreaks in sites previously negative were both associated with highest TTL (p<0.05).

## **Discussion and Conclusion**

PRRSV strains played a role in outbreak intensity in terms of TTL. Whole-herd and immediate closure were effective interventions to control the impact of PRRSV outbreaks. Collecting production, diagnostic, and intervention data is crucial for efficient PRRSV outbreak management programs.

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# COST MINIMIZATION ANALYSIS OF INTRADERMAL AND INTRAMUSCULAR ADMINISTRATION OF VACCINES IN BRAZIL

<u>D. Holtkamp</u><sup>1</sup>, S. Von Berg<sup>2</sup>, P. Moraes<sup>4</sup>, F. Ibanez<sup>3</sup>, L. Peixoto<sup>4</sup>, C. Ferronato<sup>4</sup>, L. Rossi<sup>4</sup> <sup>1</sup>Iowa State University College of Veterinary Medicine, United States <sup>2</sup>MSD Animal Health, Germany <sup>3</sup>MSD Animal Health, Chile <sup>4</sup>MSD Animal Health, Brazil

## **Background and Objectives**

Intradermal (ID) administration of vaccines is an alternative to traditional routes of administration, such as intramuscular (IM) and oral, that may result in lower costs for producers. Cost minimization analysis (CMA) is frequently used in human medicine to measure and compare the costs of different medical interventions when the efficacy of the outcomes is the same. The aim of this study was to apply CMA to compare ID and IM administration of three vaccines in growing pigs in a hypothetical 38,000 sow production system in Brazil.

## Material and Methods

Data to estimate the cost of vaccine storage, vaccination equipment, waste disposal and carcass trim loss was obtained from various sources and was representative of conditions in Brazil. It was assumed that three vaccines were administered. For the IM scenario, pigs were vaccinated intramuscularly with commercially available vaccines against porcine circovirus, Mycoplasma hyopneumoniae and orally against Lawsonia intracellularis. For the ID scenarios, pigs were intradermally vaccinated with all three vaccines.

## Results

Fewer resources were required under the ID scenario due to the smaller doses, vial sizes and packaging, and the elimination of needles and syringes. The cost of electricity to store the vaccines declined from US\$3,773 annually with IM to US\$1,078 with ID as fewer refrigerators were required. The amount of glass, plastic and cardboard waste generated declined from 16.5 metric tons annually with IM to 1.1 metric tons with ID. Pork trim loss declined from 26.8 metric tons with IM to 9.4 metric tons annually for the ID scenario. The total cost savings associated with the ID scenario compared to IM was US\$65,776 annually or US\$0.06 per pig marketed.

## **Discussion and Conclusion**

The CMA analysis demonstrates the magnitude of resource and cost savings associated with ID administration of vaccines. The cost savings for individual producers will depend on the specific circumstances that vary between countries and producers. Other costs that may be relevant include the cost of the vaccine, labor for administration and medical insurance and lost workdays. Differences in the economic value of productivity differences may be relevant if data to support differences is available.

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# CROSS-FOSTERING AFFECTS GROW-FINISHING PIG RESULTS DEPENDING ON BIRTH WEIGHT AND AGE OF CROSS-FOSTERING

<u>M. Nuyens</u><sup>1</sup>, A. Van Der Heide<sup>2</sup>, M. Steenaert<sup>3</sup> <sup>1</sup>Advee Dierenartsen, the Netherlands <sup>2</sup>Westfort Vleesproducten, the Netherlands <sup>3</sup>Boehringer Ingelheim Animal Health Netherlands bv

# **Background and Objectives**

Hyperprolific sows typically have more live born piglets than functional teats. The surplus of piglets is often cross fostered using nurse sows. The objective of this study was to analyze the effect of cross-fostering on daily gain and mortality until slaughter.

# **Material and Methods**

In a Dutch sow herd (TN70, 860 heads) piglets were individually tagged with an electronic earmark (LeeO) at day 1-2, before cross-fostering. After cross-fostering three groups were identified: Piglets from one mother within one litter (Own) Piglets from two mothers within one litter (Cross2) Piglets from three or more mothers within one litter (Cross3+)All finishing pigs were slaughtered at Westfort Meat Products. Individual Daily Gain was calculated (slaughtered weight divided by age; KG/ day). Mortality was calculated (registered causes of death excluding slaughter, divided by including slaughter; %).The dataset included all piglets born in 2022 with a registered cause of death. The dataset contained 25,830 pigs of which 1,812 were weighed at birth. Birth weight categories: Smaller (<1.06 KG; 0.25 percentile value), Middle and Bigger (>1.48KG; 0.75 percentile value). Cross-fostering age categories: 0-3 days of age (doa), 4-7 doa or 8+ doa.

# Results

All piglet's: ADG 0.526, mortality 12.1%. Bigger piglets Cross2 and Cross3+: ADG 0.546, mortality 6.6%. Middle piglets Cross2 and Cross3+: ADG 0.495, >44.7%, versus Own: ADG 0.501, 13.5%. Smaller piglets Cross2 and Cross3+: ADG 0.495, >44.7%, versus Own: ADG 0.501, 13.5%. Off all cross fostered piglets 78% was group 0-3 doa. Group 0-3 doa Bigger and Middle piglets: ADG 0.514, mortality 14.5%. Group 0-3 doa Smaller piglets: ADG 0.494, mortality 25.7%. Group 4-7 doa: ADG 0.514, mortality 21.9%. Group 8+ doa: ADG 0.497, mortality 18.0%.

# **Discussion and Conclusion**

Smaller piglets did not benefit from cross-fostering. Middle piglets in Cross3+ suffered. Bigger piglets did not suffer from cross-fostering. The 4-7 group were piglets cross fostered for doing poor for no obvious reason. The 8+ group were piglets at a sick or dead sow. The low number of piglets in the Cross-foster age didn't allow further analysis.Our conclusions are that for welfare and production results, apart from limiting cross-fostering, cross-fostering bigger piglets at early age (after intake of colostrum) is the best option.

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# MAXIMIZING THE USAGE OF NGS OUTPUTS FOR FARM-SPECIFIC PRRSV EPIDEMIOLOGICAL INVESTIGATIONS

<u>G. Trevisan</u><sup>1</sup>, M. Zeller<sup>1</sup>, G. Li<sup>1</sup>, J. Zhang<sup>1</sup>, R. Main<sup>1</sup>, G. De Souza E Silva<sup>1</sup>, P. Gauger<sup>1</sup>, D. Correia Lima Linhares<sup>1</sup> <sup>1</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

## **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) genetic diversity continues to expand. Recent years have been marked by advancements in tools and analytical capabilities for PRRSV epidemiological investigation using next-generation sequencing (NGS) outputs. This study summarizes findings related to utilizing and applying PRRSV NGS outputs on a farm or production system level.

## Material and Methods

We have developed NGS techniques and approaches for analyzing and interpreting PRRSV NGS outputs. A summary of how to use, interpret, and implicate PRRSV NGS outputs for epidemiological investigation is presented in this abstract.

## Results

When NGS detects PRRSV, the output can be a whole genome or genome fragments, i.e., contigs. Effective epidemiological investigations on a farm or production system necessitate a farm-reference strain for comparison, which can be derived from the outbreak samples or retrieved from banked samples stored in ultra-low temperatures. Serum or lung samples with a low Ct value (<22) are more likely to recover a complete PRRSV genome.

NGS is useful during the outbreak to characterize the reference PRRSV strain and in the future when unexpected drops in Ct values or clinical signs appear in the herd.

Whole-PRRSV genome sequencing allows for comparing sequences to investigate whether this virus has been detected before, track genetic evolutions over time, assess sequence similarities to vaccine viruses and other wild-type strains, and identify the occurrence of insertions, deletions, or recombinations.

Contigs are usually recovered from population samples, e.g., processing fluids, and are useful to reveal multiple virus strains if present in a sample. Contigs can also be used to differentiate between wild-type and vaccine-like strains. Wild-type sequence comparison is epidemiologically relevant if a farm reference strain is present.

Breeding herds facing outbreaks with a recombinant strain or having multiple strains circulating in the herd had higher production losses and longer time to stability, respectively.

#### **Discussion and Conclusion**

When clinical signs are unexpected, using NGS for PRRSV epidemiological investigations is valuable to farms and production systems. The NGS method is well-suited to identify cocirculating strains and detect recombination within the entire genome. Proper utilization and interpretation of whole-PRRSV genome information provide valuable insights for the decision-making process.

# NUTRITION

# NUTR-OP-01

NUTR - Nutrition

# DOES 25-HYDROXYVITAMIN D3 HAVE ANY ADVANTAGES IN RESPECT TO LAMENESS OF PIGS FED PROTEIN-AND PHOSPHORUS-REDUCED DIETS IN COMPARISON TO CONVENTIONAL VITAMIN D3?

M. Lütke-Dörhoff <sup>1</sup>, J. Schulz <sup>2</sup>, H. Westendarp <sup>1</sup>, C. Visscher <sup>3</sup>, M. Wilkens <sup>4</sup>

<sup>1</sup>Department of Animal Nutrition, Faculty of Agricultural Sciences and Landscape Architecture, Hochschule Osnabrück, Osnabrück, Germany

<sup>2</sup>Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

<sup>3</sup>Institute for Animal Nutrition, University of Veterinary Medicine Hanover, Foundation, Hanover, Germany <sup>4</sup>Institute of Animal Nutrition, Nutrition Diseases and Dietetics, University of Leipzig, Leipzig. Germany

# **Background and Objectives**

The present study aimed to compare the effect of dietary supplementation with vitamin  $D_3$  and 25-hydroxycholecalciferol (25OHD<sub>3</sub>), which has been shown to increase serum 25OHD<sub>3</sub> more efficiently, on parameters related to lameness.

## **Material and Methods**

Sows were supplemented with either 50 g/kg feed  $25OHD_3$  (25D) or vitamin  $D_3$  (VD) throughout gestation and lactation. Their offspring was assigned to four treatments: Piglets from VD sows that received 50 µg vitamin  $D_3$ /kg feed (VDvd) with the creep, rearing and fattening diet, piglets from VD sows supplemented with 50 µg 25OHD<sub>3</sub>/kg feed (VD25d), piglets from 25D sows supplemented with 50 µg vitamin  $D_3$ /kg feed (25Dvd) and piglets from 25D sows supplemented with 50 µg vitamin  $D_3$ /kg feed (25Dvd). Legs of all animals were examined for swelling and gait changes were monitored regularly during gestation, lactation, rearing and fattening. Fisher's exact test was used to analyze the qualitative parameters.

## Results

In sows supplemented with 25OHD<sub>3</sub>, we observed less gait changes 5 d a.p. and 28 d p.p. as well as an increase in standing time after feeding on 5 d a.p. Piglets from sows supplemented with  $25OHD_3$  (25D25d, 25Dvd) presented with a lower prevalence of leg swellings during rearing (6 wk, 8 wk, 10 wk) compared with VD25d and VDvd. At the age of 6 wk, we also observed a significantly lower prevalence of gait changes in the 25Dvd group in comparison to VDvd piglets. During fattening, a positive effect of  $25OHD_3$  fed to the sow on leg swellings in the offspring could only be observed at the age of 14 wk, whereas direct supplementation of the offspring with  $25OHD_3$  seemed to reduce the prevalence of gait changes at the age of 14 wk, 20 wk, and 22 wk.

# **Discussion and Conclusion**

Studies in humans and rodents suggest a stimulating effect of vitamin D metabolites on muscle cell differentiation and contractility. The lower prevalence of leg swellings and gait changes and the increased time spent standing after feeding in sows might be explained by an influence of the increased plasma concentrations of  $250HD_3$  in the 25D groups on muscle function of pigs, too.

NUTR - Nutrition

# CALCIUM CHLORIDE SUPPLEMENTATION DURING THE TRANSITION PERIOD REDUCES THE INCIDENCE OF STILLBORN PIGLETS

J. Ruampatana<sup>1</sup>, U. Yamsrikaew<sup>1</sup>, P. Preesong<sup>2</sup>, S. Nantakhruea<sup>2</sup>, M. Nuntapaitoon<sup>1</sup>

<sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>Kemin Animal Nutrition and Health Asia Pacific, Bangkok, Thailand

## **Background and Objectives**

Genetic improvement in the swine industry has led to an increased number of piglets in each litter. However, high litter size is linked to a higher number of stillborn piglets, which is frequently attributed to uterine contraction during farrowing. Calcium plays a vital role in muscular contractions during the farrowing process. The objective of this present study was to evaluate the effectiveness of calcium chloride on sow performance.

### Material and Methods

In total, 62 Landrace x Yorkshire crossbred sows were randomly divided into two groups: control (n=31) and treatment (n=31) groups. In the control group, sows were fed with a standard lactation diet, while the treatment group received the same diet supplemented with 25 g/sow/day of calcium chloride (NutriCAB<sup>TM</sup>, Kemin Industries (Thailand) Co., Ltd., Bangkok, Thailand), from 7 days before predicted farrowing date until 7 days after parturition (13.8  $\pm$  0.3 days). Sow performance, encompassing farrowing duration, total number of piglets born per litter (TB), number of piglets born alive per litter (BA), the percentage of stillborn piglets per litter (SB), percentage of mummified fetuses per litter (MM) and wean-to-first-service interval (WSI), was recorded during both working hours (8 am - 7 pm, involving farrowing and birth intervention) and non-working hours (7 pm - 8 am, with no farrowing and birth intervention).

## Results

On average, sows exhibited 14.9  $\pm$  3.6 piglets/litter for TB, 13.2  $\pm$  3.2 piglets/litter for BA, 7.3  $\pm$  9.1% for SB, and 1.6  $\pm$  3.9% for MM. Calcium chloride supplementation showed no significant impact on TB, BA, MM, farrowing duration, and WSI (P>0.05). SB in the treatment group tended to be lower than in the control group (5.4  $\pm$  1.5% vs. 8.9  $\pm$  1.6%, P=0.125). Furthermore, during non-working hours, SB in the treatment group also tended to be lower than in the control group (6.4  $\pm$  2.0% vs. 13.9  $\pm$  2.2%, P=0.062). However, no significant effect of calcium chloride on SB during working hours was found between treatment and control groups (4.5  $\pm$  2.3% vs. 3.8  $\pm$  2.3%, P=0.996).

## **Discussion and Conclusion**

In conclusion, supplementing calcium chloride during the transitional period may contribute to a reduction in SB, especially during non-working hours.

NUTR - Nutrition

# DIET RICH IN FIBERS DURING THE TRANSITION PERIOD DECREASES FARROWING DURATION

<u>B. Bracco Donatelli Muro</u><sup>1</sup>, R. Carnevale<sup>2</sup>, M.S. Monteiro<sup>3</sup>, D. Feitosa Leal<sup>4</sup>, F.A. Pereira<sup>1</sup>, C.A. Pospissil Garbossa<sup>1</sup> <sup>1</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>2</sup> Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>3</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>4</sup>Department of Population Health & Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States

## **Background and Objectives**

Transition diets rich in dietary fiber may have different beneficial effects on farrowing traits depending on the physicochemical characteristics of the fibrous ingredients used. Soluble fiber may benefit parturient sows by providing extra energy from short chain fatty acids to support the increased energy requirement around farrowing. Insoluble fiber may benefit parturient sows by preventing constipation. The aim of this study was to evaluate the effects of including a fibrous supplement composed by soluble and insoluble fiber in the transition diet of sows on farrowing traits.

## **Material and Methods**

A total of 202 sows were used. At day 90 of gestation sows were randomly allocated in one of the two groups: sows fed 2.6 kg of standard gestation diet until farrowing (CON; n=101; crude fiber = 2.25g.kg<sup>-1</sup>; NDF =10.1g.kg<sup>-1</sup>; ME = 3,210kcal.kg<sup>-1</sup>) and sows fed 2,4 kg of standard gestation diet added of 400 g of a fibrous supplement (FIB; n=101; crude fiber = 6.00g.kg<sup>-1</sup>; NDF =14.8g.kg<sup>-1</sup>; ME = 2,963kcal.kg<sup>-1</sup>). The supplement was constituted of a blend of fibrous ingredients: 40% wheat bran, 25% lignocellulose, 25% citrus pulp, and 10% guar gum. During gestation sows were fed by electronic feeder (Gestal 3G<sup>®</sup>). The animals were transferred to farrowing crates five days prior to farrowing where they were fed twice daily. Farrowing duration was calculated as the interval between the first and last piglet born. The total number of piglets born, liveborn and stillbirth rate were recorded. Variables were analyzed by ANOVA and statistical differences were set at p<0.05.

## Results

FIB females had shorter farrowing duration (p=0.01) than CON (224 vs 247min for FIB and CON, respectively; SEM = 17.5). Total born (CON = 17.5; FIB= 17.0; SEM=0.72), liveborn (CON = 16.3; FIB = 15.9; SEM=63), and percentage of stillborn (CON = 4.3%; FIB = 4.9%; SEM=0.21) piglets did not differ (p>0.05) between treatments.

## **Discussion and Conclusion**

Feeding sows a diet rich in dietary fiber during transition period is an efficient tool to decrease farrowing duration. However, decreasing farrowing duration may not be efficient in reduce stillbirth rate, especially in herds that do not have prolonged farrowing durations evidencing the multifactorial aspect of piglets' death during farrowing.

## NUTR - Nutrition

# IMPACT OF HERBAL VITAMIN C SUPPLEMENTATION ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF LACTATING SOWS AND THEIR LITTERS

G. Frederico <sup>1</sup>, C. Sol <sup>2</sup>, <u>F. Horta <sup>2</sup></u>, A.P. Pavaneli <sup>3</sup>, E. Raele <sup>2</sup>, I. Bianchi <sup>1</sup> <sup>1</sup>Catarinsense Federal Institute - Araquari, BR <sup>2</sup>Nuproxa Switzerland Ltd. <sup>3</sup>InsideSui Research Services, Insidesui, Patrocínio - MG, Brazil.

## **Background and Objectives**

During heat stress, sows show low feed intake, resulting in impaired milk production, compromising litter weight, and increasing mortality. This shows the profound impact of environmental conditions on swine productivity. This study aimed to evaluate the effects of supplementing an herbal source of vitamin C to lactating sows during lactation under heat stress conditions and to assess its impact on the productive and reproductive performance of the sows and their litters.

## Material and Methods

The study was carried out on a commercial farm in Brazil, where at noon and in the afternoon, the Temperature-Humidity Index (THI) remained within the critical range (74<THI<79), during all the trial. At 110 days of gestation, a total of 93 F1 sows (Landrace X Large White, farrowing orders from 1 to 8) were transferred and distributed to the maternity pens (according to farrowing order). Control Group (CON, n=48): received standard lactation feed without supplementation; Treatment Group (T1, n=47): received a lactation diet supplemented with herbal vitamin C until weaning, at 21d (300 g/tn, C-Power<sup>TM</sup>, Nuproxa Switzerland). This phytogenic supplement is rich in gallotannins and hydrolysable bioflavonoids in conjugated form. Data were analyzed by GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, v.9.4).

## Results

Piglet weight 24h after birth (1.58kg for CON vs. 1.60kg for T1) and the litter size (piglets/litter) kept in sows 24 h after birth (13.10 for CON vs.12.92 for T1) were not different between treatments. For sows, there were no differences in body mobilization or feed intake (FI) during lactation. Sows in T1 had lower FI per kg of litter produced (2.16kg±0.08 vs. 2.41kg±0.08; P=0.027) with higher estimation of milk production (10.96kg±0.28 vs. 9.87kg±0.28; P=0.002), and higher piglet weight at weaning (6.05kg±0.10 vs. 5.80kg±0.10; P=0.042) than CON. Moreover, T1 reported the lowest piglet mortality (0.72±0.12 vs.1.23±0.17; P=0.0145).

## **Discussion and Conclusion**

These results suggest that herbal vitamin C supplementation positively influences the productive and reproductive performance of lactating sows and their litters under heat-stress, emphasizing its potential as a nutritional strategy to mitigate the adverse effects of heat-stress in swine production.

NUTR - Nutrition

# PROBIOTIC POWER TO SUPPORT WEANING PIGLETS' PERFORMANCE AND HEALTH

L. Vande Maele <sup>1</sup>, J.Y. Mun <sup>2</sup>, V. Vandendriessche <sup>1</sup>, J.S. Kim <sup>2</sup> <sup>1</sup>Orffa Additives BV, Breda, The Netherlands <sup>2</sup>Department of Animal Industry Convergence, Kangwon National University, Chuncheon, South Korea

## **Background and Objectives**

Weaning is a very stressful moment in piglets' life, in which most importantly gut health is challenged due to the transition from milk to solid feed and an often disturbed feed intake after weaning. Securing (gut) health is of uttermost importance for good animal performance. To maintain a favourable microbial balance in the gut of weaned piglets, probiotic feed additives can be used. In this trial the effect of a specific probiotic Bacillus spp. mixture was investigated.

## Material and Methods

In total 150 weaning piglets (Landrace x Yorkshire x Duroc, average weight 6.23kg) were divided over two treatments (5 replicate pens/treatment). The control group received a 3-phase control diet (corn/soybean meal) without probiotics. The piglets in the probiotic group were supplemented with Excential ProDi-G (Orffa Additives BV) at 5x10<sup>s</sup> CFU/kg feed. Performance was measured and faecal samples were obtained to analyse nutrient digestibility (DM, GE, CP) and bacterial counts. On day 42, blood samples were collected and analysed for markers of inflammation, oxidative stress, and immunity. Data were analysed using ANOVA and post hoc Tukey test in SAS (p<0.05).

### Results

Final body weight (day 42) was significantly higher (+6%) in piglets supplemented with the probiotic (24.52kg vs. 23.07kg). Although numerically better for all three feeding phases, FCR did not differ significantly between the treatments. Digestibility showed a trend for improvement (from 76.45% to 79.23% for CP digestibility on d28, p<0.1). Probiotics resulted in significantly lower E. coli counts in faecal samples (4.73 vs. 5.47 log<sub>10</sub> CFU/g). Blood analyses demonstrated significantly reduced levels of TNF- $\alpha$ , improved SOD activity and higher IgA concentration for the probiotic group (p<0.05).

## **Discussion and Conclusion**

Excential ProDi-G, a specific Bacillus spp. probiotic mixture, can support piglets after weaning and improve their growth. This beneficial effect can be a result of improved digestibility and FCR, however due to a low number of replicates, this could not be statistically verified in this trial. Furthermore, supplementation of the probiotic influenced the gut microbial composition of weaning piglets, reducing E. coli, and ameliorated the health status of the animals, with lower inflammation, reduced oxidative stress and an improved immunity.

NUTR - Nutrition

# SUPPLEMENTATION OF POSTBIOTICS TO MULTIPAROUS SOWS THROUGHOUT GESTATION AND LACTATION PERIODS ENHANCES SOW PERFORMANCE AND LACTOGENIC IMMUNE RESPONSE

P. Kongpanna<sup>1</sup>, P. Jermsutjarit<sup>2</sup>, A. Tantituvanont<sup>3</sup>, K. Soothiluck<sup>4</sup>, U. Jamikorn<sup>5</sup>, D. Nilubol<sup>6</sup>

<sup>1</sup>Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University

<sup>2</sup>Swine Viral Evolution and Vaccine Development Research Unit, Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>3</sup>Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

4Cargill Siam Co.,Ltd, Thailand

<sup>5</sup>Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Thailand

Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

## Background and Objectives

The objectives of the study were to evaluate the effect of postbiotic Saccharomyces cerevisiae fermentation product (XPC<sub>U</sub>; Diamond V Original XPC<sup>™</sup> Ultra, USA), when supplemented in multiparous sows throughout gestation and lactation periods, on reproductive performance, and immunoglobulin (Ig) G and IgA, both total and porcine epidemic diarrhea virus (PEDV) specific, in colostrum and milk.

# Material and Methods

A total of 138 multiparous sows (Hybrid sow; Large White Landrace x Duroc) were randomly assigned into 2 groups including CON (n=66) and XPC<sub>U</sub> (n=72) groups. XPC<sub>U</sub> group was supplemented with the postbiotic at 1 kg/ton feed, from mating until weaning. Reproductive and sow production performances were compared. Piglets were individually weighed at birth and weaning. Mortality was observed daily. Colostrum, milk and serum samples were collected and assayed for the presence of PEDV specific antibody using virus neutralizing antibody assay (NAb). Total IgA and IgG, and PEDV specific IgA and IgG concentrations were measured using ELISA. Data were analyzed using t-test by SAS and significance defined at P<0.05.

# Results

The results demonstrated that XPC<sub>u</sub> supplemented sows had higher (P<0.05) litter weight (18.28 vs 14.94 kg), litter size (12.38 vs 10.85), and birth weight (1.49 vs 1.37 kg), comparted to CON. Additionally, XPC<sub>u</sub> animals had improved (P<0.05) litter weaning weight (83.83 vs 73.13 kg), number of weaned pigs (11.53 vs 9.95), and wean-to-service interval (5.88 vs 6.95 d) compared to CON. Percentage of mummified fetus, litter weight gain, weaning weight, and mortality rate were not different (P>0.05) between both treatments. A higher (P<0.05) concentration of total IgA and IgG in colostrum and milk compared to the CON was observed in XPC<sub>u</sub> group at 0, 1, 3, 5 and 7 d. XPC<sub>u</sub> animals had numerically higher (P>0.05) in PEDV specific IgG and IgA, and Nab than CON.

# **Discussion and Conclusion**

In summary, the results of the study indicated that  $XPC_{U}$  supplemented sows had significantly improved farrowing performance as demonstrated by increased litter weight, litter size, birth weight and the number of weaned pigs. Moreover,  $XPC_{U}$  pigs had increased total IgA and IgG in colostrum and milk, thus supporting to increase piglet's health and performance.

# NUTR - Nutrition

# SPIRULINA AS AN ALTERNATIVE PROTEIN SOURCE FOR SOWS – EFFECTS ON LITTER CHARACTERISTICS AND PIGLET GROWTH

W. Pendl <sup>1</sup>, P. Tschannen <sup>2</sup>, M. Terranova <sup>3</sup>, D. Kümmerlen <sup>4</sup>, K. Giller <sup>5</sup>

<sup>1</sup>Tierklinik St. Veit, Wagendorf, Austria

<sup>2</sup>Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland

<sup>3</sup>ETH Zurich, Agro Vet-Strickhof, Lindau, Switzerland.

<sup>4</sup>Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland.

<sup>5</sup>University of Hohenheim, Institute of Nutritional Sciences, Stuttgart, Germany ETH Zurich, Department of Environmental Systems Science, Zurich, Switzerland

# Background and Objectives

Since sustainability of soybean used as pig feed is being critically discussed, alternative protein sources for pig feed are needed. The microalga Arthrospira platensis (spirulina) could serve as a suitable alternative due to its particularly high contents of protein and essential amino acids.

# **Material and Methods**

Nine multiparous sows were divided in two groups and fed either a commercial control diet (CON) or an experimental diet (SPI). In the gestation diet of the SPI group, soybean meal was replaced completely while in the lactation diet, only soybean meal was reduced by 42%. One week after the start of experimental feeding, sows were artificially inseminated with semen from the same boar. At farrowing, litter size as well as birth weight of the individual piglets were determined. Piglet body weight was determined weekly until weaning at four weeks of age. Experimental data was analysed using SPSS (version 28). Litter characteristics were analysed using t-test. Piglet data was analysed using a mixed model with experimental diet, sex, and their interaction as fixed effects and litter size as random effect.

# Results

Litter size (CON:  $17\pm2.0$ ; SPI:  $15\pm0.7$  (mean±standard error of the mean)), litter weight (CON:  $21.2\pm2.59$  kg; SPI:  $21.7\pm0.66$  kg), and individual piglet birth weight (CON:  $1.23\pm0.082$  kg; SPI:  $1.45\pm0.105$  kg) were not significantly affected by diet. At one week of age, body weight was higher in SPI compared to CON piglets ( $3.1\pm0.19$  vs.  $2.5\pm0.16$  kg; p=0.014). Also at weaning, body weight was higher in SPI compared to CON piglets ( $9.0\pm0.31$  vs.  $8.1\pm0.27$  kg; p=0.026). Still, average daily gain (ADG) from birth to weaning did not significantly differ in SPI compared to CON piglets ( $0.26\pm0.011$  vs.  $0.23\pm0.009$  g).

# **Discussion and Conclusion**

The preliminary results of the present study suggest that (partially) replacing soybean meal by spirulina in sow feed is possible without impairing offspring growth and even results in heavier piglets at weaning (approx. +1 kg). Still, despite the lack of significance, litter size may have impacted on the results.

NUTR - Nutrition

# TOXICOKINETIC APPROACH TO MEASURE THE EFFICACY OF A MYCOTOXIN DEACTIVATOR AGAINST AFLATOXIN B1 AND ZEARALENONE IN PIGS

D. Preveraud <sup>1</sup>, N. Broekaert <sup>2</sup>, M. Devreese <sup>2</sup>, S. Croubels <sup>2</sup>

<sup>1</sup>Adisseo France SAS, Feed Integrity, Antony, France

<sup>2</sup>Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Belgium

## **Background and Objectives**

Zearalenone (ZEN) and aflatoxin B1 (AFB1) are mycotoxins highly prevalent worldwide and can impair the performance and health of pigs. Preventive solutions such as the use of dietary mycotoxin deactivators (MD) can help in reducing the exposure to ZEN and AFB1 in animals. The aim of this study was to perform a toxicokinetic study in pigs focusing on the plasma concentration-time profiles of these 2 toxins, alone or in combination with a MD.

## Material and Methods

The study was conducted on 12 healthy pigs (6 males/6 females) of 8 week of age with an initial average BW of 13.8 kg (ethical approval n° 2017/106). The acclimatization period was 1 week (days 1-7). The pigs were randomly allocated into 2 mixed sex treatment groups of 6 pigs per group. On day 8, after fasting for 12 hours, the pigs were given an intragastric single bolus of ZEN and AFB1 (group 1: ZEN 0.5 mg/kg BW AFB1 0.1 mg/kg BW) or the same ZEN and AFB1 challenge with a MD, Unike® Plus 0.1 g/kg BW, group 2). Oral absorption and systemic exposure of ZEN and AFB1 were evaluated by comparing the plasma toxicokinetic parameters of the glucuronide conjugate of ZEN (ZEN-GlcA) and AFB1.

## Results

For ZEA-GlcA, the area under the instrument response-time curve (AUC0-4h) for group 1 is 616.9 responses\*h whereas it decreased to a level of 379.2 response\*h with the addition of MD. Therefore, the oral bioavailability of ZEN with MD is 61.5% compared to group 1. For AFB1, the area under the plasma concentration-time curve (AUC0-4h) for group 1 is 32.3 ng/ml\*h whereas it decreased to a level of 14.3 ng/ml\*h with the addition of MD. Therefore, the oral bioavailability of AFB1 with MD is 44.3% compared to group 1.

## **Discussion and Conclusion**

Unike® Plus was able to reduce the systemic exposure to ZEN as well as AFB1. This preventive strategy is necessary to limit the appearance of clinical symptoms and therefore, can control the development of infectious diseases.

# NUTR - Nutrition

# FEED ADDITIVE PACKAGE WITH MICROENCAPSULATED ZINC ALLOWS IMPROVEMENT IN BIOMARKERS OF IMMUNITY AND INTESTINAL INTEGRITY IN PIGLETS FED WITH LOW ZNO CONTENT AND REDUCED PROTEIN.

<u>G. Ramis</u><sup>1</sup>, F. Murciano<sup>2</sup>, J. Orengo<sup>3</sup>, L. Martínez-Alarcón<sup>4</sup>, B. González-Guijarro<sup>3</sup>, A. Cuellar-Flores<sup>3</sup>, E. Landeau<sup>5</sup>, S. Benaben<sup>5</sup>

<sup>1</sup>Departamento de Producción Animal. Universidad de Murcia <sup>2</sup>Facultad de Veterinaria, Universidad de Murcia, Spain <sup>3</sup>Dpto. Producción Animal,. Facultad de Veterinaria., Universidad de Murcia, Spain <sup>4</sup>Instituto Murciano de Investigación en Biomedicina (IMIB), Murcia, Spain <sup>5</sup>JEFO EUROPE, TECHNICAL DEPARTMENT, FRANCE

## **Background and Objectives**

The EU ban on the use of zinc oxide at therapeutic levels has limited the amount that can be added to 150 ppm in piglets feeds and has led to a clear loss of gut health on farms. One of the approaches adopted is the use of microencapsulated zinc and the reduction of protein. The aim of this study was to study biomarkers indicators of intestinal integrity and intestinal immunity stimulation in piglets receiving a low protein feed diet supplemented with a feed additive.

# **Material and Methods**

Ileal tissue samples were obtained from 96 piglets distributed in three groups with base feed without zinc: one group with addition of 3000 ppm ZnO (CON+, n=32), another with addition of ZnO at 150 ppm (CON-, n=32) and the third one with addition of Jefo package (Jefo Nutrition Inc.) containing 120 pp of Zn (J, n=32). Gene expression for calprotectin, occludin, zonulin, IFN $\alpha$ , IFN $\beta$ , IL1 $\alpha$ , IL1 $\beta$ , IL6, IL8, IL10, IL12p35, IL12p40, TNF $\alpha$  and TGF $\beta$ , was assessed. A data reduction using Discriminant Function Analysis (DFA) was performed.

## Results

Differences or trend were observed for calprotectin (CON+ = 3.4, J = 4.5, CON- = 5.3 Log2 fold change; p=0.043), zonulin (CON+ = 1.9, J = 2.6, CON- = 2.6 Log2 fold change; p=0.047), IL1 $\beta$  (CON+=20.7, J = 21.6, CON-=21.9 Log2 fold change; p=0.052) and IL12p40 (CON+= 12.55, J=12.5, CON-=11.0 Log2 fold change; p=0.081) comparing CON+ with CON-. The J group only showed differences with CON+ for zonulin. DFA had a Wilk's lambda p-value of 0.019 with 70% of samples well assigned to their group.

## **Discussion and Conclusion**

Interestingly, the gene expression level of the J group was comprised between the level of the CON+ group and the CON- group which had the highest expression in all cytokines except IL12p35, which was higher in the other two groups. There appear to be three levels of immune stimulation and intestinal integrity, with the higher gene expression observed in the CON+, an intermediate one in the J group and the lower one in the CON- group. The DFA showed a clear effect of each treatment.

NUTR - Nutrition

# RELATION BETWEEN HYDRATION-RELATED PROPERTIES AND FERMENTATION PARAMETERS OF FIBROUS INGREDIENTS

<u>C.A. Pospissil Garbossa</u><sup>1</sup>, B. Bracco Donatelli Muro<sup>1</sup>, R. Carnevale<sup>2</sup>, R. Scalise Xavier De Freitas<sup>3</sup>, D. Alexsander Rojas Moreno<sup>3</sup>, I. Cláudio Da Silva Bueno<sup>3</sup>, M.S. Monteiro<sup>4</sup>, D. Feitosa Leal<sup>5</sup>, C. Abércio Da Silva<sup>6</sup>

<sup>1</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>2</sup> Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>3</sup>Faculty of Animal Science and Food Engineering, University of São Paulo

<sup>4</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>5</sup>Department of Population Health & Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States

<sup>6</sup>Department of Zootechnology, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil

# Background and Objectives

Dietary fiber (DF) is an important feed component in swine diets. DF is available to be fermented by the microorganisms of the hindgut, resulting in production of short chain fatty acids. The physicochemical properties of DF, including water-holding capacity, viscosity, and bulking are determined by the composition of monosaccharides of the ingredient leading to different effects on intestinal health and metabolism of pigs. However, the physicochemical properties of fibrous ingredients fermentability by sows' microbiota and how they interact are still poorly explored. The aim of this trial was to correlate hydration-related properties with fermentation parameters of DF sources.

## **Material and Methods**

Different sources of fibers were selected as follow: vegetables pulp, apple pulp, citrus pulp, beet pulp, guar gum, lignocellulose, DDG, soybean hull, oat bran, wheat bran, and SmartFiber® (extruded mix of tropical grasses). Water holding capacity (WHC), viscosity and bulking were selected as hydration-related properties of the dietary fibers. For the fermentation, three inoculums were prepared with cecal content of 12 sows. Samples were incubated for 96 hours. The fermentations parameter measured were in vitro dry matter degradability, maximum amount of produced gas, specific gas production rate, decay in specific gas production rate, time of maximum fermentation rate, maximum fermentation rate, lag time, partition factor, and total in vitro production of short chain fatty acids after 96 h of incubation measured by gas chromatography. The relation between the physicochemical properties and fermentation parameter was analyzed Pearson's correlation and statistical significance were set at p<0.05.

## Results

WHC, viscosity and bulking were positive correlated (p < 0.05) with specific gas production rate, maximum fermentation rate and butyrate production. There were no further correlations (p > 0.05) between hydration-related properties and fermentation parameters.

# **Discussion and Conclusion**

The significant positive correlation found among hydration-related properties and specific gas production rate, maximum fermentation rate and butyrate production supports the notion that ingredients with higher affinity for water have greater and faster fermentability compared to ingredients with less affinity for water.

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NUTR - Nutrition

# THE EFFECTS OF DIETARY NET ENERGY LEVEL ON GROW-FINISH PERFORMANCE AND CARCASS CHARACTERISTICS OF INTACT MALE MARKET PIGS IMMUNIZED AGAINST GNRF

L. Van De Weyer 1, Y. Wang 2, B. Hansen 3, S. Pollmann 4, J. Landero 5, M. Young 5, A. Aldaz 6, B. Bohrer 7

<sup>1</sup>Zoetis Canada Inc, Kirkland, QC <sup>2</sup>Dept of Anim. Sci., The Ohio State University, Columbus, Ohio <sup>3</sup>BCH Consulting LLC, Atlantic, Iowa <sup>4</sup>DSP Consulting LLC, Alpine, Utah <sup>5</sup>Gowans Feed Consulting, Wainwright, AB <sup>6</sup>ZOETIS, Parsippany, NJ, USA <sup>7</sup>Dept of Animal Sci, The Ohio State University, Columbus, Ohio

# Background and Objectives

Study objectives were to determine the effects of three different dietary net energy (NE) levels (LOW–2.216, MID–2.341, HIGH–2.466 Mcal/kg) fed during grow-finisher on live performance and carcass characteristics of intact male pigs managed with two doses of Improvest (IMP, also known as Improvac)), an injectable gonadotropin-releasing-factor-analogue, or physically-castrated (PC) male pigs.

## **Material and Methods**

A total of 1,008 pigs (504 IMP, 504 PC; 10 weeks old) were allocated by weight to 48 pens (21 IMP or PC pigs/pen) with treatments arranged as a  $2 \times 3$  factorial design with main effects of Improvest-treatment (**IMP** or **PC**) and NE-level (**LOW**, **MID**, or **HIGH**). Diets were formulated to standardized-ileal-digestible (SID) lysine-requirements of IMP or PC males. Data were analyzed with PROC MIXED of SAS, with pen as experimental unit, fixed effects of IMP-treatment, dietary NE-level and their interactions, and a random-effect of pen location.

## Results

**IMP** males consumed less feed (5.3% lower ADFI; P < 0.01), grew faster (5.1% greater ADG; P < 0.01) and were more efficient (11.2% greater G:F; P < 0.01) compared with **PC** males. Hot-carcass-weight (HCW) did not differ (P = 0.16) between **IMP** and **PC** males (attributed to 1.6% lower dressing-percentage for IMP males; P < 0.01); however, **IMP** males were leaner (0.9 mm less backfat, 0.65% greater predicted lean yield; P < 0.01) versus **PC** males. Overall, pigs fed **LOW** and **MED** NE-diets consumed more feed than **HIGH** NE-diets; pigs fed **LOW** NE-diets grew slower than pigs fed **MED** and **HIGH** NE-diets. Pigs fed **LOW** NE-diets had 4.4% lower G:F versus **MED** and 8.6% lower G:F versus **HIGH** NE-diets (P < 0.01). Pigs fed **LOW** NE-diets had 3.0 kg lighter HCW (P < 0.01) vs. **MED** diets, while pigs fed **HIGH** diets had intermediate HCW. There were no interactions between **IMP** and **NE-level** ( $P \ge 0.19$ ) for any performance or carcass characteristic measured.

## **Discussion and Conclusion**

**IMP** males achieved expected performance and carcass response levels vs. **PC** males. However, consideration should be given to potential production impacts of feeding **LOW** NE-levels when managing **IMP**-males to ensure response can be consistently maintained.

NUTR - Nutrition

## TWO IRON INJECTIONS BEFORE WEANING IMPROVED POSTWEANING GROWTH PERFORMANCE, HEMOGLOBIN LEVELS, FECAL SCORE AND MICROBIOME OF PIGS REGARDLESS OF DIETARY IRON LEVELS UNDER NATURAL DISEASE CHALLENGE

A. Johnson <sup>1</sup>, B. Dittrich <sup>1</sup>, A. Cole <sup>1</sup>, M. Prodell <sup>1</sup>, S. Fritz <sup>2</sup>, W. Li <sup>3</sup>, P. Fregulia <sup>3</sup>, W. Lyons <sup>4</sup>, <u>Y.D. Jang</u> <sup>5</sup> <sup>1</sup>University of Wisconsin-River Falls <sup>2</sup>Kansas State University <sup>3</sup>US Dairy Forage Research Center, USDA-Agricultural Research Service, Madison, WI <sup>4</sup>PHARMACOSMOS INC, WATCHUNG, NJ, USA <sup>5</sup>University of Georgia

## Background and Objectives

As hemoglobin levels may decline in the late suckling period, piglets may need an additional dose of iron before weaning. After weaning, a high level of dietary iron may result in the proliferation of pathogenic bacteria. This study was conducted to demonstrate the effects of two iron injections before weaning and iron levels in nursery diets on growth performance, hematological parameters, and fecal microbiome of pigs.

## **Material and Methods**

Seventy newborn pigs from seven sows were allotted to 4 treatments within litter and received the first dose (200 mg iron) at 2-3 days of age. At weaning (d 27-30 of age), all pigs were housed in nursery pens within their original treatments for a 27-d growth period and naturally challenged with E. coli and rotavirus. Treatments were: 1) NC: no additional iron injection and NC diet (100 ppm iron), 2) NC+IRON: an additional iron injection (200 mg) at least 5 days after the first injection + NC diet, 3) PC: no additional iron injection + PC diet (200 ppm iron), and 4) PC+IRON: an additional iron injection (200 mg) + PC diet. A common iron-dextran product was used for both intramuscular injections. Fecal microbiome composition was analyzed using amplicon sequencing targeting the V1-V9 regions of the 16S rRNA gene.

## Results

Two injections before weaning increased hemoglobin and serum iron levels but lowered the platelet count at weaning until d 13 postweaning (P<0.05). Greater postweaning body weight, growth rate (P<0.10) and feed intake, and lower fecal score (P<0.05) were observed with pigs receiving two injections compard with one injection. The ANCOM showed a higher abundance of the bacterial taxon Lactobacillales in the 2-injection group than the 1-injection group at weaning. The pigs fed the PC diet had greater hemoglobin levels (P<0.05) at d 27 postweaning and lower fecal score (P=0.09) in d 13-27 postweaning than the NC diet.

# **Discussion and Conclusion**

Two iron injections to pigs before weaning improved postweaning growth performance and hemoglobin levels, reduced fecal score, and affected the fecal microbiome, whereas an additional 100 ppm of dietary iron supplementation increased hemoglobin levels and reduced fecal score in the late nursery period but did not affect postweaning growth.

NUTR - Nutrition

# WHICH GENERAL BIOMARKERS IN PLASMA ASSOCIATE WITH GROWTH PERFORMANCE THE FIRST WEEK POST-WEANING?

L. Fabà <sup>1</sup>, L. Dieste-Pérez <sup>2</sup>, H.M.J. Van Hees <sup>1</sup>, T.G. Hulshof <sup>1</sup> <sup>1</sup>Trouw Nutrition R&D, Veerstraat 38, 5831 JN, Boxmeer, The Netherlands <sup>2</sup>Royal GD, Arnsbergstraat 7, 7418 EZ, Deventer, the Netherlands

## **Background and Objectives**

Associations between general metabolic and immune biomarkers with growth performance early post-weaning may help define the status of a weanling pig. In the present study, a list of 15 general biomarkers was used to study which biomarkers explain most of the variance on pig individual feed intake and performance during the first week post-weaning.

## Material and Methods

One plasma sample was taken at 1-week post-weaning from 24 female pigs ( $8.0 \pm 1 \text{ kg BW}$ ) from 12 different pens (2 pigs/pen) equipped with electronic feeding stations representing the wide range of average daily feed intake (ADFI, i.e., 64-424 g/d) for the first week post-weaning. Metabolites and endogenous enzymes used to describe metabolic profile and some acute immune response biomarkers were evaluated by Pearson correlations and by partial least square regression to explain the variance of individual weaning-to-sampling average daily gain (ADG) and 24-h prior to sampling feed intake (FI-24h).

## Results

The ADFI and ADG correlated negatively with creatinine (r = -0.43 and -0.54, respectively; P<0.05). Similarly, the lower the ADFI, the higher the lactate dehydrogenase (LDH, r = -0.46, P<0.05), aspartate aminotransferase (AST, r = -0.20, P=0.05), and PigMap (r = -0.39, P=0.06). Creatine phosphokinase (CPK) correlated positively with BW at sampling (P<0.05) but not with weaning BW. Both, weaning and sampling BW correlated positively with albumin, Ca, and P (P>0.05). The FI-24h correlated positively (P<0.05) with alkaline phosphatase (ALP, r = 0.48), CPK (r = 0.46), and alanine transaminase (ALT, r = 0.43). Finally, to explain week-1 ADG variance, the most important variables were ALP, haptoglobin and PigMap. To explain FI-24h, many parameters appeared similarly important i.e., LDH, PigMap, AST, creatinine, P, CKP, albumin, ALP, ALT, Ca, urea, and haptoglobin.

## **Discussion and Conclusion**

The array of biomarkers studied here represent a metabolism snapshot explaining short-term performance i.e., FI-24h prior to sampling. Likely, variance on recovering from weaning and a rapidly increasing FI by the day explain that. Otherwise, the first week ADG, as proxy for overall pig health status, is best predicted by acute inflammation markers (haptoglobin and PigMap) and ALP rather than a wide metabolic profile.

# IMMUNOLOGY AND VACCINOLOGY

# IMM-OP-01

IMM - Immunology and Vaccinology

# AN INNOVATIVE SUBUNIT VACCINE PLATFORM FOR FARM ANIMALS, VALIDATED IN SWINE AND OTHER SPECIES

<u>H. Hennemann</u>, S. Bennewitz <sup>1</sup>, U. Diesterbeck <sup>1</sup>, E. Galemou Yoga <sup>1</sup>, S. Behrens <sup>2</sup> <sup>1</sup>Verovaccines GmbH, Halle (Saale), Germany <sup>2</sup>Verovaccines GmbH, Halle (Saale), Germany, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

## **Background and Objectives**

Most veterinary vaccines licensed for pigs are inactivated vaccines. Some of these vaccines are first-generation subunit vaccines that are free of pathogens but are considered expensive to produce and only moderately immunoreactive. The biopharmaceutical company Verovaccines has developed a technology platform for yeast-based vaccines that overcomes many of the disadvantages of first-generation subunit vaccines. Data will be presented that the technology enables easy combination of vaccines, rapid development and "Same-for-All" manufacturing.

## Material and Methods

A series of recombinant Kluyveromyces lactis (K. lactis) yeast strains were generated using recombinant methods. These strains can integrate multiple protein expression cassettes, resulting in stable and inducible expression of several antigenic proteins in a single eukaryotic cell. Analytical capabilities and formulations have been developed to enable assay- and product development. A twin fermenter system in lab scale was used to develop robust fermentation protocols with high yields. These protocols were used to scale up- and downstream processes to industrial level.

# Results

The above-mentioned second-generation subunit vaccine technology was used to streamline the generation and optimization of such vaccine strains. Validation experiments in vivo on the respective target animals showed exceptional efficacy/protection (single immunization, cross-protection, generation of protective MDAs and low dosing), different ways to combine such vaccines and a positive safety profile. Data on reproducibility, testing of process chain alternatives and standardization of production resulted in a unified manufacturing concept for most, if not all K. lactis-based vaccine strains. Establishment of industrial-scale production was achieved in a series of controlled fermenter runs.

# **Discussion and Conclusion**

The novel second generation subunit vaccine technology is discussed in the context of validation data on efficacy in various species, including swine. Data from laboratory- and full-scale fermentation experiments indicate exceptional reproducibility and high scalability. Data from a vaccine development project against a porcine pathogen showed 10 to 100 times more cost-effective antigen production compared to benchmarks. After completing most of the validation work and establishing industrial scale production, Verovaccines is using its proprietary technology to bring several innovative vaccine products to market.
IMM – Immunology and Vaccinology

### A NEW GENERATION GLYCOCONJUGATE VACCINE AGAINST THE SWINE AND ZOONOTIC PATHOGEN STREPTOCOCCUS SUIS

<u>M. Segura 1</u>, T. Lowary 2, M. Gottschalk 1 1 *University of Montreal, Canada* 2 *Institute of Biological Chemistry, Academia Sinica, Taiwan* 

#### **Background and Objectives**

Streptococcus suis, mostly serotype 2, causes significant economic losses to the swine industry. In the absence of effective commercial vaccines, the incidence of disease is controlled by extensive antimicrobial use. S. suis is covered by a capsular polysaccharide (CPS), which is the only essential virulence factor. CPS is an attractive antigen but its poor immunogenicity limits its use as a vaccine. However, when conjugated to protein carriers to produce glycoconjugate vaccines, carbohydrates acquire the required immunochemical ability, as shown by their successful application in human medicine. We previously established a proof-of-concept vaccine that protects pigs against S. suis serotype 2 challenge using native CPS conjugated to tetanus toxoid. Unfortunately, glycoconjugate standard production methods are complex, resulting in high-cost vaccines. Recent advances in chemical synthesis and formulation design have spawned a new generation of carbohydrate-based vaccines, without the limitations associated with traditional glycoconjugate vaccines. In this study, we designed the first chemically-synthesized glycoconjugate vaccine against S. suis and provide proof-of-concept of its protective capacity.

#### Material and Methods

Different fragments (epitopes) of the CPS from S. suis serotype 2, ranging in size from a monosaccharide to a heptasaccharide, were selected for synthesis and were conjugated to the carrier protein CRM197 (a non-toxic mutant of diphtheria toxin). Mouse immunization pre-trials were performed for a preliminary target selection based on the capacity of conjugated-CPS fragments to induce a high anti-CPS antibody response, a diversity of IgG sub-classes and functional ability of antibodies to eliminate S. suis by opsonophagocytosis. A protection trial of selected compounds was carried out in pigs.

#### Results

Using the swine immunization and challenge model, the levels of antibodies induced by the different pre-selected conjugated-CPS fragments varied but the IgG isotype pattern was similar. Indeed, one of the conjugated-synthetic CPS fragments induced significant protection against a challenge using a high virulent strain.

#### **Discussion and Conclusion**

This study represents the first proof of concept of a new generation glycoconjugate vaccine in veterinary medicine. We also demonstrated the importance of not only chemically design the right epitope but also that clinical evaluation in the natural host is required to predict the real value of a chemically-synthesized fragment as a vaccine candidate.

#### IMM – Immunology and Vaccinology

### INFLUENCE OF MATERNAL ANTIBODIES ON THE IMMUNE RESPONSE OF YOUNG PIGLETS VACCINATED WITH A STREPTOCOCCUS SUIS SEROTYPE 2 BACTERIN

<u>M. Gottschalk</u><sup>2</sup>, M. Poulin <sup>1</sup>, L. Cloutier <sup>1</sup>, M. Segura <sup>2</sup> <sup>1</sup>Centre de Développement du Porc du Québec, Canada <sup>2</sup>University of Montreal, Canada

#### **Background and Objectives**

Streptococcus suis is one the most important bacterial pathogen affecting post-weaned piglets and is also a zoonotic agent. There is no commercial vaccine and the only alternative practitioners have is the use of autogenous vaccines (bacterins) based on the predominant strain(s) recovered in the affected farm. Depending on the farm, sows or young piglets are vaccinated; in the latter case, it is a common practice to vaccinate them at 1 and 3 weeks of age (WOA) to protect them during the nursery period (from 3-4 to 10 WOA). Although a potential interference with maternal-derived antibodies (MDA) has been suggested, it has not been proven yet. The objectives of the present study were: a) to compare the immune response of piglets vaccinated at 1 and 3 WOA (in the presence of high MDA levels) vs those vaccinated at 3 and 5 WOA (lower MDA levels); b) to use a newly developed model of colostrum-deprived conventional piglets to evaluate the interference of MDA with a bacterin-based vaccination of piglets at 1 and 3 WOA.

#### **Material and Methods**

-A bacterin was prepared using the inactivated virulent S. suis serotype 2 P1/7 strain (Emulsigen D + Aluminum hydroxide as adjuvants).

-Experiment 1: Piglets were vaccinated at 1 and 3 or 3 and 5 WOA.

-Experiment 2: Piglets with or without maternal antibodies were vaccinated at 1 and 3 WOA.

-Antibody response was measured by indirect ELISA.

#### Results

-Piglets vaccinated at 3 and 5 weeks of age induced an active antibody production clearly detected from 7 WOA.

-Piglets vaccinated at 1 and 3 weeks of age were able to stabilize the decline of MDA when compared to control non-vaccinated piglets.

-MDA only partially interferred with the immune response of piglets vaccinated at 1 and 3 WOA.

-At 7-9 WOA there were no difference in antibody levels between animals vaccinated at 1 and 3 WOA in the presence or absence of MDA.

#### **Discussion and Conclusion**

We showed that the MDA poorly interfere with the active production of antibodies: the stabilization of the decay of such MDA seems to be as effective as an active immunization at 3 and 5 weeks of age.

#### Cod: IMM-OP-04

Immunology and Vaccinology

### EFFECT OF AMOXICILLIN, CEFTIOFUR, DOXYCYCLINE, TIAMULIN AND TULATHROMYCIN ON THE ANTIBODY RESPONSE OF PIGLETS VACCINATED AGAINST LAWSONIA INTRACELLULARIS

E.M. Fonseca <sup>2</sup>, D.G. Donin <sup>1</sup>, A.N. Jesus <sup>1</sup>, A.G. Daniel <sup>2</sup>, B.C. Gomes <sup>2</sup>, C. Feronato <sup>2</sup>, <u>R. Frandoloso</u> <sup>3</sup>, S.V. Berg <sup>4</sup>, J.X. Oliveira Filho <sup>2</sup>, G.C. Alberton <sup>1</sup> <sup>1</sup>Federal University of Paraná (UFPR) <sup>2</sup>MSD Saúde Animal

<sup>2</sup>MSD Saude Animal <sup>3</sup>University of Passo Fundo (UPF) <sup>4</sup>MSD Tiergesundheit, München, Germany

#### **Background and Objectives**

The prophylactic and metaphylactic use of antimicrobials during pig husbandry is not yet prohibited in Brazil. Some molecules are used in conjunction with the vaccination procedure, and their effect can go beyond the antimicrobial boundary and affect the immune system. The objective of this study was to evaluate the effect of different antimicrobials commonly used in the nursery phase on the antibody response induced by Porcilis<sup>®</sup> lleitis, an inactivated-based vaccine formulated with Lawsonia intracellularis.

#### **Material and Methods**

A total of 144 weaned piglets were divided into 9 different groups (G1- unvaccinated and not treated with antimicrobials; G2 – vaccinated and not treated with antimicrobials; G3 to G9 – vaccinated and treated with antimicrobials (G3 - ceftiofur; G4 - tildipirosin; G5 – tulathromycin; G6 - amoxicillin; G7 - doxycycline; G8 – florfenicol and G9 – tiamulin). The piglets were vaccinated intramuscularly with a single dose (2mL) of Porcilis® lleitis (Merck Animal Health, Madison, NJ, USA) at 30 days of life. Before (D0) and after vaccination (D7, D14, D21, D28, and D35), serum samples were collected and analyzed by Flow Cytometry Antibody Test (FCAT) to detect anti-L. intracellularis (Li) IgG. The comparison of anti-Li IgG levels at different moments was performed using the two-way ANOVA test (GraphPad Prism 9.0 software).

#### Results

Anti-Li IgG levels on day D35 were significantly (p < 0.001) lower in animals that received ceftiofur (G3), doxycycline (G7) and tulathromycin (G5) compared to the group G2 that was only vaccinated. In contrast, amoxicillin (G6), florfenicol (G8), tiamulin (G9) and tildipirosin (G4) did not affect the antibody levels. The most intense negative effect (reduction of 41.15% in the IgG levels) was found in group G3, treated with ceftiofur.

#### **Discussion and Conclusion**

Our results demonstrated that the use of some antibiotics during the development of the adaptive humoral immune response can affect the serological potency of immunogenic vaccines. This negative effect may be especially important in vaccines whose antibody response needs to reach its maximum level in the early stages of nursery phase (e.g. vaccines against Glaesserella parasuis and Streptococcus suis). Future studies need to be conducted to understand whether the reduction in antibody levels observed here is temporary or permanent.

IMM – Immunology and Vaccinology

#### **ON-FARM COLOSTRUM IMMUNOGLOBULIN G MEASUREMENTS FOR SUFFICIENT IMMUNITY IN PIGLETS**

D.P. Frandsen<sup>1</sup>, C.K. Højgård<sup>1</sup>, M.B.F. Nielsen<sup>1</sup> SEGES Innovation P/S, Aarhus, Denmark

#### **Background and Objectives**

Sow colostrum provides piglets with energy and immunoglobulin G (IgG). Previously, plasma IgG of 26-28 g/L measured within 24h after birth of the piglet indicated sufficient immunity. Colostrum IgG can be indirectly estimated on-farm using Brix refractometry. The present study aimed to determine whether the concentration of IgG in piglet plasma 10-12h after birth differed when piglets received colostrum of either high or low quality based on Brix refractometry. Brix readings of  $\geq$ 25% and  $\leq$ 20% were defined as high- and low-quality colostrum, respectively.

#### **Material and Methods**

The study was performed in a commercial Danish herd. In total 62 piglets with birth weights of <900g were collected from different sows at birth before suckling. Piglets received ear tags. Even and odd numbers received high- and low-quality colostrum, respectively. The piglets were housed six together for 10-12h in incubators equipped with a heat mat and straw. Prior to the trial, colostrum was collected from five sows of high- and low-quality, respectively. Each sample was analyzed for IgG concentration using ELISA-test. Colostrum from each of the five sows was pooled and piglets within each group were given a portion at 0, 3, 6, and 9h after birth. The portion size was calculated as: 59g x body weight x 0,33 and given to piglets via a stomach tube. At 10h, 2 mL of blood were collected from an ear vein and thereafter piglets were moved to a nurse sow.

#### Results

In piglets fed colostrum of high- (Brix  $\geq$ 25%) and low-quality (Brix $\leq$ 20%), the plasma IgG concentrations were 25.7 and 8.7 g/L (P<0.0001). The mean birth weight and milk portion size in the high- and low-quality group were 717±128 and 742±11 g, and 14.7±2.1 and 14.4±2.5 mL, respectively. Furthermore, there was a linear correlation between the Brix number and the concentration of IgG in the milk (y=0.1503x+15.171).

#### **Discussion and Conclusion**

The concentration of IgG in plasma was greater in piglets receiving high- compared to low-quality colostrum, and the plasma concentration in piglets receiving high-quality colostrum indicated that piglets were sufficiently immunized 10-12h after birth. Thus, it was possible to distinguish colostrum of high and low quality using Brix refractometry.

IMM – Immunology and Vaccinology

### ADAPTATION OF THE ASFV-989 LIVE ATTENUATED VIRUS ON CONTINUOUS CELL LINE, A STEP FORWARD TO BECOME A VACCINE CANDIDATE

<u>O. Bourry</u><sup>1</sup>, M. Le Dimna<sup>1</sup>, E. Hutet<sup>1</sup>, G. Pinsard<sup>1</sup>, Y. Blanchard<sup>1</sup>, F. Paboeuf<sup>1</sup>, M. Le Potier<sup>1</sup> <sup>1</sup>Anses-Ploufragan/Plouzané/Niort Laboratory, BP 53, 22440 Ploufragan, France

#### **Background and Objectives**

African swine fever since its reintroduction in Georgia in 2007 has spread across Europe, Southeast Asia and Caribbean. Our laboratory has recently developed an experimental vaccine candidate, ASFV-989, which has proven to be safe and highly efficacious, either by intramuscular (IM) or oronasal (ON) routes. The next step to pass for further vaccine development is the adaptation of the ASFV-989-PAM grown on porcine alveolar macrophages, to a continuous cell line.

#### **Material and Methods**

The ASFV-989-PAM strain was passaged first on a co-culture of PAM/Swine Testis (ST) cells then on ST cells only. The safety and efficacy of ASFV-989-ST strain was then evaluated in 6-weeks-old SPF pigs, with groups receiving the strain either by IM or ON route. Four weeks post-immunization (pi), the animals were challenged with the Georgia 2007/1 strain. Vaccine and challenge strain viremia were monitored by specific PCRs. Antibody response was followed by ELISA and cell-mediated immunity by ELISPOT IFNg.

#### Results

After immunization, the pigs exhibited only few cases of hyperthermia and a small decrease of growth performances. Immunized animals showed a low vaccine viremia between D3 to D27pi. ASFV specific antibodies were detected from D11pi and ASFV specific cell mediated response at D13pi. Among the pigs immunized IM then challenged with the Georgia strain, 1/6 pigs developed ASF symptoms and had to be euthanatized at D8 post-challenge, when the 5 other pigs only displayed late and light hyperthermia. Only one ON immunized pig displayed hyperthermia. In ASFV-989-ST immunized pigs, we saw a strong control of the Georgia strain replication with a viremia detected in 5/6 pigs immunized IM, and only in 1/6 pigs for the ON immunized pigs.

#### **Discussion and Conclusion**

In summary, ASFV-989-ST strain presented a very good safety and efficacy for ON immunization. For IM immunization, ASF-989-ST was safer than ASFV-989-PAM but less efficient, probably due to a suboptimal dose. Therefore, ASFV-989-ST strain seems a very good vaccine candidate that can be administered oronasally, a critical feature for potential vaccination of wild boar. Further studies are needed to evaluate ASFV-989ST stability and increase its efficacy for IM vaccination.

#### IMM – Immunology and Vaccinology

### A BROADLY PROTECTIVE VACCINE AGAINST SWINE INFLUENZA A VIRUS BASED ON THE MATRIX PROTEIN 2 (M2)

<u>F.A. Zuckermann</u><sup>1</sup>, Y. Grionkova<sup>3</sup>, R.J. Husmann<sup>2</sup>, M. Pires-Alvez<sup>2</sup>, S. Storms<sup>2</sup>, S.G. Sligar<sup>3</sup> <sup>1</sup>Department of Pathobiology, University of Illinois at Urbana-Champaign; Vastimmune Biologics, Inc. Champaign, IL. <sup>2</sup>Department of Pathobiology, University of Illinois at Urbana-Champaign <sup>3</sup>Department of Biochemistry, University of Illinois at Urbana-Champaign

#### **Background and Objectives**

The diversity of haemagglutinin (HA) among influenza A viruses (IAV) circulating in swine herds poses a significant challenge for vaccine development. Notably, the matrix protein 2 (M2) of IAV is a highly conserved envelope protein. More than 98% of IAV strains circulating in American swine herds share the identical pandemic isoform of M2. M2 is a tetrameric 97 amino acid long protein that in the virus envelope. We examined the protective efficacy of a novel IAV vaccine for swine consisting of recombinant **full-length M2 protein** displayed in soluble nanoscale structures called nanodiscs (**NDs**).

#### Material and Methods

Groups of pigs (n=9 per group) were immunized at 5 weeks of age via both intranasal (IN) and intramuscular routes twice at a 4-week interval with either: M2ND; empty NDs (eND); or empty NDs mixed with M2 (eND+M2). All three formulations were adjuvanted by incorporating synthetic oligonucleotides containing CpG oligodeoxynucleotide (CpG ODNs). At seventeen days after the booster, the animals in all three groups were challenged IN with 10<sup>6</sup> plaque forming units (pfu) of A/swine/MN/2016 (H3N2). Nasal swabs (NS) collected at 0-, 3- and 5-days post challenge (PC). After euthanasia at 5 days PC lungs were scored for gross lung lesions and bronchoalveolar lavage (BAL) fluids collected. Virus load in the BAL fluids and NS were determined by pfu assay.

#### Results

NS collected at 3- and 5-days PC from animals treated with eND or eND+M2 exhibited high titers of infectious IAV. Animals immunized with M2NDs exhibited a >10-fold decrease of infectious IAV in NS collected at 3- and 5-days PC. At 5 days PC, animals treated with either eNDs or eND+M2, exhibited extensive pneumonia and a substantial viral load in BAL samples. In contrast, pigs vaccinated with the M2NDs exhibited the presence of strong protective immunity manifested by the complete absence of pneumonic lesions in the lungs in 8 of 9 pigs, and a reduced load of virus in the BAL.

#### **Discussion and Conclusion**

The immunization of swine with M2ND provided a strong level of protective immunity against swIAV. This novel vaccine offers great promise as a universal IAV vaccine against HA antigenically diverse swIAV.

#### IMM – Immunology and Vaccinology

#### PRRSV-VACCINATED, SERONEGATIVE SOWS AND MATERNALLY DERIVED ANTIBODIES: IMPACT ON PRRSV-1 VACCINE EFFECTIVENESS AND CHALLENGE OUTCOMES IN PIGLETS

J. Fiers <sup>1</sup>, D. Maes <sup>2</sup>, A. Cay <sup>1</sup>, F. Vandenbussche <sup>1</sup>, L. Mostin <sup>3</sup>, A. Parys <sup>3</sup>, M. Tignon <sup>1</sup>

<sup>1</sup>Unit Viral Re-Emerging, Enzootic and Bee Diseases, Department Infectious Diseases in Animals, Sciensano, Uccle, Belgium

<sup>2</sup>Unit of Porcine Health Management, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

<sup>3</sup>Unit Experimental Centre, Department Infectious Diseases in Animals, Sciensano, Machelen, Belgium

#### **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) remains one of the most important pathogens in the swine industry. The prevalence of multiple PRRSV-vaccinated, but seronegative sows was recently assessed in Belgium. The current research investigated the impact of this peculiar sow immune status on the vaccine effectiveness and PRRSV-1 challenge outcomes in the progeny.

#### **Material and Methods**

Two experimental studies were conducted. In study 1, eighteen piglets born from three PRRSV-seropositive sows (E+ piglets) and eighteen piglets born from three PRRSV-seronegative sows (E- piglets) were included. In each group, twelve piglets were intranasally challenged with  $10^{5.5}$  TCID<sub>50</sub> / mL of the PRRSV-1 07V063 strain at 4 weeks of age, mock-challenged piglets served as controls. Study 2 contained twenty-four E+ piglets and twenty-four E- piglets. Eight piglets in each group were non-vaccinated, intramuscular vaccinated, or intradermal vaccinated, with the same PRRSV-1 vaccine as used in the sows. Vaccination was performed at three weeks of age and at six weeks, all study piglets were challenged with the same dose as in study 1.

#### Results

E+ piglets had the presence of maternally-derived antibodies (MDAs), while these were absent in the E- piglets. In study 1, higher viremia was observed in the challenged E- piglets compared to the E+ piglets during early infection. Additionally, differences were observed in the immune responses between both groups. In study 2, interference of MDAs was observed: 46.7% vaccinated E+ piglets lacked an antibody response at 4 weeks post-vaccination. Post-challenge, lower viremia was observed in the responding E+ piglets compared to the non-responding and non-vaccinated E+ piglets. In parallel, lower viremia was observed in the vaccinated E- piglets compared to the non-vaccinated E- piglets. Higher immune responses were observed in the vaccinated E- piglets and the responding E+ piglets compared to the non-vaccinated E- piglets.

#### **Discussion and Conclusion**

Piglets born from seronegative sows lack MDAs and are less protected in the first days post-challenge. MDAs in piglets born from seropositive sows interfere with the vaccine response, resulting in a lower effectiveness after challenge. Further research is warranted to avoid the presence of seronegative sows and to overcome MDA interference in vaccinated piglets.

IMM – Immunology and Vaccinology

### IMPACT OF SOWS' IMMUNOLOGICAL STATUS ON PCV2 VIRAL CIRCULATION DURING LACTATION USING PROCESSING FLUID PCR CT VALUES IN A PRE AND POST-SOW HERD VACCINATION PROGRAM

M. Kroeger 1, C. Stewart 2, M. Schleper 3, A. Sponheim 4, E. Fano 5, P. Pineyro 6

1 Iowa State University

<sup>2</sup>Christensen Farms, MN, USA

<sup>3</sup>2Christensen Farms, MN, USA <sup>4</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN,

USA; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA <sup>5</sup>Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA; Current affiliation: Pipestone Veterinary Services, Pipestone. MN. USA

<sup>®</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

#### Background and Objectives

The use of processing fluids (PF) to detect PRRSV has increased significantly resulting in effective monitoring strategies for PRRSV circulation and shedding in sow herds. However, the applicability and interpretation of this sample matrix regarding PCV2 circulation and herd health status is poorly understood. Therefore, the objective was to assess the impact of sows' immunological status on the PCV2 viral circulation levels during lactation using PCV2 processing fluid PCR Ct values in a pre and post-sow herd vaccination program.

#### Material and Methods

Sixty sows from two 2000-head commercial sow farms were evaluated for two parities, previous and post whole sow herd vaccination. Sows were vaccinated with a commercial PCV2 vaccine eight weeks before farrowing. Serum was collected two weeks before farrowing and antibodies levels were analyzed by total PCV2 IgG (ELISA), specific PCV2a and PCV2d IgG (IFA), and neutralizing antibody (NA) titers against PCV2a and PCV2d (chimeric virus neutralization assay). No cross fostering occurred between litters. PF were collected by litter to assess PCV2 viral levels. The correlation between PF Ct values and serological results was assessed by simple linear regression.

#### Results

Prior to vaccination, sow antibody levels had no significant correlation with the amount of PCV2 in PF. Following vaccination, the average of positive PF Ct values was significantly higher than pre-vaccination PF. Sows had significantly higher IFA and NA titers following vaccination. However, ELISA, IFA and NA titers observed post vaccination were not significantly correlated to higher high PF Ct values regardless of sow parity or PCV2 subtype.

#### **Discussion and Conclusion**

Although a direct correlation could not be established, vaccination in sows resulted in significantly higher antibodies levels and significantly higher PF Ct values in positive samples. Future studies should evaluate other factors that may affect PF Ct values and examine the implication of PF Ct on downstream production parameters. Establishing a correlation between PF Ct values and sow immune status could develop more tailored sow vaccination programs prior to farrowing to achieve maximal sow immune status. Additionally, establishing a correlation between PF Ct values and downstream performance could warrant the use of PF PCR as a predictor of grower-finisher performance.

#### ANIMAL WELFARE AND ETHOLOGY

#### WEL-OP-01

WEL - Animal Welfare and Ethology

#### ATTITUDES AND BELIEFS ABOUT TAIL BITING AND DOCKING IN PIGS

<u>A. Knörr</u>, A. Bearth <sup>1</sup>, X. Zhou <sup>1</sup>, M. Siegrist <sup>1</sup> <sup>7</sup>Consumer Behavior, Institute for Environmental Decisions, ETH Zurich, Universitaetstrasse 22, 8092 Zurich, Switzerland

#### **Background and Objectives**

Tail biting in pigs is a welfare and economic problem that can be reduced by tail docking. Although the European Union (EU) introduced a prohibition of routine tail docking over three decades ago, the majority of pigs in Europe still have their tails docked. The degree to which beliefs about tail biting and docking and attitudes toward welfare contribute to this reluctance to change is explored.

#### **Material and Methods**

A quantitative survey was conducted among farm owners and employees of medium- and large-scale pig farms in Germany, Spain, Ireland, and Switzerland (N = 439). We collected self-reported data on tail biting and docking status as well as attitudes and beliefs regarding tail biting and docking.

#### Results

With the exception of Switzerland, tail docking is still widely practiced among participants (76% in EU countries vs. 1% in Switzerland). More than half of the participants (56%) had experienced tail biting in the last six months. However, 52% of participants with docked pigs perceived biting as a moderate to big challenge, while less than 30% with undocked pigs perceived it as such. The two groups held contrasting views on the merits of docking: both groups believed that pigs' welfare was higher with the corresponding docking status (t = 10.5, p < .001) and farmers with undocked pigs also felt more confident in keeping pigs with good welfare (t = -15.3, p < .001). Also, participants with docked pigs considered production risks to be lower in docked pigs, compared to those with undocked pigs (t = 9.1, p < .001). We discuss how these different beliefs contribute to these discrepancies between participants with docked and undocked pigs.

#### **Discussion and Conclusion**

Our focus in this article was not on the contentious issue of whether pigs with or without tails experience greater welfare. Rather, the present study shows that farmers' views on docking and biting depend on the status quo. What is clear, is that the implementation of EU directives prohibiting tail docking have not been successful. We argue that the involvement and information of pig farmers plays a central role in creating lasting changes.

WEL - Animal Welfare and Ethology

### EXPLORING THE GENETIC COMPONENT OF MATERNAL BEHAVIOR ON PIGLET CRUSHING IN A CRATED AND FREE FARROWING SYSTEM

<u>C. Lipori</u><sup>3</sup>, I. Reimert<sup>3</sup>, E.F. Knol<sup>1</sup>, E.A.M. Graat<sup>3</sup>, I.M. Van As<sup>2</sup>, N.M. Soede<sup>3</sup> <sup>7</sup>Topias Norsvin, Den Bosch, The Netherlands

<sup>2</sup>Denkavit Nederland BV, Voorthuizen, The Netherlands

<sup>3</sup>Adaptation Physiology Group, Department of Animal Sciences, Wageningen University & Research, Wageningen, the Netherlands

#### Background and Objectives

With a growing desire to abolish restrictive housing systems for sows during lactation, increased efforts are needed to reduce pre-weaning mortality in free farrowing (FF) systems. One essential aspect is genetic selection for high quality maternal care. Currently, breeding values for mothering ability (EBV<sub>ma</sub> (Topigs Norsvin)) are based on information derived from sows housed under restrictive conditions. This study aims to validate these breeding values under FF conditions.

#### Material and Methods

56 Healthy TN70 sows were assigned, by parity  $(3.3 \pm 2.1)$ , to either Crated (CR, i.e., crated from  $\pm 2$  days pre-farrowing to day 5 of lactation, n=28) or Free Farrowing (FF, n=28). EBV<sub>ma</sub> were provided by Topigs Norsvin (n=55). Crushing incidents were assessed through live and video observations. Data on total number of piglets born, liveborn, stillborn, mummies, non-viable piglets, and unknown deaths was collected. EBV<sub>ma</sub> (median 0.04%) was categorized as Low (-1.92% to 0.04%, n=28) and High (>0.04 to 3.08%, n=27). Data was analyzed using logistic regression with piglet as experimental unit, housing and EBV<sub>ma</sub> as fixed effects, and sow as repeated measure.

#### Results

Up to Day 5, total mortality of liveborn piglets was higher in FF compared to CR sows (14.5% vs. 6.2%, P<0.001) and FF sows also had higher piglet mortality due to crushing compared to CR sows (11.8% vs. 1.1%, P<0.001). Overall, Low EBV<sub>ma</sub> sows crushed more piglets than High EBV<sub>ma</sub> sows (9.8% vs. 3.8%, P<0.001) and both in CR and in FF sows, the odds ratio for crushing was higher in Low vs. High EBV<sub>ma</sub> sows, 8.4 (P=0.018) and 2.9 (P=0.004), respectively. There was no significant effect of housing on stillborn piglets. Yet, Low EBV<sub>ma</sub> sows had more stillborn piglets than High EBV<sub>ma</sub> sows (8.9% vs. 3.9%, P<0.001).

#### **Discussion and Conclusion**

Sows are crated to reduce the risk of crushing, and despite this controlled environment there is still noticeable genetic variation in piglet mortality. Piglet crushing is significantly higher under FF conditions, and is clearly related to EBV<sub>ma</sub>. Therefore, genetic variance estimated and utilized under FF conditions will further improve the possibilities for selection of improved maternal care. This will maximize the welfare and performance of both sows and piglets.

WEL - Animal Welfare and Ethology

#### THE ROLE OF EAR MANIPULATIONS BY PEN MATES IN PORCINE EAR NECROSIS

#### M. Malik 1, D. Maes 1

<sup>1</sup>Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Belgium

#### **Background and Objectives**

Porcine ear necrosis (PEN) is characterized by uni- or bilateral lesions of the ear tip or margin. Lesions mostly appear in piglets between the sixth and eighth week of life. Different risk factors have been described such as infections, ear biting, high stocking density, poor ventilation, mycotoxins in the feed or insufficient environment enrichment. The present study assessed the role of ear manipulations in the occurrence of PEN in weaned piglets.

#### **Material and Methods**

Three farms (A,B,C) with PEN problems in nursery piglets were selected. During the nursery period, PEN prevalence was assessed weekly. A mobile camera was placed in each pen 2m above floor level, and piglets were video recorded for 15 minutes. Video recordings were made on day two after weaning, and then weekly during the nursery period. At the end of the nursery period, pens with the highest and lowest ear lesions prevalence were chosen, and the videos of these pens were used to assess the number of oral manipulation events on penmates.

#### Results

The overall highest prevalence of PEN on farms A, B and C was 58%, 87% and 93%, respectively. The max prevalence of PEN per pen ranged from 12% to 100%. Therefore, 15 pens with highest, and 8 pens with low PEN prevalence were evaluated. This implied observations from 710 piglets, and in total 50 hours of recordings. The number of ear manipulations in pens with high and low PEN prevalence was: 186±20 vs. 113±36 on farm A, 160±31 vs. 84 on farm B, and 164±17 vs. 105±1 on farm C. The ear manipulation occurred before the first animals developed ear lesions, and the peak in number of ear manipulations in each farm was followed by a peak prevalence of ear lesions 2 weeks later.

#### **Discussion and Conclusion**

The present study shows that oral ear manipulations of penmates plays an important role in the development of PEN. The dynamic of manipulations in time corresponds to the dynamic of prevalence increase. Providing sufficient enrichment in the pens might direct the chewing behaviour of the piglets away from their penmates, and therefore decrease PEN problems in nursery pigs.

#### WEL - Animal Welfare and Ethology

### ECONOMIC IMPACTS AND WELFARE CONSIDERATIONS IN SWINE PRODUCTION: A COMPARATIVE ANALYSIS OF GESTATION CRATE AND GROUP HOUSING SYSTEMS

L.K.S. Alves 1, C. Raineri 2, C.A. Pospissil Garbossa 3, M.D. Pairis-Garcia 4

<sup>1</sup>Swine Research Laboratory, Department of Nutrition and Animal Production, Faculty of Veterinary Medicine and Animal Science, University of São Paulo

<sup>2</sup>Agribusiness Studies Laboratory, Department of Animal Science, College of Veterinary Medicine, Federal University of Uberlândia

<sup>3</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>4</sup>Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

#### Background and Objectives

The global swine industry commonly utilizes gestation crate systems on sow farms to optimize space and minimize resource allocation. However, global legislation, driven by societal concerns, has restricted gestation crate based on animal welfare. This study aimed to estimate the production costs and profitability associated with sows' farms utilizing either gestation crates (GC) or group housing pens (GH).

#### Material and Methods

A mathematical model was developed to calculate production costs and profitability for a 2,300-sow farm in São Paulo/Brazil with both a GC and GH layout. Sows housed in GC, remained in the crate throughout gestation, while the GH system moved sows to pens with Electronic Sow Feeders 35 days post-insemination. Cost (variable cost; VC, fixed operating; FOC, and Opportunity costs; CC) and profitability variables were evaluated. An analysis of sensitivity was conducted to evaluate how a 1% improvement in farrowing rate, number of born alive piglets, and slaughter weight impacted economic results in both scenarios, applying Ceteris paribus concept.

#### Results

The GC had a production cost per kilogram of US\$ 1.36 compared to US\$ 1.40 in GH, representing a 2.78% cost increase. Variable costs were similar between systems, but FOC was 10.45% and CC was 18.83% higher in GH compared to GC. Although revenue was the same for both systems, economic profit was 94.27% lower for the GH compared to GC, despite being positive for both. In this scenario, the GH system would need to produce 33 (2.83%) more fattened pigs/week than the GC system to achieve the breakeven point. During the sensitivity analysis, a 1% improvement in farrowing rate resulted in a 141.35% increase in profit margin (PM) for the GH. A 1% surge in the number of born alive piglets led to a 162.09% PM increase, and a 1% growth in slaughter weight resulted in a 534.42% greater profit for the GH system.

#### **Discussion and Conclusion**

The GC system demonstrated economic advantages over GH due to increased investment and associated expenses in the latter. Nevertheless, profitability can still be achieved in GH, but additional factors, including management, technological integration, and genetic selection, must be considered to optimize output.

WEL - Animal Welfare and Ethology

#### PREVALENCE OF AND RISK FACTORS FOR TAIL LESIONS IN NURSERY PIGS

<u>A. Valros</u><sup>1</sup>, M. Tuominen-Brinkas<sup>1</sup>, H. Koskikallio<sup>1</sup>, K. Ahlqvist<sup>1</sup>, M. Heinonen<sup>1</sup>, C. Munsterhjelm<sup>1</sup> <sup>1</sup>Department of Production Animal Medicine & Research Centre for Animal Welfare, Faculty of Veterinary Medicine, Helsinki, 00014, Finland

#### **Background and Objectives**

Tail biting is a common, multifactorial behavioural problem in pigs. Tail lesions evaluated at slaughter have been suggested as an animal-based measure of lifetime pig welfare. To facilitate the development of such a measure, it is important to understand how the different production phases contribute to the total occurrence of tail lesions. Thus, our aim was to evaluate the prevalence of tail lesions during the nursery period on commercial farms. In addition, we assessed potential risk factors for tail biting at this production phase.

#### **Material and Methods**

We performed one-day visits to ten Finnish farms, rearing undocked nursery pigs from weaning at about 4 weeks until approximately 9-11 weeks. On each farm visit 2-15 rooms in the growing unit (81 rooms in total) representing different age groups of pigs (1-2, 3-4- or 5-6-weeks post-weaning) were randomly selected. In each room, every pen ((1,217 pens in total, 56-240 pens per farm) was scored as having no pigs with tail lesions, or lesions in > 10% of the pigs (TB pens). A risk assessment protocol was performed in at 35 rooms with a TB pen, representing the three different age groups.

#### Results

The percentage of TB pens in the pooled data increased with age of the pigs: 11% of all pens at 1-2 weeks, 24% at 3-4 weeks and 38% at 5-6 weeks after weaning. Commonly occurring potential risk factors included not all pigs being able to eat at the same time (74% of rooms), sneezing (69%), rooting material not destructible (69%), pigs of different size (65%), subordinate pigs cannot always reach food or water (52%), diarrhoea (41%) and rooting material not always present (39%).

#### **Discussion and Conclusion**

These results indicate that prevalence of tail biting lesions increases with age in nursery pigs. Identified potential risk factors are similar as in earlier studies, including elements of resource competition, health problems and insufficient enrichment material. The high level of tail biting and the commonness of risk factors in the nursery unit underlines the importance to address problems at this production stage to ensure more pigs reach slaughter with intact tails.

#### PRECISION LIVESTOCK FARMING

#### PLF-OP-01

#### PLF - Precision Livestock Farming

### DEVELOPING A WEAN-QUALITY SCORE TO FORECAST NURSERY MORTALITY: A MACHINE-LEARNING APPROACH

<u>E. Magalhaes</u><sup>1</sup>, G. Trevisan<sup>1</sup>, C. Wang<sup>2</sup>, D. Correia Lima Linhares<sup>1</sup>, G. De Souza E Silva<sup>1</sup> <sup>1</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine <sup>2</sup>Iowa State University Department of Statistics

#### **Background and Objectives**

Swine nursery mortality is a key performance indicator (KPI) of optimal productivity in the nursery phase. Although the importance of the pre-weaning phase on the downstream nursery performance is also known, this information is not utilized to predict the nursery performance regarding survivability rate. Thus, this study aimed to create a wean-quality score for groups of weaned pigs and forecast their downstream nursery mortality, based on variables available at the weaning event.

#### **Material and Methods**

This study used retrospective data from 1,723 groups of pigs marketed within a US swine production system to develop a Wean-Quality Score (WQS) using a Random Forest (RF) machine learning (ML) model to predict groups having high (>5.3%) or low (<5.3%) nursery mortality. A total of 12 variables from the pre-weaning phase were included as predictors. High mortality groups represented 25% of the groups among the study population with the highest mortality values (n=431), and the remaining 75% groups were of low mortality (n=1,292). The RF model was trained using a 10-fold cross-validation process, and later the predictive performance was tested on the test set of data by forecasting the mortality of the groups in the dataset as high or low nursery mortality. The model performance on accurately predicting groups as high or low nursery mortality was then assessed by calculating the accuracy (proportion of groups correctly predicted), specificity, and sensitivity.

#### Results

The RF model had an accuracy of 90.7% in accurately predicting groups with high (>5.3%) or low (<5.3%) nursery mortality. The values for sensitivity (84.62%), specificity (92.48%), positive predicted value (76.74%), and negative predicted value (95.35%) also demonstrated relatively high forecasting capability, especially for accurately predicting high nursery mortality groups. Also, it was observed that most groups with a high wean-quality score (8-10) were clustered as low mortality, and the majority of the low wean-quality score (0-2) groups had high nursery mortality.

#### **Discussion and Conclusion**

The machine-learning approach described in this study demonstrates the predictive capability of creating a Wean-Quality Score (WQS) for commercial groups of pigs, using information available at weaning to forecast the downstream performance of weaned pigs, which offers a practical tool for the decision-making process.

#### PLF - Precision Livestock Farming

#### LIFETIME RFID EAR TAG RETENTION IN A COMMERCIAL & FIELD-BASED RESEARCH TRIAL

#### H. Schwecke <sup>1</sup>, M. Pieters <sup>2</sup>

<sup>1</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

<sup>2</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

#### **Background and Objectives**

Ear tags are commonly used as an identification in research and commercial pigs. Radio frequency identification (RFID) tags are a type of smart label that uses electromagnetic fields for identification and tracking, and can store a range of information. Thus, RFID tags have been in swine research in recent years. However, information on the retention of RFID tags in pigs in large-scale commercial conditions is limited. Therefore, the objective of this study was to determine the retention rate of RFID tags in swine in commercial barn settings.

#### Material and Methods

A subset of 6,500 pigs (from a larger study), were born over several weeks at a sow farm and received one RFID ear tag in their left ear, and ear notches in their right ear as a second form of identification, within 24 hours of birth. Pigs were weaned and housed at one of three nursery and finishing sites (A, B, and C) or one of two wean-to-finish operations (D and E). Pigs were observed weekly from birth until 21-23 weeks, at which time live pigs with ear tags were scanned and inventoried. Pigs with only ear notches were manually recorded. Ear tag retention was calculated based on the starting number of ear tagged pigs at each location and was evaluated at the room and pen level.

#### Results

The percentage of pigs retaining RFID tags at 21-23 weeks in each barn were: A 97%, B 96%, C 96%, D 68%, and E 93%. Evaluation at the pen level identified the largest loss of ear tags to specific pen(s).

#### **Discussion and Conclusion**

Overall, the RFID ear tag retention rate was high and only lower at the two wean-to-finish sites. The highest loss of ear tags was identified within specific pens. In one instance, the majority of ear tag losses were in one single pen. Therefore, it could be hypothesized that the high ear tag loss was associated to pen-specific conditions, as management practices were similar for the entire sites. Detailed investigations are needed to determine the factors that may influence ear tag retention in commercial pig production.

#### PLF - Precision Livestock Farming

#### A PHEROMONE-ASSISTED ENRICHMENT DEVICE ALLOWS PIGS TO SELF-VACCINATE FOR SALMONELLA

R. Robbins 1, C. Archer 2, L. Gimenez-Lirola 3, J.C. Mora-Diaz 4, J. Mcglone 5

<sup>1</sup>PIC North America, Hendersonville, TN 37075

<sup>2</sup>College of Food, Agricultural and Research Services, University of Minnesota

<sup>3</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA

<sup>4</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

<sup>5</sup>Laboratory of Animal Behavior, Physiology and Welfare, Texas Tech University

#### **Background and Objectives**

Individual oral administration (drench) is a method to deliver animal health products but requires more labor. The objective was to determine if, with minimal human effort, pigs will self-vaccinate with an oral Salmonella vaccine. Secondly, we sought to determine if Salmonella exposure resulted in quantifiable isotype-specific antibody responses indicative of recent inoculation.

#### **Material and Methods**

Three groups were evaluated: control (not vaccinated), drench by hand per label (positive control), or vaccine selfadministered (by the pigs) requiring no human-pig contact. Four, 15 week, PRRSV, SECD, IAV-S and Mycoplasma hyopneumoniae negative pigs were each allotted 20 ft<sup>2</sup>. A prototype environmental enrichment (EE) device containing oral live-attenuated Salmonella vaccine and maternal pheromone as an attractant was mounted for 5 h. The device had a push-plate that encouraged rooting and pushing without training. When a pig pushed on the face plate, it sprayed vaccine to the pig's mouth and face. Individual oral fluid and serum samples were collected at -7, 14-, and 21-days postvaccination (dpv) and tested using the IDEXX Swine Salmonella Ab Test adapted to an indirect format for isotypespecific IgG and IgA antibody detection. Feces was collected at -7, 1, 3, 7, 14 and 21 dpv for Salmonella enrichment and culture.

#### Results

All fecal samples were negative for Salmonella by enrichment and culture. All pigs in the self-vaccination group visited the sprayer and were sprayed at least once in the first 80 min after placement. Pigs that were in the hand-drenched or self-administration treatments had increased IgG and IgA serum and oral fluid concentrations at most time points (LSMeans, p<0.05). Controls had little or no IgG or IgA at all time points.

#### **Discussion and Conclusion**

A pen-mounted EE sprayer allowed group-housed pigs to self-administer an oral vaccine in an efficacious manner. We show the utility of adapting a commercial ELISA kit into an indirect format for isotype-specific detection of Salmonella antibody in both serum and oral fluid. Knowing both IgG and IgA can distinguish recent from previous infection or vaccination, which can aid in on-farm diagnoses. This device provided EE, and it can be used to improve animal health with minimal human labor. A smart-barn can incorporate the EE device so products could be added and operated remotely.

#### PLF - Precision Livestock Farming

#### A MODEL TO PREDICT WATER CONSUMPTION IN GROWING PIGS

M. Marroco<sup>1</sup>, A. Fernández-Fontelo<sup>1</sup>, J. Segalés<sup>2</sup>, B. Garcia-Morante<sup>2</sup>

<sup>1</sup>Departament de Matemàtiques, Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193, Spain.

<sup>2</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain.

#### **Background and Objectives**

Animal well-being can be measured indirectly using behavioural indicators; a change in the drinking pattern is, for instance, one of the first signs in sick pigs. Automated monitoring of water consumption could therefore provide a sensitive tool for real-time, continuous monitoring of the presence of pre-clinical and sub-clinical diseases in pigs. The current study aimed to develop a robust learning model for the prediction of the drinking behaviour of growing pigs.

#### **Material and Methods**

Retrospective water consumption data from 30 batches of fattening pigs with no signs of apparent disease from one Spanish commercial farm were used. Each batch of pigs was housed in units of 687±7 pigs, which were managed all-in/all-out. Data was recorded per unit using a water flowmeter and stored in the computer as the litres consumed in a one-hour period. Then, the total water consumption in the unit was divided among the number of pigs across the fattening period (L/pig/hour). Data were modelled by means of an autoregressive moving average (ARMA) model to account for the auto-correlated structure of the hourly-recorded water consumption data. Variables such as temperature, humidity, feed consumption, and days of fattening were incorporated as covariates in the ARMA model. The model also introduced a variable considering the water consumption of other batches on the same hour and fattening day.

#### Results

Total water consumption rate increased throughout the fattening period, and it was associated with a circadian rhythm, which was modelled by the sum of four harmonic components (i.e., sinusoidal waves representing 24-, 12-, 8-, and 6-day hours). As a result, the model could predict well the hourly water intake in a new batch of pigs, as the validation of the model showed that the recorded observations over the last period of the study were within the predicted 95% confidence interval.

#### **Discussion and Conclusion**

An ARMA model including seasonal components, historic water intake observations, and external variables has proven to be well suited for modelling the water consumption behaviour over time. The monitoring of such pattern is a prerequisite for utilizing drinking behaviour for an early detection of health and welfare issues in growing pigs.

#### PLF - Precision Livestock Farming

## RELATIONSHIP BETWEEN ENVIRONMENTAL, RESPIRATORY HEALTH STATUS AND PRODUCTION PERFORMANCE PARAMETERS IN FULLY MONITORED GROWING SWINE FARMS: A PRECISION LIVESTOCK CROSS-FARMING STUDY

L. Kurschilgen <sup>1</sup>, C. Alonso Garcia-Mochales <sup>2</sup>, <u>M. Steenaert</u> <sup>3</sup>, F. Gonzalvo <sup>4</sup> <sup>1</sup>Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH, Germany <sup>3</sup>Boehringer Ingelheim AH the Netherlands BV <sup>4</sup>Boehringer Ingelheim Animal Health Spain

#### **Background and Objectives**

The objectives of this study were to evaluate the relationship between respiratory health status, measured by a soundmonitoring system, with environment and key production performance indicators such as daily water usage, and feed consumption, as well as daily weight gain and feed intake, registered across multiple farms.

#### Material and Methods

A convenient subset of 4 sound-monitored finishing farms (SoundTalks<sup>®</sup>\_monitors = 18; Npigs/room = 477-1031 pigs) in Spain and The Netherlands were included in the study. Inclusion criteria were continuous data registered at a pen or room level for  $\ge$ 4 months for relative humidity/temperature, water intake, feed intake, as well as weight gain. To ensure cross-farm analysis, all continuous data were adjusted to daily inventory and pig age at a pen level and related to a sound-monitor zone for respiratory health analysis (pens under the sound-monitor, 20m diameter). After the stationary state of the time series was achieved, Granger causality analysis was used to investigate the relationship and strength (% likelihood) of that relationship between variables within and across farms and output plotted ranging from lag 1 to lag 4 (day 1-4).

#### Results

A total of 126 months of data were evaluated for the study. "Across-farm analysis" demonstrated that changes in farmtemperature impacted respiratory status (ReHS) being lag 2 the highest value (17%) and water usage (lag1 and 2, at 18%). Changes on water intake impacted ReHS only on lag4 (18%), however changes detected first in feed intake had highest impact on ReHS in all 4lags (10-14%). Results "within farm", demonstrated significant difference between buildings (with regards of strength of the relationship between variables and occurrence) in 1 of the 4 farms with multiple buildings and between monitors of the same farms in those in which data was obtained as a pen level.

#### **Discussion and Conclusion**

Results from this study demonstrated, for the first time, the relationship (occurrence and strength) between respiratory health and changes in environmental/production parameters. Precision livestock farming technologies, such us those based on sound, could provide this precise feedback for producers to choose the best intervention in order to prevent such impact and to achieve higher health and production performance.



# **FLASH TALKS**



#### FLASH TALKS

#### FTP-OP-01

#### BBD – Bacteriology and Bacterial Diseases

### ESTABLISHMENT OF AN EXPERIMENTAL PIG MODEL FOR THE INDUCTION OF STAPHYLOCOCCUS HYICUS SKIN INFECTION

K. Matiašková<sup>1</sup>, E. Jeklová<sup>1</sup>, M. Zouharová<sup>1</sup>, K. Nedbalcová<sup>1</sup>, J. Matiašovic<sup>1</sup>, M. Faldyna<sup>1</sup> Veterinary Research Institute (VRI) Brno, Czech Republic

#### **Background and Objectives**

The bacterium Staphylococcus hyicus is a commensal of skin in pigs, but it can cause a serious disease called exudative epidermitis. Affected piglets showing skin lesions can be treated with systemic antibiotics. However, the increasing antimicrobial resistance of S. hyicus isolates makes treatment difficult. So, it is necessary to look for other therapeutic alternatives. The aim of this study was to establish a porcine challenge model of skin infection with S. hyicus for the purpose of planned testing of the effectiveness of an alternative medicinal product intended for a local application to the infected skin.

#### **Material and Methods**

At D0, six superficial skin defects (5x5 cm) were created by scratching epidermis and creating of a grid with a needle on the back of each 35-day-old piglet (six pigs in total) and inoculated with 3 ml of S. hyicus in two different concentrations (each in 3 pigs). On every pig, three field strains (CAPM6346, CAPM6689 and CAPM 6690) were tested in duplicate. Every day for the duration of the experiment, pigs were monitored and the rectal temperature was measured. At D4, D9 and D14, visual evaluation of wounds (presence of erythema, scabs, oedema, exudate) and indirect imprints of defects were performed.

#### Results

Purulent secretion was not present. Crusts were present from D9 and were present in higher amount in wounds infected with strain CAPM6689 and CAPM4346 than in wounds infected with CAPM6690. It seems that a lower concentration of bacteria led to the lower secretion and creation of crusts. Moreover, based on results of bacterial cultivation of indirect imprints, S. hyicus was present in higher amounts in wound defects infected with the lower concentration of bacteria, especially at the end of the experiment.

#### **Discussion and Conclusion**

Taken together, the strain CAPM6689 is the best choice and the lower concentration (10<sup>s</sup>/ml) seems to be enough for the infection development. This project NeoGiANT has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N<sup>o</sup> 101036768. All procedures involving animals were approved by the Branch Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic (approval protocol No. MZe 2396).

#### HHM - Herd Health Management

### ASSESSING PRRSV-1 DETECTION DYNAMICS POST-OUTBREAK AND IMPLEMENTATION OF A TARGETED VACCINATION STRATEGY IN A BREEDING HERD

Z. Tomic<sup>1</sup>, V. Miljkovic<sup>1</sup>, G. Lopez-Moreno<sup>2</sup>, R. Morgenstern<sup>3</sup> <sup>1</sup>Boehringer Ingelheim Serbia d.o.o. <sup>2</sup>Boehringer Ingelheim RCV GmbH & Co KG <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH, Germany

#### **Background and Objectives**

PRRSV infection can significantly impact reproductive performance in a previously naive farm. The aim of this study was to monitor a control program for PRRSV-1 in pre-weaning piglets using three different types of samples: processing fluids (PF) from newborn piglets, serum from due-to-wean piglets, and oral fluid from growing pigs.

#### **Material and Methods**

The study was conducted on a PRRS-naive, nucleus farm with 650 sows. After observing clinical signs suggestive of PRRSv, the virus was detected through diagnostic testing in week 44 of 2020. Vaccination and management and biosecurity measures, began in week 51 of 2020 with double mass vaccination (four weeks apart) of the breeding herd, including all gilts in GDU with ReproCyc® PRRS EU. Vaccination of the breeding herd was repeated every 4 months. Piglets were also vaccinated with Ingelvac PRRSFLEX®EU from 14 days of age until weaning. Vaccination of piglets continued from week 52/2020 until 40/2021 with weekly batch vaccination at or shortly before weaning at three to four weeks of age. To monitor PRRSV-1 in pre-weaning piglets, weekly processing fluid samples (N=36) were collected from week 29 of 2021 until week 36 of 2022, with the number of samples collected varying from 30 to 60 to detect at least one positive sample when the prevalence was 10% or lower with a 95% confidence. From week 36 of 2021 and onwards, weekly oral fluids were collected to assess PRRSV circulation in the growing pig population.

#### Results

PRRSV was found in processing fluids in 2 out of 75 samples (2.66%), corresponding to weeks 20 and 52 of 2021. From serum samples, only one positive sample was detected from week 36 of 2021. All collected oral fluids at the end of the nursery period were negative by RT-PCR.

#### **Discussion and Conclusion**

These monitoring results provide evidence of a successful control strategy, in which PRRSV was no longer detected in collected samples as early as 14 weeks post sow vaccination, with the exception of 2 weeks during a 2-year monitoring program.

#### IMM – Immunology and Vaccinology

#### A LOOK BEHIND THE SCENES OF ROTAVIRUSES

<u>M. Harzer</u><sup>1</sup>, B. Euring <sup>1</sup>, K. Heenemann <sup>2</sup>, A. Rückner <sup>3</sup>, T.W. Vahlenkamp <sup>1</sup> <sup>1</sup>Centre for Infectious Diseases, Institute of Virology, Faculty of Veterinary Medicine, University of Leipzig, Germany <sup>2</sup>Center for Infectious Diseases, Institute of Virology, Faculty of Veterinary Medicine, University of Leipzig <sup>3</sup>Klinikum St. Georg gGmbH, Delitzscher Str. 141, 04129 Leipzig, Germany

#### **Background and Objectives**

Diarrhea is one of the most common diseases in piglets. This affects both the profitability of piglet production and the well-being of the pigs. Despite prophylactic and therapeutic interventions, diarrhea still occurs frequently in the first few days of life, significantly affecting the average daily weight gain of the affected animals. Diarrheal diseases are a multifactorial event in which not only nutritional and climatic factors but also various pathogens, e.g. viruses, bacteria and parasites, play an important role. Some veterinary practices report individual cases in which there is no improvement in the clinic despite the implementation of management measures and adapted vaccination regimes. A retrospective analysis was performed in order to detect in which of these individual cases rotaviruses represent a factor that should not be underestimated/a crucial factor.

#### **Material and Methods**

Molecular biological and serological data from 2018 to the present were analyzed. The focus was on the integrated consideration of all data with the inclusion of co-infections.

#### Results

It was shown that rotaviruses can circulate in herds over a long term despite the use of specific vaccines. Herd-specific factors determine the extent and timing of the clinic. Besides general hygiene measures, the quality of maternally transmitted immune protection via colostrum or milk is a decisive factor. In addition to the immunological status, the detected rotavirus strain sometimes also has an impact on the success of veterinary interventions.

#### **Discussion and Conclusion**

The immunological status of the dams should be assessed at various points in the reproductive cycle when the use of "suitable" vaccines does not show the intended effect. This can lead to adjustments in management if necessary. Certain sero-/genotypes are detected within group A rotaviruses, as well as group C rotaviruses, for which no sero-/genotype-specific vaccines currently exist. New therapeutic and prophylactic methods need to be developed here.

#### MIS - Miscellaneous and Clinical cases

#### RABIES IN PIGS: CLINICAL REPORT AND DIAGNOSTIC CHALLENGES

S. Davi Traverso 1, J. Cristani 2, W. Consoni 3, C.S. Wisser 4

<sup>1</sup>Department of Veterinary Medicine, Universidade Estadual de Santa Catarina, UDESC, Lages - SC, Brazil. <sup>2</sup>Animal Production and Food Department, Universidade do Estado de Santa Catarina UDESC, Brazil <sup>3</sup>Cooperalfa Agribusiness

<sup>4</sup>Department of Veterinary Medicine, Universidade do estado de Santa Catarina, UDESC, Lages - SC, Brazil.

#### **Background and Objectives**

Rabies is an acute viral disease, rare in pigs, and with low economic relevance in swine farming, but is a public health issue due to the risk of human contamination. This study aimed to report the occurrence of rabies in pigs, provide information on the diagnosis of the disease in this species, and warn that rabies should always be considered as a differential diagnosis for neurological diseases in endemic areas.

#### Material and Methods

### Two pigs with nervous symptoms were necropsied by autonomous veterinarians and the clinical history and samples, preserved in formalin, were sent for analysis.

#### Results

Pigs were raised for subsistence in areas where cattle died from rabies. The affected pigs had anorexia, hindlimb paralysis, lateral recumbency, and paddling movements. One animal died within 4 days of clinical evolution and the other was sacrificed 2 days after the onset of clinical signs. Histological examination of the brain revealed individual neuronal necrosis, multifocal gliosis, and meningeal and perivascular lymphoplasmacytic infiltrate. Intracytoplasmic inclusions weren't observed in the portions evaluated. Immunohistochemistry was performed in the telencephalon, trunk, and cerebellum regions and showed intense staining for rabies antigens of neurons in the brainstem, moderate staining in the telencephalon, and absence in the cerebellum.

#### **Discussion and Conclusion**

The low ocurrence of rabies in pigs, the similar clinical signes with other neurological diseases and the absence of Negri corpuscles in this species point to the complexities involved in the diagnosis of rabies in swines, which ranges from clinical suspicion to the correct collection of samples for analysis and laboratory tests. The diagnosis of pig rabies was performed by the IHC, although is not the official technique for diagnosing rabies, but it was effective in concluding the diagnosis and, together with the histological lesions, was extremely important for the differential diagnosis of other encephalitides in pigs. Furthermore, like cerebellum samples in both cases did not show IHC staining, we recommend that the spinal cord and brainstem should always be collected for histological analysis for pig rabies diagnosis, since only the cerebral cortex and especially the cerebellum can increase the chances of a false-negative result.

MIS – Miscellaneous and Clinical cases

### ATTITUDES TOWARDS THE POTENTIAL USE OF NEEDLELESS INJECTION DEVICES (HIPRADERMIC®) IN THAI PIG FARMS

P.P. Pwint Thu <sup>1</sup>, A. Buakhiew <sup>2</sup>, C. Ananratanakul <sup>2</sup>, N. Deelum <sup>2</sup>, N. Chavaranggul <sup>2</sup>, J. Saewong <sup>2</sup>, S. Tantanasarn <sup>2</sup>, T. Sittikorn <sup>2</sup>, F.D.M. Botet <sup>3</sup>, <u>N. Am-In <sup>4</sup></u>

<sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>HIPRA Bangkok Thailand

<sup>3</sup>HIPRA Amer Spain

<sup>4</sup>Center of Excellence in Swine Reproduction, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Thailand

#### Background and Objectives

Despite multiple studies supporting needle-free injection devices, in Thailand, these devices are not widely used. In response to the lack of popularity of these vaccination tools, a survey examined attitudes and variables that favor their use. This study aimed to assess needleless injection users' perceptions.

#### **Material and Methods**

To accomplish this, an intricately planned survey was developed, with the specific goal of exploring the viewpoint of managers regarding procurement decisions within the context of swine farming. The survey additionally evaluates the current practices, knowledge base, and attitudes prevalent among swine practitioners in Thailand. A carefully designed questionnaire was distributed through Google Forms platform scoring system ranging from 1-10 (indicating the least to the most favorable responses). The survey was administered to a targeted sample of 136 Thai swine practitioners and 57 Thai swine farm managers, overseeing swine farms with a sow population between 500-10,000 sows.

#### Results

According to the research, 74.3% of practitioners emphasized equipment safety the most, a 9.2% margin. A substantial connection (p=0.04) was found between farm worker skill and product safety and dependability. Practitioners value personal preferences (9.1 points) and product reliability (9.1). In contrast, 79% of managers expressed serious worries about food safety (9.5 points), carcass quality (9.4 points), and animal welfare (9.4 points). The majority of responders (63.8%) prioritized finding solutions to vaccine difficulties, particularly side effects and needle residues (9.4 points). Nearly 98% of firm managers believe needleless injection devices improve operational efficiency due to their traceability.

#### **Discussion and Conclusion**

The preliminary findings of the survey showed a substantial divergence in perspectives between practitioners and managers within the Thailand swine farming sector. Managers prioritized product quality and business image, on the other hand workers preferred safety rules. These nuanced perspectives show intricacies inherent in the industry's decision-making processes. Nevertheless, a consensus among both company managers and practitioners regarding the advantageous nature of needleless injection devices, which were perceived as instrumental in enhancing daily farm operations. These insights, which shade light on stakeholder attitudes and concerns, may help increase the usage of needle-free devices in the swine farming in Thailand.

MIS - Miscellaneous and Clinical cases

#### A SURVEY OF BIOSECURITY MEASURES ON COMMERCIAL FARMS IN SOUTHERN AFRICA

#### D. Mostert 1

<sup>1</sup>CS Vet Veterinary Consultancy, Pretoria, South Africa

#### **Background and Objectives**

African Swine Fever virus is endemic in the southern parts of the African continent. This necessitates strict biosecurity measures need to be maintained to ensure that these farms remain free of the virus and other disease. The survey aimed to ascertain which biosecurity measures pig farming units have in place and evaluate the operational success thereof.

#### **Material and Methods**

The survey was done on 58 units, which including multisite operations and integrated businesses. The survey was done by a physical visit as well as a comprehensive questionnaire. The criteria included the physical biosecurity infrastructure, biosecurity risks in the surroundings, and the degree to which these measures were maintained daily. The assessment outcomes were ranked into four categories: low risk, acceptable risk, moderate risk, and high risk. Other characteristics such as health status, management years of experience, the age of the farm, and number of animals present, were also noted.

#### Results

While the region is not considered pig-dense, the units tend to be in areas where there are multiple units, with 63% of units having at least one other unit within 25km. Of these, half were within 5km of an informal pig trading market. While 97% of units were only accessible through a shower-in system, 61% of these had errors in the operational protocol, decreasing the benefit of good infrastructure. 88% of the unit's feed was delivered over the fence and contained no animal products. 59% of the units had no disinfection protocol for incoming goods or equipment. While high-health farms scored better on average, the age of the farm and years of management experience did not alter the risk significantly.

#### **Discussion and Conclusion**

While the general infrastructure is good, only relying on infrastructure to ensure biosecurity is not enough – practical biosecurity protocols need to be practiced consistently. Implementing monitoring systems to ensure compliance with protocols must be prioritized and an ongoing risk assessment process is needed to ensure biosecurity risks are managed well.

MIS - Miscellaneous and Clinical cases

#### MANAGEMENT AFTER ASF OUTBREAK IN A FINISHER FARM: A REAL STORY

<u>P. Poolperm</u><sup>1</sup>, N. Phetphloy <sup>2</sup> <sup>1</sup>Professional Training and Consultant, Co.LTD., Thailand <sup>2</sup>Porg Co.,LTD. Bangkok, Thailand

#### **Background and Objectives**

African Swine Fever (ASF) is a highly contagious viral disease causing highly economic losses to the pig industry. ASFv is highly tolerated in environment after its outbreak in a farm, making it more challenging to eradicate from affected farms. Total depopulation on an infected farm is the only means of controlling the outbreak. However, for small-scale pig farms, the goal is not only to eradicate the virus but also to safely restock and restart their operations. This study aims to evaluate the effectiveness of biosecurity measures implemented on a pig farm that had previously experienced an ASF outbreak, focusing on the successful reintroduction of pigs and the resumption of pig farming activities.

#### **Material and Methods**

The study farm consisted of three open-housing buildings, prior to the outbreak, each building was raised 500 finisher pigs. Upon detection of clinically ill pigs, the farm owner initiated a complete depopulation of all pigs. Healthy pigs were tested using PCR, and all negative pigs were transported to a slaughterhouse under strict biosecurity protocols. Clinically ill pigs were euthanized and buried away from the farm. The farm underwent extensive renovation, including paving new solid floor, the construction of a closed-tunnel system, and thorough disinfection at least three times. Environmental swabs from all buildings were tested for ASFV, and after negative results were obtained, the farm owner introduce nursery piglets from an ASF-negative farm. However, the number of pigs per building was reduced to 400 (1.2 pigs/sq.m). Since the reintroduction of pigs 1.5 years ago, no further clinical cases of ASF have been observed.

#### Results

Following the farm's renovation and the reintroduction of pigs, the performance of finisher pigs has significantly improved. Average daily gain (ADG) ranges from 843-999 g/d, feed conversion ratio (FCR) is between 2.35-2.50, and mortality rates are below 2%.

#### **Discussion and Conclusion**

The biosecurity measures implemented on this farm, including the conversion of open-housing buildings to a closed-housing system, rigorous disinfection procedures, restricted worker access to the buildings, and reduced stocking density, played a crucial role in the successful reintroduction of pigs following an ASF outbreak. These findings underscore the importance of comprehensive biosecurity measures in controlling ASF and facilitating the recovery of affected farms.

MIS - Miscellaneous and Clinical cases

### ASSESSMENT OF RISK FACTORS FOR SPREADING OF AFRICAN SWINE FEVER IN BACKYARD PIG HOLDINGS IN THE BELGRADE CITY AREA

<u>B. Kureljušić</u><sup>1</sup>, B. Savić<sup>1</sup>, B. Milovanović<sup>1</sup>, N. Jezdimirović<sup>1</sup>, D. Glišić<sup>2</sup>, J. Prodanov Radulović<sup>3</sup>, V. Milićević<sup>1</sup> <sup>1</sup>Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia <sup>2</sup>Institute of Veterinary Medicine of Serbia, Janisa Janulisa 14, 11000 Belgrade, Serbia <sup>3</sup>Scientific Veterinary Institute "Novi Sad", Serbia

#### **Background and Objectives**

African swine fever (ASF) was first detected in Serbia in 2019 in the city Belgrade. The first outbreak was resolved by implementing a stamping out policy. The second occurrence was recorded in June of 2023. Within a wave of ASF spread westward in the county included more infected holdings. In this investigation, we assessed the main epidemiological risk factors for ASF persistence and in the Belgrade area.

#### Material and Methods

In total 89 backyard pig holdings were visited and epidemiological investigations were carried out using questionnaires. All the investigated pig units were backyards or just a few small-scale farms.

#### Results

The most holdings did not satisfy even the minimum of biosecurity measures. The average number of pigs per holding was 3,07 pigs. Most of the holdings had only fatteners (59,55%) and usually were used for self-consumption. The 61,8% of holdings were located in the hunting area or close to 1000 meters from the hunting ground. Close proximity to other holdings with pigs at a distance of up to 100 meters was recorded in 60,67% of holdings. Other species at the holdings included usually dogs, sheep, cats etc. were present in 76,4% of holdings. Moving of pigs was noticed in only 11,24%, whereas moving of people in 53,93% of holdings. Home slaughtering was practised in 87,64% of holdings, usually for their own consumption. Fences were present in 57,3% of holdings. Regarding feeding, usually, the pigs were fed extensively with swill in 57,3% of cases, with grains from local grounds in 91,01% and with freshly mowed grass in 64,04%.

#### **Discussion and Conclusion**

During the epidemiological investigations, different answers from the owners about possible sources of virus introduction were obtained. Usually, one of the most important factors is human activity which can transmit the virus over a very long distance. On the other hand as a source of infection, other animals may have possible roles in mechanic transmission for example: stray dogs and cats, rodents, birds, and insects. In most cases, the source of infection remains unknown. Different types of traditional extensive pig farms with no or very low biosecurity in combination with human activity play pivotal roles in ASF spreading in Belgrade.

NUTR - Nutrition

#### DIVERSE, FIBROUS CREEP DIET INCREASES THE AMOUNT OF EATERS DURING SUCKLING PERIOD.

<u>K. Goris</u><sup>1</sup>, M. Decoux <sup>1</sup>, C. Vanderhaeghe <sup>1</sup>, J. De Laat <sup>1</sup>, G. Mantovani <sup>1</sup>, R. Faris <sup>2</sup> <sup>1</sup>Cargill Animal Nutrition, Rotterdam, The Netherlands <sup>2</sup>Cargill Animal Nutrition, Elk River, MN

#### Background and Objectives

Piglets that eat solid feed early during suckling period tend to develop better and have higher post-weaning performances. However, we know that a considerable number of suckling piglets are not consuming creep feed before weaning. Evidence supports that diversified diets stimulate the number of eaters. In our study we aimed to evaluate the effect of a highly fibrous and diverse creep feed (NN, Neopigg Nutriplay®) against a conventional creep feed (CON) on the prewean feed intake of individual piglets within a litter.

#### Material and Methods

Litters of 18 gilts (TN70 x TNselect) were equally distributed and randomly allocated to one of two treatments (n= 9 litters) in a randomized complete block design. Block was considered paired sows, whose litters were swopped daily to minimize the effect of sow milk yield on experiment outcome. The experimental diets were provided from second day of age (d 0) until weaning (d 19) and using two pan feeders to guarantee ad libitum access to feed. Individual body weights (d0 and d19) and number of eaters per litter (d9 and d16) were registered. Data were analyzed using the Ime4 package of R 3.4.1 with the fixed effect of treatment and random effect of block.

#### Results

There were, 10% (P=0.0062) and 34% (P<0.0001) more eaters in NN compared to CON treatment, at d9 and d16 of the trial, respectively. Although total feed intake was slightly increased, no significant differences between the 2 treatments were seen in total litter intake nor in daily growth until weaning. However, the NN treatment had more pigs weighing > 5.5 kg at weaning (84%), compared to CON (75%) (P=0.079).

#### **Discussion and Conclusion**

In conclusion, this study showed that fibrous and diverse creep feed increases the amount of eaters before weaning significantly and gives a tendency for lower amount of light (< 5,5kg) weaned piglets. These data suggest that diversified feed is an innovative method to help reduce feed neophobia of piglets around weaning.

#### PAR - Parasitology and Parasitic Diseases

#### **REDUCTION OF ASCARIS SUUM IN ORGANIC PIG FARMS - A FIELD STUDY**

#### H. Nienhoff <sup>1</sup>, S. Döring <sup>2</sup>

<sup>1</sup>LUFA Nord-West, Swine Health Service, Ammerländer Heerstr. 123, D-26129 Oldenburg <sup>2</sup>Chamber of Agriculture Lower Saxony, Oldenburg, Germany

#### **Background and Objectives**

Control of ascaris sum is one of the most challenging issues in organic pig farming. A high burden of roundworms can be an animal welfare as well as economic issue. Therefore, special attention must be paid to timely, regular and targeted prevention and control of roundworms.

#### **Material and Methods**

In this study five organic pig farms were accompanied by the swine health service from 2019 to 2021. In the first step an individual management und treatment plan was developed for each farm, followed by three farm visits per year to check the implementation. Per visit five manure samples were taken and checked for ascaris suum by flotation. In addition the slaughter findings on the livers were evaluated also three times / year. The results were discussed with the farmer and adaptations were made.

#### Results

Measurement measures were eg. changing to AIAO (Operation / Manure Axis / Animal Group), dividing age groups, intense manure removal, cleaning and disinfecting and strategic deworming. The manure samples were mostly negative on ascaris sum (96%). Evaluation of liver findings (milk spots) at slaughter showed a reduction after implementation of management measures from 98% to -16%. Highest rate of slaughter findings was 59,8%, lowest 0%. Only three of the five farms were able to implement the management measures. All three farms, who implemented the measures had, a high reduction of liver findings at slaughter (98%, 96%, 80%). A handbook for the management of ascaris sum in organic farms was written and released.

#### **Discussion and Conclusion**

Ascaris sum is a big problem in organic farms. Measurement measures can reduce the burden very well. Farmers are often not willing or able to implement management measures. Effective management measures in this study were: AIAO (Operation / Manure Axis / Animal Group), regular manure removal, separating different groups, after moving animals out: manure removal, washing and disinfecting, intense and regular strategic deworming. To measure the success of acaris sum reduction slaughter findings are much more suitable than manure samples.

**REP** - Reproduction

#### BIRTH DYNAMICS IN HYPERPROLIFIC SOWS WITH AND WITHOUT PARTURITION INDUCTION

H. Haller 1, L. Wahl 1, J. Kauffold 1

<sup>1</sup>Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, University of Leipzig, 04103 Leipzig, Germany

#### **Background and Objectives**

Induction of parturition with PGF2 $\alpha$  is a common procedure usually being done on gestation day 114. Earlier induction may cause the delivery of weak/ less viable piglets. Concomitant to an increased litter size in hyperprolific sows, gestation length has increased to  $\ge$  116 days on many farms, bearing the risk of a "too early" induction if done on day 114 of gestation. This study was aimed to address this issue.

#### Material and Methods

The study was done on a farrow-wean farm including 210 hyperprolific Yorkshire x Landrace sows allocated to three groups: A) spontaneous farrowing on gestation day 114 (n = 70); B1) no farrowing until PM of gestation day 114 treated with 2 ml cloprostenol farrowing 12 – 24 h (n = 63) or B2) farrowing >24 h (n = 77) post cloprostenol. If necessary, manual obstetrics (MO) were applied. Duration of parturition, piglet expulsion intervals, MO and litter characteristics were recorded.

#### Results

Litter sizes were higher in A than in B2 sows (21.2 vs. 18.9 piglets; p = 0.006; for B1 19.5), as was litter weight (24,104 g vs. 21,210 g; p = 0.007; for B1 22,273 g). Piglet birth weights, piglet expulsion intervals and stillbirth rates did not differ significantly between groups. Parturition took longer in A and B1 than in B2 sows (269.7, 260.9 and 217.0 min, respectively; p < 0.03). The percentage of births requiring MO was lower in B2 (64.9%) than in A (81.4%, p = 0.025; for B1 74.6%).

#### **Discussion and Conclusion**

Interestingly, larger litters were born earlier than smaller litters but were not associated with reduced piglet weights or increasing stillbirth rates. A comparison of the results toward dynamics of spontaneous vs. induced births is somehow biased due to different litter sizes known to effect e.g. MO and stillbirth rate. Since both parameters were similar in spontaneously farrowing and birth induced sows, results of this study indicate that a birth induction protocol as employed in this study, i.e. cloprostenol PM on gestation day 114, is appropriate in hyperprolific sows.

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

#### WOUNDS ON UMBILICAL OUTPOUCHINGS OF SLAUGHTER PIGS

<u>T.B. Jensen</u><sup>1</sup>, B.M. Jørgensen <sup>2</sup>, A. Birch <sup>3</sup>, J.S. Pelck <sup>1</sup>, N.P. Nielsen <sup>4</sup>, H.E. Jensen <sup>2</sup> <sup>1</sup>SEGES Innovation, Axeltorv 3, Copenhagen, Denmark <sup>2</sup>University of Copenhagen, Ridebanevej 9, Copenhagen, Denmark <sup>3</sup>Ø-Vet A/S, Køberupvej 33, Næstved, Denmark <sup>4</sup>Danish Agriculture and Food Council, Axeltorv 3, Copenhagen, Denmark

#### **Background and Objectives**

In Denmark, pigs with wounds on umbilical outpouchings (UOs) are not fit for transport. These pigs are euthanized which affects sustainability and the farmer's economy. We investigated if clinical signs registered before transport were associated with the pathological severity and risk of rupture of the wounds.

#### **Material and Methods**

The study included 87 slaughter pigs with wounds up to 4 cm in diameter on UOs. For each pig, we registered: shape of the UO (spherical, elongated), location of the wound (ventral, not ventral), the size of the wound (cm) and the depth of the wound (superficial, not superficial). The pigs were transported to the abattoir in separate compartments (five in each) and for maximum 2 hours. The UOs were sent for pathological examination at University of Copenhagen. The distance between the surface of the wound and the abdominal cavity was measured, which may be indicative for the risk of wound rupture.

#### Results

The mean distance from the wound to the abdominal cavity was 2,27 cm, and 95 % of the pigs had a distance of at least 1 cm. One wound had ruptured causing access to the abdominal cavity. Results showed that the size of the wound was associated with the distance from the wound to the abdominal cavity. This association was dependent on the shape of the UO. If the UO was elongated (p=0,22), no statistically significant association was found. However, if the UO was spherical the association was statistically significant (p=0,035) – with the distance being shorter as the wounds became larger.

The shape of the UO could indicate the pathological morphology of the UOs (hernia, cyst, or a combination). The probability of an UO being a hernia when the UO was elongated was 86 %, whereas the probability was 58 % when the UO was spherical.

#### **Discussion and Conclusion**

In conclusion, it seems that most wounds on UOs were not at risk of rupturing during the given transportation conditions. However, histological examinations of the wounds and UO tissue need to be carried out for further verification. The results may be used to reassess the risk of transporting pigs with wounds on the UO.

#### VVD - Virology and Viral Diseases

### PORCINE CIRCOVIRUS 2 (PCV2) SURVEILLANCE – LONGITUDINAL SAMPLING TO ASSESS PCV2 FLOW DYNAMICS VIA DIAGNOSTICS AND PERFORMANCE DATA

<u>G. Greaves</u><sup>1</sup>, C. Werth<sup>1</sup>, C. Lichty<sup>1</sup>, B. Dewolf<sup>1</sup>, M. Misener<sup>1</sup> <sup>1</sup>South West Ontario Veterinary Services, Stratford, Ontario, Canada

#### **Background and Objectives**

In Canada, there have been recent cases of porcine circovirus diseases (PCVD) diagnosed on farm, despite utilizing commercial Porcine circovirus 2 (PCV2) vaccines. The cause of PCVD is likely multifactorial, including concomitant infections or vaccine application errors. This study used a longitudinal sampling approach to monitor PCV2 throughout production flow and examine its relationship with performance parameters.

#### Material and Methods

In 2021, PCV2 was monitored longitudinally via PCV2 PCR testing across twenty commercial pig farms ranging from 400-2950 sows. The genetics, PCV2&3 vaccination status, PRRS health status, and baseline production performance varied amongst study farms. At each sow farm cohorts were created - defined as 4-weeks of production - and 1-week of pooled processing fluids (PF) were collected per cohort. Subsequently, in the nursery, pooled oral fluids (OF) were collected at 2- and 6-weeks post-entry for each cohort. At finishing, pooled OF were collected at 2-weeks post-entry. Production parameters were recorded for each cohort, during each phase of production. Each farm enrolled up to 13 cohorts.

#### Results

Sows were vaccinated for PCV2 in 10% of herds, while all herds vaccinated gilts. Across all cohorts, 31% of PF, 35% of early-nursery OF, 35% of late-nursery OF, and 52% of finishing OF were positive for PCV2 on PCR. Positive PCV2 PCR Ct values ranged from 25.1-36.8 for PF (mean 32.3), 24.6-36.9 for early-nursery OF (mean 32.2), 19.5-36.8 for late-nursery OF (mean 30.8), and 18.4-36.9 for finishing OF (mean 30.0). In cohorts with samples from all phases of production, 10% remained PCR positive for PCV2 from birth to finishing. PCV2 PCR positive Ct values were significantly different across flows for sow and finishing sampling timepoints (p<0.05). Reproductive parameters, nursery gain and mortality, and finishing mortality have remained stable in all herds and no clinical signs of PCVD were reported thus far.

#### **Discussion and Conclusion**

Longitudinal PCV2 sampling of Canadian swine herds illustrated substantial variability in PCV2 virus throughout production, despite stable performance parameters. These data suggest that monitoring PCV2 over time may provide context in herds experiencing viral instability; however, PCR Ct values alone were insufficient at predicting clinical instability and PCVD in this study.

WEL - Animal Welfare and Ethology

CONSEQUENCES OF NO TAIL DOCKING IN PIGS IN BRAZIL II: HUMAN-ANIMAL INTERACTION AND PRODUCTIVE PERFORMANCE.

<u>J. Rego Ribas</u><sup>1</sup>, J. Grajales-Cedeño<sup>1</sup>, I. Gianeis<sup>1</sup>, V. S. Sobral<sup>2</sup>, M. Paranhos Da Costa<sup>1</sup> <sup>1</sup>UNESP Jaboticabal <sup>2</sup>Agroceres PIC

#### **Background and Objectives**

A norm from the Brazilian Ministry of Agriculture, Livestock and Supply recommends avoiding piglets' tail docking, but it allows doing it by cutting off the final third of the tail until the third day of life. This study aimed to evaluate the impact of piglets' tail docking in fear toward humans and performance throughout the growing and finishing phases.

#### **Material and Methods**

A total of 854 piglets of synthetic Pietran line were randomly located into two treatments: tail-docking the third final part of the tail (DT, n = 442) and no tail docking (NDT, n = 412). The human-animal interactions were scored according to Welfare Quality® methodology by scoring (1) when curious behavior and absence of fear was shown by the pigs, and (2) when more than 60% of them showed signs of fear (hugging at the bottom of the pen, running away from the observer, and vocalizing). The pigs' body weights were recorded in the nursery and finishing phases. Contingency plans were adopted when cases of cannibalism were detected. Human-animal interactions were assessed by using the chi-square test and body weight by using linear mixed models.

#### Results

In the nursery phase, NDT piglets, which did not require the implementation of the contingency plan, demonstrated fewer fear reactions during the interactions with humans than DT piglets (41.30% vs. 58.00%, respectively; p<0. 05). In contrast, NDT piglets which required the adoption of the contingency plan exhibited higher levels of fear (44.44%) than did DT piglets (0%; p<0.05). In the finishing phase, there were no significant differences in the fear reactions between the treatments (p>0.05), regardless of whether the animals needed a contingency plan. The body weight of DT and NDT piglets in the farrowing phase ( $2.50\pm0.02$  and  $2.49\pm0.02$  kg, respectively), nursery ( $24.6\pm0.43$  and  $25.0\pm0.43$  kg, respectively) and finishing ( $109.0\pm0.92$  and  $110.0\pm0.94$  kg, respectively) did not differ (p>0.05).

#### **Discussion and Conclusion**

This study showed that tail docking can affect the fear of piglets, especially during the nursery phase, without affecting their performance. Fear response is an important indicator to improve human-animal interactions on the farm and during transportation.



# CHAIRED POSTER PRESENTATIONS



#### VIROLOGY AND VIRAL DISEASES

#### VVD-CP-01

VVD – Virology and Viral Diseases

### RECOMMENDATIONS FOR SAMPLING AND SHIPMENT OF MATERIALS FOR PCR-TESTS FOR SURVEILLANCE OF PRRS-VIRUS IN DANISH HERDS

E.O. Nielsen 1, L.K. Kvisgaard 2, C.K. Hjulsager 3, A. Droce 4, L.E. Larsen 5

SEGES Innovation P/S, Livestock, Axeltorv 3, DK-1609 Copenhagen V, Denmark

<sup>2</sup>University of Copenhagen, Institute for Veterinary and Animal Sciences, Stigbøjlen 4, DK-1870 Frederiksberg C, Denmark

<sup>3</sup>Statens Serum Institute, Copenhagen, Denmark

<sup>4</sup>L&F Diagnostic laboratory, Danish Agriculture & Food Council, Denmark

<sup>5</sup>University of Copenhagen, Dpt. of Veterinary and Animal Sciences, Grønnegårdsvej 2, DK-1870 Frederiksberg C, Denmark

#### Background and Objectives

A program to reduce the prevalence of porcine reproduction and respiratory syndrome (PRRS) in Denmark was initiated in 2022. The efforts to eliminate PRRS-virus demands more RT-qPCR-tests for PRRSV using a variety of sample materials including non-invasive samples like Processing Fluid (PF), Tongue Tip Fluid (TTF), and oral fluid (OF). The aim of the project was to develop validated recommendations for optimal collection, storage and transport of the different sampling material for PRRSV detection to avoid false negative test results.

#### Material and Methods

The influence of storage temperature and time on the decay of PRRSV RNA in OF and PF was tested by spike of negative sampling material with a PRRSV-1 cell culture isolate. The spiked materials were stored at a combination of different temperatures and times and finally extracted viral RNA was tested by RT-qPCR to monitor the impact of the different processes.

#### Results

The results showed that storage of serum, OF and PF at 4°C; -20°C and -80°C for up to 30 days had limited impact on the Cq value of the RT-qPCR. One round of freeze-thaw had limited impact of the Cq value whereas 2-3 rounds had a more pronounced negative effect especially on OF samples. In contrast, storage at room temperature (RT) for just one day resulted in a significant increase in Cq values for all sampling materials, showing a severe impairment of detection of PRRS-virus.

#### **Discussion and Conclusion**

Correct handling of samples for PCR analyses are essential to avoid false-negative test results. Based on the results of this study we recommend that PF and TTF samples should be frozen at -20 °C immediately after sampling and then be thawed at a temperature not exceeding 4-8 °C before the fluid is collected. Previously, we also detected a relative pronounced negative impact of storage of serum at RT and therefore we recommend keeping all samples for RT-qPCR cooled as soon as possible after sampling. Samples should also be kept cooled during transportation to the laboratory and therefore styrofoam cool box and ice packs are advised for all types of material.

#### VVD-CP-02

VVD – Virology and Viral Diseases

#### REPEAT OFFENDERS: PRRSV-2 CLINICAL RE-BREAKS FROM A WHOLE GENOME PERSPECTIVE

#### J. Baker <sup>1</sup>, A. Rovira <sup>2</sup>, K. Vanderwaal <sup>1</sup>

<sup>1</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA <sup>2</sup>Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA

#### **Background and Objectives**

Some sow farms in the USA experience repeated clinical outbreaks caused by PRRSV-2 viruses with near-identical sequences. The ability of a very closely related virus to induce a second clinical outbreak of disease (a "re-break") suggests that the virus has changed, allowing it to reinfect a herd with preexisting "homologous" immunity. The objective of this study is to generate and characterize PRRSV whole genomes from viral isolates associated with clinical re-breaks on single sow farms and to examine amino acid changes that could be related to viral immune escape in field settings.

#### **Material and Methods**

A production system shared weekly abortion counts, which were evaluated using statistical process control methods, and weekly processing fluid qPCR data to identify clinical PRRSV-2 breaks on single sow farms. If a subsequent clinical break took place within 3-12 months and the ORF5 gene sequence obtained from both breaks was ≥97% similar, it was considered a "clinical re-break". Upon fulfilling these criteria, viral isolates from these defined clinical re-breaks were utilized for whole genome sequencing.

#### Results

Suitable viral isolates were available for 13 clinical re-break events. The median number of days between re-breaks was 213.5 days (IQR = 159.8-230.8). ORF1a/1b were the most variable, with median p-distances (between re-break pairs) of 1.85% (IQR: 0.53-4.23%) and 2.25% (IQR: 0.45-8.28%), respectively. ORF5 was the most variable structural protein encoding gene, with a median p-distance of 1.60% (IQR: 0.675-1.98%). Re-break pairs had several amino acid positions within GP5 where non-synonymous mutations occurred more frequently than expected. Additionally, proteins E and GP2-4 (encoded by ORFs2-4), included numerous sites with frequent non-synonymous mutations within re-break pairs, though no expected rate could be calculated outside of ORF5.

#### **Discussion and Conclusion**

Overall, our analysis identified repeated clinical outbreaks of PRRSV attributable to closely related, yet distinct viral variants. Sites identified in this study are consistent with previous work, including amino acid positions undergoing positive selection or those associated with increased resistance to neutralization by antisera generated against a parent or vaccine strain. To our knowledge, this is amongst the first evidence that sites associated with immune escape experimentally also occur in field scenarios where immune escape is suspected.
#### VVD – Virology and Viral Diseases

## A DECADE-LONG RETROSPECTIVE STUDY ON PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) ASSOCIATED FACTORS TO TIME-TO-STABILITY AND TIME-IN-POSITIVE IN UNITED STATES BREEDING HERDS

X. Yue 1, M. Kikuti 1, M. Melini 1, C. Corzo 2 1 University of Minnesota 2 Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

#### **Background and Objectives**

Porcine epidemic diarrhea virus (PEDV) was first identified in the United States (US) in April 2013. The epidemic period lasted for approximately 1.5 years, transitioning to endemic levels. Little published work on how long a sow herd remains PEDV-positive (i.e., time-in-positive, TIP) and the time needed to achieve a stable status (i.e., time-to-stability, TTS). The objective of this study was to estimate the PEDV TIP and TTS in the US breeding herds and assess the associated recorded factors.

## Material and Methods

PEDV monitoring data originating from 1028 US herds between May 1, 2013 and June 30, 2023 were obtained from the Morrison Swine Health Monitoring Project. TIP was calculated as the number of weeks that a herd stayed PED status 1 (positive unstable); TTS as the number of weeks in status 1 before changing to 2 (undergoing elimination) or 2fvi (ongoing gilt field virus exposure). Both status 2 and 2fvi herds required to be absent of clinical signs and no detectable virus for a minimum of 4 weeks. The association of TIP and TTS with recorded factors were assessed using mixed-effects Cox proportional hazards models in both epidemic periods.

#### Results

This study included 466 PEDV breaks, in which 236 breaks corresponded to the epidemic period (from May 1, 2013 to December 31, 2014) and 230 breaks to the endemic period (January 1, 2015 to June 30, 2023). Overall, the median TIPs in the PEDV epidemic and endemic period were 25 and 17 weeks, respectively. The median TTS in the epidemic period was 24 weeks, and 14 weeks in the endemic period. Cox model showed that during the endemic period, herds that experienced 2 breaks stabilized sooner than herds that never broke (hazard ratios 2.79). Whether the site experienced a PED outbreak within the previous 6 months was not associated with either TIP or TTS during either study periods.

#### **Discussion and Conclusion**

The US industry has effectively been able to attack PEDV as herds are getting cleaned faster. Such information is timely as the industry considers whether this pathogen can be eradicated from the US swine population.

VVD – Virology and Viral Diseases

## EFFECT OF STABILIZERS ON THE DETECTION OF SWINE INFLUENZA A VIRUS (SWIAV) IN SPIKED ORAL FLUIDS AND FIELD SAMPLES

J. Stadler 1, K. Grau 1, M. Ritzmann 1, K. Lillie-Jaschniski 2, A. Graaf-Rau 3, T. Harder 3

<sup>1</sup>Clinic for Swine at the Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-Universität München, Oberschleißheim, Germany <sup>2</sup>Ceva Tiergesundheit GmbH

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut (FLI), Suedufer 10, 17493 Greifswald-Insel Riems, Germany

#### **Background and Objectives**

Previous studies have highlighted the suitability of group samples for swIAV monitoring. However, nasal swabs still remain the preferable sampling material for identification of swIAV subtypes. The aim of the present study was to evaluate if detection and subtyping of swIAV can be enhanced in group samples by the addition of stabilizers.

#### **Material and Methods**

Oral fluids were collected from influenza negative pigs and spiked with swIAV isolates of subtypes H1avN2 or H1pdmN2 to viral load measured by RT-qPCR of Ct-25 and Ct-32, respectively. Replicas of spiked samples were stored either at 4 °C or at room temperature for 72h, 7 days and 14 days. Additionally, two different saliva stabilizers were added to spiked oral fluid specimens at a ratio of 3:1 and compared to control samples. The stabilizers used were PrimeStore® MTM (Longhorn Vaccines & Diagnostics LLC) and the Sigma-Virocult® MW950S (Check Diagnostics GmbH, Westerau, Germany). In the frame of additional field investigations environmental samples were obtained from a swIAV positive farm and suspended in the aforementioned media. Samples were analyzed for IAV genome and samples with ct-values ≤32 were subtyped by multiplex RT-qPCR.

## Results

At 14 days spiked oral fluid samples suspended in Sigma-Virocult® were still positive regardless of the isolate and storage temperature. In contrast, PrimeStore® suspended samples spiked with a low viral load of H1pdmN2 were negative when stored at room temperature from 72h onwards and control samples spiked with low viral loads from 72h onwards regardless of the isolate and storage temperature. Ct-values were significantly lower in samples suspended with Sigma-Virocult® and PrimeStore® compared to control samples (p<0.01). In general, Ct-values were significantly (p<0.01) lower in samples stored at 4 °C compared to samples stored at room temperature. Under field conditions 9/10 environmental samples suspended with Sigma-Virocult®, 4/10 with PrimeStore® and 4/10 control samples yielded RT-qPCR positive results.

#### **Discussion and Conclusion**

Addition of stabilizers improved detection and subtyping of swIAV over time under both laboratory and field conditions. Sigma-Virocult® resulted in the highest percentage of swIAV positive samples. In addition, storage of samples under cool conditions is highly recommendable to improve molecular swIAV diagnostics.

VVD – Virology and Viral Diseases

## EVOLUTION AND ATTENUATION OF PORCINE EPIDEMIC DIARRHEA VIRUS FOLLOWING IN VITRO AND IN VIVO PROPAGATION

P. Jermsutjarit <sup>1</sup>, S. Mebumroong <sup>1</sup>, P. Watcharavongtip <sup>1</sup>, H. Lin <sup>2</sup>, A. Tantituvanont <sup>3</sup>, K. Kaeoket <sup>4</sup>, P. Piñeyro <sup>5</sup>, <u>D.</u> <u>Nilubol</u> <sup>1</sup>

<sup>1</sup>Swine Viral Evolution and Vaccine Development Research Unit, Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>MSD Animal Health Innovation Pte Ltd, Singapore

<sup>3</sup>Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

<sup>4</sup>Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand

<sup>5</sup>Veterinary Diagnostic Laboratory, Veterinary Diagnostic and Production Animal Medicine, Iowa State University, IA, USA

### Background and Objectives

Feedback is a common practice in swine production where sows are inoculated with macerated intestinal material from diarrheic piglets to generate maternal antibody against Porcine epidemic diarrhea virus (PEDV). To maintain a constant supply of this material, colostrum deprived piglets are often inoculated with material derived from sick piglets and then sacrificed. This practice of inoculating PEDV in piglets to regenerate feedback material might influence the genetic evolution and attenuation of PEDV. The study was conducted to evaluate evolutionary rate and attenuation following serial in vitro and in vivo propagation.

#### Material and Methods

PED-JPFP0-PJ, Passage 0 (P0), was isolated from infected pigs and serially subcultured in Vero cells for 5 consecutive passages, designated P1-P5. P0, P2 and P5 were further orally inoculated into 3-day-old piglets. At 24 hours post inoculation, intestines of each passage (F1), were collected, and subsequently sub-passaged in piglets for 2 additional passages (F2-F3). Virus titration, VH:CD ratios, and immunohistochemistry were evaluated. S and ORF3 genes were genetically characterized.

#### Results

The results demonstrated that virus titer and virulence were negatively correlated with increased passages, both in vitro and in vivo. Significantly increased substitution rate was observed in higher passages, especially P5F3 in which seven aa changes at positions 223, 291, 317, 607, 694, 1,114 and 1,199, with reduced N-linked glycan were observed. These changes are associated with receptor bindings in which could involve in attenuation. In addition, the evolutionary rate of S gene was higher than that of ORF3.

### **Discussion and Conclusion**

The serial passage of PEDV, both in vitro and in vivo, results in its genetic alteration, accelerating the rate of genetic change. The findings in this study suggest that aa changes in the RBD region caused by serial passage might decrease virus binding ability and viral virulence decrease at high passage levels. This might explain anecdotal observations that endemically infected herds become stable over time, possibly due to natural attenuation of the virus in hosts. Changes in the RBD region of S gene are an important factor in predicting virulence of SARS-CoV-2 and our findings may also be applied to other novel coronaviruses in future.

VVD – Virology and Viral Diseases

# DEVELOPMENT, EVALUATION, AND CLINICAL APPLICATION OF PRRSV-2 VACCINE-LIKE REAL-TIME RT-PCR ASSAYS

G. Rawal <sup>1</sup>, K. Krueger <sup>1</sup>, W. Yim-Im <sup>1</sup>, G. Li <sup>1</sup>, P. Gauger <sup>1</sup>, M. Almeida <sup>1</sup>, E. Aljets <sup>1</sup>, <u>J. Zhang</u> <sup>1</sup> <sup>1</sup>Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, United States

### **Background and Objectives**

For molecular detection of PRRSV, the initial step typically involves testing samples using a PRRSV screening PCR that targets the conserved genomic regions and can detect both wild-type and vaccine strains. To discern whether the detected PRRSV is a vaccine-like or wild-type strain, ORF5 sequencing via the Sanger method or whole-genome sequencing through next-generation sequencing (NGS) technology is generally conducted. However, NGS is costly and time-intensive, and ORF5 sequencing alone cannot rule out the presence of recombinant viruses.

#### **Material and Methods**

In this study, we developed and validated singleplex real-time RT-PCRs for specific detection of six PRRSV-2 MLV vaccines (IngelvacMLV, IngelvacATP, Fostera, PrimePac, Prevacent, and PRRSGard) and a 4-plex real-time RT-PCR (IngelvacMLV-Fostera-Prevacent-XIPC) including the internal positive control XIPC for detecting and distinguishing the three most commonly used vaccines in the USA (Prevacent, IngelvacMLV, and Fostera).

#### Results

The vaccine-like PCRs and the reference PCR (VetMAX<sup>™</sup> PRRSV NA&EU, Thermo Fisher Scientific) did not cross-react with non-PRRSV swine viral and bacterial pathogens. The limits of detection of vaccine-like PCRs ranged from 25 to 50 genomic copies/reaction. Through the testing of 531 clinical samples and comparison with the reference PCR, the diagnostic sensitivity, specificity, and agreement were in the respective ranges of 94.67-100%, 100%, and 97.78-100% for singleplex PCRs and 94.94-100%, 100%, and 97.78-100% for the 4-plex PCR. Further validation involved testing 45 PRRSV-2 isolates representing various genetic lineages/sublineages with both the screening and vaccine-specific PCRs, followed by verification through sequencing.

## **Discussion and Conclusion**

These vaccine-like PCRs provide an additional tool for molecularly characterizing PRRSV, particularly when used in conjunction with screening PCR and ORF5 sequencing. The combined screening and vaccine-like PCRs can not only discern the presence or absence of a vaccine-like virus but also aid in revealing the coexistence of wild-type and vaccine-like viruses in a sample. In instances where conflicts arise between vaccine-like PCRs (targeting the ORF1a region) and ORF5 sequencing results, it is advisable to flag the sample for additional characterization through NGS to investigate possible recombination. Moreover, these vaccine-specific PCRs prove valuable in determining whether samples collected post challenge in a vaccination/challenge study contain the vaccine virus.

VVD – Virology and Viral Diseases

## EFFECT OF PCV2 VACCINATION IN SOW'S REPRODUCTION PARAMETERS IN A SPF FARM

J. Zhang <sup>1</sup>, A. Zeng <sup>1</sup>, <u>R. Krejci</u> <sup>2</sup>, P. Mazerolles <sup>2</sup>, J. Carr <sup>3</sup> <sup>1</sup>Ceva Animal Health China <sup>2</sup>Ceva Animal Health France <sup>3</sup>Carrsconsulting.com

## **Background and Objectives**

Porcine circovirus type 2 (PCV2) is ubiquitous in domestic pig farms causing the economically-important porcine circovirus diseases (PCVD). Previous research had showed porcine embryos were susceptible to PCV2 infection, which might lead to embryonic death, stillbirth, or foetus mummification. The aim of this study was to evaluate the effect of sow vaccination on the reproductive parameters in a SPF farm.

#### **Material and Methods**

The trial was conducted in a genetic breeding farm with one week batch management in northern China. The farm was free of ASFV, PRRSV, ADV, CSFV, APP, M.hyo, FMDV and Mange, etc. The sows hadn't received any PCV2 vaccination before. No PCVD clinical symptoms had been observed in the farm, however viremia of PCV2 had been found in foetus and sequenced as PCV2d strain. Sows were vaccinated with Circovac<sup>®</sup> (inactivated PCV2 vaccine, 2ml/dose, I.M.) 3 weeks before farrowing from December 2019. Related reproductive parameters were recorded and compared with the historical record, corresponding to the 3 months previous to the beginning of the vaccination by T student test. In order to discriminate the genetic variation, three lines of purebred sows were conducted separate statistics and analysis.

#### Results

A statistically significant difference (p<0.05) was observed in born alive between vaccinated and non-vaccinated sows of Landrace (+0.9) and Duroc (+0.8). Sows of Landrace genetic had an increase of 0.6 born healthy (defined as >0.8kg BW) after vaccination, significant different (p<0.05). Mummified per litter showed reduction in Landrace (-0.09), Yorkshire (-0.05) and Duroc females (-1.79) after vaccination, significant different (p<0.05). Other parameters between vaccinated and non-vaccinated sows from different genetic lines were not statistically significant (p<0.05).

### **Discussion and Conclusion**

During this study, it can be observed an improvement of the sows' reproductive parameters after vaccination, especially in Landrace genetic animals which was corresponding to previous research.

VVD – Virology and Viral Diseases

## EFFECT OF THE IMPLEMENTATION OF RESPIPORC FLUPAN ON REPRODUCTIVE PERFORMANCE ON A PIG FARM IN THE UNITED KINGDOM

<u>T. Hill</u><sup>1</sup>, E. Velazquez <sup>2</sup>, C. Gale <sup>2</sup> <sup>1</sup>The George Veterinary Group, Malmesbury, UK <sup>2</sup>Ceva Animal Health Ltd, High Wycombe, UK

### **Background and Objectives**

Swine Influenza virus (swIAV) is one of the primary viral agents involved in the Porcine Respiratory Disease Complex (PRDC). Swine Influenza has been recognised as a disease which results in significant economic losses for pig farmers, primarily due to decreased average daily gain in infected pigs and reduced reproductive performance. The objective of this study was to assess the effect of vaccination against swIAV on a pig farm with low conception rate.

#### **Material and Methods**

A farrow to finish farm located in the South West of the UK had historically low reproductive performance, particularly in the gilt herd, including low conception rate, low piglet viability, low birthweights and early farrowing, but did not observe clear respiratory clinical signs in any of the production stages. Hemagglutination Inhibition Test was performed in blood samples from gilts and seroconversion for pandemic (H1N1) 2009 was observed in 90% of the animals tested. Conception rate figures were recorded 10 batches pre and 10 batches post implementation of a primary vaccination (2 doses, 3 weeks apart) with Respiporc FLUpan H1N1 (Ceva Animal Health, Libourne, France), and were analysed. No other vaccinations or management changes were introduced during this period.

#### Results

Pre vaccination conception rate in the overall herd, including both sows and gilts, was 79.4%, with a value of 77.9% in the gilt herd. Post vaccination, there was a significant increase in both overall conception rate, as well as gilt herd conception rate, with values of 93.5% (P<0.05) and 95% (P<0.05) respectively. Improvements in piglet viability and birthweights was also observed by the farmer, as well as a reduction in early farrowing.

#### **Discussion and Conclusion**

This study demonstrated that vaccination against swIAV in the breeding herd can have a positive effect on the reproductive performance on infected farms, with notable improvement in the gilt herd. Similar conclusions were observed in a large number of farms, where reproductive parameters such as return to oestrus, abortion and piglets born alive were improved after vaccination.

## VETERINARY PUBLIC HEALTH AND SUSTAINABLE PIG PRODUCTION

## VPH-CP-01

VPH – One Health: Veterinary Public Health and Sustainable Pig Production

# EFFECT OF IMPROVED FEED EFFICIENCY BY LAWSONIA INTRACELLULARIS VACCINATION ON PHOSPHORUS EMISSIONS IN PIG PRODUCTION

<u>R. Tabeling</u><sup>1</sup>, F. Von Und Zur Muehlen<sup>1</sup>, C. Renken<sup>1</sup> <sup>1</sup>Intervet Deutschland GmbH, MSD Animal Health, Unterschleißheim, Germany

## **Background and Objectives**

Phosphorus (P) is an essential element for pigs, especially for bone formation. Due to its ability to accelerate the eutrophication process, upper P limits are defined for German agriculture and pig farms must fulfil an individual nutrient management plan. The aim of this report was to illustrate the effect of the enhanced pig performance by Lawsonia intracellularis vaccination together with different feed rations (defined P content classes) on the P-excretion.

## **Material and Methods**

Performance data (i.e. feed conversion ratio; FCR) from field observations of 9 farms with a history of subclinical or clinical ileitis was recorded in non-vaccinated (NV) and vaccinated (PL) (intramuscularly/intradermally Porcilis®Lawsonia/ID; at 3-11 weeks of age) batches. NV batches, used as historical control, were compared to PL batches after implementing Lawsonia intracellularis vaccination to control ileitis. P excretion was calculated with the official manual from the Lower Saxony Chamber of Agriculture using four different P containing rations (g P/kg 88% dry matter: "universal feed" 5.1; "P reduced" 4.6; "P greatly reduced" 4.3; "P very greatly reduced" 4.1).

#### Results

Calculation of the P excretion in PL batches showed a mean reduction by 5.7-6.4% (P in feed 5.1-4.1 g) with a maximum of 17.1%, when compared to NV batches. On the farm (NV batch) with the most unfavorable FCR and assumed highest P content in feed (FCR 3.07; 5.1 g P/kg FM), P excretion reached 10.5 g/kg LW or 1012 g/96 kg weight gain, while the most favorable practical case (PL FCR 2.51; 4.1 g P/kg FM) could decrease P excretion to a level of 5.2 g P/kg LW or 503.9 g P/96 kg weight gain. The P excretion between these two scenarios has thus halved.

## **Discussion and Conclusion**

Under the conditions of this field observation, it has been shown that P excretion in pig farming can be reduced markedly by improving performance data with Lawsonia intracellularis vaccination and by modulating P content in the ration. This data suggests that Lawsonia intracellularis vaccination together with the feed design has a high potential for improving sustainability with particular interest for farms in very pig dense areas.

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## THE IMPORTANCE OF THE DRINKER MODEL IN WATER USE AND THE ENVIRONMENTAL IMPACT IN THE NURSERY PHASE

<u>G. Schlindwein Da Silva<sup>1</sup></u>, J. Cristani<sup>2</sup>, C. Pellis<sup>3</sup>, J. Bona Preisler<sup>3</sup>, N. Rampon Cendron<sup>3</sup>, N. Rigo<sup>3</sup>, S. Ribeiro Krasilchik<sup>1</sup>, S. Davi Traverso<sup>2</sup>

<sup>1</sup>Animal Science Postgraduate Program, Santa Catarina State University, Lages, Brazil <sup>2</sup>Department of Veterinary Medicine, Santa Catarina State University, Lages, Brazil

<sup>3</sup>College of Veterinary Medicine, Santa Catarina State University, Lages, Brazil

## **Background and Objectives**

The type of drinker used can directly affect the post-weaning water and nutritional fasting time. Furthermore, the type of drinker can lead to increased water wastage. Therefore, this study aimed to compare the effect of an ecological drinker with a swing drinker model on total water use, manure production, and growth performance of nursery pigs at a commercial farm.

## Material and Methods

1104 weaned piglets with an average age of 28.32 days and 8.2 kg were used in a 35-day study and divided into three experimental treatments: Treatment 1 (T1) - Ecological drinker with a constant water level model fixed in the center of the pen with two drinkers for 46 animals per pen. Treatment 2 (T2) - The same model as T1, but with four drinkers for 46 animals per pen. Treatment 3 (T3) - Swinging nipple drinker model with four drinkers for 46 animals per pen.

## Results

The water use in the drinkers (L/pig/day) was higher in T3 (7.27) than in T1 and T2, 2.69 and 2.55 (P<0.0001). There was no difference in water use in the feeders (P>0.05). Water: feed ratio was higher in T3 than in T1 and T2, 13.85, 6.4, and 6.02 L/kg (P<0.0001). Water: gain ratio was higher in T3 than in T1 and T2 (18.22, 8.46 e 7.75 L/kg) (P<0.0001). Regarding the manure production, T1 and T2 presented lower volumes than T3, 1.78, 1.69, and 6.59 L/pig/day (P<0.0001). Furthermore, the manure in T1 and T2 had a higher total solids content than T3 (5.81, 5.83, and 0.589% with P<0.0001). The coefficient of variation in body weight at 35 days was lower in T2 than in T3, 15.5 and 18.5% (P<0.05), and the same in T1, 17.8% (P>0.05). The average daily gain was higher in T3 (0.454 kg) than in T1 (0.432 kg) (P<0.05) and the same in T2 (0.434 kg) (P>0.05). There was no difference in the feed conversion ratio (P>0.05).

#### **Discussion and Conclusion**

The study found that using an ecological drinker led to lower total water use without affecting growth performance. This resulted in less water wastage and a lower volume of manure produced, reducing environmental impact and delivering more uniform weight per pen.

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### ALTERNATIVE TO ZNO TO ESTABLISH BALANCED INTESTINAL MICROBIOTA FOR WEANING PIGLETS

A. Juhász <sup>1</sup>, Z. Mayer <sup>1</sup>, K. Posta <sup>1</sup>, K. Tso <sup>2</sup>, <u>V. Molnár-Nagy</u> <sup>2</sup>, Z. Bata <sup>2</sup> <sup>1</sup>Hungarian University of Agriculture and Life Sciences, Institute of Genetics and Biotechnology, Gödöllö, Hungary <sup>2</sup>Dr. Bata Ltd, Ócsa, Hungary

#### **Background and Objectives**

A wide range of phytobiotic feed additives are available on the market claiming to have beneficial effects on the growth of the piglets and to promote the development of a balanced microflora. The present study investigated the effects of a phytobiotic-prebiotic feed additive (PPFA) containing curcumin, wheat germ, and chicory on the growth performance and on the intestinal microflora composition of weaning piglets.

#### **Material and Methods**

A total of 110 weaned piglets were involved in two consecutive experiments at Herceghalom, Hungary. Three treatments were applied: a negative control; a positive control, supplemented with 3.1 kg/t Zinc-oxide (ZnO); and a trial group, supplemented with 1 kg/t PPFA. Piglets were weighed individually, feed intake was recorded on a pen basis, and feed conversion ratio (FCR) were calculated for all treatment groups. Fecal samples were collected and analyzed for lactic acid-, coliform-, total aerobic and anaerobic bacteria. Additionally, the abundance of the bacterial communities of the fecal samples were measured by high-throughput sequencing on an Illumina MiSeq platform.

#### Results

There was no significant difference in the final body weight and average daily gain of the trial and positive control groups, and both groups showed significantly better results compared to the negative control. The FCR of the trial group was significantly improved compared to both controls. Both the PPFA and ZnO were able to significantly reduce the amount of coliforms after weaning. Even though ZnO reduced the amount of coliforms more efficiently than the trial feed additive, it also reduced the amount of potentially beneficial bacteria. In the trial and positive control groups, the relative abundance of Enterobacteriaceae decreased by 85% and 88% between 3 weeks and 6 weeks of age, while in the negative control group a slight increase occurred. Lactobacillaceae were more abundant in the trial group (29.98%) than in the positive (8.67%) or in the negative (22.45%) control groups at 6 weeks of age.

### **Discussion and Conclusion**

In summary, this study demonstrated that both the PPFA and ZnO are effective additives in reducing the relative abundance of Enterobacteriaceae, including E. coli responsible for post weaning diarrhea. The PPFAs - in contrast to ZnO - also promoted the development of a balanced gut system.

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## EFFECTS ON THE CARBON FOOTPRINT OF PORK BY AN INFECTION WITH LAWSONIA INTRACELLULARIS

<u>J. Gickel</u><sup>1</sup>, A. Abd El-Wahab<sup>2</sup>, J. Hankel<sup>2</sup>, C. Visscher<sup>2</sup> <sup>1</sup>University of Veterinary Medicine Hannover, Foundation, Science and Innovation for Sustainable Poultry Production (WING)

<sup>2</sup>University of Veterinary Medicine Hannover, Foundation, Institute for Animal Nutrition

#### **Background and Objectives**

The carbon footprint (CF) of pork is highly linked to the performance. As an infection with Lawsonia intracellularis (LI) could result in a worse performance the aim of this research was to determine its effects and those of a vaccination on the CF of fattening pigs in this specific phase.

#### **Material and Methods**

Based on a literature research the effects on performance (live weight, average daily gain [ADG], feed conversion ratio [FCR]) and mortality where monitored. Trials with complete data set were used to determine the CF (CO₂ eq per kg live weight) using Opteinics<sup>™</sup> (software application conducting life cycle assessments for farming animals). Therefore, it was assumed that all pigs got the same diet based on wheat, barley (both from Germany) and soybean meal (Brasil). Further consumptions (e.g. energy/fuels) and former/later stages of production where excluded.

#### Results

In total 19 trials (2004-2022) were included. One directly addressed the effects of LI infection, two the effect of a LI vacciantion under trial conditions and 16 the effect of a vaccination under field conditions. The group with an LI challenge showed a lower ADG and a higher FCR compared to its not infected control groups (mortality not displayed), resulting in a 9.3 % higher CF. Focusing on the effects of a vaccination under field conditions the ADG showed a mean increase of 2.6% compared to related control groups (epidemic infected); the mean FCR was 2.8% lower. The mean mortality decreased from 3.7% to 2.7%. The CF comparing vaccinated groups to epidemic infected groups showed reductions between 0.6 % and 30.1%.

#### **Discussion and Conclusion**

Focusing on trials from Europe only very few data deal with the performance of pigs in relation to the LI infection and/or vacciantion. Nevertheless, it could be concluded from this small amount of suitable publications, that the carbon footprint of the trial period increased markedly with a challenge or rather decreased following vaccination. Therefore, data management and health monitoring of the pigs is and has to become an even more important part to develop sustainable pork production.

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VPH - One Health: Veterinary Public Health and Sustainable Pig Production

### TRANSMISSION DYNAMICS MODELLING OF HEPATITIS E VIRUS IN COMPARTMENTALIZED PIG FARMS REVEAL BETWEEN BATCH AND BETWEEN COMPARTMENT TRANSMISSION AS POTENTIAL EFFECTIVE ON FARM CONTROL TARGET

M. Meester <sup>1</sup>, <u>T. Tobias</u> <sup>2</sup>, C. Valenzuela Agüí <sup>6</sup>, R. Hakze <sup>3</sup>, C. Guinat <sup>4</sup>, M. Bouwknegt <sup>5</sup>, E. Fischer <sup>1</sup>, F. Harders <sup>3</sup>, L. Du Plessis <sup>6</sup>, T. Stadler <sup>6</sup>, A. Stegeman <sup>1</sup>, W. Van Der Poel <sup>3</sup>

<sup>1</sup>Utrecht University, Faculty of Veterinary Medicine, Department of Population Health Sciences – division Farm Animal Health

<sup>2</sup>Royal GD, Deventer, The Netherlands & Utrecht University, Faculty of Veterinary Medicine, Dept. Population Health Sciences

<sup>3</sup>Wageningen Bioveterinary Research, Lelystad, the Netherlands

<sup>4</sup>IHAP, Université de Toulouse, INRAE, ENVT, 31076, Toulouse, France

<sup>5</sup>Vion Food, Boxtel, the Netherlands

<sup>6</sup>Department of Biosystems Science and Engineering, ETH Zürich, Basel, Switzerland

#### **Background and Objectives**

In Europe, consumption of pork products attributes mostly to hepatitis E virus (HEV) gt3 infections in humans (EFSA, 2017). On farm control strategies can contribute to control. As most pig farms are endemically infected, control must target transmission within farms. The hierarchical structure of pig farms calls for understanding transmission dynamics across pens and compartments as epidemiological units. We aimed to study and quantify transmission of HEV among pens of pigs within and between farm compartments, and between successive batches.

## Material and Methods

A longitudinal study was conducted in two farms, totalling 72 pens of fattening pigs, by weekly sample collection for two consecutive batches (40 weeks). One whole genome sequence for most pens was obtained. Next, phylodynamic multi-type-birth-death models were constructed to model transmission dynamics while taking into account the population structure. As such, quantification of the onset of infection, duration of infectiousness per pen and the effective reproduction number ( $R_e$ ) of transmission between epidemiological units could be established.

## Results

The onset of infection in pens started ~2.5 weeks after arrival of pigs in farm A and after 12 weeks in farm B. The mean duration of infectiousness per pen was 7.5 weeks (IQR 5-9 weeks). In compartments affected by HEV, all pens became infected, with a median  $R_e$  of 3.6 (95% highest posterior density interval (HPDI) 1.3–6.7). Moreover, between-compartment transmission occurred within farm A, shown by infections by multiple HEV strains, with median  $R_e$  of 0.13 (95% HPDI 0.03–0.28). In farm B, no between-compartment transmission was observed, as two out of three sampled compartments remained HEV negative in all batches.

### **Discussion and Conclusion**

Clustering of sequences from successive batches of the same compartment, and a lower evolutionary rate of HEV in the latest batch, suggest infection of pigs by older HEV strains that persisted in the environment, thereby indicating between-batch transmission. HEV quickly spread between pens within compartments. Spread between compartments and batches occurs less efficiently. HEV infection in compartments of farm B was presumably prevented by adherence to stringent biosecurity measures. These findings emphasize the critical role of biosecurity measures on the level of compartments and batches to reduce HEV infection within farms and contribute to on farm control of HEV.

VPH – One Health: Veterinary Public Health and Sustainable Pig Production

# PREVALENCE OF ANTIMICROBIAL-RESISTANT BACTERIA IN PIG BARNS AFTER CLEANING AND DISINFECTION PROCEDURES

<u>A. Perrucci</u>, M.C. Stella<sup>1</sup>, P. Robino<sup>1</sup>, P. Nebbia<sup>1</sup>, A. Scollo<sup>1</sup> <sup>1</sup> Department of Veterinary Science, University of Torino, Italy

#### **Background and Objectives**

Cleaning and disinfection (C&D) of livestock environments is gaining importance as a biosecurity measure. Due to a wide variety of germs in livestock environments, through C&D it is important to interrupt and minimize the transmission of pathogens from a group of animals to the next one. Considering that antimicrobial-resistance is one of the top-10 global public health threats, C&D are essential to reduce the selective pressure on microorganisms and their persistence in the environment. The aim of the study was to investigate the prevalence of antimicrobial-resistant microorganisms after C&D in swine barns, focusing on livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) and extended-spectrum  $\beta$ -lactamase producing Escherichia coli (ESBL-E. coli), as sentinels of antibiotic resistance.

### Material and Methods

Sixteen swine Italian herds (four environmental swabs per herd) were tested after C&D. The bacteriological investigation was conducted with a phenotypic identification using a selective medium supplemented with antibiotic and identified by MALDI-TOF MS analysis. Finally resistant Staphylococci underwent genotypic identification using mecA gene detection, while ESBL-E. coli was phenotypically confirmed through the combination disc test method according to EUCAST.

#### Results

After C&D, not only were LA-MRSA (12,5% on farms) and ESBL-E. coli (12,5%) detected, but also several other species of resistant Staphilococci (81,5%): S. sciuri (43,75%), S. saprophyticus (37,5%), S. xylosus (25%), S. cohinii (18,75%), S. equorum (12,5%), S. epidermidis (12,5%), S. haemolyticus (6,25%) Moreover, other resistant microorganisms were detected: Pseudomonas putida, Citrobacter freundii, Pseudomonas spp., Aerococcus viridans, Enterobacter spp..

#### **Discussion and Conclusion**

Some of the microorganisms identified in pig barns after C&D in the present study are considered reservoir of resistant genes and have an exceptional ability to accumulate resistance traits; many of these antibiotic-resistant bacteria have been reported as human pathogens capable of causing nosocomial infections. Their persistence on inanimate surfaces has been described as source of the infections, this should be of great concern not only in hospitals, but also in pig farms environments. In the context of the One Health approach, these results suggest the need to focus on the development of tailored C&D procedures in pig farms, as host of threatening pathogens also for the workers' health.

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

## CAN POOR DISINFECTION PRACTICES IN PIG FARMING PREDISPOSE THE ENVIRONMENTAL MAINTENANCE OF ANTIMICROBIAL-RESISTANCE?

<u>V. Vincenti</u><sup>3</sup>, C. Marra<sup>2</sup>, R. Novara<sup>3</sup>, C. Caruso<sup>1</sup>, S. Zoppi<sup>2</sup>, A. Scollo<sup>3</sup> <sup>1</sup>Azienda Sanitaria Locale CN1 (ASL-CN1), Cuneo, Italy <sup>2</sup>Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d' Aosta, Torino, Italy <sup>3</sup>Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Grugliasco, Italy

### **Background and Objectives**

Cleaning and disinfection procedures (C&D) in pig farms increasingly represent fundamental aspects of farm biosecurity. At the same time, it is known that pig farms may be colonized by several antibiotic-resistant bacteria that represent a threat both for animals and human health. Implementation of farm sanitation practices could play a key role in limiting the spread of these bacterial populations. Aim of this work was to evaluate the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum beta-lactamases/AmpC producing Enterobacteriaceae (ESBL/AmpC) in pig farm environment before and after C&D, and to investigate their possible association with dirty environment, as expressed by the Staphylococcus spp., Enterococcus spp. and total mesophilic counts.

#### **Material and Methods**

The evaluation was conducted in 20 fattening swine herds in North-Western Italy. During the trial, 5 environmental swabs for each farm were taken before and after C&D of the barns. MRSA and ESBL/AmpC were evaluated by a chromogenic screen, whereas the total mesophilic count was evaluated on plates incubated at 30°C; Staphylococcus spp. was incubated at 37°C; Enterococcus spp. at 42°C. Prevalences before and after C&D were compared using Fisher's exact test; comparison between bacterial counts and frequency of MRSA and ESBL/AmpC colonies were evaluated by Kuscall-Wallis test.

## Results

Prevalences of ESBL/AmpC and MRSA in dirty environments were 45 and 55%, respectively. After C&D, only prevalence of ESBL/AmpC decreased significantly (20%; p=0.0012). Prevalence of MRSA remained challenging (45%; p>0.05). Surprisingly, in absence of MRSA and ESBL/AmpC colonies, Enterococcus spp. count showed the highest values (p=0.018 and 0.025, respectively). In absence of MRSA, also Staphylococcus spp. and total mesophilic counts showed the highest values (p=0.015 and 0.008).

## **Discussion and Conclusion**

MRSA and ESBL/AmpC were investigated as considered sentinel bacteria for monitoring antimicrobial-resistance in livestock and humans. Their high prevalence in pig barns after C&D is challenging. In particular, C&D seemed to be unable to decrease the prevalence of MRSA. The findings suggest that tailored and more targeted C&D protocols might be needed in order to improve their efficacy and to counteract the antimicrobial-resistance spread.

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

## EFFECTS OF VACCINATING FATTENING GILTS AGAINST ENDOGENOUS GNRH (GONADOTROPIN-RELEASING-HORMONE) ON PRODUCTIVITY IN TWO COMMERCIAL FARMS IN BRAZIL

C. Da Silva <sup>1</sup>, E. Poleze <sup>2</sup>, C. Pazinato Dias <sup>3</sup>, <u>A. Aldaz</u> <sup>4</sup> <sup>1</sup>Universidade Estadual de Londrina, Londrina, Paraná, Brazil <sup>2</sup>Zoetis, Sao Paulo, SP, Brazil <sup>3</sup>Akei Animal Research, Fartura, SP, Brazil <sup>4</sup>Zoetis, Parsippany, NJ, USA

#### **Background and Objectives**

Immunizing gilts against GnRH suppresses temporarily the ovarian function, production of steroids and estrus, and undesirable associated behaviors. Vaccinated gilts are calmer and have a more consistent feed, resulting in higher market weights. This report summarizes the secondary benefits of vaccinating fattening gilts in Southern Brazil.

### Material and Methods

Two commercial finishing farms A and B, with 840 and 1170 fattening 9–10-week-old gilts, respectively. All animals were individually weighed on arrival (initial weights farm A: 24.68kg ±2.67; farm B: 27.46kg ±2.83) and randomly allocated to one of two treatments: half pigs received Vivax/Improvac, Zoetis (V=vaccinated) 96 and 28 days before harvest, and the other half not-treated (C=controls). Experimental unit was the pen, with 35 pigs/pen (farm A) and 45 pigs/pen (farm B), resulting in 12 and 13 replicas per treatment, respectively. Pigs were fed ad-libitum. Primary end point was daily weight gain (DWG), secondary feed conversion rate (FCR). Daily feed intake (DFI), initial and final weights (FW) were measured by pen for the duration of the study (farm A: 101 days, farm B:103 days). Data were analyzed separately (ANOVA) for each farm using the General Linear Model considering treatment effects and means evaluated by Student's t test.

#### Results

For farm A, DFI (2.090 vs 2.005 kg/day, P=0.148) and FCR (2.000 and 2.002, P=0.967) were not statistically different between treatments. However, DWG and FW were higher for vaccinated gilts, 1.044 vs 1.001kg/day (p=0.001), and 131.26 vs 126.83kg/day (P=0.003). For farm B, only FCR was similar between treatments (2.200 and 2.222, P=0.366). DFI, DWG and FW were better for vaccinated gilts (V), 2.300 vs 2.388kg/day (P=0.05), 1.074 vs 1.045kg/day (P=0.03), and 138.17 vs 135.12kg (P=0.010), respectively. High variation coefficients found for DFI and FCR may explain why DFI in farm A was numerically but not-statistically better for vaccinates, while ADG was superior.

## **Discussion and Conclusion**

Vaccination has a secondary indirect positive effect improving ADG while not having a negative impact on FCR. Immunization against GnRH may be a useful management tool to prevent the negative effects of sexual maturation of fattening gilts on wellbeing and productivity.

## REPRODUCTION

#### REP-CP-01

**REP** - Reproduction

## SOWS WEANING SURPLUS PIGLETS: CHARACTERISTICS AND SUBSEQUENT PERFORMANCE

<u>G. Zanin</u><sup>1</sup>, L. Dos Santos<sup>1</sup>, R. Dalmina Rech<sup>1</sup>, F. Bortolozzo<sup>1</sup>, R. Ulguim<sup>1</sup>, A.P. Gonçalves Mellagi<sup>1</sup> <sup>1</sup>Universidade Federal do Rio grande do Sul

### **Background and Objectives**

In hyperprolificacy scenarios, strategies such as cross-fostering with piglets exceeding the number of teats are interesting to ensure production sustainability. This study aimed to identify the sow characteristics to wean more piglets and assist new protocols to select sows capable of nursing large litters.

### Material and Methods

Litters (n=254) were cross-fostered with one piglet exceeding the functional teats of pluriparous sows. The following variables were evaluated at cross-fostering (D0): sow parity order (PO), number of functional teats (FT), caliper and backfat thickness (BT); milk yield (MY) on D5 and D20; in the subsequent cycle: weaning-to-estrus interval (WEI), farrowing rate (FR), and live born (LB). Additionally, sows were divided into classes according to the number of piglets dead or removed throughout lactation: (W+1) sows that did not lose piglets; (W-1) sows that lost one; (W-2) sows that lost two, and (W-3) sows that lost  $\ge$ 3 piglets. Statistical analysis was performed using the GLIMMIX procedure (SAS 9.4), and means were compared by the Tukey-Kramer test. Logistic regression models determined the odds ratio for each response variable to wean one surplus piglet. Statistical significance was considered when P<0.05.

### Results

**W+1** sows were younger than **W-2** and **W-3** (3.32, 4.14, and 4.03, respectively;  $P \le 0.03$ ), with no difference to **W-1** (P=0.31). PO $\le 4$  sows were up to 5.0 times able to wean a surplus piglet than sows PO $\ge 5$  (P<0.01). There were no differences (P $\ge 0.08$ ) among groups for BT (11.27 mm), caliper (10.09) and FT (14.60). **W+1** sows produced more milk until D5 than **W-3** (450.81 and 377.63 g/day/piglet, respectively; P<0.01). However, there was no difference among the groups in MY until D20 (604.78 g/day/piglet, P=0.84). Regarding subsequent performance, no differences were observed among groups (P $\ge 0.06$ ) for WEI (5.36d), FR (87.01%), and LB (15.73 piglets).

#### **Discussion and Conclusion**

Selecting younger sows with high early lactation milk yield is important to wean one piglet exceeding the number of teats. Sows in their second and third lactation produce more milk than in their fifth (Etienne et al., 1998). Therefore, strategies aiming to increase milk yield at early lactation are an opportunity to enable the weaning of more piglets.

**REP** - Reproduction

## ENHANCING OXYGENATION IN NEONATAL PIGLETS TO REDUCE PRE-WEANING MORTALITY

G. Naruekatpichai<sup>1</sup>, J. Deesuwan<sup>1</sup>, N. Pinyocheep<sup>1</sup>, B. Bunyakida<sup>1</sup>, A. Koharn<sup>1</sup>, R. Boonprakob<sup>1</sup>, T. Padet<sup>2</sup>, <u>N. Am-In<sup>2</sup></u> <sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>Center of Excellence in Swine Reproduction, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Thailand

#### **Background and Objectives**

Oxygen can boost piglets' vitality and reduce pre-weaning mortality. However, knowledge concerning the effects of oxygen supplementation in newborn piglets raised under tropical climates are lacking. Thus, the aims of the present study were to investigate the prevalence of hypoxia in newborn piglets and to study the effect of enhancing oxygenation in newborn piglets on piglet pre-weaning mortality rate.

#### **Material and Methods**

Data were collected from 40 Landrace x Yorkshire crossbred sows with parities 1-5 and 600 piglets. Sows were divided into control (20) and treatment (20) groups. Piglets from the treatment group were placed in a 21% oxygen chamber for 10 min after birth. Piglets' ear vein blood glucose was measured 8 h postpartum. Birth weight, 24-hour postpartum weight, and colostrum intake were measured for each piglet. Piglets died at 3 and 21 days of breastfeeding before weaning. The colostrum consumption, birth weight, weaning weight, 3-day and 21-day piglet mortality rate were compared between groups using student t test.

#### Results

Piglet birth weight averaged  $1.34 \pm 0.36$  kg in the control group and  $1.27 \pm 0.32$  kg in the treatment group (P = 0.03). The two groups did not differ in blood oxygen saturation and colostrum consumption (P>0.05). Interestingly, piglets delivered after 11th birth numbers in the treatment group consumed more colostrum than the control group (426 ± 1.9 g vs. 409 ± 1.7 g, P = 0.001). Piglets in the control group had greater mortality rates after 3 days (15.7% vs. 13.0%, P < 0.05) and 21 days (20.7% vs. 17.4%, P < 0.05) compared to the treatment group.

## **Discussion and Conclusion**

Colostrum consumption was improved in the piglets received enhanced oxygenation immediately after birth especially among those born after the 11<sup>th</sup> birth numbers. The study identified mortality gaps. The therapy group showed lower 3-day and preweaning mortality than the control group. These findings suggest the treatment procedure may increase early piglet survival. The therapy increased colostrum intake, and piglet survival. These findings improve piglet growth comprehension and may improve swine management and welfare. Further research may be needed to understand the processes and long-term effects of the data.

**REP** - Reproduction

## AN OUTBREAK OF PRRSV IN A GERMAN BOAR STUD: A RETROSPECTIVE ANALYSIS OF PRRSV SHEDDING IN BOAR SEMEN

<u>J. Aundrup</u><sup>1</sup>, C. Lüken<sup>2</sup>, K. Heenemann<sup>3</sup>, T.W. Vahlenkamp<sup>3</sup>, I. Hennig-Pauka<sup>4</sup> <sup>1</sup>Swine Health Service, LUFA Nord-West, Institute for Animal Health, Oldenburg, Germany <sup>2</sup>LUFA Nord-West, Institute for Animal Health, Oldenburg, Germany <sup>3</sup>Centre for Infectious Diseases, Institute of Virology, Faculty of Veterinary Medicine, University of Leipzig, Germany <sup>4</sup>Field Station for Epidemiology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

#### **Background and Objectives**

The porcine reproductive and respiratory syndrome virus (PRRSV) causes high economical costs due to reduced productivity and losses in pig production. PRRSV can infect sow herds through various routes. One risk factor is the transmission of PRRSV through artificial insemination with infected semen. In this retrospective and longitudinal field study PRRSV was detected by RT-PCR in fresh semen. Duration and profile of PRRSV shedding in semen was reconstructed by field data.

#### **Material and Methods**

In total, 354 boars of different breeds were housed in the stud. After PRRSV introduction and further routine semen collection, each fresh semen sample was tested by RT-qPCR. Data were assessed retrospectively considering diagnostic results from the defined first day of outbreak (p.o. = 1) until day 83 p.o. For evaluation of the shedding period, only boars from which at least four semen samples had been tested for PRRSV were considered.

#### Results

The following profiles could be defined:

No shedding (n): no virus detected in fresh semen samples

Shedding of PRRSV (s): virus detection in at least one semen sample

Intermittent shedding (is): intermittent detection of PRRSV in a minimum of 4 samples but with negative samples in between

Permanent (ps): PRRSV was found in at least the last two semen samples without PRRSV-negative samples in between (maximum until day 83 p.o.)

A total of 2184 semen samples from 336 boars were evaluated, with a range of 1 to 17 semen testings per boar. In 144 boars no virus (n) could be detected by RT–qPCR (42.8%), while in 192 (57.2%) at least one testing was positive for PRRSV (s), of these 28 boars (8.4%) have shed PRRSV in semen by at least in the last two time points of examination (ps). Intermittent shedding (is) was detected in 7 boars (2.0%). The positive boars showed shedding periods of a minimum of 2 days until day 83 p.o., the average period of shedding was 35 days.

### **Discussion and Conclusion**

This study reveals the complexity of PRRSV infection and shedding dynamics within a boar stud. Four individual shedding profiles could be identified.

#### **REP** - Reproduction

## DIFFERENCES IN AGE, WEIGHT, BACKFAT THICKNESS AND LOIN DEPTH AT FIRST MATING IN EIGHT DIFFERENT GENETIC LINES DURING ALL SEASONS

P. Sánchez-Giménez <sup>1</sup>, H. González-Ramiro <sup>1</sup>, R. Fernández-Rodríguez <sup>1</sup>, A. Martínez-Moreno <sup>1</sup>, L. Martínez-Alarcón <sup>2</sup>, G. Ramis <sup>3</sup>

<sup>1</sup>AGROPOR, Murcia, Spain

<sup>2</sup>Instituto Murciano de Investigación en Biomedicina (IMIB), Murcia, Spain

<sup>3</sup>Dpto. Producción Animal, Facultad de Veterinaria., Universidad de Murcia, Spain

## **Background and Objectives**

The condition in which we breed and introduce gilts into the breeding herd is key to maintaining the health and productive stability of the herd. The objective of this study was to determine the differences in age (AFM), weight (WFM), backfat thickness (BFT) and loin depth (LD) at first mating in eight different genetic lines during all seasons.

#### **Material and Methods**

The data of 10,694 gilts were collected in 5 commercial farms during fifteen months (November 2021- January 2023), located in Murcia, Southeastern Spain. They share health status, vaccination and management guidelines and all feed. They are introduced at 30 weeks of age and housed in the same conditions. The age and the weight at first mating was recorded, using a digital scale CIMA Control Pig (CIMA, Italy), and the BFT and LD with an SV-2 ultrasound scanner (ProVetScan, Spain).Depending on the value of BFT obtained, the genetic lines were divided into two morphotypes: FAT (≥15 mm of average) and LEAN (<15 mm of average).

#### Results

Differences were found for AFM, WFM, BFT and LD comparing all data from LEAN and FAT morphotypes for all the seasons comparisons (p<0.001 for all parameters). Moreover, AFM was significantly higher in spring for FAT gilts (p<0.001), and in summer and autumn for LEAN ones (p<0.001), WFM was higher in summer and autumn for FAT (p<0.001) and in spring for LEAN (p<0.001), BFT lowest in spring and highest in autumn for FAT and lowest in summer and autumn (p<0.001) and LD lowest in spring and summer (p<0.001) in FAT and lowest in summer and highest in winter (p<0.001) for LEAN.

## **Discussion and Conclusion**

There is no common criterion to establish optimal values to obtain the best yields in gilts. From this work, done in a subtropical area, we can conclude that the influence of these factors is greatly influenced by the morphotype and the season. LEAN morphotypes have difficulties to grow and reach puberty in summer. However, FAT gilts have less variation throughout the year. For this reason, with LEAN morphotypes, specific management and nutrition plans should be implemented during the summer/fall to reduce non-productive days to first mating.

**REP** - Reproduction

# PIGLETS BORN LATER IN THE FARROWING HAVE LESSER VITALITY AND INCREASED BLOOD PH AND LACTATE

<u>B. Bracco Donatelli Muro</u><sup>1</sup>, R. Carnevale<sup>2</sup>, A. Augusto Justo<sup>4</sup>, M.S. Monteiro<sup>3</sup>, N.D.A.C. Gomes<sup>1</sup>, C. Tavares Meloto <sup>4</sup>, M. Ambroso Adib Donato Henriques<sup>4</sup>, A. Nunes Reis<sup>4</sup>, G. Salvador Cardoso<sup>4</sup>, E. Marlon Pereira<sup>1</sup>, E. Correia Da Silva<sup>1</sup>, L. Braselino Borges<sup>4</sup>, C.A. Pospissil Garbossa<sup>1</sup>

<sup>1</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>2</sup> Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>3</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>4</sup>Department of Veterinary Medicine, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, SP, Brazil

## Background and Objectives

Genetic selection aiming for high prolificacy has caused both an increase in the duration of farrowing and in the time of expulsion of piglets that are born close to the end of the farrowing. Due to repeated uterine contractions the last piglets born have higher chance of having the umbilical cord compressed or ruptured, and the consequence is a lack of oxygen supply and decreased vitality. Reduced oxygenation causes a drop in heart rate and initiates anaerobic metabolism, which collectively reduces blood pH and increases blood lactate. The aim of the present study was to assess if piglet birth order, vitality lactate and blood pH at birth are associated.

### Material and Methods

Thirteen gilts had their farrowing monitored. The birth order of each piglet was recorded. To assess piglet vitality, an Apgar score considering respiratory latency, hearth rate, snout skin color, latency to stand up and meconium staining, was used. Lactate and blood pH level were measured in a commercial hemogasometer (Blood gas analyzer cobas® b 221- Roche), from a blood sample collected from the umbilical artery, immediately after birth. Linear regressions were performed to assess associations between variables and statistical differences were set at  $p \le 0.05$ .

## Results

Piglet birth order and Apgar score were negatively (p<0.05) associated. Piglet birth order and Apgar Score were negatively associated (p<0.05) with blood pH and lactate levels.

## **Discussion and Conclusion**

Piglet blood lactate is as an indicator of fetal hypoxia, which decreases piglet vitality at birth. Piglets of higher birth order faces more challenges to adapt to early extra uterine life, have decreased vitality which can impair colostrum intake e survivability

**REP** - Reproduction

## THE ANTIMICROBIAL PEPTIDE: ITS POSSIBILITY FOR A REPLACEMENT OF ANTIBIOTIC IN BOAR SEMEN EXTENDER

K. Keeratikunakorn 1, P. Chanapiwat 1, R. Aunpad 2, N. Ngamwongsatit 1, K. Kaeoket 1

<sup>1</sup>Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand

<sup>2</sup>Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Rangsit Campus, Klonglaung, Pathumthani 12120, Thailand

#### **Background and Objectives**

In the swine industry, artificial insemination (AI), is a common method to preserve liquid boar semen. After insemination, bacterial contamination will negatively impact the quality of the semen, reducing the fertility rate and increasing the risk of endometritis in sows and gilts as well as embryonic or fetal death. For the purpose of inhibit bacterial development and limit the adverse impacts of contamination, many antibiotics are usually combined with semen extender. However, consideration of antibiotic-resistant bacteria in pig farm and the concerning of resistant gene transfer to human via direct contact or pork product, a rationale use of antibiotics should be considered. Many antimicrobial peptides (AMP) have been shown to inhibit growth of both gram positive and negative bacteria. In the present study, we were evaluated the possibility of AMP to inhibit bacteria isolated from fresh boar semen in order to replace antibiotic contained in boar semen extender by using AMP.

## Material and Methods

Both Gram-positive and Gram-negative bacteria that were isolated from swine semen are inhibited by PA-13. Seven fresh boar semen samples were collected, each one diluted with semen extender containing antibiotics (positive control) or with none (negative control), and the PA-13 was mixed in the semen extender without antibiotics at different concentrations (15.625, 31.25, and 62.5  $\mu$ g/mL), and stored at 18°C. After storage, the total bacteria concentration was measured as well after 0, 24, 36, 48, and 72 hours.

#### Results

The positive control group had the lowest total bacterial count at 0, 24, 36, 48, and 72 hours, whereas the negative control group had the highest total bacterial count. Comparing bacterial count during 0 and 36 hours among AMP groups, AMP at a concentration of 31.25 µg/mL showed lowest bacterial count (log0.56-log2.88 CFU/mL).

## **Discussion and Conclusion**

PA-13 has ability to inhibit bacterial growth in boar semen for at least 24 h. This indicates that PA-13 could be an acceptable replacement for an antimicrobial peptide to be used as a substitute antibacterial agent for boar semen extender. However, more research on the impact on the boar semen quality and fertility on pig farm is needed.

#### **REP** - Reproduction

## THE EFFECT OF LONG-TIME TRANSPORTATION OF EXTENDED BOAR SEMEN ON THE MOTILITY TRAITS: A FEASIBILITY OF BEING A NEW FERTILITY PREDICTOR

#### P. Chanapiwat 1, K. Kaeoket 1

<sup>1</sup>Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand

#### **Background and Objectives**

Preservation of boar semen by diluting with extender is technique to extend life span of spermatozoa before artificial insemination. In some countries, farms have boar stud inside the farm. Fresh semen was diluted with short/medium/long term semen extenders and carried with Styrofoam box/temperature-control box in a short distance. This may cause no effect on semen quality before artificial insemination. However, in many countries, breeder farms have no boar stud, they need to buy an extended fresh boar semen from other studs. Thus, the semen extenders used for semen dilution and long-time transportation may affect the extended semen quality before artificial insemination on farm. Therefore, we performed in vitro experiments that imitated movement of water/turbulent flow of diluted semen during the long-time transportation by using orbital shaker to evaluate the semen quality in different extenders used and among different times of shaking.

### **Material and Methods**

Boar semen was collected from pig farm and diluted with medium-term (M-III, Minitube, Germany) and long-term extender (Androstar®-Plus, Minitube, Germany) to meet a concentration of 3 x 10° spermatozoa/ 100 ml. The diluted boar semen samples were filled into semen tubes with a volume of 100 ml. The semen tubes were placed on the tray of Shaker (IKA® KS 100, Shaker, Staufen, Germany) at 80 RPM which was settle in the control refrigerator at 15 °C. The semen tubes were placed on the Shaker for 0 (no shaking), 2, 4, 6 and 8 hours. The motility patterns (i.e. Total Motility, Progressive Motility, Circular Motility and Immotile) were examined by CASA (AdroVision®, Minitube, Germany).

## Results

Percentage of TM, PM, CM and IM in diluted semen for M-III and Androstar®Plus were 88.5 vs 92.53, 83.17 vs 90.28, 31.92 vs 45.49, respectively. After shaking for 2 hours, the CM decreased dramatically to 21.72 (M-III) vs 38.64 (Androstar®-Plus). Moreover, after 6 hours, the CM and IM of M-III and Androstar®Plus also gradually decreased to 17.23 vs 23.65 and 21.96 vs 15.38, respectively.

### **Discussion and Conclusion**

The results showed that circular motility and immotile are a good predictor of semen fertility after transportation.

## MISCELLANEOUS AND CLINICAL CASES

## MIS-CP-01

MIS - Miscellaneous and Clinical cases

## BOTRYOMYCOSIS OF SWINE MAMMARY DERMAL TISSUE, A RE-ACQUAINTANCE

E. Rooijakkers <sup>1</sup>, M. Kanters <sup>1</sup>, N. De Bruijn <sup>2</sup>, <u>T. Tobias <sup>3</sup></u> <sup>1</sup>De Varkenspraktijk, the Netherlands <sup>2</sup>Royal GD, Deventer, The Netherlands <sup>3</sup>Royal GD, Arnsbergstraat 7, 7418 EZ, Deventer, the Netherlands

#### **Background and Objectives**

Since several years, an organic 400 sow farrow-finish farm experiences cases of apparent pustular dermatitis in sows, with peak prevalence reaching 30%. Sows affected are of random parity and generally in the third trimester of gestation. Pustules develop on and just dorsal of the mammae. This case describes the diagnostic workup and the successive intervention strategies.

#### **Material and Methods**

The telephone-consultancy "de Veekijker" was consulted to aid in designing the diagnostic process. A clinical case description was set and (histo)pathologic and microbiological (aerobic and anaerobic) examination of fresh and formalin-fixed tissue biopsies obtained at slaughter was performed.

#### Results

The mammary skin of affected sows had red pustules developing to firm nodules of 1-2 cm in diameter. Some pustules resolve, others increase in size. During lactation, pustules can ulcerate and develop draining tracts. Clinically, the presumptive diagnosis of 'Actinomycosis' was made, but lesions do not resemble textbook cases. Histologically, lesions consist of multifocal granulomatous to pyogranulomatous inflammatory processes in the dermis and subcutis. The central necrotic debris had an intense neutrophilic inflammatory infiltrate, accompanied by radially arranged deeply eosinophilic amorphous protein, known as the 'Splendore Hoeppli' phenomenon, around large bacterial colonies. Culture revealed two strains of Staphylococcus aureus with different antimicrobial susceptibility, as well as Streptococcus dysgalactiae ssp. dysgalactiae. Anaerobic culture revealed absence of growth.

#### **Discussion and Conclusion**

The diagnosis was best defined as 'Botryomycosis' of mammary dermal tissue with micro-trauma as the likely cause of the pustules. The suspected pathogenesis is an infection in relation to wound contamination or trauma by lacerations, bites, or puncture wounds with foreign bodies. In this specific case, inspection of housing revealed straw bedding as prime suspect for the pustules. It was recommended to remove and clean the litter bedding. The house was disinfected. In addition, selection strategies for sows were amended. The current incidence is reduced but long term follow-up is needed. Reporting this case aims to raise awareness 1) of variation in appearance of apparent well known conditions 2) that such lesions can contain multiple bacterial species and 3) to show that biopsies taken at the slaughter can serve purpose to investigate conditions of the integuments.

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## PATHOLOGICAL FINDINGS IN CLAWS FROM 21 LAME SOWS

<u>H. Bak</u><sup>1</sup>, S. Haugegaard <sup>2</sup>, L.U. Hansen <sup>1</sup> <sup>1</sup>SEGES Innovation P/S, Aarhus, Denmark <sup>2</sup>Danish Agriculture and Food Council, Kjellerup, Denmark

## **Background and Objectives**

Lameness is a common problem for sows in production herds, and several cases of lameness are caused by claw lesions. This study investigated the pathological lesions in claws of 21 lame sows with clinical signs of claw problems.

## Material and Methods

Four sow herds were selected based on a history of claw problems. In each herd, 2-12 moderate to severely lame sows were euthanized, and the distal part of the affected leg was submitted for autopsy with thorough description and photo documentation of macroscopical lesions. If indicated, specimens were submitted for histological examination. From one herd, samples from claw lesions and surroundings were submitted for bacteriological examination (culture and DNA-sequencing).

## Results

Twenty-one feet were autopsied. Nine feet had an abscess inside the claw, 4 had necrotizing laminitis, 4 had traumatic lesions without infection, 3 had reddening in the bones' growth lines, and for the last foot, no lesions that could explain the lameness were found. For the infected claws, a point of entrance was identified, e.g. a crack in the white line. Histological examination of bones with reddening showed bleeding that was found to be a physiological reaction typical for young sows. The degree of lameness and the severity of pathological lesions was not correlated.Bacteriological examination by amplicon sequencing showed that Fusobacterium accounted for 33% of the bacteria in the abscesses and 4% of the infections found in the connective tissue of the claws, while they made up only 0-0.08% of the bacteria found in the surroundings. Contrary, the 5 most frequently found bacteria in the surroundings were not found in any abscesses and only accounted for 0.09 of bacteria found in connective tissue.

#### **Discussion and Conclusion**

The present study revealed severe and irreversible pathological lesions in the claws of lame sows, also when the sows showed only moderate lameness. Bacteriological examinations did not indicate that it is the presence of specific bacteria in the surroundings that causes infections in the claws. Instead, the findings indicate that claw injuries constitute a point of entrance and therefore, claw infections should be prevented by keeping the claws intact.

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## EFFECT OF PASTEURELLA MULTOCIDA ON PERFORMANCE OF A SOW HERD.

<u>A. Labuscagne 1</u> <sup>1</sup>BVSc, MMedVet(Suil), CSVet

## Background and Objectives

A high health sow unit in the Eastern part of South Africa had a history of a very variable farrowing rate as well as a higher-than-expected sow mortality rate. At a point the herd expanded by a third, increasing the number of gilts in the herd significantly. When this happened, we started seeing the following clinical signs: acute lameness, with animals showing very high levels of pain, abortions, returns and if not treated promptly, most of these animals would die. There were no neurological or respiratory symptoms. If treated with antibiotics and anti-inflammatories, the response was slow and affected animals were often culled after recovery. Most animals affected were gilts, but older sows were also affected.

#### Material and Methods

Samples were taken on two occasions of livers, spleens, kidneys, joint fluid and lungs and sent for aerobic as well as anaerobic bacteriological culture.

#### Results

On both occasions the were no bacterial growth from liver, spleen, kidney or lung samples but on both occasions Pasteurella multocida from the joint fluid. Capsular typing identified it as Pasteurella multocida type A. The antibiogram on both occasions indicated that this specific bacterium was sensitive to most antibiotics.

### **Discussion and Conclusion**

Because P. multocida was isolated from the joint fluid, we decided that it is probably the causative agent of our clinical symptoms. Our treatment plan included treating the entire sow herd at the same time with in-feed Amoxycillin for 10 days and after that all sows were treated with amoxycillin for 7 days post-weaning. All in-coming gilts are vaccinated with an autogenous P. multocida vaccine at 23 and 27 weeks of age. All sows and gilts are vaccinated 7 days after farrowing. Since vaccination has started, the farrowing rate has stabilised to consistently be above 92%, and sow mortality has dropped below 6%. P. multocida has probably been a subclinical problem for years in this herd, but because more gilts were introduced into the herd for the expansion, it became a clinical problem.

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## CASE REPORT OF A CLINICAL PULMONARY MANIFESTATION OF ASCARIS SUUM IN A FINISHING PIG HERD

<u>I. Spiekermeier</u><sup>1</sup>, M. Beumer<sup>1</sup>, M. Heimann<sup>2</sup>, D. Meyer<sup>2</sup> <sup>1</sup>SAN Group Biotech Germany GmbH, Hoeltinghausen, Germany <sup>2</sup>BeVet GmbH, Bevern, Germany

## **Background and Objectives**

Ascaris suum, commonly referred to as the pig roundworm, represents a nematode species with a widespread global appearance, even in regions with high hygiene standards implemented within pig farming. Although roundworm infections frequently lack obvious clinical manifestations and are primarily detected by post-mortem examinations at slaughter, a multitude of research studies highlights the economic impact of these infestations, particularly in terms of diminished feed conversion efficiency. Nevertheless, in the presented case report, conspicuous clinical pathology was instigated by the migration of larval stages of Ascaris suum through the pulmonary system.

#### Material and Methods

The diagnosis of Ascaris suum infestation in this particular case relied on a comprehensive assessment that encompassed clinical, pathological and histological observations. Differentially relevant pathogens were excluded by applying bacteriological and molecular biological diagnostic techniques. Due to the prepatent phase of the infestation, fecal flotation analysis was not conducted.

#### Results

The initial clinical observations combined with the gross anatomical examination of the lungs and liver, led to the tentative clinical diagnosis of Ascaris suum infection. Subsequent histological analysis confirmed the preliminary diagnosis by detecting ascarid larvae in the lung tissues. All pathogens tested as part of the differential diagnosis yielded negative results, except for Porcine-Circovirus-2.

#### **Discussion and Conclusion**

Although the clinical appearance within the respiratory tract is not a leading symptom in cases of Ascaris suum infection, it is essential to establish the infection, particularly if clinical manifestations and macroscopic alterations in the pulmonary and hepatic tissues are evident. To confirm the tentative diagnosis at this stage of infection, histological methods are preferable, as flotation techniques are unsuitable due to the prepatent period of the parasite.

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## EXPLORING THE RELATIONSHIP BETWEEN CLINICAL LAWSONIA INTRACELLULARIS INFECTION AND HEMORRHAGIC BOWEL SYNDROME: A RETROSPECTIVE ANALYSIS OF POST-MORTEM EXAMINATIONS

H. Kreutzmann<sup>1</sup>, K. Junker<sup>1</sup>, C. Sanderman<sup>1</sup>, S. Klarenbeek<sup>1</sup>, T. Tobias<sup>1</sup> Royal GD, Deventer, The Netherlands

### **Background and Objectives**

The pathogenesis of Hemorrhagic Bowel Syndrome (HBS) is not well understood. An inquiry has been raised by veterinarians and stakeholders regarding a potential correlation between HBS and clinical Lawsonia intracellularis (L.i.) infection. This study aims to elucidate the occurrence of HBS throughout the year in combination with clinical L.i. infections in post-mortem examination submissions from Dutch pig farms at Royal GD.

## **Material and Methods**

For this analysis, results from all pigs necropsied at GD over the past five years (November 2018 to October 2023) were included. A qPCR and or Warthin-Starry staining is performed either upon request or when suspicion of infection was raised based on macroscopic or histological findings. Cases were filtered to encompass those with HBS, which also involved manifest torsio mesenterialis/intestinalis and colon torsion. Within the study population, three groups were formed: animals without macroscopic signs of L.i. infection without further investigation, animals in which a clinical L.i. infection could not be confirmed through further examination (histology of ileum/colon, Warthin-Starry-staining, and/or qPCR with <10^5 bacteria/g feces), and a group with confirmed clinical L.i. infection. Furthermore, a distinction was made between sows/boars and younger animals, and the occurrences were linked to the months in order to investigate potential seasonal effects.

#### Results

Out of 10,516 total submissions, HBS was identified in 202 cases in pigs ≥25kg. Out of these, 127 evaluations relied only on macroscopic evaluation. In 68 cases, clinical L.i. infection could not be confirmed through further examination, and only in seven cases, clinically relevant amounts of L.i. were detected. The relative risk to detect HBS in September/October is 9.59 compared to the remaining time of the year.

#### **Discussion and Conclusion**

Within the HBS case study population, clinical L.i. infection was only proven in the minority of HBS cases. The clustering of these cases in autumn may indicate other etiological factors such as feeding freshly harvested grains and temperature fluctuations. The effects of a resolved L.i. infection have not been investigated. This could potentially contribute to adverse effects on the intestinal microbiome, with HBS as a consequence. Further studies are essential to comprehensively explore this aspect.

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## VETERINARY STUDENTS' INTEREST IN PORCINE HEALTH MANAGEMENT, A GLOBAL SURVEY

<u>K.S. Pedersen</u><sup>1</sup>, A. Ladinig <sup>2</sup>, R. Guedes <sup>3</sup>, G. Almond <sup>4</sup> <sup>1</sup>University of Copenhagen <sup>2</sup>University Clinic for Swine, University of Veterinary Medicine Vienna, Austria <sup>3</sup> Federal University of Minas Gerais

<sup>4</sup>College of Veterinary Medicine, North Carolina State University

## **Background and Objectives**

Over the last 10 years, we have seen a decrease in students with interest in porcine health management (PHM) in Denmark. Anecdotal evidence supports that similar trends are occurring in other European countries and there is a concern that we will see a shortage of veterinarians that want to work with pigs. Our objectives were to obtain global estimates for the proportion of universities that have experienced a change in the interest in PHM over the last 10 years, and to obtain global estimates for the proportion of veterinary students who want to pursue a career in PHM. A third objective was to get an idea on the reasons behind these changes.

#### **Material and Methods**

An online questionnaire was made using SurveyXact and distributed by e-mail to colleagues teaching PHM at veterinary Universities/Faculties.

#### Results

Responses were received from May to October 2023 from a total of 73 Universities in 35 countries in North and South America, Africa, Asia, Europe and Australia. At 26% of the Universities, PHM was not a mandatory part of the veterinary curriculum. Most of the Universities (88%) had less than 10% of students interested in PHM. Half (52%) of the Universities had not experienced any change in the number of interested students, while 16% had seen an increase and 32% had seen a decrease over the last 10 years. The opinions concerning reasons for the decreased number of students included: students do not have an interest in large animal veterinary medicine; fewer students have firsthand knowledge on pig farming through family and friends; students think pig production has low animal welfare; students think the salary and working conditions are less attractive compared to other veterinary disciplines; sustainability is important to young people, and pig production is not considered sustainable.

#### **Discussion and Conclusion**

Approximately 1/3 of the Universities had experienced fewer PHM interested veterinary students which may result in a shortage of veterinarians. Differences exist between countries, even between countries with a strong tradition in pig production. An obvious question is what explains such trends and do some Universities do something special to recruit veterinary students to PHM.

## BACTERIOLOGY AND BACTERIAL DISEASES

#### BBD-CP-01

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### EVOLUTION OF BACTERIAL CONTAMINATION IN SEMEN DOSES

<u>A. Climente-Retornano</u><sup>2</sup>, A. Vicente-Carrillo<sup>1</sup>, N. Mendoza-Magén<sup>2</sup>, P. Soler-Llorens<sup>2</sup>, C. Giménez-Laita<sup>2</sup>, I. Franco-Castillo<sup>2</sup>, ú. Alvarez-Martín<sup>2</sup>, R. Ausejo-Marcos<sup>2</sup>

<sup>1</sup>CienclAnova, Investigación y Desarrollo Magapor A.I.E., Zaragoza, Spain

<sup>2</sup>Department of Biotechnology R&D, Magapor S.L., Ejea de los Caballeros, Spain

## **Background and Objectives**

Artificial insemination (AI), a common practice in swine reproduction and so, has reduced the spreading of venereal-borne diseases, but the contamination of the doses cannot be neglected as these pathogens reduce the quality of the sperm and can be transmitted to the sows. This contamination happens usually during collection operations and in vitro handling of ejaculates. The aim of this research is to review the evolution of the semen quality, focusing on the microbiology parameters of the doses in the last nine years in Spain.

#### **Material and Methods**

Over 37000 AI-doses, belonging to four different breeds, were obtained from the databases of the Monitoring and Quality Control Plan (PSCCS), performed in Magapor S.L., since 2014 to 2022. The seminal doses were obtained with the double glove technique and the sperm quality parameters, along with the microbial contamination, were analysed upon the first 48h post extraction. Total aerobic mesophilic microorganisms were incubated, and the isolated bacteria were identified by Gram staining and biochemical methods. Statistical analysis was performed using StatView.

#### Results

The sperm quality and the microbiological status has improved during the analysed period. Since 2014, the percentage of contaminated doses with aerobic bacteria, molds and yeast, has decreased, except for the year 2020, in which the contamination increased, probably related to the pandemic. Interestingly, the percentage of contaminated samples differ during the seasons, being at its highest during summer and autumn and at its lowest during winter and spring. Besides, the worst sperm quality was generally observed during the warmer months. The most common genera identified were Pseudomonas spp., Burkholderia spp., Serratia spp., Acitenobacter spp., Providencia spp. and Alcaligenes spp.

## **Discussion and Conclusion**

The AI-swine industry efforts to improve the AI-doses quality has been reflected in the microbiological status, where the contamination has been reduced to almost half the value obtained in 2014. On the other hand, warmer seasons worsen the quality of the sperm and increases the probability of contamination, so more precautions must be taken during the hottest months. In agreement with the literature, the most common bacteria genera found in the contaminated samples were Pseudomonas, Burkholderia and Serratia.

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## FACTORS ASSOCIATED WITH CASES OF BACTERIAL NEONATAL DIARRHOEA IN THAI FARMS

J. Miguel <sup>1</sup>, D. Giralt <sup>1</sup>, A. Buakhiew <sup>2</sup>, C. Ananratanakul <sup>2</sup>, S. Krasaesub <sup>2</sup>, <u>S. Traiyarach <sup>1</sup></u> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA Thailand, Bangkok

## **Background and Objectives**

Neonatal diarrhoea (ND) is a disease with a significant productive and economic cost. Its prevention and control need a multifactorial approach based on preventive measures and other management such as proper cleaning and disinfection or the control of environmental conditions. The objective of this study was to evaluate possible associated factors with ND and the effectiveness of antibiotics as preventive and controlling tools used in Thailand.

## Material and Methods

A total of 189 pooled diarrhoea samples (0–28-day old piglets) from 49 farms (between 150-12,000 sows) were analysed during 2023. Only 6 farms were using a vaccine for the prevention of E. coli and C. perfringens type C. Samples from diarrhoeic piglets were collected using FTA<sup>®</sup> ELUTE cards and sent to HIPRA DIAGNOS Thailand. A multiplex quantitative PCR was performed to detect toxins for pathogenic factors for E. coli, C. perfringens type A and C, and C. difficile. The association between toxins and variables such as the use of antibiotics in feed, piglets treated with antibiotics, sow-parity, or piglet-age were evaluated. A Chi-squared test was used to compare the positivity rate of the bacteria with associated factors.

#### Results

Antibiotics in sow feed or the administration of antibiotics to piglets did not result in a lower positivity rate in any of the toxins. Piglets born from gilts showed a higher positivity to C. difficile and CpA. Farms with over 3,000 sows had a higher incidence of C. difficile. In relation to age, piglets younger than 10 days old had higher positivity rate for different pathogenic factors than those over 10 days of age.

### **Discussion and Conclusion**

According to the results observed, the inclusion of antibiotics in sow feed or in piglet treatment was not associated with a lower incidence of agents causing neonatal diarrhoea. These findings would be in accordance with previous data from Thailand that reported antimicrobial resistance for C. perfringens and E. coli in cases of ND. The results are in accordance with previous studies regarding piglet age and parity as risk factors. In conclusion, alternative tools to antibiotics for ND control in Thailand such as the use of vaccines should be evaluated.

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MESOMYCOPLASMA HYOPNEUMONIAE INFECTS AND DISRUPTS THE ORGANOTYPIC AIR-LIQUID INTERFACE (ALI) PORCINE-DERIVED RESPIRATORY EPITHELIAL CELLS (PRECS) CULTURE.

A.F. Castillo-Espinoza <sup>2</sup>, R.K. Nelli <sup>2</sup>, J.C. Mora-Diaz <sup>2</sup>, R. Rauh <sup>1</sup>, N.C. Twu <sup>2</sup>, L. Yen <sup>2</sup>, S. Parameswaran <sup>2</sup>, <u>L.G.</u> <u>Gimenez-Lirola <sup>2</sup></u>

<sup>1</sup>Tetracore, Inc., Rockville, MD, USA

<sup>2</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

#### **Background and Objectives**

Mesomycoplasma hyopneumoniae (Mhp) is a respiratory pathogen that requires alternative models mirroring the complexity of the pig airway for reliable infection studies. This study evaluates the suitability of air-liquid interface porcine respiratory epithelial cells (ALI-PRECs) culture model for Mhp.

#### Material and Methods

Trachea (six-week-old CD/CD pig) was dissected, washed, and enzymatically digested to isolate PRECs, which were seeded into transwells and cultured under ALI conditions for 4 weeks until complete differentiation. ALI-PRECs were inoculated with Mhp strain 232 (10<sup>5</sup>, 10<sup>6</sup>, 10<sup>7</sup> CCU/ml), along with mock-inoculated controls, and incubated for 2 h vs. 5 h at 37°C, 5% CO<sub>2</sub>. After inoculum removal, ALI-PRECs were incubated for 24, 48, and 72 h post-inoculation (hpi). The outcome of the infection was assessed via microscopy, immunofluorescence (IFA), quantitative analysis of DAPI and FITC-labelled Mhp P46 protein staining (Image J software), and Mph qPCR in both pseudostratified epithelia and subnatants of ALI-PRECs.

#### Results

Progressive loss of epithelial integrity, rounding, clustering, and detachment of cells, decreased cilia, and ciliostasis were observed in all Mhp-inoculated ALI-PRECs by 24 hpi. DAPI staining revealed a significant (p<0.05) reduction of the number of PRECs after Mhp-inoculation in a dose-, time of exposure, and time post-exposure-dependent manner. Mhp P46 was detected by IFA at 24 hpi (10<sup>6</sup> and 10<sup>7</sup> CCU/ml) or 72 hpi (10<sup>5</sup> CCU/ml). Co-localization of DAPI and Mhp P46 was detected by IFA in areas pre-identified as ciliary niches on ALI-PRECs under bright field microscopy, indicating Mhp affinity towards ciliated cells. Mhp DNA was detected by qPCR in all Mhp-inoculated transwells by 24 hpi, regardless of the infectious dose and time of exposure. Mhp DNA was only detected in subnatants of ALI-PRECs inoculated with the highest Mhp dose of 10<sup>7</sup> CCU/ml by 24 hpi (5 h of exposure) or 48 hpi (2 h of exposure), indicating complete disruption of the epithelia. Mock-inoculated ALI-PRECs remained normal and tested Mhp negative by IFA and qPCR throughout the study.

#### **Discussion and Conclusion**

Mhp binds and infects ciliated cells of organotypic ALI-PRECs cultures, disrupting the integrity of the epithelial barrier and ciliary function. The immunopathological mechanisms underlying ALI-PRECs damage during Mhp infection are being evaluated.

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## POLYARTHRITIS - SIGNIFICANCE OF STREPTOCOCCUS DYSGALACTIAE IN JOINT SAMPLES

<u>M. Beumer</u><sup>1</sup>, S. Freytag<sup>1</sup>, I. Spiekermeier<sup>1</sup>, C. Helmer<sup>1</sup> <sup>7</sup>SAN Group Biotech Germany GmbH, Hoeltinghausen, Germany

## Background and Objectives

Streptococcus spp. are gram-positive coccoid bacteria of which several strains reside in tonsils, intestine and genital tract of clinically healthy pigs. However, some species may have pathogenic potential, such as Streptococcus suis or Streptococcus dysgalactiae. One leading symptom is arthritis. If streptococcal infection is suspected, Streptococcus suis is usually thought of directly, as it is by far the most important streptococcal pathogen in swine worldwide. However, many other streptococci can also play a significant role and cause similar symptoms. Streptococcus dysgalactiae is one of the more common Streptococcus suis-like pathogens. Two subspecies have been described in swine: Streptococcus dysgalactiae und Streptococcus dysgalactiae subsp. equisimilis. The aim of these analyses is to obtain an overview of the prevalence of Streptococcus dysgalactiae and to assess its clinical relevance in cases of arthritis in piglets.

#### **Material and Methods**

205 joint swabs from piglets with pathogen findings in culture from 2022 were evaluated regarding the prevalence of Streptococcus dysgalactiae. Species identification was carried out using Matrix-assisted laser desorption/ionization (MALDI). Analyses were deepened regarding the differentiation between Streptococcus dysgalactiae subsp. dysgalactiae and Streptococcus dysgalactiae subsp. equisimilis using 16S rRNA sequencing.

#### Results

Among all pathogen findings in culture, the prevalence of Streptococcus dysgalactiae is 11%. When differentiating between the two subspecies, it is noticeable that both subspecies are found in approximately equal proportions (6% Streptococcus dysgalactiae subsp. equisimilis und 5% Streptococcus dysgalactiae subsp. dysgalactiae). Further results will be displayed in the poster.

### **Discussion and Conclusion**

The results demonstrate that Streptococcus dysgalactiae is a relevant pathogen for polyarthritis in piglets. It should therefore be borne in mind that Streptococcus suis is not the only Streptococcus spp. that can be the cause of polyarthritis. Streptococcus dysgalactiae is becoming increasingly important, so that in addition to antibiotic measures and due to the desire to minimise the use of antibiotics, the implementation of a herd-specific, autogenous vaccination against Streptococcus dysgalactiae might be considered.

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## UNRAVELING THE IMPACT OF STREPTOCOCCUS SUIS DISEASE ON BACTERIAL COMMUNITIES IN PIGS

J. Santos Streauslin<sup>1</sup>, A. Anilkumar Vinithakumari<sup>2</sup>, N. Macedo<sup>3</sup>, D. Nielsen<sup>4</sup>, O. Sahin<sup>5</sup>, B. Arruda<sup>6</sup>

<sup>1</sup> Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>2</sup>Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>3</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

<sup>4</sup>Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, United States Department of Agriculture, Agricultural Research Service, Ames, Iowa, USA

<sup>5</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

<sup>e</sup>Virus and Prion Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA

#### **Background and Objectives**

Streptococcus suis is a frequent colonizer of the upper respiratory tract of clinically healthy pigs and an important pathogen in swine production, causing significant economic losses in farms with S. suis outbreaks. This study aimed to study the microbial composition of samples collected from pigs with confirmed S. suis disease and pen-matched controls to assess the potential impact of other bacterial etiologies on the pathogenesis of S. suis.

#### Material and Methods

Samples including tonsil, ileal content, and colon content were obtained during necropsy of fourteen pigs with histologic confirmation of S. suis disease and twenty clinically healthy pigs across 7 commercial farms in Iowa and Minnesota. DNA extraction and 16S gene (V4) sequencing were performed to identify bacterial composition. Qiime2 was employed for data processing, with amplicon sequence variants (ASV) compared to SILVA databases. Alpha and beta diversities were calculated using CHAO1, ACE, Shannon, Simpson, and Bray-Curtis metrics. Linear discriminant effect size (LEfSe) analysis was applied to identify taxonomic differences. Microbiome impact was evaluated through comparisons of samples from control vs. diseased pigs.

#### Results

Alpha and beta diversity revealed no significant differences within the clinical evaluation metrics of the sample types. LEfSe analysis identified 120 significant differences in the samples tested. In particular, the tonsil microbiota of healthy pigs exhibited an increased abundance of Streptococcus, Actinobacillus, and Moraxella genera, while Pasteurella, Peptostreptococcus, and Gemella were more abundant in the tonsils of diseased pigs. Additionally, Terrisporobacter and Alloprevotella genera were identified as significantly enriched in the ileum and colon microbiota of healthy pigs, respectively. In contrast, Lactobacillus and Prevotella genera were identified in higher abundance in the ileum and colon samples of diseased pigs.

#### **Discussion and Conclusion**

Our project aligns with previous research, noting a lack of differences in alpha and beta diversity among samples from healthy and diseased pigs. However, similar to other studies, we observed variations in microbial composition among samples. Our results revealed an increased abundance of specific genera in both diseased and healthy groups. This observation suggests that these particular genera may play a crucial role in shaping the porcine microbiota, potentially influencing the dynamics of S. suis disease.

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## CHARACTERIZATION OF A PASTEURELLA MULTOCIDA TYPE A STRAIN ASSOCIATED WITH A SEVERE PNEUMONIA OUTBREAK IN GILTS

S. Kähl<sup>1</sup>, H. Haller<sup>2</sup>, S. Hanstein<sup>1</sup>, M. Dembowski<sup>3</sup>, R. Ulrich<sup>3</sup>, P. Kutzer<sup>4</sup>, J. Kauffold<sup>2</sup>, C.G. Baums<sup>1</sup>

<sup>1</sup>Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, Leipzig University, 04103 Leipzig, Germany

<sup>2</sup>Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, University of Leipzig, 04103 Leipzig, Germany <sup>3</sup>Institute of Veterinary Pathology, Faculty of Veterinary Medicine, Leipzig University, 04103 Leipzig, Germany <sup>4</sup>Berlin-Brandenburg State Laboratory - Institute for Food, Drugs, Epizootics and the Environment, 15236 Frankfurt (Oder), Germany

## Background and Objectives

Though Pasteurella multocida (P. multocida) is described as a causative agent of severe pneumonia in pigs, it is not considered a major pathogen in Germany. This study was initiated by an outbreak of a severe disease in gilts associated with high mortality during lactation. This study aimed to characterize the associated P. multocida isolates.

#### **Material and Methods**

Of the affected breeding farm (3.300 sows, own replacement) three gilts with cough and fever were examined pathomorphologically and microbiologically. P. multocida isolates from their lungs were genotypically characterized by PCR targeting different virulence-associated factors. Survival of different P. multocida isolates from pigs was compared in whole blood survival assays to assess the ability to survive in blood of pigs of different age classes and herds.

## Results

Pathomorphology revealed a moderate to severe, acute to chronic, multifocal, suppurative bronchopneumonia with abscesses in the three gilts. Microbiological cultures of lung samples revealed Trueperella pyogenes in all three gilts and P. multocida with very mucous colonies in two of the three gilts, whereas other pathogens (ASF, ESF, SuHV-1, PCV-2, PCMV, PRRSV, SIV, Mycoplasma hyopneumoniae, PRCV) were not detectable. P. multocida lung isolates were confirmed as capsular type A. Genes encoding nine of eleven tested virulence-associated factors involved in adherence (ptfA), iron acquisition (tonB, hgbA), sialometabolism (nanH, nanB), and immune evasion (ompA, ompH, oma87, plpB) were detected. In comparison to a porcine hemorrhagic septicemia (HS) causing P. multocida strain, the present isolate showed even higher proliferation rates in whole blood drawn from gilts of an uninfected herd. In contrast, the outbreak strain was killed in blood collected from gilts of the original herd after the outbreak in accordance with bactericidal immunity after convalescence.

#### **Discussion and Conclusion**

This study reports an outbreak of acute pulmonal disease in gilts with P. multocida type A as putative primary pathogen. This suggestive claim is based on the facts that i) other e.g. major viral pathogens causing pneumonia were not detected, ii) lungs of affected gilts with severe bronchopneumonia were culturally positive for the same P. multocida strain, iii) all isolates showed a very prominent mucous phenotype, and finally, iv) the profile of numerous virulence-associated factors and proliferation in blood of uninfected gilts.

#### BBD – Bacteriology and Bacterial Diseases

# DETECTION OF GENES RELATED TO VIRULENCE FACTORS IN PASTEURELLA MULTOCIDA ISOLATES IN BRAZIL

G. Pinheiro Paes <sup>1</sup>, J. Viktor De Paula Barros Baêta <sup>1</sup>, <u>L. Fernando Dos Santos <sup>1</sup></u>, D. Lúcio Dos Santos <sup>1</sup>, W. Vieira Guimarães <sup>1</sup>, J. Lúcio Dos Santos <sup>1</sup> <sup>1</sup>*MICROVET - Microbiologia Veterinária Especial* 

#### Background and Objectives

Pasteurellosis is one of the most economically important diseases of swine, caused by Pasteurella multocida (P. multocida) capsular types A and D. Due to its genetic and pathogenic variability, P. multocida is capable of producing fibrinous pleuritis and suppurative bronchopneumonia as well as pericarditis and even septicemia. Epidemiological analysis of the organism based on serotyping and typing of virulence gene patterns allows the identification of highly pathogenic strains. The objective of this work was to characterize the pathological lesions in pigs and the association with the typing pattern of virulence genes present in strains of P. multocida isolated in Brazil.

### **Material and Methods**

A total of 1059 swine Pasteurella multocida isolates from the Veterinary Diagnostic Laboratory of Microvet database, during the period from January 2022 to June 2023, were included in this study. The isolates originated from 9 Brazilian States and were predominantly derived from the lower respiratory tract of sick pigs. All isolates were subjected to capsular serotyping by multiplex PCR. From, 120 isolates from animals at finishing were selected for analysis to identify genes related to 10 virulence factors and pathological characterization of lung lesions.

#### Results

The results demonstrated predominant isolation of Pasteurella multocida in animals at finishing (79.7%) and predominance of P. multocida type A (68.6%). And the distribution of isolation were abscess samples (12.9%), pericarditis (10%), pleuritis (22.9%) and lung (54.2%). Six genes related to virulence factors (ompA, ompH, oma 87, exbB, exbD and ptfA) were present in 100% of the samples and the toxA gene was not found in any of the samples evaluated. The pfhA gene was identified in only 9.2% of the samples.

#### **Discussion and Conclusion**

Despite the variety of virulence factors detected with high frequency among the isolates in this study, further evaluations should be explored for analyzes of swine strains of P. multocida in order to better understand these pathogens. The intense lesions described in the histopathological evaluations of lung samples, from which P. multocida strains carrying the pfhA virulence genes were isolated, demonstrate that this gene can be considered a characteristic marker of highly pathogenic P. multocida strains.

#### BBD – Bacteriology and Bacterial Diseases

## ETEC GENETIC AND MORPHOLOGIC CHARACTERIZATION FROM ISU-VDL PORCINE CASES FROM 2018 TO 2022

#### R. C. Paiva 1, E. Burrough 1, N. Macedo 1, P. Pineyro 1, M. Almeida 1

<sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

#### **Background and Objectives**

Data from the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) has shown changes in the prevalence of porcine enterotoxigenic E. coli (ETEC) isolates in recent years. This study aimed to characterize ETEC by virotype from ISU-VDL isolates to provide contemporary E. coli genetic characteristics to swine veterinarians, producers, and stakeholders.

#### Material and Methods

All isolates included in this study (4,188) from 35 different US states were associated with ETEC and clinical disease. The criteria included histopathologic examination and colonization of villi by coccobacilli. Laboratory testing included bacterial culture and genotyping based on gene detection by gel-based PCR. A descriptive analysis was performed to characterize adhesins and virotype prevalence, the colony morphology, the specimen, and the growth of the isolates.

#### Results

The total number of isolates was 514, 684, 803, 1190, and 997 in 2018, 2019, 2020, 2021, and 2022, respectively; F18 and F4 adhesins were the most prevalent (66% and 19%, respectively), represented by 87%, 86%, 78%, 85%, and 89% of the isolates in 2018, 2019, 2020, 2021, and 2022; the percentage of F4 isolates decreased over study period (31%, 30%, 20%, 14%, and 10% in 2018, 2019, 2020, 2021, and 2022); the percentage of F18 isolates increased (55%, 55%, 58%, 70%, and 79% in 2018, 2019, 2020, 2021, and 2022, respectively); The most prevalent F4 virotype was F4:PAA:LT:STa:STb:EAST1; The most prevalent F18 virotype was F18:LT:STa:STb:Stx2e. The colony morphology of the isolates was classified as smooth/mucoid (53%), smooth (31%), intermediate (3%), lactose negative (1%), or rough (1%), and 12% was not specified. 40% of the isolates were classified as high growth, 25% moderate, 22% heavy, 5% low, and 8% were not specified.

#### **Discussion and Conclusion**

Understanding the ETEC isolate characteristics across different regions and contemporary virotypes might help to define novel strategies to prevent and control this important porcine enteric disease. The virulence gene characterization is important to determine pathogenicity. Morphologic and growth classification might help to consolidate lab data for future selection criteria of isolates. This study demonstrated important factors related to ETEC that might help the swine industry in a better understanding of this enteric disease.

## HERD HEALTH MANAGEMENT

## HHM-CP-01

#### HHM - Herd Health Management

## EFFECT OF DIFFERENT FEED ADDITIVES TO IMMUNE RESPONSE, OXIDATIVE STRESS AND WEIGHT GAIN IN POSTWEANING PIGS USING PIGMARKSAL SALIVARY BIOMARKERS.

J. Sánchez <sup>1</sup>, A. Miralles-Chorro <sup>1</sup>, M. Matas-Quintanilla <sup>2</sup>, P. Fuentes <sup>1</sup>, <u>A.M. Gutiérrez</u><sup>2</sup> <sup>1</sup>CefuSA,30840, Alhama de Murcia, Murcia, Spain. <sup>2</sup>BioVetMed Research Group, Department of Animal Medicine and Surgery, Veterinary School, University of Murcia, 30100, Espinardo, Murcia, Spain

#### **Background and Objectives**

Porcine health is influenced not only by infections but also by factors such as stress, local inflammation, injuries, management conditions, and environment, among others. In recent years, the use of saliva samples in pigs for diagnostic purposes has expanded due to its various advantages, including easy and non-invasive sampling. The aim of this study is to examine how the use of specific feed additives affects immune response, oxidative status and weight gain.

#### Material and Methods

Three groups of post-weaning pigs on a farm in southeastern Spain were created: a control group, a group with additive 1, and another with additive 2. The feed composition was similar in all groups, with the only difference being the inclusion of the additive. Saliva from 10 healthy and 10 diseased pigs from each group after 5- and 8-weeks were collected, and their individual weights were recorded. The concentration of inflammatory (Adenosine deaminase (ADA) and S100A12), acute phase reaction (C-reactive protein (CRP) and Haptoglobin (Hp)) and oxidative status (Total antioxidant capacity (TAC) and Total oxidant status (TOS)) biomarkers was analyzed using previously optimized assays. A 2-way ANOVA was used to analyzed differences between groups.

#### Results

After 5 weeks, the levels of ADA and TAC were higher in the group of animals feed with additive 2 in comparison to controls in both healthy and diseased pigs. Moreover, animals from additive 1 resulted in a lower weight loss in diseased pigs compared to the other groups but showed higher level of CRP and Hp. At the end of the study, after 8 weeks at post-weaning phase, the high levels of CRP and S100A12 observed in the diseased animals in the control group did not appear in the groups of animals fed with additives. In the additive 2 animals a lower weight loss in diseased animals was observed.

#### **Discussion and Conclusion**

The addition of specific additives to feed can be an effective tool for reducing weight loss in diseased animals during post-weaning stages, as well as maintaining proper oxidant status and a lower acute-phase response. (Granted by PID2020-116310RB-I00; pigmarksal.com)
#### HHM - Herd Health Management

### GIS BASED RISKS OF INTRODUCTION OF PRRS, MYCOPLASMA HYOPNEUMONIAE AND ACTINOBACILLUS PLEUROPNEUMONIAE INTO DANISH PIG HERDS

J. Dahl <sup>1</sup>, <u>B. Lorenzen <sup>2</sup></u> <sup>1</sup>Danish Agriculture and Food Council, Axeltorv 3, Copenhagen, Denmark <sup>2</sup>Danish Agriculture & Food Council, Aarhus, Denmark

#### **Background and Objectives**

It has been shown that PRRS, Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae serotype 2 are able to be transmitted from neighbouring, infected herds to naïve herds. The Danish SPF system has data on presence of these infections for a large part of the Danish pig populations, including 80 per cent of all Danish sows, and the Geographical Information System coordinates of the herds.

The Geographical Information System, GIS, models for predicting the transmission probabilities under Danish conditions have been updated.

#### **Material and Methods**

Three different datasets were constructed, one for each infection. Each observation represented a sow herd negative for the infection at the beginning of the period. We only included herds with more than 50 sows. We followed the herds for one year in two periods. The status of the sow herd at the end of each of the years 2020 and 2021 was the outcome.

For each herd, the number of heat-producing units were calculated based on number of sows, weaners and finishers. The distance to the sow herd was calculated for all herds in Denmark. The infection pressure from each positive neighbouring herd was calculated, based on the heat producing units, divided by distance between the sow herd and neighbour. It was investigated, if including an index for east-west-location improved the model. Finally, the infection pressure for all neighbours were added together.

Many logistic regression models were explored to achieve the optimal prediction.

#### Results

The optimal prediction of the risk of infection with PRRS and Mycoplasma hyopneumoniae was obtained by including herds up to 5,000 meters, weighted by east-west-orientation and the herd size of the sow herd as predictors. For Actinobacillus pleuropneumoniae serotype 2 including herds up to 1,000 meters gave an optimal model, without including east-west or herd-size of the sow herd.

#### **Discussion and Conclusion**

Models for predicting the probabilities of infection for PRRS, Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae serotype 2 were updated and a GIS map is available for veterinarians and pig producers as a standard decision support tool when planning a depopulation/repopulation and is an important tool in the Danish PRRS reduction program

#### HHM - Herd Health Management

# PREDICTION OF PIGLET THROUGHPUT AFTER LIVE VIRUS EXPOSURE IN BREEDING HERDS UNDERGOING PRRS ELIMINATION

E. Paladino<sup>1</sup>, C. Corzo<sup>2</sup>, A. Betlach<sup>3</sup>, A. Rendahl<sup>5</sup>, M. Pieters<sup>4</sup>, R. Singer<sup>5</sup>

<sup>1</sup>University of Minnesota

<sup>2</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA <sup>3</sup>Swine Vet Center, St Peter, MN, USA

<sup>4</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

<sup>5</sup>Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

#### Background and Objectives

PRRS elimination via whole herd exposure with live-resident virus is a common strategy. However, live-virus inoculation (LVI) can precipitate a "storm" of abortions and fetal death due to vertical transmission. The goal is to wean virus-free pigs and recover baseline production rapidly, although it can first increase losses. The objective of this study was to quantify production losses and predict the number of weaned pigs following the implementation of this strategy.

#### Material and Methods

Twelve farrow-to-wean farms (single system) were enrolled. Farms experienced PRRS outbreaks between 2021-2022 and implemented a load-close-expose approach with LVI. Production records were obtained and a linear mixed model was fitted to explore the role of covariates on the average number of weaned piglets, as a function of gestation week, after exposing pregnant sows to the protocol. Predictors included were: i)previous PRRS herd status, ii)interval (time between outbreak and LVI), iii)herd size, iv)parity, and v)interactions with pregnancy week. Estimated marginal means were calculated and reported alongside 95% confidence intervals.

#### Results

Records from 28,331 pregnant sows exposed to LVI were analyzed. Statistically significant predictors included PRRS status, parity, and interactions of both interval and parity with pregnancy week. Overall, average number of weaned piglets was higher in females of lower parity orders, the ones exposed early in gestation, and also in farms with previous exposure to PRRS. Sows at 16-weeks gestation at the moment of LVI exposure weaned on average 5.45 piglets (95%CI:4.01,6.90), whereas those at the first week weaned 11.62 (95%CI:11,3,11.94). Sows at 10-weeks, weaned 6.82 (95%CI:5.45,8.19).

#### **Discussion and Conclusion**

LVI has been demonstrated as an effective strategy to aid virus elimination sooner than other interventions, although its implementation may initially increase losses in both late-term gestation and older females, resulting in reduced productivity. In the short-term, farms with longer intervals between outbreak and LVI presented higher averages of weaned piglets. Moreover, our results suggest that previous herd immunity plays a role in mitigating losses. For the next steps, the long-term effect of LVI on productivity will be assessed, as a function of gestation period stratified by the aforementioned predictors.

HHM - Herd Health Management

#### SWINE INFLUENZA A VIRUS AND ITS CO-INFECTIONS - A PERSPECTIVE FROM SIX EUROPEAN COUNTRIES

M.V. Agerlin <sup>1</sup>, G. Simon <sup>5</sup>, E.M. Mateu De Antonio <sup>6</sup>, H.E. Everett <sup>2</sup>, C. Chiapponi <sup>7</sup>, T. Harder <sup>4</sup>, G. Dauphin <sup>3</sup>, C. Fablet <sup>5</sup>, P. Ryt-Hansen <sup>1</sup>, G.E. Martin Valls <sup>6</sup>, B. Mollett <sup>2</sup>, L. Soliani <sup>7</sup>, A. Graaf-Rau <sup>4</sup>, S. Hervé <sup>5</sup>, L. Coronado <sup>6</sup>, S. Leetham <sup>2</sup>, A. Luppi <sup>7</sup>, K. Lillie-Jaschniski <sup>3</sup>, S. Thiroux <sup>5</sup>, S. Gorin <sup>5</sup>, G. Richard <sup>5</sup>, C. Deblanc <sup>5</sup>, M. Andraud <sup>5</sup>, A. Prosperi <sup>7</sup>, N. Rose <sup>5</sup>, N.R. Weber <sup>8</sup>, N.B. Goecke <sup>1</sup>, L.E. Larsen <sup>1</sup>

<sup>1</sup>University of Copenhagen (UCPH), Institute for Veterinary and Animal Sciences, Frederiksberg, Denmark

<sup>2</sup>Animal and Plant Health Agency (APHA), Virology, New Haw, United Kingdom

<sup>3</sup>Ceva Santé Animale S.A., Libourne, France

<sup>4</sup>Friedrich-Loeffler-Institut (FLI), Institute of Diagnostic Virology, Greifswald-Insel Riems, Germany

<sup>5</sup>French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Ploufragan-Plouzané-Niort Laboratory, Ploufragan, France

<sup>6</sup>Universitat Autònoma de Barcelona (UAB), Sanitat i Anatomia Animals, Cerdanyola del Vallès, Spain

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy

\*Veterinary & Quality Services, Danish Agriculture & Food Council F.m.b.a, Denmark

#### **Background and Objectives**

Swine influenza A virus (swIAV) is known to be part of the porcine respiratory disease complex (PRDC) involving both bacteria and viruses. In large herds, swIAV has been shown to be endemically circulating due to the constant supply of naïve piglets. Apart from the direct pathogenic effect of swIAV, infection may pave the way for secondary viral and bacterial infections, however, the impact of swIAV on co-infections has only been sparsely studied. The aim of this study was to investigate the presence and prevalence of co-infections in persistently swIAV infected herds in six European countries.

#### Material and Methods

Longitudinal studies were carried out in pig herds with circulating swIAV in France, Italy, Spain, the United Kingdom, Germany, and Denmark. In each country, two batches in two herds were followed from the farrowing to the end of the nursery phase, and nasal swab samples were collected from both sows and piglets at different time points. Selected swabs (n=1,089) were tested for 12 known porcine pathogens using a high-throughput real-time PCR platform (BioMark HD, Standard BioTools).

#### Results

SwIAV was detected in all 12 batches (two batches per country). Tests of nasal swab samples revealed a low-moderate prevalence of Porcine Circovirus type 2 (0%-31%), Porcine Circovirus type 3 (5%-38%), Porcine Respirovirus-1 (0%-38%), Swine orthopneumovirus (5%-43%), and Actinobacillus pleuropneumoniae (0%-42%) and a high prevalence of Haemophilus parasuis (69%-100%), and Streptococcus suis serotype 2 (96%-100%) in all six countries. The prevalence of Bordetella bronchiseptica (0%-61%), Mycoplasma hyorhinis (55%-89%), and Porcine Cytomegalovirus (36%-90%) differed among the six countries and Mycoplasma hyopneumoniae was not detected in any of the countries and Pasteurella multocida (31%-62%) was detected in approximately half of the samples.

#### **Discussion and Conclusion**

The prevalence of specific co-infections varied between the countries and batches. Still, overall the results showed that almost all known PRDC pathogens are detected in swIAV infected herds, and therefore, may impact the health, welfare and productivity in the herds. These results further support the clinical impact of swIAV, however, case-control studies are needed to quantify the impact of swIAV in PRDC clinical disease.

#### HHM – Herd Health Management

### PIG INDUSTRY STAKEHOLDERS' INSIGHTS INTO DATA TOOLS FOR PIG HEALTH AND WELFARE MANAGEMENT: AN ONLINE SURVEY IN SPAIN

B. Garcia-Morante 1, X. Zhou 2, M. Sibila 1, C. Vilalta 1, A. Bearth 2, J. Segalés 1

<sup>1</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain. <sup>2</sup>Consumer Behavior, Institute for Environmental Decisions, ETH Zurich, Universitaetstrasse 22, 8092 Zurich, Switzerland.

#### **Background and Objectives**

Good data utilization with application of data tools can help the swine industry to tackle the challenges regarding pig health and welfare. However, stakeholders' needs for data tools are still unknown. Therefore, this study aimed to explore the status quo of data collection and application of data tools on pig farms and uncover stakeholder's needs for pig health and welfare management data tools.

#### Material and Methods

An online survey was conducted in Spain to investigate how pig industry stakeholders (n=108) collect data at farm level, their attitudes towards technology and their evaluation towards two simulated dashboards: 1) a benchmarking tool that visualized pig mortality on an individual farm with time series; and 2) an early warning tool that visualized cough counts per pen in real time on a specific farm.

#### Results

Spanish stakeholders reported unequal levels of collecting data on farms. For instance, 52% (56/108) stakeholders reported manual entry of mortality data into the data management system, and 30% (32/108) indicated paper-based recording. Overall, the stakeholders were positive towards the application of technology for pig disease management (75%, 81/108). Regarding the evaluation towards the mortality benchmarking dashboard, most stakeholders perceived it as somewhat useful (42%, 45/108) and very useful (37%, 40/108). However, the lack of context information, difficulties in data interpretation, and missing flexibility in selecting time intervals undermined the perceived usefulness of this data tool. Similarly, most stakeholders perceived the early warning dashboard based on cough data as very useful (47%, 51/108) and somewhat useful (30%, 32/108). However, labour required to maintain this data tool and the lack of confidence in the reliability of the warning function to early detect respiratory diseases decreased stakeholders' perceived usefulness of this data tool.

#### **Discussion and Conclusion**

Pig industry stakeholders were positive towards the implementation of tools focused on data collection, analysis and visualization for pig health and welfare management, but currently their needs are not fulfilled. This study provides stakeholders' insights into the requirements of such tools, which should contribute to the development of data-driven decision support tools that could potentially fulfil the stakeholders' needs.

HHM - Herd Health Management

### A PRACTICAL APPROACH TO CONTROL PRRSV SUBCLINICAL INFECTION BY DATA MANAGEMENT AND ROUTINE PRRSV MONITORING FOLLOWING PRRS VACCINATION

<u>B. Goesten</u><sup>1</sup>, F. Von Und Zur Muehlen<sup>2</sup> <sup>1</sup>LH Vet group, TPB Blumberg, Luckower Damm 1a, 16306, Casekow - Germany <sup>2</sup>Intervet Deutschland GmbH, MSD Animal Health, Unterschleißheim, Germany

#### **Background and Objectives**

PRRSV causes reproductive failure in sows and is well known for decreasing reproductive performance in pig herds. Its clinical presentation may range from acute outbreaks to subclinical infection (i.e. reduced alive born piglets and piglet viability). In this field case, it was aimed to improve reproductive performance in a farm with subclinical disease by vaccination of sows and gilts intradermally against PRRSV.

#### **Material and Methods**

The field case took place in a herd of 4.500 sows in east Germany. PRRSV routine monitoring (PCR) was performed in 30 sows (pooled by 5) on a quarterly basis. From 2022 onwards, all pools tested negative. In March 2023, one of the pools tested positive (Ct 23.45) in the absence of clinical signs or low performance of sows. However, suckling piglets suffered from neonatal diarrhea. To avoid spreading of PRRSV and secure sow performance, Porcilis® PRRS (intradermal) was applied to gilts and sows from April 2023 onwards. PRRSV load was monitored every three months in serum from sows (n=30) and processing fluids from newborn piglets using PCR. In addition, historical reproductive parameters were available on farm and were used to monitor the effect of these interventions in two periods: historical data before (January-June 2022) and after (April-September 2023) vaccination. The presence or absence of neonatal diarrhea in piglets was recorded at group level.

#### Results

After virus detection and subsequent PRRS vaccination, PRRS-related clinical disease was not observed. After implementation of intradermal vaccination, no further virus was detected. Surprisingly, distinct production parameters improved compared to the historical period of the previous year: abortion rate -0.4%, total born piglets +0.2/litter, dead piglets -0.3/litter, mummies -0.1/litter. After vaccination, diarrhea in suckling piglets was hardly seen anymore.

#### **Discussion and Conclusion**

The outcome of a PRRSV infection can cause a wide spectrum of clinical signs. In this farm, a subclinical course with focus on impaired piglet viability was observed. Only with the combination of routine monitoring and detailed data management it was possible to detect subclinical course and to potentially prevent a shift to a severe clinical outbreak. An improvement of reproductive performance was observed after implementation of PRRS (intradermal) vaccination.

HHM - Herd Health Management

### CHARACTERIZATION OF CIRCULATING MYCOPLASMA HYOPNEUMONIAE VARIANTS USING MULTIPLE-LOCUS VARIABLE NUMBER TANDEM REPEAT ANALYSIS AND P146 GENE SEQUENCING

A. Betlach <sup>1</sup>, D. Marthaler <sup>2</sup>, E. Fano <sup>3</sup>, <u>M. Pieters <sup>4</sup></u>

<sup>1</sup>Swine Vet Center, St Peter, MN, USA

<sup>2</sup>INDICAL Bioscience Inc, FL, USA

<sup>3</sup>Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA; Current affiliation: Pipestone Veterinary Services, Pipestone, MN, USA

<sup>4</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

#### Background and Objectives

Molecular characterization of Mycoplasma hyopneumoniae (Mhp) is essential to link pathogen transmission, understand genetic diversity, and to support control and elimination efforts. However, limited information is available on the interpretation and comparison of Mhp molecular characterization, along with the epidemiological insight inferred. The aim of this study was to characterize and compare Mhp variants in the Midwestern United States using MLVA and P146 gene sequencing.

#### Material and Methods

A total of 160 clinical Mhp PCR positive samples were used for the study. Samples were collected between 2013 and 2018 from 84 unique swine herds in five production systems in the Midwestern US, along with epidemiological data. P146 sequencing and MLVA typing of P97 and P146, were performed from the genetic material. The techniques were compared based on assay sensitivity, discriminatory power, and epidemiological insight. Agreement in assay outcome was measured using Cohen's kappa index. Discriminatory power was estimated via Simpson's and Hunter-Gaston diversity indexes. Logistic regression models were conducted to estimate the odds of obtaining a MLVA type or P146 sequence based on Mhp PCR Ct values. Epidemiological variables towards Mhp diversity were evaluated by employing principal coordinates analysis plots.

#### Results

The sensitivity of MLVA and P146 sequencing was 88.1% (141/160) and 78.7% (126/160), respectively. Fair agreement ( $\kappa$ =0.34; 95% CI: 0.13-0.55) for assay outcome between the two molecular techniques was obtained. A higher discriminatory power was calculated for MLVA (D=0.899) than for P146 sequencing (D=0.844; p-value<0.01). Regardless of technique, a significant relationship (p-value<0.001) between Ct value and assay outcome was observed. Similar epidemiological insight was inferred from both molecular techniques, in which Mhp diversity was significantly associated with pig production flow. Variation in MLVA type and P146 sequences was minimally explained by production stage, state, and year.

#### **Discussion and Conclusion**

Results of this study showed differences in assay sensitivity and discriminatory power, favoring the utilization of MLVA for the characterization of Mhp diversity compared to P146 sequencing. Depending on the question at hand, the construction of phylogenetic trees using P146 sequencing may be utilized to further infer evolutionary relationships among Mhp variants. Additional research is warranted to expand the knowledge of Mhp diversity and molecular characterization.

HHM - Herd Health Management

#### DETECTION OF DIFFERENT AGENTS INVOLVED IN NEONATAL DIARRHOEA CASES IN CHINESE FARMS

J. Miguel <sup>1</sup>, T. Yang <sup>2</sup>, <u>W. Fei <sup>2</sup></u>, M. Wu <sup>1</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA China, Beijing

#### **Background and Objectives**

Neonatal diarrhoea (ND) in pigs is multifactorial. The differential diagnosis of the multiple agents involved must be considered. The aim of this study was to evaluate the prevalence of bacterial and viral agents on farms suffering from neonatal diarrhoea problems in China.

#### **Material and Methods**

Pooled fecal samples from 217 litters (sampling age 0-28 days), from 57 farms (600-15,000 sows) suffering from neonatal diarrhoea were evaluated between 2022 and 2023. Samples were collected using FTA Elute Cards and subsequently sent to HIPRA Diagnos China. A multiplex PCR test was performed to detect genes encoding F4, F5, F6 adhesion factors and LT toxin of Enterotoxigenic Escherichia coli; A and B toxins of C. difficile; The viral agents were evaluated in 27 of the farms (93 litters).

#### Results

92% of the samples analysed corresponded to piglets less than 14 days old. E. coli was detected on 63% of the farms. F4 and LT-toxin were the most frequent pathogenic factors, representing 43% and 51% in the positive cases, and 36% of positive samples were positive to Both toxins were detected in 54% of the positive cases. C. perfringens type A was found on 100% of the farms, in 46% of cases with a Ct value lower than 26, Regarding viral agents, PED was detected on 18% of the farms. Rotavirus A on 59% and C on 29%. Only 18% of the farms were positive for both Rotaviruses. In terms of coinfection, when viral and bacterial agents were evaluated on 81% of the farms 3 or more agents were detected.

#### **Discussion and Conclusion**

According to the results observed, bacterial agents are more prevalent than viral ones in piglet neonatal diarrhoea episodes in China. Furthermore, in most of the Chinese farms a multidisciplinary approach to control neonatal diarrhoeas is needed as more than one agent can be coinfecting the piglet. This study provides a database for field surveillance in China, which in turn will have an impact on the selection of suitable vaccines to control piglet neonatal diarrhoea.

### NUTRITION

#### NUTR-CP-01

NUTR - Nutrition

### DIETARY FIBER INCLUSION IN TRANSITION DIET MODULATES SOWS' BODY CONDITION AT FARROWING AND WEANING

<u>B. Bracco Donatelli Muro</u><sup>4</sup>, R. Carnevale <sup>1</sup>, M.S. Monteiro <sup>2</sup>, D. Feitosa Leal <sup>3</sup>, F.A. Pereira <sup>4</sup>, C.A. Pospissil Garbossa <sup>4</sup> <sup>1</sup> Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>2</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>3</sup>Department of Population Health & Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States

<sup>4</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

#### Background and Objectives

Improper maternal body condition is amidst the main causes of poor reproductive health, decreased longevity, and increased perinatal mortality. Thus, the aim of this study was to evaluate the effects of including a fibrous supplement in the transition diet on sows' corporal composition until weaning.

#### Material and Methods

A total of 202 females were used. At day 90 of gestation sows were randomly allocated in one of the groups: sows fed 2.6kg of standard gestation diet until 5 days post-farrowing (CON; n=101; crude fiber=2.25g.kg<sup>-1</sup>; NDF=10.1g.kg<sup>-1</sup>; ME=3,210 kcal.kg<sup>-1</sup>) and sows fed 2.4kg of standard gestation diet added of 400g of a fibrous supplement until 5 days post-farrowing (FIB; n=101; crude fiber=6.00 g.kg<sup>-1</sup>; NDF=14.8g.kg<sup>-1</sup>; ME=2,963 kcal.kg<sup>-1</sup>). The daily ME ingested by each sow was 3,000kcal.kg<sup>-1</sup> and 3,043kcal.kg<sup>-1</sup> in groups FIB and CON, respectively. The supplement was a blend of fibrous ingredients: 40% wheat bran, 25% lignocellulose, 25% citrus pulp, and 10% guar gum. During gestation sows were fed by electronic feeder. The animals were transferred to farrowing crates five days prior to farrowing where they were fed twice daily. During lactation all sows were fed the same lactation diet ad libtum. Body weight (BW) was evaluated at day 90 and 113 (pre-farrowing) of gestation and at weaning (21days of lactation). Backfat thickness (BFT) and loin depth (LD) were measure by ultrasound at the same timepoints. Variables were analyzed by ANOVA and statistical differences were set at p<0.05.

#### Results

At day 90 of gestation, the BW, BFT and LD were all similar (p>0.05) between groups. At pre-farrowing, the BW (CON=277 kg; FIB=271 kg; SEM=1.8) and LD (CON=61.1mm; FIB=58.9mm; SEM=0.7) were greater (p<0.05) in sows from CON, whilst the BFT (CON=17.2 mm; FIB=16.8mm; SEM=0.4) was similar (p>0.05) between groups. At weaning, LD was greater (p<0.05) in sows from FIB (CON=51.4mm; FIB=53.0mm; SEM=0.6), whilst the BFT (CON=14.3 mm; FIB=14.2mm; SEM=0.6) and BW (CON=239kg; FIB=237kg; SEM=2.1) were similar (p>0.05) between groups.

#### **Discussion and Conclusion**

Feeding sows with transition diet rich in fiber may modulate sows' metabolism even with similar energy intake. These effects in metabolism led to changes in body composition at farrowing and weaning

NUTR - Nutrition

### EARLY POST-NATAL NUTRITION WITH AN ISOTONIC PROTEIN DRINK REDUCED PIGLET PRE-WEANING MORTALITY BY 22% - AN ANALYSIS OF 91 STUDIES

<u>S.G. Buzoianu</u><sup>1</sup>, C. Putrino<sup>1</sup>, A.M. Firth<sup>1</sup> <sup>1</sup>Tonisity International Ltd

#### **Background and Objectives**

Pre-weaning mortality (PWM) is a major welfare and economic concern in pig production. The InterPig Consortium reports that globally, PWM is ~14% (10%-20%). PWM is perceived by the industry as a hard-to-improve parameter and most occurs in the first week post-birth. Causes are multi-factorial, but nutritional issues are extremely common. Since the neonatal piglet intestine grows exponentially in the first 3-4 weeks of life, any nutritional intervention should logically be implemented early, target intestinal development and require minimum labour. Tonisity Px (TPX) is an isotonic protein drink containing key nutrients that support enterocyte metabolism and development. Our aim was to investigate the effects of TPX on piglet pre-weaning mortality in a large multi-national dataset.

#### Material and Methods

PWM data was collected on 94,947 Control pigs and 90,007 TPX pigs, from 91 studies in 23 countries. All studies were performed on commercial farms from 2015 to 2023. Litters were randomly assigned at farrowing to the Control or TPX group. Within studies, both groups were housed concomitantly in the same rooms and were balanced for litter size, sow parity, and genetics. TPX piglets received TPX from day 2 to 8 of life in round pans for voluntary consumption. Controls received no additional supplementation. The primary outcome measured was PWM by treatment group within study. Treatment, genetics and continent were fixed effects and study was a random effect. Data were analysed using PROC GLIMMIX in SAS 9.4, and are presented as LS means.

#### Results

Overall, TPX pigs had a 22% lower PWM than Controls (8.3% vs 10.7%; P<0.0001). This means 24 pigs saved per 1000 born or 0.74 extra pigs/sow/year. Compared to Controls, TPX PWM was lower by 24% in Europe (8.1% vs 10.7%; P<0.0001), 15% in the USA (10.6% vs 12.5%; P<0.0001), 20% in South America (9.0% vs 11.3%; P<0.0001), 32% in Asia (5.4% vs 8.0%; P<0.0001) and 20% in Africa (9.1% vs 11.4%; P=0.26). TPX pigs also had lower PWM regardless of genetics (Danbred, PIC, Hypor or Topigs).

#### **Discussion and Conclusion**

These results demonstrate that Tonisity Px administered from day 2 to 8 of life is a viable intervention to reduce PWM.

NUTR - Nutrition

### FIBRE DEGRADING ENZYME AND PROBIOTIC COMBINATION EFFECTIVELY IMPROVE PIGLET PERFORMANCE AND SUPPORT INTESTINAL DEVELOPMENT

<u>N. Soares</u><sup>1</sup>, T. Poeikhampha<sup>2</sup> <sup>1</sup>Huvepharma NV, Belgium <sup>2</sup>Department of Animal Science, Kasetsart University

#### **Background and Objectives**

Weaned piglets are under significant nutritional stress which can result in impaired zootechnical performance and gastrointestinal environment disruption. Nutritional tools to support piglets' stress challenges are key to achieve optimal animal performance. The aim of this study was to evaluate the effect of a fibre degrading enzymatic complex, xylanase based (Hostazym® X, Huvepharma) combined with a Clostridium butyricum based probiotic (Top Gut®, Huvepharma) on the performance and small intestine morphology of weaned piglets.

#### **Material and Methods**

30 weaned piglets (7kg body weight), crossbred Duroc x Large White, were allocated to 3 different dietary treatments. Trial period was from day 1 after weaning till 42 days. Treatments consisted in 2 phases feeding (pre-starter and starter), pelleted feed: basal diet (maize, broken rice, soybean meal based) formulated according breed nutritional requirements (T1: Control); basal diet supplemented with 100g/t fibre degrading enzymatic complex (T2: Enzyme); basal diet supplemented with Enzyme (100 g/t feed) and 500 g/t Clostridium butyricum probiotic (T3: Enzyme + Probiotic). Piglets were fed ad libitum. Measured performance parameters: body weight, BW, feed intake, FI, feed conversion, FCR and feed cost to gain: FCG). Samples for intestinal morphology (duodenum, jejunum, ileum) were taken at day 43.

#### Results

At the end of the trial statistically significant differences (p<0.05) were found for FCR, Enzyme versus Control (1.670 versus 1.748) and Enzyme + Probiotic versus control (1.561 versus 1.748). FCG improved compared to control by 4% and 10% for Enzyme and Enzyme + Probiotic, respectively.Intestinal morphology was significantly (p<0.05) improved by treatment. When compared to Control, Enzyme + Probiotic treatment increased duodenum and jejunum villi height (541 versus 645 µm and 522 versus 639 µm, respectively) and villi:crypt ratio at jejunum, 235 vs 268.

#### **Discussion and Conclusion**

Supplementation of piglets diets with an enzymatic complex, Hostazym® X and a Clostridium butyricum probiotic, Top Gut®, significantly improves animals' zootechnical performance with positive feed cost to gain outcome and supports the development of the intestinal wall.

NUTR - Nutrition

# EFFECT OF GRUEL AND TONISITY PX ON WEIGHT GAIN AND MORTALITY DURING THE POST-WEANING PERIOD

<u>F. Vangroenweghe</u><sup>1</sup>, E. Folens<sup>2</sup> <sup>1</sup>Elanco - BU Food Animals - Benelux <sup>2</sup>BU Swine & Ruminants, Elanco Benelux, Elanco Animal Health

#### **Background and Objectives**

The transition to weaning and dry feed intake is a well-documented problem in pig management. Decreased feed intake after weaning results in a post-weaning growth dip that causes significant production losses and has negative impacts on gut health. An isotonic protein-electrolyte solution (Tonisity Px; Tonisity International) containing electrolytes, bio-active amino acids and proteins, and flavoring substances supports the intestinal function. It was hypothesized providing a 3% Px solution to make a gruel with creep feed could result in improved feed intake post-weaning and improved health.

#### **Material and Methods**

The study was performed on a farrow-to-finish sow herd using 4-week batch-management system and weaning at 21 days of age. A total of 40 sows and their respective litters (481 piglets) were enrolled in the study and randomized in two groups (Control, Px). The piglets in the Px group received 500 ml of a 3% Px solution daily for 3 days pre-weaning. After weaning, the piglets received 100 ml per piglet of a 3% Px solution on top of the creep feed for 3 days. Piglets were weighed at weaning (d0), at 4 weeks post-weaning (d28) and at the end of nursery (d49). Mortality was recorded during the post-weaning period. Data was analyzed in SAS 9.4.

#### Results

At weaning, piglets in both groups had similar weaning weights. At 28 days post-weaning, piglets in the Px group weighed 0.49 kg more than the Control piglets (+4%; P = 0.29). At the end of nursery (d49), piglets in the Px group had 1.92 kg more weight as compared to Control piglets (+8%; P < 0.001). In the Px group, mortality was reduced by 35% (P = 0.32) as compared to the Control group (4.1% vs. 6.3%; Px and Control group, respectively).

#### **Discussion and Conclusion**

Tonisity Px administered on-top of the creep feed from day 3 prior to weaning to day 3 post-weaning resulted in significantly increased weight gain (+1.92 kg at d49 post-weaning).

#### NUTR - Nutrition

### ALGAE-DERIVED $\beta$ -GLUCAN SUPPLEMENTATION IN LATE GESTATION ENHANCED COLOSTRUM IMMUNOGLOBULIN AND PIGLET PERFORMANCE

<u>J. Ruampatana</u><sup>1</sup>, U. Yamsrikaew<sup>1</sup>, P. Preesong<sup>2</sup>, S. Nantakhruea<sup>2</sup>, M. Nuntapaitoon<sup>1</sup> <sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand <sup>2</sup>Kemin Animal Nutrition and Health Asia Pacific, Bangkok, Thailand

#### Background and Objectives

The expansion of litter sizes due to genetic selection, combined with teat limitations, heightens the competition for colostrum among piglets, negatively impacting their health and overall performance. Enhancing colostrum quality through immunoglobulin enhancement has proven to be beneficial. Algae-derived  $\beta$ -glucan, a common feed additive, is known for promoting piglet growth and serving as an immunostimulant. The objective of this study was to evaluate the effectiveness of algae-derived  $\beta$ -glucan on colostrum immunoglobulin concentration and piglet performance.

#### Material and Methods

In total, 62 Landrace x Yorkshire crossbred sows were randomly divided into two groups: Control (n = 31) and Treatment (n = 31) groups. Both sow groups were fed with a standard lactation diet and the treatment sows group was supplemented with 1 g/sow/day of algae-derived  $\beta$ -glucan (ALETA<sup>TM</sup>, Kemin Industries Thailand Co. Ltd.) from 30 days before predicted farrowing date until 21 days after parturition (54.5 ± 4.7 days). Piglets were individually weighed on day 0 (< 1 h) and 1 (24 h) after birth for calculating the colostrum yield (CY) using a predictive equation. The piglets were also weighed on day 21 (W<sub>21D</sub>) and weaning (W<sub>w</sub>) (33.5 ± 4.2 days). Colostrum samples were pooled from all teats within 1 h after the onset of farrowing to assess the immunoglobulin G (IgG) concentration using a Brix refractometer (Pocket PAL-1 refractometer, Atago, Tokyo, Japan).

#### Results

On average, the CY was  $4.4 \pm 0.8$  kg/day and there were no significant differences between treatments (P > 0.05). However, the IgG in colostrum was higher in the treatment group compared to the control ( $39.2 \pm 2.5\%$  vs.  $32.6 \pm 2.6\%$ , P = 0.010). Furthermore, W<sub>21D</sub> showed a tendency to be higher in the treatment group compared to control ( $5.2 \pm 0.1$  kg vs.  $4.8 \pm 0.1$  kg, P = 0.070). The W<sub>w</sub> in the treatment group was higher than the control group ( $7.8 \pm 0.1$  kg vs.  $7.5 \pm 0.1$  kg, P = 0.018).

#### **Discussion and Conclusion**

In conclusion, supplementing algae-derived β-glucan during late gestation may increase colostrum quality and positively impact piglet performance.

**NUTR - Nutrition** 

#### PHYSICOCHEMICAL PROPERTIES OF SOLUBLE AND INSOLUBLE DIETARY FIBER SOURCES

<u>B. Bracco Donatelli Muro</u><sup>1</sup>, R. Carnevale<sup>2</sup>, M.S. Monteiro<sup>3</sup>, R. Scalise Xavier De Freitas<sup>4</sup>, I. Cláudio Da Silva Bueno<sup>4</sup>, D. Alexsander Rojas Moreno<sup>4</sup>, D. Feitosa Leal<sup>5</sup>, F.A. Pereira<sup>1</sup>, C. Abércio Da Silva<sup>6</sup>, C.A. Pospissil Garbossa<sup>1</sup> <sup>1</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>2</sup> Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>3</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>4</sup>Faculty of Animal Science and Food Engineering, University of São Paulo

<sup>5</sup>Department of Population Health & Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States

<sup>6</sup>Department of Zootechnology, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil

#### Background and Objectives

Dietary fiber (DF) plays a pivotal role in promoting the sustainability of swine production through nutritional interventions The physicochemical properties of DF are determined by the composition of monosaccharides leading to different effects on intestinal health and metabolism of pigs. However, the physicochemical properties of fibrous ingredients are still poorly explored. The aim of this trial was to evaluate hydration-related properties of five sources of soluble fibers and five sources of insoluble fibers.

#### **Material and Methods**

Vegetables pulp, apple pulp, citrus pulp, beet pulp, and guar gum were selected as sources of soluble fibers and lignocellulose, DDG, soybean hull, wheat bran, and SmartFiber® (extruded mix of tropical grasses) as sources of insoluble fibers. Water holding capacity (WHC) was measured by mixing a sample of 0.5 g (W1) of each ingredient with 10 mL of distilled water. The wet samples were weighed (W2) dried and weighed again to obtain the final weight (W3). WHC was calculated as: WHC = (W2 – W3) / W1. Viscosity measurements were performed in a Brookfield LVF and RVT models using the adaptors UL and 4 (Brookfield Lab) and maintaining the samples at a shear rate of  $30 - 100 \text{ s}^{-1}$ . Bulking was measured by mixing a sample of 1.0 g of each ingredient with 10mL of distilled water. The bulking was calculated as: bulking = of volume occupied by sample / initial weight. Variables were analyzed by ANOVA using Tukey as post-hoc test. Statistical differences were set at p<0.05.

#### Results

Guar gum had the highest (p<0.05) values for all the hydration-related properties, while DDG had the lowest (p<0.05) values for all variables. Beet pulp and citrus pulp followed guar gum as ingredients with high WHC. Wheat bran, SmartFiber®, and beet pulp viscosity was similar with DDG (p>0.05) with the lowest (p<0.05) values. DDG had the lowest (p<0.05) bulking, whilst beet pulp and apple pulp followed guar gum as ingredients with high bulking.

#### **Discussion and Conclusion**

Ingredients rich in soluble fibers had higher values of hydration-related properties. This indicates a potential to provide metabolic and physiological benefits to sows, such as distention of the gastrointestinal tract, decreasing the felling of hunger and increasing the welfare

NUTR - Nutrition

### PERFORMANCE GROWING AND FINISHING PIGS SUPPLEMENTED WITH AN ALTERNATIVE SOURCE OF PHOSPHORUS AS A REPLACEMENT TO DICALCIUM PHOSPHATE

<u>A.L.B. Mezzina</u><sup>1</sup>, N.D.A.C. Gomes <sup>1</sup>, F.A. Coelho <sup>1</sup>, L.K.S. Alves <sup>1</sup>, M.S. Monteiro <sup>2</sup>, C.A. Pospissil Garbossa <sup>1</sup> <sup>1</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil <sup>2</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

#### Background and Objectives

Phosphorus is the most expensive nutrient after energy and protein. The phosphorus contained in foods of plant origin is in the form of phytate, making it unavailable to pigs. The most common source for supplementation is dicalcium phosphate, a non-renewable resource with high cost and excessive excretion of the mineral into the environment. The objective of this study was to evaluate the use of Tricinophós<sup>®</sup> (Nutrivet Brazil, São Carlos, SP), an alternative source of phosphorus, to replace dicalcium phosphate in the diet on the performance of growing and finishing pigs.

#### Material and Methods

It was used 72 gilts and 72 barrows with 70 days of age and average weight of 23 ±2.87 kg. The experimental design was in randomized blocks (weight and gender) with six treatments and eight replications, being (CON) control – dicalcium phosphate, and for the following treatments it was included an alternative source of phosphorus: (TP300) 0.300 kg/ton; (TP350) 0.350 kg/ton; (TP400) 0.400 kg/ton; (TP450) 0.450 kg/ton and (TP500) 0.500 kg/ton. To evaluate performance, the animals were weighed individually at 21, 39, 63, and 91 days of experiment. From these data, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion (FC) were calculated. Statistical analyzes were analyzed by ANOVA using the SAS MIXED procedure and the means compared by the Tukey test at 5% significance.

#### Results

The replacement of dicalcium phosphate in the first 21 days didn't impact (P>0.05) ADG and ADFI, however, statistical difference was found for FC (P<0.05), with better FC in CON compared to all treated groups. TP350 and TP450 improved (P<0.05) the FC from 63 to 91 days when compared to CON. From 21 to 63 days, the inclusion of the alternative source didn't show any harm effect (P>0.05) to performance. When evaluating the total experimental period, we observed that the inclusion of different levels of the alternative source of phosphorus didn't change the performance of the animals (P>0.05).

#### **Discussion and Conclusion**

According to the results presented, dicalcium phosphate can be replaced by Tricinophós<sup>®</sup> in the diet of pigs in the growing and finishing phase, without causing losses to performance.

**NUTR - Nutrition** 

### SUPPLEMENTATION OF A β-MANNANASE ENZYME TO DIETS WITH A REDUCED NE CONTENT SUPPORTS POST-WEANING PIGLET PERFORMANCE DURING A PRRSV OUTBREAK UNDER FIELD CONDITIONS

<u>F. Vangroenweghe</u><sup>1</sup>, A. De Bruijn<sup>2</sup>, G. Vandenbussche<sup>3</sup>, P. Joye<sup>3</sup> <sup>1</sup>Elanco - BU Food Animals - Benelux <sup>2</sup>BU Nutritional Health, Elanco Benelux, Elanco Animal Health <sup>3</sup>Voeders Decadt NV, Meulebroekenlaan 21, Staden, BELGIUM

#### **Background and Objectives**

 $\beta$ -Mannans - strongly anti-nutritive polysaccharide fibers - are found in many vegetable feed ingredients. In common swine diets, the content of soluble  $\beta$ -mannans is estimated to range between 0.15 to 0.40%. In vitro studies have demonstrated that as little as 0.05% of soluble  $\beta$ -mannan content in feed can elicit a strong innate immune response. Hemicell HT (Elanco Animal Health) is a  $\beta$ -mannanase enzyme to supplement animal feed which breaks down  $\beta$ -mannans. This field study compared pig performance on a control diet to a reformulated diet with lower energy content – 55-65 kcal/kg net energy (NE) reduction – including a  $\beta$ -mannanase enzyme during a PRRSV outbreak under field conditions.

#### Material and Methods

A six-week feeding trial was conducted on a commercial post-weaning facility with DanBred x Belgian Piétrain piglets (n = 1502; 30 piglets per pen; 24 replicates per group) weaned at 21 days of age. Standard three-phase control diets (0.34%  $\beta$ -mannan) were compared to reformulated diets (0.35%  $\beta$ -mannan) with an energy reduction of 55-65 kcal NE/kg and inclusion of a  $\beta$ -mannanase enzyme (Hemicell HT<sup>M</sup>; Elanco) at 300 g/tonne. Standard production data were collected. The data were analyzed using JMP 15.0 statistical program.

#### Results

Overall, performance data did not differ significantly (P > 0.05) between treatment groups during the post-weaning period. Mortality was only numerically, but not significantly higher in the Control (5.3%) as compared to the Enzyme-treated group (3.8%). The effect of Enzyme supplementation was beneficial in both light-weight and heavy-weight piglets to maintain ADWG and reduce mortality during a PRRSV outbreak. Hemicell HT had an overall benefit of  $\in$  3.59 per piglet and  $\in$  5.18 per tonne of feed due to the NE reduction.

#### **Discussion and Conclusion**

The current trial demonstrated that the inclusion of Hemicell HT in reformulated diets with a lower energy content (55 – 65 kcal NE/kg of feed) was able to maintain production performance in post-weaned piglets, suffering from an active PRRSV circulation and secondary S. suis meningitis, with an economic benefit. The inclusion of Hemicell HT had an overall benefit of  $\in$  3.59 per piglet and  $\in$  5.18 per tonne of feed due to the 55 – 65 kcal/kg NE reduction.

### IMMUNOLOGY AND VACCINOLOGY

#### IMM-CP-01

IMM - Immunology and Vaccinology

# LACK OF CROSS REACTIVITY BETWEEN PCV2 AND PCV3 ANTIBODIES FOLLOWING EXPERIMENTAL INOCULATION

<u>M. Kroeger</u><sup>1</sup>, P. Pineyro<sup>2</sup> <sup>1</sup>Iowa State University <sup>2</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

#### **Background and Objectives**

PCV2 and PCV3 are endemic globally and concomitant circulation in swine systems and coinfection are described commonly. Both pathogens can cause reproductive failure and systemic infection. Given their genetic similarity, the potential of antibody cross reactivity has been speculated following natural infection and PCV2 vaccination. Therefore, the objective was to evaluate the humoral PCV2 and PCV3 cross reactivity following seroconversion in PCV2 and PCV3 experimentally inoculated pigs.

#### Material and Methods

Twelve 5-week old cesarean-derived colostrum deprived pigs were intramuscularly and intranasally inoculated with 2mL of PCV3 tissue homogenate (Ct=13, 1.04x10<sup>11</sup> genomic copy numbers/mL) on days 0 and 7. Serum was collected on days 0, 28, and 42. Seven 7-week-old commercial pigs were inoculated intramuscularly and intranasally with 1 mL of PCV2d (5 log10/2ml dose) on day 0. Serum was collected on days 0, 14, and 28. For both studies, serum was analyzed for PCV2 and PCV3 Cap antibodies by IFA and ELISA and PCV2 and PCV3 viremia by qPCR.

#### Results

In the PCV3 inoculation study, all pigs displayed PCV3 viremia on days 28 and 42 while having no PCV2 viremia throughout the study. All pigs had detectable PCV3 Cap antibodies by day 28 reaching maximum titers of 1:4,096 by IFA. However, PCV2 IFA titers were less than 1:160 and the PCV2 ELISA S/P ratios were below the positive cutoff on days 0, 28, and 42, suggesting a negative PCV2 antibody status. In the PCV2 inoculation study, all pigs displayed PCV2 viremia on days 14 and 28 while having no PCV3 viremia. All pigs had PCV2 ELISA S/P ratios above the positive cutoff on day 28. However, the PCV3 IFA titers were less than 1:160, suggesting a negative PCV3 antibody status.

#### **Discussion and Conclusion**

These results suggest that antibody response is virus specific and antibodies generated against the Cap protein do not cross react. Techniques used in this study measure total antibodies. Therefore, future studies should examine the potential of cross protection of the two viruses following infection.

IMM - Immunology and Vaccinology

#### ROLE OF IGM IN PORCINE IMMUNITY AGAINST STREPTOCOCCUS SUIS

A.K. Breitfelder <sup>1</sup>, S. Anne-Marie <sup>2</sup>, C.G. Baums <sup>1</sup>, G. Alber <sup>2</sup>, J. Kauffold <sup>3</sup>, H.L. Sigmarsson <sup>3</sup>, T. Sattler <sup>3</sup>, U. Müller <sup>2</sup>, M. Eschke <sup>2</sup>

<sup>1</sup>Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, University of Leipzig, Germany

<sup>2</sup>Institute of Immunology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, University of Leipzig, Germany <sup>3</sup>Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, University of Leipzig, Germany

#### **Background and Objectives**

Streptococcus suis (S. suis) is one of the most important porcine pathogens affecting mostly weaners, but also a successful colonizer of mucosal surfaces. The cysteine-protease Ide<sub>Ssuis</sub> is host and isotype-specific as it specifically cleaves porcine IgM, but Ide<sub>Ssuis</sub> is also able to cleave the IgM B cell receptor (BCR). Cleavage of soluble IgM by Ide<sub>Ssuis</sub> has been shown to promote survival of S. suis in porcine blood and to be a novel complement evasion mechanism as it reduces C3b deposition on the bacterial surface. Previous studies demonstrated an essential role of S. suis-specific IgM in host defense (IgM-complement-oxidative burst-axis). Furthermore, immunization with Ide<sub>Ssuis</sub> protects against mortality after cps 2 infection. This study aims to further characterize the early B-cell response against S. suis and modulation by Ide<sub>Ssuis</sub>.

#### **Material and Methods**

S. suis-specific IgM and IgG of 4 and 8-week-old piglets was investigated in serum by ELISA. The survival of S. suis in porcine blood was studied in bactericidal assays. IgM was cleaved by Ide<sub>Ssuis</sub> to investigate the role of IgM. Flow cytometry analysis was used to identify IgM B cell receptor (BCR) cleavage by rIde<sub>Ssuis</sub> and characterize B cell populations. The number of IgM-secreting cells after treatment with rIde<sub>Ssuis</sub> was investigated by ELISpot.

#### Results

A significant increase of S. suis-specific IgM in blood of 4 to 8-week-old piglets was concomitant with an increase in the frequency of non-conventional B-1-like cells capable of constitutive IgM production. Currently, it is investigated whether IgM antibodies secreted by individual B cell subpopulations are able to recognize S. suis and in particular capsule components. IgM BCR cleavage by rIde<sub>Ssuis</sub> results in a decrease of the number of IgM secreting cells and a long-term inhibition of both BCR-independent and -dependent activation of intracellular signaling.

#### **Discussion and Conclusion**

Results highlight the important role of the early B cell response, and IgM in particular, in providing immunity against S. suis, especially at the time when maternal IgG decline. IgM BCR cleavage does not only result in an immediate but also in a longer lasting interference with B cell function. These findings suggest a modulation of antigen-dependent B cell responses by S. suis through Ide<sub>Stuis</sub> expression.

IMM – Immunology and Vaccinology

### ANTIGENICITY ANALYSIS AS A TOOL TO PREDICT CROSS-PROTECTION BETWEEN CLINICAL STRAINS OF GLAESSERELLA PARASUIS

<u>M. Tamanini</u><sup>1</sup>, A.P. Mori<sup>1</sup>, R. Petri<sup>1</sup>, D. Baldasso<sup>2</sup>, J.A. Guizzo<sup>2</sup>, L.C. Kreutz<sup>3</sup>, R. Frandoloso<sup>3</sup> <sup>1</sup>Vaxxinova, Vargem Grande Paulista, Brazil <sup>2</sup>AFK Imunotech, Passo Fundo, Brazil <sup>3</sup>University of Passo Fundo & AFK Imunotech. Passo Fundo. Brazil

#### Background and Objectives

Glaesserella parasuis (Gps) is a phenotypically complex bacterium that causes Glässer's disease (GD) in pigs. The occurrence of GD can be prevented by using vaccines. Recently, we observed outbreaks of GD caused by strains of Gps belonging to the same serovar (SV) used in the vaccine. Motivated by this observation, we carried out the antigenic characterization of a panel of clinical isolates of Gps using a flow cytometry antigenic assay (FCAA).

#### Material and Methods

A total of 20 clinical isolates of Gps SV 5 (n = 10) and SV 12 (n = 10) were included in this study. The strains were isolated from piglets (35 - 52 days old) and molecularly classified according to their capsular type. For the antigenicity analysis, the isolates were incubated with reference porcine sera against Gps SV5 and SV12 and analyzed by FCAA (Frandoloso et al., 2023). Additionally, we analyzed the cross-reactivity between SV5 and SV12 by incubating Gps SV12 isolates with the anti-Gps SV5 antiserum.

#### Results

Our results demonstrated the existence of antigenic variation within specific Gps serogroups. Antigenically, the isolates belonging to SV5 were classified into 3 different clusters, and the similarity of cluster I with cluster II and III was 49% and 12%, respectively. Lower variation was observed within SV12 that comprised 2 antigenic clusters, with an antigenic similarity of 42% between them. Interestingly, of the total number of SV12 strains, only 2 were highly recognized by the antiserum against SV5.

#### **Discussion and Conclusion**

In this work we demonstrate that the complexity of Gps goes beyond its capsular type, and that the molecular typing of this agent is insufficient to predict protection based on classic vaccines. The antigenic variation reported here may explain the failure of some vaccines to induce protection even when there is serotype equivalence between the vaccine and the clinical strain. We also demonstrated low cross-reactivity between SV5 and SV12. Additionally, we highlight that FCAA is based on the use of live bacteria and porcine anti-Gps serum; therefore, it mimics conditions similar to those encountered in the host.

#### IMM – Immunology and Vaccinology

# EVALUATION OF DIFFERENT SCHEDULES OF SOW VACCINATION WITH AN AUTOGENOUS STREPTOCOCCUS SUIS SEROTYPE 1 BACTERIN

<u>G. Freymüller</u><sup>1</sup>, S. Lehnert<sup>1</sup>, C. Unterweger<sup>2</sup>, A. Ladinig<sup>2</sup>, T. Vogelmayr<sup>3</sup>, C.G. Baums<sup>1</sup>, L. Mayer<sup>1</sup> <sup>1</sup>Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Veterinary Faculty, University of Leipzig, Leipzig,

Germany

<sup>2</sup>University Clinic for Swine, University of Veterinary Medicine Vienna, Austria <sup>3</sup>Traunkreis Vet Clinic GmbH

#### **Background and Objectives**

Streptococcus suis is a major pathogen in piglets causing mainly meningitis and arthritis. Mucosal surfaces are considered to be the

most important entry sites of invasive infections. Autogenous S. suis bacterins are commonly used to control disease but field studies

comparing different vaccination schedules are scarce. The objective of this study was to read out immunogenicities of different bacterin

vaccination schedules designed to protect suckling piglets.

#### Material and Methods

Field vaccination trials were conducted within a closed herd of 160 Austrian Large White and Austrian F1 sows after experiencing a

major S. suis serotype 1 outbreak in suckling piglets. Sows were vaccinated prior service and/or prior farrowing with a bacterin of this

outbreak strain including an oil-in-water adjuvant. Levels of IgG, IgM and IgA binding to S. suis cps1 were determined in colostrum

and serum samples using different in-house ELISAs. Survival of S. suis cps1 in blood samples was investigated in vitro to assess

opsonizing antibodies.

#### Results

Preparturient intramuscular prime-boost vaccination with a S. suis cps1 (sly+, mrp+, epf+) bacterin elicited significantly increased levels

of colostrum IgG but not IgM binding to the surface of the homologous strain. Accordingly, specific serum IgG levels were increased in

the 2nd and 4th but not in the 6th week-of-life in piglets of these sows. Increased IgG levels were associated with decreased proliferation

of S. suis cps1 in blood of 2-week-old maternally vaccinated piglets. The increase of IgM binding to S. suis cps1 between the 4th

and 6th week of life was comparable between piglets fostered by vaccinated and non-vaccinated sows. Different vaccination protocols

including intranasal or intramuscular boost vaccination prior farrowing after intramuscular prime-boost vaccination prior service failed

to induce increased levels of specific IgA.

#### **Discussion and Conclusion**

Intramuscular prime-boost vaccination of sows with a S. suis cps 1 bacterin is sufficient to induce significantly increased IgG levels

and opsonizing antibodies restricting proliferation of this highly virulent genotype in blood of suckling piglets. However, future studies

are needed to identify vaccination schedules or application routes leading to specific mucosal immunity as proven by increased specific

IgA levels.

#### IMM - Immunology and Vaccinology

### IMPACT ON PRODUCTIVE PARAMETERS IN FARROWING AND POST-WEANING PERIOD OF PIGLETS AFTER SOW VACCINATION AGAINST SWINE INFLUENZA A VIRUS

S. Cárceles <sup>1</sup>, P. Sánchez-Giménez <sup>2</sup>, á. Guerrero <sup>2</sup>, <u>D. Espigares</u> <sup>1</sup> <sup>1</sup>Ceva Salud Animal, Barcelona, Spain <sup>2</sup>AGROPOR, Murcia, Spain

#### **Background and Objectives**

Swine influenza A virus (SIV) is one of the most important respiratory pathogens in pig farms causing important productive and economic losses. Sow vaccination prevents clinical signs of SIV infection in sows and piglets. This study aimed to evaluate the effect of the implementation of sow vaccination on farrowing productive parameters and on post-weaning period of piglets.

#### Material and Methods

The study was carried out on a 2000 sows farrow farm. Piglets showed respiratory clinical signs in suckling and post-weaning period. Confirmation of SIV infection by nasal swabs and subtype characterization was performed (H1huN2). Herd vaccination protocol was implemented by applying two doses of Respiporc Flu3 (RF3) with an interval of 3 weeks. Afterwards, gilts were vaccinated in quarantine and multiparous every 6 months. Productive parameters from litters during suckling period and piglets during nursery period coming from no vaccinated (NV) or vaccinated (RF3) sows were registered during the same period in two consecutive years. Total-born (TB-L), born-alive (BA-L), dead-born (%DB-L), mummified (%MM-L), weaned per litter (W-L) and mortality (%ML) during lactation were recorded. In nursery period weight at the beginning (WB) and at the end (WE), mortality rate (%MN), economic feed conversion rate (EFCR) and antibiotic cost/piglet (€Ab-piglet) were registered. Significant differences were considered when p<0.05.

#### Results

A total of 6440 litters were included (3225 NV, 3215 RF3). TB-L and BA-L were significantly higher in RF3 compared to NV, [TB-L:(NV:14.07;RF3:14.92)-TA-L: (NV:12.74;RF3:13.59]. %DB-L (NV:9.12;RF3:8.10),%MM-L(NV:0.986;RF3:0.823), %ML (NV:16.47; RF3:15.94) and W-L (NV:10.66;RF3:11.04) showed numerically differences comparing litters from vaccinated or not vaccinated sows. Regarding nursery period, there were statistical differences for WB in NV scenario (NV: 5.97;RF3:5.61), no differences were found for WE (NV:23.30;RF3:21.37). %MN, EFCR and  $\epsilon$ Ab-piglet were significantly better in RF3 group [(NV:11.35;RF3:3.19), (NV:1.76;RF3:1.61), (NV:1.25;RF3:0.81), respectively]

#### **Discussion and Conclusion**

On the conditions of this study, sow vaccination against SIV significantly improved productive parameters during lactation and nursery period and showed to be an efficient strategy for increasing productive efficiency in SIV infected farms.

#### IMM - Immunology and Vaccinology

### MUCOSAL AND SYSTEMIC ANTIBODY RESPONSES TO INFLUENZA A VIRUS FOLLOWING LIVE ATTENUATED INFLUENZA VIRUS OR REPLICON PARTICLE VACCINE

<u>M. Wymore Brand</u><sup>1</sup>, C. Kunzler Souza<sup>1</sup>, B. Arruda<sup>1</sup>, A. Vincent Baker<sup>1</sup> <sup>1</sup>Agricultural Research Service United States Department of Agriculture

#### **Background and Objectives**

Swine influenza A virus (IAV) vaccines in the United States include adjuvanted inactivated virus vaccines and adjuvanted and non-adjuvanted IAV-based replicon particle vaccines (RP). Mucosal live attenuated influenza virus vaccines (LAIV) have been demonstrated to induce mucosal immunity but are not available commercially. Non-adjuvanted hemagglutinin (HA)-RP vaccines generate homologous systemic HA antibody, but little is known how they compare to LAIV in induction of mucosal immunity. The present study compared RP and LAIV induction of mucosal antibody responses following heterologous virus infection.

#### Material and Methods

Pigs were vaccinated with experimental pandemic H1N1 LAIV or experimental, non-adjuvanted H1-RP, challenged with a heterologous delta-1 H1N2, and necropsied at 5 or 21 days post infection (DPI). Serum was evaluated for hemagglutinin inhibition (HI) titers and IgG and IgA whole-IAV (IAVw)-specific ELISAs. Nasal swabs were collected at 3 and 5 DPI for virus titration. Bronchoalveolar lavage fluid (BALF) was collected at necropsy for virus titration and IgG, total IgA (tIgA), and secretory IgA (sIgA) IAVw and HA-specific ELISA.

#### Results

H1-RP-vaccinated pigs had lower macroscopic lesion scores but similar virus titers to non-vaccine challenge (NV/CH) pigs. LAIV-vaccinated pigs had similar macroscopic lesion scores compared to NV/CH pigs but minimal virus titers. Both H1-RP and LAIV vaccines induced homologous HI and IAVw-specific serum antibody. In 5 DPI BALF, both groups of vaccinated pigs had homologous IAVw and HA-specific IgG, but only LAIV-vaccinated pigs had homologous IAVw and HA-specific IgG, but only LAIV-vaccinated pigs. By 21 DPI, homologous IAVw and HA-specific IgG, and sIgA, and low levels of heterologous IAVw-specific IgG, tIgA, and sIgA. By 21 DPI, homologous IAVw and HA-specific tIgA and sIgA was present in the BALF of H1-RP-vaccinated pigs.

#### **Discussion and Conclusion**

LAIV limited viral shedding and load while the H1-RP limited macroscopic lung lesions. Both vaccines induced similar homologous systemic antibody and BALF IgG, while only LAIV had detectable IAVw and HA-specific tIgA and sIgA in 5 DPI BALF. Stimulation of mucosal antibodies are important to limit viral shedding and disease particularly against heterologous infection. However cellular immune responses are also an important component of vaccine protection. A better understanding of mucosal correlates of protection including assessments on cellular immunity will aid in the development of improved swine IAV vaccine strategies.

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#### PRRSV-SPECIFIC IMMUNE RESPONSES AT THE MATERNAL-FETAL INTERFACE DURING LATE GESTATION

<u>M.R. Stas</u><sup>1</sup>, H. Kreutzmann <sup>1</sup>, K.H. Mair <sup>2</sup>, M. Koch <sup>1</sup>, C. Knecht <sup>1</sup>, M. Stadler <sup>2</sup>, K.A. Van Dongen <sup>2</sup>, A. Saalmüller <sup>2</sup>, T. Rümenapf <sup>3</sup>, W. Gerner <sup>4</sup>, A. Ladinig <sup>1</sup> <sup>1</sup>University Clinic for Swine, University of Veterinary Medicine Vienna, Austria <sup>2</sup>Institute of Immunology, University of Veterinary Medicine Vienna, Austria <sup>3</sup>Institute of Virology, University of Veterinary Medicine Vienna, Austria <sup>4</sup>The Pirbright Institute, Woking, United Kingdom

#### **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped RNA virus that causes reproductive failure, especially during late gestation. Research addressing the in utero immune response is sparse. Our previous ex vivo phenotyping experiments highlighted a local increase of early effector T cell phenotypes following PRRSV infection. Therefore, T cell phenotypes and their function at the maternal-fetal interface were investigated in an experimental PRRSV-infection model.

#### **Material and Methods**

Two groups of pregnant gilts (n = 5) were either infected with the PRRSV-1 field isolate AUT15-33 ( $5x10^5$  TCID<sub>50</sub>, intranasal) or sham-inoculated at day 85 of gestation. Twenty-one days post infection, all gilts were euthanized and the preservation status of each individual fetus was assessed. Tissues from the maternal endometrium (ME) and fetal placenta (FP) were collected, tested for viral loads by qRT-PCR, and mononuclear cells isolated to assess T cell phenotypes and proliferation (Ki-67). Cytokine production of T cells was investigated following in vitro re-stimulation with AUT15-33-derived peptide pools.

#### Results

Fetuses from PRRSV-infected gilts showed an impaired preservation status which coincided with the presence of PRRSV in the ME and FP. Ex vivo phenotyping revealed a strong increase in CD8 T cells in the ME and FP from PRRSV-infected gilts compared to controls, whereas no significant changes were observed for CD4 T cells. Ki-67 staining revealed a significant increase in proliferating Ki-67<sup>+</sup> CD8 T cells with an early effector phenotype (perforin<sup>+</sup>CD27<sup>+</sup>) following infection at both sides of the maternal-fetal interface. No differences were observed for proliferating Ki-67<sup>+</sup> CD4 T cells between the two groups; however, an increase of central memory cells (CD8 $\alpha^+$ CD27<sup>+</sup>) within Ki67<sup>+</sup> CD4 T cells was found. PRRSV-specific CD4 and CD8 T cells were identified in both ME and FP, based on the production of IFN- $\gamma$  and TNF- $\alpha$  following peptide restimulation.

#### **Discussion and Conclusion**

Data on the local T cell responses indicate that CD8 T cells are strongly involved in combatting PRRSV, even in the compartmentally separated FP. However, their activation may cause immunopathogenesis instead of protection. Further functional and in situ investigations are required to corroborate this hypothesis.

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### A FIELD TRIAL ON THE EFFICACY IN PROTECTING WEANED PIGLETS AGAINST TWO PRDC PATHOGENS USING A VACCINE AGAINST NON-PROGRESSIVE AND PROGRESSIVE ATROPHIC RHINITIS

H. Lichterfeld <sup>1</sup>, S. Trittmacher <sup>1</sup>, B. Wegner <sup>2</sup>, H. Blömer <sup>2</sup>, <u>T. Kornhoff <sup>2</sup></u>, S. Cros <sup>3</sup>, J. Montané Giralt <sup>3</sup>, I. Ballarà <sup>3</sup>, I. Gale <sup>3</sup>, I. Hennig-Pauka <sup>1</sup>

<sup>1</sup>Field Station for Epidemiology, University of Veterinary Medicine, Hannover, Foundation, Germany <sup>2</sup>Veterinary Practice Dümmerland, Steinfeld, Oldenburg, Germany <sup>3</sup>HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

Bordetella (B.) bronchiseptica and Pasteurella (P.) multocida are bacteria of the porcine respiratory disease complex (PRDC). They can facilitate the invasion of other pathogens and might interact with them synergistically. Vaccination against these opportunistic bacteria might reduce infection pressure in the population and improve respiratory health. In this field study, improvement of respiratory disease after passive transfer of colostrum antibodies from vaccinated sows to their piglets was assessed by clinical scoring.

#### **Material and Methods**

Six subsequent groups of nursery pigs alternating from Rhiniseng<sup>®</sup> vaccinated (experimental) and not vaccinated (control) sows with a 1-week-interval shift were included in the study. The vaccination schedule consisted of two doses at six and three weeks before farrowing. The selected farm, boasting 1200 sows, was PRRSV and M. hyopneumoniae free but had a history of respiratory issues in 3 - 4-week-old suckling piglets. Subsequent lung diagnostics of affected pigs revealed the presence of both B. bronchiseptica and P. multocida. For each of the six groups, one compartment with 8 pens was examined over seven weeks for the coughing and sneezing index. These indices were analysed using a linear mixed-effect model, wherein the weaning location and compartment were treated as random effects.

#### Results

Animals born from vaccinated sows showed significantly lower coughing indices at 5, 9 and 10 weeks of age and significantly lower sneezing indices at 6 weeks of age.

#### **Discussion and Conclusion**

In this field study, sow vaccination with Rhiniseng<sup>®</sup> was effective in improving respiratory health in nursery piglets.

### ANIMAL WELFARE AND ETHOLOGY

#### WEL-CP-01

WEL - Animal Welfare and Ethology

# EFFECT OF PIG SYNTHETIC PHEROMONES AND POSITIVE HANDLING OF SOWS ON THE PRODUCTIVITY OF NURSERY PIGS

<u>D. De Meyer</u><sup>1</sup>, I. Chantziaras<sup>2</sup>, A. Amalraj<sup>2</sup>, D. Maes<sup>2</sup> <sup>1</sup>Vedanko, Belgium <sup>2</sup>Department of Internal Medicine, Reproduction and Population Medicine, Ghent University, Belgium

#### **Background and Objectives**

Weaning is an important stress event in the life of pigs, increasing the risk for health problems and reduced performance. The release of pheromones to mimic maternal presence in nurseries may create a familiar environment for the piglets and alleviate the negative effects of weaning stress. This study investigated the effect of synthetic pheromones on the performance of nursery pigs. The effect of positive handling of sows in the farrowing unit on the performance of the offspring in the nursery was also investigated.

#### **Material and Methods**

The study was performed in a commercial pig farm, including 24 batches of weaned piglets (216 per batch). Half of the piglets originated from sows exposed to positive handling, namely, music was played from 6.00 am to 6.00 pm when the sows entered the farrowing unit until weaning, and backscratching was performed upon entering the farrowing unit until farrowing. In the nursery, half of the batches were randomly allocated to treatment, and half served as controls. Piglets of treated groups were exposed to a synthetic analog of the maternal pig appeasing pheromone (PAP) (SecurePig®, Signs, Avignon France). The product consisted of a gel block from which the pheromones diffused slowly in the room. Different performance parameters were measured during the nursery period.

#### Results

Neither the sow treatment nor the treatment with pheromones significantly influenced the performance of the piglets during the nursery period (P>0.05). The average daily gain (318 vs. 305 g/day), feed conversion ratio (1.66 vs. 1.68), and the number of antimicrobial treatment days (16.9 vs. 17.9 days) were numerically better in the pigs exposed to the pheromones compared to the control groups, mortality however was numerically (4.4 vs. 2.2%) higher in the treated groups.

#### **Discussion and Conclusion**

In the present farm, pigs exposed to the pheromone treatment during the nursery did not show significant increases in performance. Further research is warranted to investigate more farms, including farms where piglets suffer from aggression problems in the post-weaning period.

#### WEL - Animal Welfare and Ethology

### DIFFERENTIAL GENE EXPRESSION IN THE LIVER OF SUCKLING PIGLETS WITH DIFFERENT DEGREES OF SWINE INFLAMMATION AND NECROSIS SYNDROME

<u>K. Gerhards</u><sup>1</sup>, S. Becker<sup>2</sup>, H. Willems<sup>2</sup>, G. Reiner<sup>2</sup> <sup>1</sup>Justus-Liebig-University Giessen <sup>2</sup>Department for Veterinary Clinical Sciences, Justus-Liebig-University Giessen, Frankfurter Strasse 112, Giessen, Germany

#### Background and Objectives

Swine inflammation and necrosis syndrome (SINS) can occur with high prevalence in pigs of different ages. The syndrome affects the tail, ears, teats, coronary bands, claws and heels and affects the well-being of involved animals. The primarily endogenous syndrome leads to vasculitis, thrombosis and intimal proliferation in the affected animals. Signs of SINS appear to be triggered by intestinal and liver disorders and can be modified by a wide range of external factors. The aim of the present study was to investigate the liver transcriptome in piglets with different degrees of the syndrome, in order to identify practice-relevant associations of liver gene regulation with the syndrome.

#### Material and Methods

For transcriptome analysis, samples were taken from 24 piglets, 8 animals each from Duroc, SINS-resistant Pietrain and SINS-susceptible Pietrain boars. The piglets were selected in such a way that 4 animals with lowest SINS scores and 4 piglets with highest SINS scores out of each boar group were analysed. The piglets were clinically scored on their 3rd day of life. The RNA was extracted, purified and hybridised with a porcine transcriptome microarray (GeneChip). The results were validated by qPCR.

#### Results

The transcriptomes of the offspring of Duroc and Pietrain boars were significantly different. In addition, 126 genes were differentially expressed depending on the SINS status, with at least a 2-fold difference between piglets, independent of the boars' breed. SINS was associated with the elevated expression of 61 specific genes and the reduction in expression of 63 further genes. Some of the differentially expressed genes were associated with inflammation, vasculitis and necrosis pathways. Among them were CRP, GYPA, S100A12, and LIPK.

#### **Discussion and Conclusion**

The markedly different gene expression in piglets with different degrees of SINS proves that, in addition to the external clinical signs, liver metabolism is severely impaired towards the acute phase and inflammation. The associated genes provide valuable information on the pathogenesis of the syndrome and could serve in future studies to develop new practical diagnostic tools.

#### WEL - Animal Welfare and Ethology

# NEST MATERIAL DECREASES SHAW CHEWING, BAR BITING AND CAGE INTERACTION AROUND FARROWING IN CRATED GILTS

M.S. Monteiro <sup>1</sup>, B. Bracco Donatelli Muro <sup>2</sup>, <u>R. Carnevale</u> <sup>3</sup>, M.V.B. Nicolino <sup>1</sup>, A.L.B. Mezzina <sup>2</sup>, H.R. Geremias <sup>2</sup>, T.M.P. Lima <sup>2</sup>, C. Veloso <sup>2</sup>, C.A. Pospissil Garbossa <sup>2</sup>

<sup>1</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>2</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>3</sup> Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

#### **Background and Objectives**

Even though domestication has occurred, research results indicates that modern sows still exhibit a strong instinct to construct nests before the farrowing process. However, in current pig farming, due to limited space or materials, sows cannot satisfy this natural behavior, causing distress and frustration, jeopardizing animal welfare. This study aimed to compare the behavior of crated gilts with access or not to a biodegradable, water soluble and edible nesting material.

#### **Material and Methods**

Animals were kept in crates for the total duration of the trial. Farrowing was synchronized with cloprostenol, all gilts received a split dose protocol on day 113 of gestation. Gilts were randomly allocated at entrance of the farrowing room in one of the two groups: CON (n = 6; gilts that did not receive any material) and NES (n = 6; gilts that received nesting material). Behavior observation started for both groups 7 hours from first cloprostenol dose, and at this moment NES gilts had access to the nesting material. Behavior observation was performed continuously until the onset of farrowing (birth of the first piglet). The behaviors recorded were standing, lying and sitting, grouped as postural changes; interaction with nesting material and snout-floor, analyzed as nesting building behavior and bar biting, cage interaction, sham chewing, analyzed as steryotyped behavior. Results are presented as the percentage of time the gilts spent doing a group of behavior. Variables were analyzed with generalized linear models and statistical differences were set at p < 0.05.

#### Results

The postural changes were similar between groups (p>0.05). The nest building behavior was increased (p < 0.05) for NES gilts (3.37% and 13.37% for CON and NES gilts, respectively), while sterotype were increased (p < 0.05) for CON gilts (11.13% and 4.56% for CON and NES gilts, respectively).

#### **Discussion and Conclusion**

Even sows with no access to farrowing material showed the behavior of nest building, showing how important is this action for the species. The results of decreased sham chewing and interactions with the cage and increased nest building behavior, support the notion that supplying sows with nesting material is effective in promoting a better welfare around farrowing.

WEL - Animal Welfare and Ethology

#### ACOUSTIC ENVIRONMENTAL ENRICHMENT FOR ENHANCED ANIMAL WELFARE IN SWINE

H. Fried <sup>1</sup>, S. Becker <sup>1</sup>, G. Reiner <sup>1</sup>

<sup>1</sup>Department for Veterinary Clinical Sciences, Justus-Liebig-University Giessen, Frankfurter Strasse 112, Giessen, Germany

#### Background and Objectives

The keeping of pigs often leads to reduced activity and boredom in the animals. At the same time, social interactions are often characterised by antagonistic behaviour. Numerous studies have shown that this problem can be alleviated by improving environmental enrichment. The aim of the present study was to investigate the effect of acoustic environmental enrichment on the overall activity and antagonistic behaviour of weaners.

#### Material and Methods

The effect of music was tested against sounds of the sea, forest sounds and human voices on the radio. For extended characterisation, two different quiet pieces of music (Antonio Vivaldi, Summer; Pachelbel, Canon in D) were tested with 3 instrumental variations (strings, brass, piano) each. The behavioural patterns of each pig before, after and during the auditory impressions were assigned to the exact number and duration of seconds. In total, over 6 hours of video footage was recorded for each pig. This meant that raw data of over 730 hours per run was recorded and compiled into over 15,000 data records.

#### Results

During acoustic stimulation, significantly fewer negatively associated behavioral patterns such as fighting, biting each other, mounting or belly nosing were shown. Music as acoustic stimulation performed better than other acoustic stimuli. Calm music performed better than energetic music. Human voices that could only be heard on the radio particularly attracted the pigs' attention and significantly stimulated them to listen and to stop any current activity.

#### **Discussion and Conclusion**

Pigs of that age seem to prefer acoustic stimulation in the form of calm classical music and brass instruments. The acoustic stimuli could be used to abruptly end current behaviour, including antagonistic behaviour. But overall, antagonistic behaviour was also significantly regulated by acoustic stimuli and especially by calm music, and the overall activity of the animals was positively influenced. Calm music can be easily and inexpensively realised in the compartments via loudspeakers. It could help to counteract antagonistic behaviour and promote animal welfare in praxiy.

#### WEL - Animal Welfare and Ethology

#### EVALUATION OF THE EFFICACY AND SAFETY OF PROCAINE HYDROCHLORIDE PLUS EPINEPHRINE APPLIED INTO THE TESTICLE FOR ANALGESIA BEFORE CASTRATION OF PIGLETS AGED UP TO 7 DAYS

<u>A. Rostalski</u><sup>1</sup>, L. Naderer<sup>2</sup>, C. Schneider<sup>2</sup>, A. Randt<sup>3</sup>, K. Hellmann<sup>4</sup> <sup>1</sup>Doctor of Veterinary Medicine, Head of Swine, Bavarian Animal Health Service (TGD Bayern e.V.) <sup>2</sup>Klifovet <sup>3</sup>CEO TGD Bayern e.V. <sup>4</sup>CEO, Head of Klifovet

#### **Background and Objectives**

Pain elimination during piglet castration is required by law in the EU. As the necessity is given for surgical intervention in preventing boar taint, the opinion about legal methods differ in European countries. Sweden and Denmark prefer local anesthesia applied into the testicles by the farmer, Germany solely accepts general anesthesia requirering either a veterinarian or a certificate of competence for the farmer. While general anesthesia is stressful for younger piglets, intratesticular injections are recalled as painful and noneffective. The aim of the study was to evaluate the effectiveness and safety of local anesthesia under field conditions, using an EU-wide authorised medical product.

#### **Material and Methods**

In a double-blinded, placebo-controlled, randomized and blocked field study on an Austrian breeding farm of 200 sows, 126 healthy male piglets aged up to 7 days were divided into 4 groups and injected with three different doses (0,25/0,5/0,75 ml) of procaine hydrochloride plus epinephrine tartrate (Pronestesic™ 40 mg/ml, Veyx) into one testicle (G1-3) and a similar volume of 0,9% NaCl-solution into the remaining one (G4). Castration followed after 10 minutes while defensive behaviour, vocalisation (volume and character) and resistance of the cremaster muscle was evaluated during removal of each testicle in three phases (skin cut, pulling on and cutting off the spermatic cord). The healing process was scored after 24 hours and 10 days. All obtained variables were descriptive and piled into study groups. Continuous variables were statistically analyzed with avg, sd, sample size, median, quartils, min and max. For categorical and binary variables absolute and relative frequencies were displayed.

#### Results

The defensive behaviour differed highly significant (p<0,001) between treated (G1-3) and untreated testicles (G4), the vocalisation exhibited similar results (p<0,1-0,001). The tone of the cremaster muscle was also significantly reduced (p<0,009-0,002) between G1-3 and G4. Best results were scored with a volume of 0,75 ml Pronestesic<sup>TM</sup> (G3) per testicle. The healing process was completely undisturbed.

#### **Discussion and Conclusion**

Pronestesic<sup>™</sup> applied intratesticularly interrupts nociception during castration significantly and has no negative impact on the healing process. Combined with "low stress handling" it`s a benefit for animal welfare accompaniing an unwanted but still necessary surgical intervention.

#### WEL - Animal Welfare and Ethology

#### DEVELOPMENT OF THE UDDER IN SOWS DURING FOUR LACTATIONS

<u>F. Thorup 1</u> <sup>1</sup>SEGES Innovation, Agro Food Park 15, 8200 Aarhus

#### Background and Objectives

Sows need many functional teats to wean many piglets. We followed gilts from farrowing their first litter until the end of 4<sup>th</sup> lactation learning what influences the capacity of the specific teat to nurse a piglet.

#### **Material and Methods**

From first farrowing, 445 DanBred LY gilts in three herds were followed until culling or weaning their 4<sup>th</sup> litter. The number of live piglets was registered at farrowing, after litter adjustment, at castration (around day 4), on day 21, and if the sow was used as a nurse sow also when weaning the nurse litter. Functionality of each milk gland was evaluated at farrowing, on day 21 and if weaning a nurse litter.

#### Results

In the three herds, 147, 130 and 166 gilts were included, and 90, 39 and 86 sows weaned their 4<sup>th</sup> litter. Liveborn averaged 16.2; 18.5; 18.7 and 17.9 in parity one to four. The sows were given 14.6; 14.8; 14.7 and 14.2 piglets to nurse in parity one to four. At farrowing, the sows had averagely 14.3 teats in all four parities, of which 98% were rated functional. When milk glands were evaluated 21 days after farrowing, the frequency of functional teats had dropped to 92, 92, 91 and 90% in the four parities. Among gilts finishing at least 2 parities, the gilts had 4119 functional and 303 nonfunctional milk glands on day 21. Only 27% (82 of these nonfunctional glands) were still nonfunctional on day 21 in 2<sup>nd</sup> lactation, while 221 of the previously nonfunctional glands were rated as functional. By day 21 in 2<sup>nd</sup> parity, 281 glands (7%) of the previously 4119 functional glands were now nonfunctional.

#### **Discussion and Conclusion**

The low frequency of milk glands that were nonfunctional in two consecutive lactations indicates that nonfunctional milk glands are not the primary factor explaining the number of piglets nursed. Thus, after farrowing a sow should be given the number of piglets that fits the actual number of functional milk glands, and not the number of piglets the sow weaned in the previous lactation.

WEL - Animal Welfare and Ethology

### COMPARISON OF LABOUR TIME, COST, ADVANTAGES/DISADVANTAGES OF FOUR METHODS TO EVALUATE THE GESTATING SOWS' FIGHT INTENSITY

<u>E. Cantaloube</u><sup>1</sup>, A. Taktak<sup>1</sup>, L. Gautier<sup>1</sup>, T. Gin<sup>1</sup> <sup>1</sup>Chêne Vert, 4 rue Théodore Botrel, 22600 Loudéac FRANCE

#### **Background and Objectives**

Evaluation of gestating sows' aggression intensity is useful to fix a mixing problem. We used four methods: audio recording, video recording, observations in situ and a skin lesion score. The aim of this study was to evaluate labour time, cost, advantages/disadvantages of each method to determine which one is most effective for a regular use.

#### Material and Methods

110 sows were mixed in 4 rooms. From 30min to 30h after mixing we measured: the number of fights (NF) observed with cameras (Method1 video recording), the number of shouts and feet tramples (FT) with dictaphones (Method2 audio recording). NF was also noted in situ 30min, 24 and 36h after mixing by an observer during 10min per pen (Method3). The same observer gave a skin lesion score for each sow before, 24 and 48h after mixing (Method4).

For all methods, we noted the global time (GT) spent. The total cost (TC) of each method was calculated multiplying the time spent with the cost of an engineer and adding it with the cost of the material. GT and TC was evaluated per sow.

#### Results

GT and TC were 9minutes and 3€ for audio recording, 16minutes and 15€ for video recording, 4minutes and 1,15€ for the observations in situ and 3minutes and 0,8€ for the skin lesion score. Observations in situ had the benefit that it doesn't required material but it only allowed short periods of observations. Video recording had the advantages of seeing longer periods and skipping sow sleeping times. The limits were the investment in cameras, heavy video files, need of a visible individual identification of the sow. Compared to camera, audio recording was less expensive, files were lighter, and we didn't need a good luminosity. It had the disadvantage of being a collective and not individual measure. Skin lesion score didn't need any material and wasn't time consuming.

#### **Discussion and Conclusion**

We recommend using skin lesion score regarding cost and time. Camera recording is a method of interest but need to be automatically analysed and the material is still quite expansive. Audio recording has to be improved to have measures at pen level.

### PRECISION LIVESTOCK FARMING

#### PLF-CP-01

PLF - Precision Livestock Farming

## DATA AND TECHNOLOGY APPLICATION IN PIG HEALTH AND WELFARE MANAGEMENT: A FOCUS GROUP STUDY OF VETERINARIANS IN IRELAND, THE NETHERLANDS AND SPAIN

X. Zhou <sup>1</sup>, B. Garcia-Morante <sup>2</sup>, A. Burrell <sup>3</sup>, L. Dieste-Pérez <sup>4</sup>, K. Eenink <sup>4</sup>, C. Correia-Gomes <sup>3</sup>, J. Segalés <sup>2</sup>, M. Sibila <sup>2</sup>, T. Tobias <sup>4</sup>, <u>C. Vilalta <sup>2</sup></u>, A. Bearth <sup>1</sup>

<sup>1</sup>Consumer Behavior, Institute for Environmental Decisions, ETH Zurich, Universitaetstrasse 22, 8092 Zurich, Switzerland <sup>2</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain. <sup>3</sup>Animal Health Ireland, 2–5 The Archways, Carrick on Shannon, Co. Leitrim, Ireland N41 WN27

\*Animal Health Ireland, 2–5 The Archways, Carrick on Shannon, Co. Leithim, Ireland N41 WN \*Royal GD, Arnsbergstraat 7, 7418 EZ, Deventer, the Netherlands

\*Royal GD, Amspergstraat 7, 7418 EZ, Deventer, the Nethenands

#### Background and Objectives

Data-driven strategies can facilitate stakeholders' decision-making and on-farm health management. Such strategies should meet stakeholder needs, including pig veterinarians amongst others. Henceforth, this study aimed to understand the current status of veterinarians' data utilization and their needs for data tools to support daily work.

#### **Material and Methods**

Focus group discussions were completed in Ireland (n = 8 participants), the Netherlands (n = 7), and Spain (n = 12) to investigate veterinarians' goals in pig health management, the status quo of using data and technology for management, and their needs regarding data tools. Thematic analysis was applied to analyse the transcribed data from these discussions.

#### Results

One of the principal goals of veterinarians is to control/prevent disease in pigs and improve their health and welfare. Management of diseases with the reduction of antibiotic use was identified as an ongoing challenge. Veterinarians believed that data-driven strategies, specifically using data tools, are beneficial for pig health and welfare management, but several barriers were recognized. At the farm level, many data are still manually collected and recorded, leading to the delay or lack of data access. Additionally, extra investment cost, poor internet connection and the lack of technical skills and time of farmers might hinder some farms to employ the data tools. At the veterinarian level, a user-friendly and intuitive data tool for sharing, managing, and analyzing data is needed. Specifically, veterinarians needed an integrated data tool that links with different data sources to overview the health information of pigs. For short-term purposes, an early warning tool or a dashboard visualizing disease prevalence across a geographical map could help to detect and control outbreaks of infectious diseases. For long-term purpose, data tools that constantly visualize data from farms and slaughterhouses in time series could be helpful to track the pig health status and facilitate decision-making.

#### **Discussion and Conclusion**

This study uncovered barriers to data utilization from the perspectives of veterinarians. To facilitate data utilization by transforming raw bytes into useful information, tools that allow intuitive analysis and visualization with standardized and timely updated information are in high demand by veterinarians.

#### PLF - Precision Livestock Farming

#### MACHINE LEARNING ACCURATELY PREDICTS INFECTION IN SWINE FARMS

A. Halev <sup>1</sup>, B. Martínez-López <sup>2</sup>, M.J. Clavijo <sup>3</sup>, C. Gonzalez-crespo <sup>4</sup>, J. Kim <sup>1</sup>, C. Huang <sup>7</sup>, S. Krantz <sup>6</sup>, R. Robbins <sup>5</sup>, X. Liu

<sup>1</sup>Department of Mathematics, University of California, Davis, CA, USA
<sup>2</sup>University of California, Davis
<sup>3</sup>Veterinary Diagnostic and Population Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, Pig Improvement Company, PIC®, Hendersonville, TN, USA
<sup>4</sup>Center for Animal Disease Modeling and Surveillance (CADMS), School of Veterinary Medicine, University of California, Davis, CA, USA
<sup>5</sup>PIC North America, Hendersonville, TN 37075
<sup>6</sup>Tosh Farms, Henry, TN USA
<sup>7</sup>Department of Computer Science, University of California, Davis, CA, USA

### **Background and Objectives**

Harnessing the power of artificial intelligence (AI) and successfully incorporating it into everyday life can be a dilemma. We previously laid out the potential for machine learning (ML) applied to swine health and production data streams, like diagnostic results and animal movement, to predict infectious disease outbreaks. In our study, we design a model to predict infection using three disease pathways: direct contact, indirect contact, and local area spread.

#### **Material and Methods**

We consider six ML models with different combinations of the features to determine the model that generalizes and performs best. Specifically, we consider logistic regression, support vector machines, decision trees, gradient boosting, and random forests in both systems; in system B, we also consider a neural network model (auto encoder MLP) to handle distribution shift. The data was extracted from both internal swine farm collection and external sources, which allowed us to model various factors contributing to disease occurrence, including historical as well as current environmental, climatic and farm-level specific factors.

#### Results

The global disease model created with system A data to classify any positive or negative case had 85% balanced accuracy. The disease specific models created with system B data to predict infections of PEDV, PRRSV, Mycolasma hyopneumoniae (MHP) and IAV had balanced accuracies ranging from 58-74%. In system A, distance of nearby farms, total piglet inventory, the number of pigs weaned per mated female per year (PWMFY), pregnancy rates and average gilt pool inventory (total gilt days per days in period). In system B, test rates, wind direction and speed, sow gestation feed, incoming swine movements, and biosecurity data are important predictors. Biosecurity data proves important for PRRSV, MHP and PEDV: the employee access point plan is valuable in PRRSV and PEDV prediction, and the carcass disposal plan is valuable in PEDV and MHP prediction.

#### **Discussion and Conclusion**

This work considered production systems as a whole, including both sow and nursery/finishing farms in both systems. In addition, analysis and comparison of two distinct farm production systems with differing levels of data availability are presented, and showed that machine learning is able to predict positive samples in both of these contexts.

#### PLF - Precision Livestock Farming

# SOUND-MONITORING VISUALIZATION OF AN HP-PRRS VIRUS STRAIN OUTBREAK AFTER LATERAL INFECTION IN A SPANISH NURSERY

S. Figueras <sup>1</sup>, T. Guiu <sup>2</sup>, <u>C. Alonso</u> <sup>3</sup>, F. Erusalimsky <sup>4</sup> <sup>1</sup>Boehringer Ingelheim Animal Health España <sup>2</sup>Piensos Costa S.A, Fraga, Huesca, Spain <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH, Germany <sup>4</sup>SoundTalks, Leuven, Belgium

#### **Background and Objectives**

SoundTalks<sup>®</sup>, a cloud-based 24/7 artificial intelligence sound sensor technology, provides the Respiratory Health Status (ReHS) metric, well demonstrated to be the swine industry gold standard for respiratory clinical signs evaluation. This study aimed to visualize and measure the respiratory health impact due to the inclusion and circulation of a highly pathogenic PRRS virus (HP PRRSv) strain in a nursery facility sound-monitored.

#### **Material and Methods**

The nursery has 4 buildings, 8 rooms/building equipped with 1 sound-monitoring device and 500 piglets/room. In total 16000 animals growing from 6 to 18 kgs during 6 weeks before been transferred to finishing sites. All piglets came from the same sow herd. The nursery has been positive to the "resident" type 1 PRRSv strain (monitored by PRRS\_ORF5 sequencing) since it is being sound monitored by monthly oral fluids samples. In November 2022 a new strain was detected. ORF5 sequencing revealed a 99% homology with an HP PRRSv. Daily ReHS as well as daily alarm status (i.e. green healthy days, vs yellow/red alarmed days) for all rooms were consolidate and analyzed for the study.

#### Results

After the outbreak the days in green color decreased 39% whereas yellow alert and red alerts increased 100% and 56% respectively compared to the period before the outbreak. Analysis of the heatmap overtime reveals a higher proportion of days in red after the new infection occurred. Pigs remained in red alarm period for the entire batch from November 23 to June 23 being the hot months (July 23 and August 23) better months for the respiratory pathology. This season-related impact on ReHS observation is shown in the heatmap. Since January 22 to April 22 more red and yellow alerts whereas from June 22 to September 22 more days in green were detected.

#### **Discussion and Conclusion**

A sound-based real-time monitoring technology provides an objective method to analyze and visualize the impact of respiratory pathogens. In this case, we could clearly see how a high virulence PRRS virus strain can enhance the severity of the respiratory distress in nursery piglets.

#### PLF - Precision Livestock Farming

# RESPIRATORY HEALTH STATUS, MEASURED BY A SOUND-BASED TECHNOLOGY, IMPACTS GROWTH OF FINISHING PIGS

V. Thuring <sup>1</sup>, <u>M. Steenaert <sup>2</sup></u>, C. Alonso <sup>3</sup>, H. Prüst <sup>2</sup> <sup>1</sup>Coöperatie VARKENSARTSEN <sup>2</sup>Boehringer Ingelheim AH the Netherlands BV <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH, Germany

#### **Background and Objectives**

Respiratory health in modern commercial pig husbandry is a major concern worldwide. SoundTalks® is a cloud-based sensor technology that monitors 24/7 the sound emitted from pigs. Based on artificial intelligence, this technology processes the sound data collected at the farm and transforms it into a metric (ranging between 0 and 100) that represents the animals' respiratory health status (ReHS). When the ReHS value falls below a certain threshold, the system emits early warnings (yellow/red alerts) allowing producers to intervene prior to clinical signs of respiratory disease are evident for care givers. Despite some initial studies on the impact of ReHS on production performance the impact of these early disease signals is not yet fully understood. The goal of this study was to further investigate the relationship between ReHS, average daily gain (ADG) and mortality in finishing pigs under field conditions.

#### **Material and Methods**

In a Dutch farrow-to-finish herd 3 different finishing batches, sound-monitored with SoundTalks®, were included in the study. Each batch was placed in 2 rooms (n=150/room). Pigs from 4 pens/room were individually weighed at 4 and 12 weeks after placement (weeks on feed). Linear regression models were used to study the association between production variables and respiratory health (i.e., average ReHS value, SoundTalks alarms, yellow and red monitor color and combinations).

#### Results

A total of 445 animals were monitored during the study. Two out of three batches had respiratory alarms (avg 18.5%, min 8%, max 23%) however mortality was similar in all groups (avg 1.1% min 0.0%, max 2.7%) with no relevant differences between rooms. A high correlation was observed between batch average ReHS value and ADG (R<sup>2</sup>=0.746, p=0.023).

#### **Discussion and Conclusion**

Results from this study demonstrated that 5% increase in respiratory alarms impacted in 10gr difference in the ADG at this age of animals, impact not fully previously demonstrated in other settings at this animal stage. The demonstrated impact seen in this study supports the implementation of interventions as early as possible to combat disease outbreaks. A cloud-based sensor technology that monitors respiratory health 24/7 in an objective way is a tool that could help producers in the field.

PLF - Precision Livestock Farming

### RESPIRATORY HEALTH STATUS IMPACT ON PRODUCTION PERFORMANCE MEASURED BY SOUND-MONITORING DEVICE IN A FATTENING FARM IN HUNGARY

K. Kerenyi <sup>1</sup>, B. Woeller <sup>1</sup>, B. Kartalik <sup>1</sup>, <u>G. Lopez-Moreno <sup>1</sup></u>, C. Alonso <sup>2</sup> <sup>1</sup>Boehringer Ingelheim RCV GmbH & Co KG <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany

#### Background and Objectives

Swine production is currently facing challenges to improve animal welfare, reduce antibiotic usage, and optimize resource utilization. Respiratory disease remains the primary health concern for growing pigs, with losses estimated to be as high as \$10 per pig. This has prompted producers to explore innovative tools. One such tool is a sound monitoring device (SoundTalks®) that continuously evaluates pig sounds, providing a Respiratory Health Status (ReHS) score ranging from 0 (lowest) to 100 (highest). When the ReHS value falls below a threshold, an alarm is triggered (yellow/red light). This cutting-edge sound monitoring device has proven its ability to detect respiratory health issues up to 5 days earlier than farmworkers, allowing for early intervention and ultimately leading to reduced antibiotic usage. Despite all these advantages, such technology has never been tested under Hungarian farming conditions. Therefore, the objectives of this study were to describe the ReHS status and to assess the correlation between ReHS, production parameters and diagnostic results.

#### **Material and Methods**

A Hungarian fattening farm (N=5,000 pigs) installed eight sound monitors in two barns (1 and 2; N=1.600pigs). Oral fluid samples were collected from pigs when the monitors displayed a red alarm to investigate the associated pathogens. Production data was consolidated from the monitored barns and associations between alarms and respiratory health status evaluated.

#### Results

Significant differences in ReHS (average score and % green days) were observed between Barn 1 (ReHS average=78; 91%) and Barn 2 (ReHS average=51; 35%)(p value <0.05). Barn 1 had 1.4% lower mortality, 72 grams higher daily weight gain, and 0.03 lower feed conversion units compared to Barn 2. Diagnostic results from collected samples of both barns tested positive for IAV, M.hyo, and APP.

#### **Discussion and Conclusion**

This report represents the first implementation of a sound monitoring system on a Hungarian farm. SoundTalks® demonstrated reliable results, with alerts observed during the growth period aligning with clinical observations, diagnostic results, and, most importantly, production performance parameters measuring disease impact between barns. The adoption of a sound monitoring system in growing pigs can lead to increased animal welfare, enhanced production performance, and more precise antibiotic usage.



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# **POSTERS**
# VIROLOGY AND VIRAL DISEASES

# VVD-PP-01

VVD - Virology and Viral Diseases

# QUANTIFICATION OF EXCRETION AND VIREMIA OF ORTHORUBULAVIRUS SUIS

<u>J.F. Rivera-Benitez</u><sup>1</sup>, J. De La Luz-Armendáriz<sup>2</sup>, K. González-Solórzano<sup>2</sup> <sup>1</sup>*INIFAP* <sup>2</sup>*Facultad de Medicina Veterinaria y Zootecnia. UNAM.* 

## Background and Objectives

Orthorubulavirus suis (ORVS) causes blue eye disease (BED), it is a virus considered endemic in Mexico. BED has been identified since 1980 and has not been completely eradicated or understood. Various studies have been carried out, however, excretion, viremia and viral load during persistent infection have not been analyzed. The objective of this study was to identify and quantify excretion and viremia during prolonged ORVS infection.

#### **Material and Methods**

Twenty-five six-week-old pigs were inoculated, clinical follow-up was performed, and nasal, oral/tonsillary swab samples, and PBMCs were collected at variable intervals until 380 days post-infection (dpi). An immunosuppressive agent was applied at 240 and 365 dpi. The samples were analyzed with a qRT-PCR.

## Results

Viral excretion was identified through the nasal and oral routes from the third day post-infection. Excretion lasted 120 dpi, decreasing to negative values between 150 and 280 dpi; however, a new excretion was recorded between 310 and 380 dpi (associated with induced immunosuppression). Through the oral route, excretion was identified throughout the evaluated period, the highest concentration was recorded at 5 dpi. In the viremia evaluated in the PBMCs, the highest viral load occurred on average at 70 dpi, variable values were identified during the experimentation, however, a prolonged and intermittent viremia was observed, when positive samples were recorded up to 360 dpi.

#### **Discussion and Conclusion**

BED was long considered a self-limiting disease, which disappeared in a period of around six months. This concept is correct in the sense of the clinical signs associated with the infection, however, in terms of the infection it would not be appropriate. ORVS infection is established as persistent in various organs of the nervous, respiratory, lymphatic and reproductive systems. The distribution of the viral load identified in the present study indicates that the infection pattern is chronic, maintaining a high viral load in the first days post-infection and subsequently decreasing until it disappears in some type of sample. These results point out the importance of epidemiological surveillance of one of the most important diseases at the national level, as it is exclusive to Mexico. Financing FONSEC SADER-CONACYT 2017-06-292826.

VVD – Virology and Viral Diseases

# CLINICAL OUTCOMES FOLLOWING EXPERIMENTAL CHALLENGE WITH A HIGHLY PATHOGENIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS STRAIN IN NURSERY PIGS

<u>A.M. Stoian</u><sup>1</sup>, P. Curto<sup>1</sup>, A. Pey<sup>2</sup>, A. Trabal<sup>2</sup>, E. Aulinas<sup>2</sup>, J. Baliellas<sup>3</sup>, L. Fraile<sup>1</sup> <sup>1</sup>University of Lleida, Lleida, Spain <sup>2</sup>AM Animalia, Bianya SL, Spain <sup>3</sup>Grup de Saneiament Porci. Lleida. Spain

#### **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) remains arguably the most important infectious disease affecting the global pig industry. The emergence of highly pathogenic strains, such as the Rosalia strain, poses significant challenges to effective disease control worldwide. Despite the observation of this highly virulent PRRSV strain under field conditions, its comprehensive characterization under experimental settings is lacking. Thus, the main objective of this study was to assess the clinical outcome resulting from the infection of nursery piglets with the high-virulence type I PRRSV Rosalia strain under two challenge routes.

# Material and Methods

Twenty-five 8-weeks-old PRRSV-free pigs were divided into three groups. Group 1 (n=5) remained PRRSV-negative throughout the study, while group 2 (n=10, IN group) and group 3 (n=10, IM group) were challenged with a high-virulence PRRSV strain ( $1x10^5$  TCID<sub>50</sub>/mI), via intranasal and intramuscular routes, respectively. For nine weeks, the pigs were examined daily for clinical signs: behavior, body condition, dyspnea, cough, nasal discharge, and digestive signs. In addition, rectal temperatures (RT) were recorded daily for the first 10 days post-challenge, and afterward, only if fever signs persisted (RT>39.5°C).

#### Results

In both groups, animals exhibited prolonged and severe pyrexia (RT>40.5°C) with peak rectal temperatures observed at 10- and 11-days post-challenge The IM-infected pigs showed acute respiratory distress, severe depression, lethargy and cyanotic ears, while the IN-challenged pigs exhibited mainly prolonged and moderate dyspnea. Limb edema and growth retardation were observed in both challenged groups, with higher severity in the IM group. Overall, clinical scores calculated based on RT, behavior, body condition, cough, dyspnea, and nasal discharge were higher for the IM-challenged pigs, particularly for RT and behavior. Finally, the mortality rates in the IM and IN groups were 100% and 30%, respectively.

### **Discussion and Conclusion**

Differences in clinical presentation were observed based on the challenge route. Intramuscular virus administration led to acute clinical cases with high mortality rates, intranasal administration allowed for recovery with lower mortality rates, even in the presence of moderate and persistent clinical signs. These results underscore the importance of correct management practices to prevent iatrogenic transmission on farms.

VVD – Virology and Viral Diseases

# EFFICACY OF A PRRSV LIVE VACCINE BASED ON THE NATURAL NONPATHOGENIC G16X STRAIN IN SWINE OPERATIONS IN MEXICO.

<u>H. Lara</u><sup>1</sup>, J.A. Torres Islas<sup>1</sup>, D. Sarfati<sup>1</sup>, B. Lozano<sup>1</sup>, F. Zuckermann<sup>2</sup>, A. Carranza<sup>1</sup> <sup>1</sup>Laboratorio Avimex S.A. de C.V. <sup>2</sup>University of Illinois at Urbana-Champaing

### **Background and Objectives**

During the period of 2021-2023, the efficacy of a PRRS live virus vaccine based on the G16X nonpathogenic strain was assessed in commercial swine operations located in different regions in Mexico.

#### Material and Methods

19 operations in different regions of Mexico involving 219,230 piglets, that previously were using a commercial PRRS modified live virus (MLV) vaccine were enrolled in the trial. All the farms switched to the G16X live virus vaccine. The vaccine administration in 12 farms was by the intranasal route (IN) on the 3<sup>rd</sup> day of age (DA), 6 farms vaccinated intramuscularly (IM) or IN (after weaning) between the 3<sup>rd</sup> and the 4<sup>th</sup> week of age (WA) and in one farm, the vaccine was administered IM at 10<sup>th</sup> WA. The main parameter to assess vaccine efficacy was the average pig mortality rate for 6 months prior to beginning the use of the G16X vaccine, as compared to the mortality rate observed afterwards. The mortality rates in each farm at either 10<sup>th</sup> WA or the end of the production cycle were compared to the respective historical mortality rate.

### Results

The vaccinated pigs didn't present any adverse clinical effects after the application of the vaccine. Exposure to 30 field viruses with 21 distinct RFLP patterns and <88% nucleotide or aminoacidic sequence homology to the G16X virus were detected. A reduction in mortality rate was registered in all the farms, when compared with their historical mortality rate, being the difference of 6.85% when the vaccine was administered at 3<sup>rd</sup> DA, 6.57% when administered at 3<sup>rd</sup> and the 4<sup>th</sup> WA and 4.19% when administered in at 10<sup>th</sup> WA, representing a percentual decrease of mortality that ranged from 36% to 55% (statistically significant at p<0.05 between historical and G16X mortality of each group by Chi-squared).

## **Discussion and Conclusion**

The vaccine based on the nonpathogenic strain G16X proved to be a more efficacious immunoprophylactic tool than the MLV vaccines to reduce mortality rates in farms with a high prevalence of PRRSV located in various regions of Mexico.

#### VVD – Virology and Viral Diseases

# ACCURACY OF TONGUE TIPS AND OTHER WELFARE-FRIENDLY POSTMORTEM SAMPLES FOR DETECTING PRRSV

M. Kikuti <sup>1</sup>, M. Melini <sup>1</sup>, X. Yue <sup>1</sup>, M. Culhane <sup>2</sup>, <u>C. Corzo <sup>3</sup></u> <sup>1</sup>University of Minnesota <sup>2</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN <sup>3</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

#### **Background and Objectives**

Specimens collected from dead pigs are a welfare-friendly and possibly cost-effective active surveillance method for PRRSV detection. We aimed to determine whether postmortem sampling of suckling piglets allows for the detection of PRRSV and determining the sensitivity of each postmortem specimen studied.

# **Material and Methods**

Two farrow-to-wean farms undergoing a wild-type PRRS outbreak were conveniently selected. Samples were collected at each farm at approximately 8 and 20 weeks after the outbreak detection. Postmortem sampling of piglets consisted of individual swabs of the nasal, oral, and rectal cavities, tongue tips, and intracardiac blood. All specimens were stored and tested individually for PRRSV by rRT-PCR. Additionally, we collected blood via jugular venipuncture live piglets. Sera from live piglets were tested for PRRSV by rRT-PCR in pools of five, sera in positive pools were then tested individually. Sera from live piglets were used to estimate the within-farm PRRS prevalence. The sensitivity and agreement of tongue tips and oral, nasal, and rectal swabs were calculated using the postmortem intracardiac sera as the assumed gold standard.

#### Results

PRRSV was detected by rRT-PCR in 79% of live piglets' sera on farm 1 visit 1 (F1V1), 10% on farm 1 visit 2 (F1V2), 63% on farm 2 visit 1 (F2V1), and 0% on farm 2 visit 2 (F2V2). Overall, oral swab sensitivity when compared to the same dead animal's serum was 90.9%, 84.8% for nasal swabs, 75.8% for rectal swabs, and 97.0% for tongue tips. Compared to sera, agreement ranged from 70.2% for tongue tips (kappa 0.45, p<0.001) to 89.6% for oral and nasal swabs (kappa 0.77, p<0.001).

## **Discussion and Conclusion**

Postmortem sampling of piglets to detect PRRSV is a welfare-friendly alternative. Any of the assessed specimen types were capable of identifying PRRSV on F1V1, F1V2, and F2V1. PRRSV was identified in F2V2 through serum and tongue tips, possibly by due to environmental contamination. Although this might be an issue when trying to assess within-herd prevalence, having highly sensitive detection methods, even those that detect environmental contamination, might represent an improvement in detecting presence of the virus on a farm.

VVD – Virology and Viral Diseases

# ASSESSMENT OF MECHANICAL TRANSMISSION OF AFRICAN SWINE FEVER VIRUS BY FLIES IN PENINSULAR MALAYSIA

J.X. Lee <sup>1</sup>, P.T. Ooi <sup>2</sup>, M.W.C. Fong <sup>2</sup>, M.I. Nur Mahiza <sup>2</sup>, A.R. Yasmin <sup>2</sup>, S.E. Low <sup>2</sup>, S.X. Lau <sup>2</sup>, B. Sia <sup>3</sup>, D. Ang <sup>3</sup>, S. Soh <sup>3</sup>, K.Y. Kam <sup>1</sup>, E.H.C. Cheah <sup>1</sup>, <u>R.L.H. Yong <sup>1</sup></u>

<sup>1</sup>Boehringer Ingelheim (Malaysia) Sdn. Bhd. <sup>2</sup>Faculty of Veterinary Medicine, Universiti Putra Malaysia <sup>3</sup>Agritech Enterprise Sdn. Bhd, Malaysia

### **Background and Objectives**

African swine fever (ASF) is a fatal viral disease that generate substantial economic losses in the swine industry worldwide due to its high morbidity and mortality. It is caused by Asfivirus which affects both wild boars and domestic pigs. Sources of ASFV transmission have been extensively studied to reduce the risk of ASF infection in swine farms. Aside from contaminated fomites or infected pigs as source of ASF infection in Malaysia, potential mechanical vectors such as flies, rats or birds are not well-studied. Hence, the objective of this study is to assess the mechanical transmission of ASFV by flies in Peninsular Malaysia.

# **Material and Methods**

The study was conducted in 4 swine farms from different state in Peninsular Malaysia. 1 farm was ASF negative upon sample collection whereas another 3 farms were post-ASF outbreak within 6 months. Flies from sewage pool, feed house, finisher pens and sow pens were collected using 4 sticky fly traps in each location over 24 hours. 5 flies from each location were pooled and real-time PCR was conducted to detect the presence of ASF DNA virus in the flies using IDEXX RealPCR\* ASFV DNA test with positive validity of Ct value <38.

#### Results

A total number of 2254 flies were collected from this study. Farm 1 collected 403 flies, Farm 2, 3 and 4 collected 67, 1450 and 334 flies respectively. Flies from sewage pool of Farm 1 & 2 found positive with Ct value of 35.39 and 36.77 respectively. Besides that, the flies from feed house of Farm 3 tested positive with Ct value of 36.73. Flies collected from sow pen of Farm 4 also tested positive with Ct value of 34.29.

#### **Discussion and Conclusion**

Positive detection of ASF virus in flies conclude that flies are potential mechanical vectors of ASFV for both ASF negative and post-ASF outbreak farms. However, there is no significant difference in densities of flies in different areas of the farm due to different farm status and insufficient samples collected from each farm. Nevertheless, a cost-effective fly control measure should be implemented to reduce the risk of ASF transmission via flies.

VVD – Virology and Viral Diseases

#### COMBATING ASFV: OPTIMIZING ALLOCATION OF SAMPLE COLLECTORS FOR RAPID OUTBREAK RESPONSE

<u>J.A. Galvis</u><sup>1</sup>, Y.S. Muhammed <sup>2</sup>, A. Sykes <sup>1</sup>, D. Roberts <sup>2</sup>, G. Machado <sup>1</sup> <sup>1</sup>Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

<sup>2</sup>Department of Computer Science, North Carolina State University, Raleigh, NC, USA,

#### **Background and Objectives**

The introduction of the African swine fever (ASF) into previously uninfected countries inflicted devastating economic consequences. To mitigate the risk of widespread outbreaks, timely detection and effective intervention are crucial. However, significant challenges exist concerning the resources and time required to identify ASF-positive farms. This study aims to establish a methodology for determining the optimal number of samplers and the time frame required to identify ASF-positive farms promptly.

## **Material and Methods**

We have developed a novel tool, EpiSAT, to identify the daily and overall number of certified sample collectors required in an ASF outbreak considering the necessary biosecurity and sample schema the USDA requires. EpiSAT estimates the number of samplers needed accounting for 1) sampling variables, which considers the total number of available samplers, working hours/day/sampler, sampling duration, time to farms, and time for laboratory dropoff of samples; 2) laboratory capacity including total samples processed/day and screening protocol (individual or pooled sample processing); 3) outbreak size, number of samples/day, sample priority (e.g. sample first farms in an infected zone), biosecurity restrictions (e.g. same sampler visits no more than one sow farm/day).

#### Results

To illustrate EpiSAT, we simulated ASF spread in a swine-dense region of the U.S. for 140 days. On average, the epidemic spanned 70 days (Cl95%=69-72), in which farms required 6,745 samples (Cl95%=6,433-7,057). Using a laboratory capacity of 1,000 samples/day, we estimated the time to complete sampling with 100 and 300 sampler collectors and a screening protocol of one sample and five pooled samples. Our results showed that with 100 samplers, sampling would take 101 days (Cl95%=98-104), while with 300 sampling would be completed in 72 days (Cl95%=71-73). For the screening protocol of one sample, it took 222 days (Cl95%=214-230) to process all the samples, independent of the number of samplers. Conversely, with a pool of five samples, this time was reduced to 102 days (Cl95%=100-104) with 100 samplers and 77 days (Cl95%=76-78) with 300 samplers.

# **Discussion and Conclusion**

EpiSAT effectively addresses the challenge of organizing necessary resources for controlling a potential ASF outbreak, thereby enhancing disease preparedness plans.

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# PHYLOGENETIC ANALYSE OF ORF5 GENES OF PRRSV-1 STRAINS CIRCULATING IN ITALY

<u>M. Ustulin</u><sup>1</sup>, L. Ferino <sup>1</sup>, C. Zanon <sup>1</sup>, C. Targhetta <sup>1</sup>, D. Vio <sup>1</sup> <sup>1</sup>Istituto Zooprofilattico Sperimentale delle Venezie

#### **Background and Objectives**

Sequencing and analysis of ORF5 gene of PRRS virus has become common for epidemiologic surveillance of PRRSV strains circulating among farms. In recent years, the availability of ORF5 genes published in public database, allows extensive comparison among strains of different geographical origins, and therefore to evaluate viral variability among Countries or production areas. Results of the phylogenetic analysis of ORF5 sequences collected during a period of 5 years of PRRS monitoring in Italy, are described in this study.

### Material and Methods

The present analysis include 481 PRRSV-1 ORF5 sequences collected during routine monitoring between years 2019 and 2023. In the phylogenetic tree are included ORF5 sequences from vaccines strains and 134 sequences of various geographical origins registered in Genbank. ORF5 sequence of prototype PRRSV-2 strain (VR-2332), was included as outgroup. Sequences were aligned with MAFFT re source and Maximum likelihood phylogenetic tree calculated using IQ-TREE v.1.6.6.

#### Results

All sequences were confirmed to belong to subtype 1 of PRRSV-1, known as the only one circulating in Western Europe (Balka et al., 2018). The sequences appeared to be divided in 3 main clusters; most of the sequences correlate with those classified by Balka et al. (2018) as lineage 3 of subtype 1, appeared in Italy at the end of the '90. Our study suggests this lineage to be prevalent in Italy. However, occasional presence of sequences clustering with Lelystad virus ORF5 suggests that strains similar to the prototype or vaccine derived, still circulates.

## **Discussion and Conclusion**

Phylogenetic analysis of PRRS strains, associated with geographical data and information about animal transfer, can be a useful epidemiological tool for the monitoring of PRRS strain. It allows evaluating the variability and evolution of circulating viral strains on a farm, production chain or regional basis. Moreover, the appearance of new a strain in the farm can highline a fail in biosecurity measures. Despite sequencing of ORF5 is commonly used, it can give only limited information, considered the gene represents only 5% of PRRS-1 genome (Zimmerman et al., 2019). In the future, the development of standardized NGS protocols will give access to more robust and informative data.

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## STREAMLINING PRRSV-2 ORF5 CLASSIFICATION USING NEXTCLADE

M. Zeller <sup>2</sup>, J. Chang <sup>1</sup>, R. Main <sup>2</sup>, P. Gauger <sup>2</sup>, <u>G. Trevisan <sup>2</sup></u>, J. Zhang <sup>2</sup> <sup>1</sup>Fred Hutchinson Cancer Center <sup>2</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

#### **Background and Objectives**

The ability to unambiguously identify porcine reproductive and respiratory syndrome virus (PRRSV) strains through a consistent nomenclature is quintessential for tracking viruses and for communication and response to viral disease outbreaks. While a standard framework for PRRSV-2 identification and classification based on genetic lineage has been established and refined, challenges persist in terms of access to standard reference sequences and consistency in classification assignment methodologies.

# **Material and Methods**

Yim-im et al. 2023 provided the latest refinement to the PRRSV-2 ORF5-based lineage nomenclature, extending previous nomenclatures. Scaffold sequences representing this updated lineage classification, including historical and contemporary vaccine sequences, were combined with metadata representing year, country, and RFLP of the sequence. These sequences were processed through the Nextclade pipeline using VR2332 as a reference strain for rooting and comparison, resulting in a Nextclade compatible dataset.

#### Results

PRRSView The resultant classifier be accessed through а link the can on homepage (PRRSView, https://prrsv.vdl.iastate.edu/). This classifier functions the same as other classifiers hosted by the Nextclade core group and can provide genetic-based PRRSV-2 ORF5 classifications on demand. Nextclade provides additional sequence metrics such as classification quality and notable mutations relative to the reference sequence. The submitted query sequences are appended to the reference tree using phylogenetic placement, allowing for comparison to nearby sequences of reference viruses and vaccine strains. The tree can display metadata about the genotype, sample collection year, country and state, and RFLP of the reference and vaccine sequence set for comparison. Importantly, although Nextclade is a webtool, the sequences are not uploaded to a server, ensuring the confidentiality of all analyses to the user.

#### **Discussion and Conclusion**

The implemented PRRSV Nextclade dataset provides a transparent and consistent platform for streamlining PRRSV-2 ORF5 lineage classification. The convenient accessibility, ease of use, and public nature of the platform allows diagnosticians, veterinarians, producers, and researchers to assign a common nomenclature to their PRRSV-2 ORF5 sequences.

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# A HISTONE-LIKE PROTEIN OF AFRICAN SWINE FEVER VIRUS INHIBITS TYPE I IFN SIGNALING BY TARGETING KPNAS/IMPORTINS

#### N. Gamage 1, L. Jong-Soo 1

<sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

#### **Background and Objectives**

African swine fever (ASF) is a highly contagious and devastating viral disease affecting domestic and wild swine, resulting in a 100% mortality rate. It is caused by the African Swine Fever Virus (ASFV), a large DNA arbovirus belongs to the family Asfaviridae. The worldwide pork industry confronts substantial repercussions due to African Swine Fever (ASF), resulting in socio-economic and environmental consequences. Developing a safe and effective vaccine against ASFV has been challenging because the immune regulatory functions of many ASFV proteins that contribute to its pathogenicity remain unknown. This study investigated how A104R, a eukaryotic histone-like homolog of ASFV, evades host antiviral responses by targeting the nuclear translocation of interferon regulatory factor 3 (IRF3) and signal transducer and activator of transcription 1 (STAT1).

# Material and Methods

A large-scale mass spectrometry analysis and interferon  $\beta$  (IFN- $\beta$ ), luciferase assays were conducted to determine the target of the ASFV A104R protein. Immunoprecipitation and immunofluorescence assays confirmed the A104R protein interaction with KPNA3,4 and 6. DNA virus replication was evaluated by infecting GFP-tagged ADV, HSV, and VACV viruses into A104R stably expressing porcine macrophages (PAMs), MA104 cells, and transiently transfected PK-15 cells. IFN- $\beta$ , pro-inflammatory cytokines and chemokines secretion were assessed by ELISA. A Fractionation assay kit was used to isolate cytoplasmic and nuclear fractions.

#### Results

We specifically explored A104R interaction with nuclear localization signals (NLS) of  $\alpha$ -karyopherin (KPNA) proteins. Interestingly, A104R established interactions with KPNA3 and KPNA4, disrupting the nuclear translocation of IRF3 and inhibiting type I interferon (IFN-I) production. Additionally, A104R interaction with KPNA6 hindered the translocation of tyrosine-phosphorylated STAT1 (PY-STAT1). In particular, we elaborate the interaction of A104R with the unique C-terminal nonclassical nuclear localization signal (ncNLS) region of KPNA6, which suppresses downstream antiviral immune responses.

## **Discussion and Conclusion**

This dual inhibition results in the suppression of type 1 interferon activity, effectively undermining the host's antiviral response. It holds significant implications for understanding and addressing ASFV infections, offering insights for developing therapeutic strategies against this deadly disease. [National Research Foundation (2021R1A6A1A03045495) and Ministry of Environment (NIWDC-2021-SP-02)]

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# ABNORMAL REPRODUCTIVE DISORDERS INCREASING IN BREEDING PIG HERDS WITH THE INVOLVEMENT OF NOVEL PATHOGENS

T.N.T. Ngo <sup>4</sup>, M.N. Nguyen <sup>1</sup>, R. Thanawongnuwech <sup>2</sup>, T.T.N. Bui <sup>3</sup>, T.P.T. Nguyen <sup>4</sup>, T.T.N. Nguyen <sup>4</sup>, T.T. Nguyen <sup>4</sup>, <u>T.D.</u> <u>Do</u> <sup>4</sup>

<sup>1</sup>Center for Genetics and Reproductive Health (CGRH), School of Medicine, National University HCMC, Vietnam <sup>2</sup>Faculty of Veterinary Science, Chulalongkorn University, Thailand <sup>3</sup>College of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam

<sup>4</sup>Faculty of Animal Science and Veterinary Medicine, Nong Lam University, HCMC, Vietnam

#### **Background and Objectives**

Reproductive failure in sows is a critical concern in pig breeding, involving both infectious and non-infectious factors. With the expansion of modern pig farms, co-infections are on the rise, impacting swine health. Recent studies associate porcine circovirus type 3 (PCV3) with reproductive disorders, and Mycoplasma suis (M. suis) is known to cause significant consequences during the pregnant period.

## **Material and Methods**

A survey was conducted on nine breeding pig farms experiencing reproductive disorders, collecting a total of 216 samples (whole blood samples from sows, and 82 tissue samples from fetuses (mummified, stillborn) and weak-born piglets. PCR/RT-PCR techniques were employed to detect ASFV, PCV2, PCV3, PCV4, PPV, M. suis, PRRSV, CSFV and PRV. Whole genome sequencing of PCV3 and sequencing of the partial 16S rRNA gene of M. suis were performed. Genetic diversity analysis was conducted on PCV3 and M. suis in farms where these pathogens were detected. Besides, clinical and pathological evaluation of the case of interest was performed if necessary.

#### Results

M. suis was prevalent in all surveyed farms, followed by PCV3, PRRS, PCV2, with the absence of other reproductive disorder pathogens such as ASFV, PCV4, PPV, CSFV, PRV, and JEV. Co-infection by two pathogens, particularly suis and PCV3 accounted for the highest infectious rate (31.94%). M. suis and PCV3 infection rates were highest in weak-born piglets (64%), followed by whole blood in sow samples (32.09%), stillborn (19.35%), and mummified (15.38%). PCV3b was identified as the prominent genotype. The eight M. suis 16S rRNA sequences shared high identity each other and with those of M. suis isolated from pigs reported from other countries. The clinical and pathological findings of the cases from the farms surveyed in this study also supported evidence of the pathogenic role of M. suis and PCV3.

# **Discussion and Conclusion**

M. suis and PCV3 emerged as predominant pathogens in the surveyed breeding pig herds, with the absence of certain pathogens. The co-infection of PCV3 and M. suis prompts further investigation into their combined impact on swine reproductive health. This study presents novel insights into the prevalence of these pathogens in Vietnam and emphasizes the need for ongoing research in understanding their role in reproductive disorders.

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# ASSESSMENT OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) FARM SURFACE CONTAMINATION THROUGH ENVIRONMENTAL SAMPLING

<u>M. Melini</u>, M. Kikuti<sup>2</sup>, M. Torremorell <sup>3</sup>, L. Bruner <sup>4</sup>, C. Stahl <sup>5</sup>, B. Roggow <sup>5</sup>, B. Leuwerke <sup>4</sup>, M. Allerson <sup>6</sup>, K. O'Brien <sup>6</sup>, M. Schwartz <sup>2</sup>, C. Corzo <sup>2</sup>

<sup>1</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

<sup>2</sup> Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA
<sup>3</sup> College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA
<sup>4</sup> Swine Vet Center, St Peter, MN, USA

<sup>5</sup>Fairmont Vet Clinic, Fairmont, Minnesota, United States

<sup>e</sup>Holden Farms, Northfield, MN, USA

## **Background and Objectives**

When porcine reproductive and respiratory syndrome virus (PRRSv) infected pigs shed the virus it may contaminate surfaces which can lead to potential indirect transmission. Data related to on-farm surface contamination with PRRSv is scarce. This study aimed to investigate whether PRRSv RNA could be detected on surfaces inside and outside pig barns housing PRRSv-positive pigs

#### **Material and Methods**

negative control farm. A total of 169 environmental samples were collected using a previously validated environmental sampling protocol where doorknobs, exhaust fans, floors among others were sampled. Presence of RNA was assessed via RT-PCR while virus viability was attempted in a subset of samples through cell culture.

#### Results

Nineteen (13%) out of 143 samples from positive farms yielded positive results with Ct values ranging between 25.4 and 37.0. Out of the 19 positive samples, 79% originated from non-porous surfaces such as plastic, and metal, and 21% from porous materials such as concrete. Eight (42%) of the positive samples originated from exhaust fan cones from four farms with a Ct value ranging from 30.3 to 37.0. Three doorknobs at four farms tested RT-PCR positive with Ct values ranging between 31.7 and 36.4. The remaining positive samples originated from surfaces such as ante-room floors and mortality carts/sleds with Ct values ranging from 25.4 to 35.4. Virus isolation attempts on two samples with ct values of 25.4 and 28.7 did not yield positive results.

# **Discussion and Conclusion**

Detecting PRRSV RNA was possible on surfaces that employees come in contact with frequently such as doorknobs and flooring. Almost half (42%) of the RT-PCR positive results originated from exhaust fan cones which suggests that viral particles originating from animals were becoming airborne and perhaps exiting the barn. Failing to detect viable virus is related to the fact that virus remains on such surface for a long period of time and loses its viability. This study provides evidence that virus can be found in non-pig areas and that biocontainment efforts to prevent the spread of PRRSV from infected farms need to be further investigated.

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## EFFECT OF FREEZE-THAW ON THE DETECTION OF PRRSV RNA BY PCR

B. Munguia-Ramirez <sup>1</sup>, B. Armenta-Leyva <sup>1</sup>, A. Henao-Diaz <sup>2</sup>, F. Ye <sup>3</sup>, K. Doolittle <sup>4</sup>, S. Zimmerman <sup>4</sup>, L. Gimenez-Lirola <sup>5</sup>, <u>J.</u> Zimmerman <sup>6</sup>

<sup>1</sup>Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.

<sup>2</sup>PIC Latinoamérica, México.

<sup>3</sup>Department of Statistics, Iowa State University, Ames, IA, USA.

<sup>4</sup>IDEXX Laboratories, Inc., USA <sup>5</sup>Innoceleris LLC

<sup>®</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA

#### **Background and Objectives**

Sample re-testing is a common practice in diagnostic and research to confirm PCR results or test for additional pathogens. This requires exposing samples to repeated freeze-thaw cycles, but the effect of these conditions on viral nucleic acid detection is largely unexplored. Thus, the aim of this study was to quantify the effect of freeze-thaw cycles on the detection of PRRSV RNA and a porcine RNA internal sample control (ISC) in serum, oral fluid, and feces.

## **Material and Methods**

Serum samples in the study were from pigs experimentally inoculated with wild-type PRRSV (n = 5). Oral fluids (n = 6) and feces (n = 5) were from individually housed pigs vaccinated with Ingelvac<sup>®</sup> PRRS MLV. Each sample was exposed to one of 4 treatments (2, 5, 10, or 15 freeze-thaw cycles) and then tested in technical triplicates (n = 132) for PRRSV and the ISC by RT-qPCR (IDEXX Laboratories, Inc.). RT-qPCR Cqs were re-expressed as a function of PCR efficiency, i.e., efficiency standardized Cqs (ECqs) and cube root transformed for analysis. The freeze-thaw effect was measured using a mixed-effects regression model (MRM) in R v.4.2.1.

#### Results

In serum, no freeze-thaw effect was observed for either PRRSV or ISC ECqs (p > 0.05, MRM). In oral fluids, freeze-thaw cycles affected both PRRSV and ISC RNAs (p < 0.05, MRM), with a PRRSV ECq loss of 0.05 (5.4%), 0.13 (13.4%), 0.27 (26.8%), and 0.40 (40.2%) at 2, 5, 10, and 15 freeze-thaw cycles, respectively. In feces, no freeze-thaw effect was observed in either PRRSV or ISC ECqs (p > 0.05, MRM).

#### **Discussion and Conclusion**

This study showed that freeze-thaw cycles had no detectable impact on PRRSV or ISC RNAs in serum and feces but significantly affected oral fluids, i.e., decreased PRRSV RNA concentration per freeze-thaw cycle. Thus, it is recommended to minimize freeze-thaw cycles in oral fluids until tested. It should be noted that these results apply to PRRSV and a proprietary ISC and that further studies are needed to address the effect of freeze-thaw on other pathogens and matrices.

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#### EFFECT OF SAMPLE HANDLING ON PRRSV DETECTION BY RT-QPCR: SHOULD WE BE CONCERNED?

B. Munguia-Ramirez <sup>1</sup>, B. Armenta-Leyva <sup>1</sup>, A. Henao-Diaz <sup>2</sup>, F. Ye <sup>3</sup>, K. Doolittle <sup>4</sup>, S. Zimmerman <sup>4</sup>, L. Gimenez-Lirola <sup>1</sup>, <u>J.</u> Zimmerman <sup>1</sup>

<sup>1</sup>Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA. <sup>2</sup>PIC Latinoamérica, México. <sup>3</sup>Department of Statistics, Iowa State University, Ames, IA, USA. <sup>4</sup>IDEXX Laboratories, Inc., USA

## **Background and Objectives**

Decision-making for PRRSV surveillance relies on the accuracy of PCR testing. During sample collection, storage, and transport, viral nucleic acids are exposed to adverse conditions that compromise their integrity and affect PCR results (false negatives). This study aimed to quantify the effect of storage temperature x time on detecting PRRSV RNA in serum, oral fluids, and feces.

## Material and Methods

Serum samples (n = 5) in this study were from pigs experimentally inoculated with wild-type PRRSV. Oral fluids (n = 5) and feces (n = 5) were from individually housed pigs vaccinated with Ingelvac<sup>®</sup> PRRS MLV. Each sample was divided into 28 aliquots and exposed to one combination of (temperature x time) treatment: 4, 10, 20, or 30°C for 24, 48, 72, 96, 120, 144, or 168 hr. Thereafter, samples (n = 420) were tested by RT-qPCR (IDEXX Laboratories, Inc.) to detect PRRSV RNA. RT-qPCR Cqs were re-expressed as efficiency standardized Cqs (ECqs), and the effect of (temperature x time) was analyzed by a mixed-effects regression model (MRM) using R v.4.2.1.

#### Results

In serum, PRRSV RNA was stable at 4, 10, and 20°C (p > 0.05, MRM), but a significant effect was detected at 30°C: PRRSV ECq loss of 0.11 (5.3%) every 24 hr. In oral fluids, PRRSV RNA was affected by all treatments (p < 0.05, MRM), with a daily PRRSV ECq loss of 0.03 (6.3%), 0.05 (9.4%), 0.07 (13.6%), and 0.08 (16.6%) at 4, 10, 20, and 30°C respectively. In feces, PRRSV RNA was also affected by all treatments, with a daily PRRSV ECq loss of 0.05 (6.0%), 0.06 (7.9%), 0.05 (14.3%), and 0.05 (15.3%) at 4, 10, 20, and 30°C respectively.

#### **Discussion and Conclusion**

The effect of storage temperature x time on PRRSV RNA was specimen-dependent, with serum being the most robust specimen type. PRRSV RNA in oral fluid and feces was significantly affected even at 4°C when stored  $\ge$  24 hr. Based on this data, serum samples should be stored at <20°C to optimize PRRSV RNA detection. Oral fluid and feces should be frozen in a non-self-defrosting freezer until tested to prevent loss of detectable PRRSV RNA.

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# EFFECT OF SOW VACCINATION AGAINST PORCINE CIRCOVIRUS TYPE 2 (PCV2) ON PCV2 VIREMIA DOWNSTREAM IN GROWING PIGS

<u>J. Wu</u><sup>1</sup>, K. Wang<sup>1</sup> <sup>7</sup>Swine Business Unit, Zoetis China, Shanghai, P.R. China

#### **Background and Objectives**

Commercial porcine circovirus type 2 (PCV2) vaccines have been widely used on pig farms. The aim of this study was to investigate the effect of sow PCV2 vaccination timing on PCV2 viremia downstream in growing pigs of a commercial farm.

## **Material and Methods**

The study was carried out in a two-site farm with 3,000 sows in China. The production parameters of the sow farm were stable and monthly processing fluids qPCR results showed no detection of PRRSV and PCV2. The downstream pig flow in the wean-to-finish site reported good performance, but the presence of PCV2 subclinical infection had been identified. A PCV2-and-Mycoplasma hyopneumoniae bivalent vaccine (Fostera PCV MH, Zoetis) was given 3 weeks pre-farrowing in sows and to 3-week-old piglets. A change of vaccination timing was implemented, and sows started to get the bivalent vaccine 3 weeks after farrowing, while the protocol for piglets remained the same. Right before and one year after the change, pigs aged 3-week (before PCV2 vaccination), 10-week, 14-week, 18-week and 22-week were blood-sampled (4 batches before the change and 4 batches after the change), 30 serum samples were collected at each time point for each batch. All samples were tested for PCV2 viremia by qPCR. In addition, 3-week-old piglets samples were tested for antibodies using Synbiotics SERELISA PCV2 Ab Mono Blocking ELISA.

#### Results

For the anti-PCV2 antibody titers in 3-week-old piglets, before the change of sow vaccination timing, 80% of the samples had a saturation titer (+2484), after the change, a significant decrease was observed (1062±869.1). Both the percentage of PCV2-DNA positive sera and viral loads in the positive sera decreased during fattening period, 10-week-old: 11.7%, 10<sup>2.5±0.3</sup> vs 0.8%, 10<sup>1.7</sup>, 14-week-old: 9.2%, 10<sup>2.6±0.9</sup>vs 2.5%, 10<sup>2.1±0.3</sup>, 18-week-old: 3.3%, 10<sup>2.5±0.3</sup> vs 1.6%, 10<sup>2.4±0.3</sup>, 22-week-old: 5.0%, 10<sup>2.5±1.5</sup> vs 0.8%, 10<sup>2.5</sup>.

## **Discussion and Conclusion**

After changing the vaccination timing, the seroprevalence, and the level of PCV2 viremia downstream in fattening pigs was reduced, suggesting that the decrease of maternal antibody may have helped PCV2 vaccine effectiveness. However, more studies are needed to fully elucidate the impact of the sow PCV2 vaccination timing or strategy and PCV2 viremia downstream in growing pigs.

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# PIGS CO-INFECTED WITH MULTIPLE INFLUENZA A VIRUS SUBTYPES UNDER FIELD CONDITIONS

<u>J. Alvarez-Norambuena</u><sup>1</sup>, C. Li<sup>1</sup>, Y. My<sup>2</sup>, M. Culhane<sup>3</sup>, M. Torremorell<sup>2</sup> <sup>1</sup>University of Minnesota <sup>2</sup>University of Minnesota College of Veterinary Medicine <sup>3</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

## **Background and Objectives**

Influenza A virus (IAV) is common in pigs and multiple strains and subtypes of IAV can be found co-circulating in infected herds. IAV is also a public health risk due to the risk of interspecies transmission that may result in pandemics. There is limited understanding of factors that drive reassortment in pigs and few studies have attempted to study farm management features impacting reassortment. Consequently, this study aimed to describe the within-farm detection of IAV subtypes at the individual pig level as a first step to evaluate factors involved in the emergence of IAV reassortants in pigs.

## **Material and Methods**

Herds from one pig flow from a pork production company were recruited in the study. Pigs from three sow farms were commingled at weaning into all-in/all-out nursery and finishers facilities. Sixty pigs were identified at weaning, and at 4, 8, and 16 weeks of age for a total of 240 pigs per cohort. Pigs were sampled by collecting nasal swabs (n= 720) for detection of IAV by RT-qPCR targeting the IAV matrix gene. A subset of IAV RT-qPCR positive nasal swabs (n= 272) were further subtyped targeting the IAV H1 and H3, and N1 and N2 genes.

#### Results

Overall, IAV was detected by RT-qPCR in 36.3% pigs included in the study. At weaning, 37.2% of pigs tested positive. The percentage IAV RT-qPCR positive pigs increased to 58.3% in the nursery at 3-5 weeks of age, declining to 33.9% at 7-8 weeks of age, and was the lowest at 16 weeks of age in the finisher, when 15.6% tested positive. There was evidence of more than one IAV subtype circulating in pigs from all three cohorts. Among the samples tested, 18.8% tested positive for H1 and H3, or N1 and N2 or H1, H3, N1, and N2, which indicated co-detection of multiple IAV subtypes from the same individual animal.

## **Discussion and Conclusion**

This study highlights the commonality of finding pigs co-infected with multiple IAV subtypes from weaning to finishing. The highest percentage of pigs infected with multiple IAV subtypes was observed at weaning and throughout the nursery phase creating favorable conditions for reassortment to occur.

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# SWINE INFLUENZA A TYPING RESULTS IN 11 EUROPEAN COUNTRIES FROM JANUARY 2020 TO SEPTEMBER 2023

K. Lillie-Jaschniski 1, M. Köchling 2, T. Revesz 3, E. Pileri 4, A. Jardin 5, E. Velazguez 6, M. Albin 7, C. Casanovas 8, F. Costa 9, R. Panek 10, B. Cornelis 11, E. De Jonghe 12

<sup>1</sup>Ceva Santé Animale, Libourne, France <sup>2</sup>Ceva Tiergesundheit GmbH. Düsseldorf. Germanv <sup>3</sup>Ceva-Phylaxia Zrt., Budapest, Hungary 4CEVA Animal Health, Italy 5Ceva Santé Animale, 33 500 Libourne, France 6Ceva Animal Health UK

<sup>7</sup>Ceva Animal Health A/S, Vejle, Denmark <sup>8</sup>Ceva Salud Animal, Barcelona, Spain

°Ceva Saúde Animal, Lda - Portugal

<sup>10</sup>Ceva Animal Health Poland, Warsaw, Poland

11Ceva Santé Animale. Naaldwiik. the Netherlands

<sup>12</sup>Ceva Santé Animale, Brussels, Belgium

# **Background and Objectives**

Swine influenza A virus (swIAV) infection has huge impact on pig health in all stages of production. It is part of the porcine respiratory disease complex (PRDC) and besides that can cause reproductive disorders in sow herds. Due to trade of pigs or human introduction influenza viruses involved in disease on farms can change and subtypes can vary within the regions. The aim of this study is to give an overview on passive Influenza surveillance in 11 European countries over the past four vears.

#### **Material and Methods**

This report summarizes Influenza A subtyping results of the years 2020, 2021, 2022, 2023 (Q1-Q3) on farm level in UK. DE, ES, PT, BE, NL, IT, FR, PL, HU and DK. Nasal swabs, oral fluids, bronchoalveolar lavage or lung tissue were either taken in cases of acute influenza -like symptoms or in cases of unclear, persistent respiratory or reproductive symptoms. Samples were analyzed for influenza A by real-time PCR. Subtyping of samples with a ct-value <30 was done by multiplex real-time PCR.

## Results

Overall, in 2751 swIAV samplings it was possible to identify subtypes. In the investigated period, most farms were positive for H1avN2 (29.0%) followed by H1avN1 (27.2%). From year 2020 to 2023, the percentage of farms testing positive for H1pdmN1 increased from 10.9% positive farms in 2020 to 15.3% in 2023. Over the whole period H1huN2 could be detected in 12% and H1pdmN2 in 10.7% of the cases. The lowest detection rates were in H1huN1 (3.1%), H3N2 (2.6%) and H3N1 (0.5%). A huge difference between countries and regions could be seen. For example, in DK and HU no H1hu subtypes could be detected. In DK the two pandemic subtypes and H1avN2 played the dominant role, whereas in UK mainly H1huN2 and H1pdmN1 were detected

### **Discussion and Conclusion**

The frequencies of swIAV subtypes all over Europe have slightly changed from 2020 to 2023. There are huge differences regarding the proportion of subtypes within different European countries. Knowing which strains are circulating on farms and in which stages of production, is important to be able to implement the right vaccination regime.

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## A NEW CLASSICAL SWINE FEVER INDIRECT ELISA FOR HERD PROFILING AND VACCINATION MONITORING

L. Olagnon <sup>1</sup>, M. Milano <sup>1</sup>, <u>A. Limozin</u> <sup>1</sup>, L. Comtet <sup>1</sup>, P. Pourquier <sup>1</sup> <sup>1</sup>IDvet – Innovative Diagnostics, Grabels, France

#### **Background and Objectives**

Classical swine fever (CSF), caused by the CSF virus (CSFV) in the Pestivirus genus, is highly contagious among pigs and shares genetic similarities with ruminant pestiviruses. The acute form presents with fever, depression, and hemorrhages, often resulting in high morbidity and mortality. The milder chronic form occasionally sees recovery in mature animals. Transplacental infection with low-virulence strains leads to persistently infected piglets, contributing to virus spread.

Commonly, serological tests like serum neutralization or ELISA are used for monitoring CSFV antibodies. IDvet offers a competitive ELISA (cELISA), the ID Screen® CSF Competition ELISA, for detecting anti-CSFV E2-glycoprotein antibodies. Additionally, a new kit, the ID Screen® Classical Swine Fever E2 Indirect ELISA (iELISA), is introduced, facilitating the assessment of anti-CSFV E2-glycoprotein antibody titers in swine serum or plasma. This enables improved quantification of the E2 response post-vaccination or natural infection.

## **Material and Methods**

The diagnostic specificity was assessed with 247 swine samples from CSFV-free herds in France. Diagnostic sensitivity was assessed using 179 swine sera from vaccinated herds in Asia. Of these, 92 samples were tested with the new ID Screen® Classical Swine Fever E2 Indirect ELISA (iELISA), a competitive ELISA (cELISA), and commercial Kit A. Additionally, serum samples from pigs vaccinated with C-strain and recombinant E2 subunit vaccines were serially diluted and tested with all ELISAs.

#### Results

Measured specificity was 98.4%, Cl<sub>95%</sub> [95.9-99.4], n=247. Measured sensitivity was 99.4% (Cl95%: 96.8% - 99.9%), n=179. Out of the 92 vaccinated samples, 90, 89 and 60 samples were found positive with the cELISA, the iELISA, and Kit A, respectively.

The C-strain vaccinated sample were found positive until dilution 1:32 with the 3 ELISAs. The recombinant E2 subunit vaccinated sample were found positive until dilution 1:256, 1:128 and 1:32 with the cELISA, the iELISA and Kit A, respectively.

#### **Discussion and Conclusion**

The ID Screen® CSF Indirect ELISA exhibits high specificity, effectively identifying anti-CSFV E2 antibodies, and demonstrates high analytical sensitivity, outperforming Kit A. This newly introduced iELISA proves to be a reliable tool for detecting pig antibodies against CSFV.

VVD – Virology and Viral Diseases

# A NEW RT- PCR FOR OF CLASSICAL SWINE FEVER VIRUS DETECTION IN SWINE AND WILD BOAR, ALLOWING FOR PARALLEL TESTING WITH THE ID GENE ASF QPCRS.

A. Greatrex <sup>1</sup>, L. Despois <sup>1</sup>, L. Savarit <sup>1</sup>, E. Bianchini <sup>1</sup>, <u>A. Limozin <sup>1</sup></u>, L. Comtet <sup>1</sup>, P. Pourquier <sup>1</sup> <sup>1</sup>IDvet – Innovative Diagnostics, Grabels, France

#### **Background and Objectives**

Classical swine fever (CSF) is a contagious viral disease affecting domestic and wild swine, caused by the CSF Virus (CSFV), a single-stranded RNA virus belonging to the genus pestivirus, like Bovine Viral Diarrhea Virus (BVDV) and Border Disease Virus (BDV) which affects cattle and sheep respectively.

Swift implementation of control measures relies on reliable and accurate diagnostics to detect and prevent CSFV spread. Innovative Diagnostics introduce hereafter a rapid and specific RT-qPCR, the ID GENE™ Classical Swine Fever Virus duplex, for detecting CSFV RNA. Its protocol is compatible with our ID Gene™ African Swine Fever qPCR kits, enabling parallel testing for CSFV and ASFV from the same extracts. This is particularly valuable as both diseases share similar clinical patterns.

## Material and Methods

The test, targeting the non-5' UTR region, was conducted according to manufacturer's instructions and yielded results in 65 minutes following a rapid amplification protocol. Analytical specificity was confirmed with 21 CSFV strains from FLI, 11 CSFV isolates from ANSES, and 62 other pathogens, including BVDV and porcine reproductive and respiratory syndrome virus. The Limit of Detection (LDPCR) used a synthetic RNA fragment, and the Method Detection Limit (MDL) involved swine samples (blood,serum,spleen) spiked with the modified live virus vaccine (PESTIFFA - Boehringer). Diagnostic specificity and sensitivity were assessed using 66 samples.

#### Results

The ID Gene qPCR reliably detected all CSFV strains with 100% inclusivity and exclusivity, showing no cross-reaction with other pathogens. The LDPCR was 4 copies/PCR (95%), and the MDL on swine blood was 4.103 copies/ml using the ID Gene™ Mag Fast Extraction Kit. Diagnostic sensitivity and specificity were both measured at 100%.

## **Discussion and Conclusion**

The RT-PCR kit demonstrates excellent analytical performance and user-friendliness, including a single reaction mix with an internal control. For differential diagnosis, samples can be concurrently processed with our ASF PCRs (ID Gene® African Swine Fever Duplex and ID Gene® African Swine Fever Triplex) on the same plate. The kit has received approval in Germany from the Friedrich-Loeffler-Institut (FLI C-106). When combined with the ID Screen® CSF Competition ELISA for detecting anti-E2 CSFV antibodies, Innovative Diagnostics provides a comprehensive solution for CSF diagnosis and vaccination monitoring.

VVD – Virology and Viral Diseases

# A RELIABLE REAL-TIME RT-PCR FULL SOLUTION FOR THE DETECTION OF PRRS VIRUS FROM COMMON TO INNOVATIVE SAMPLE TYPES

<u>J. Charrot</u><sup>1</sup>, M. Lelièvre <sup>1</sup>, Y. Zein-Phillipson <sup>2</sup>, S. Moine <sup>1</sup> <sup>7</sup>Thermo Fisher Scientific, Lissieu, France <sup>2</sup>Thermo Fisher Scientific, California - US

## **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases that has brought significant economic losses to the swine industry worldwide. This disease is caused by a single stranded positive-sense RNA enveloped virus which leads to greater heterogeneity of the nucleotide sequence between individual strains. For more than 20 years, Thermofisher is engaged in PRRS virus (PRRSV) diagnosis: the high mutation rate of PRRSV is addressed through regular monitoring of circulating strains over Europe and USA using sequencing technologies.

## **Material and Methods**

It led to several design updates of our Real-Time RT-PCR PRRSV detection kit to efficiently cover circulating viruses including relatively novel strains variety (1-1-4 lineage 1C, USA) and Highly Pathogenic strain (HP-PRRSV, China).VetMAX<sup>™</sup> PRRSV EU & NA 3.0 Kit has been updated in 2022 to keep high diagnostic sensitivity by identifying conserved regions for both EU & NA primers and probes assays.

Considering the evolution of herd management, we have also updated our workflows to better meet our user's needs. Current methods of detecting PRRSV in boar semen are time-consuming, laborious, or not sensitive enough. Our objective was to develop a reliable and sensitive nucleic acid purification to directly detect PRRSV in boar semen.

#### Results

Positive field semen samples were collected in Europe and the optimization of the protocol led to a gain of sensitivity on real infected samples.

Additionally, we have validated a new application to our new VetMAX<sup>™</sup> Kit, detecting PRRSV from the processing fluids (PF). The use of PF was first described in 2018 (USA) and was demonstrated to provide a higher herd-level sensitivity compared with blood samples. Results observed for both EU and NA PRRSV detection from PF show that the virus is detectable in in this matrix and 100% concordant with routine diagnostic methods used, such as testing on serum or blood samples.

## **Discussion and Conclusion**

The evolution of herd management practices leads to explore new monitoring solution like environmental testing. Therefore, air sampling can be considered as a sample of interest for PRRSV detection.

Preliminary studies show a real correlation between individual serum testing and detection of the virus in the housing of infected animals.

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# ALTERNATIVES TO TRADITIONAL BLOOD SAMPLING TO MONITOR PRRSV CIRCULATION AT FARROWING: A COMPARISON BETWEEN WHOLE BLOOD SAMPLES, BLOOD SWABS, TONGUE FLUID AND TAIL FLUID

X. Sola <sup>1</sup>, J. Thomson <sup>2</sup>, S. Shanahan <sup>3</sup>, <u>R. Del Pozo Sacristán <sup>3</sup></u> <sup>1</sup>Holmefield Farm services Limited, UK <sup>2</sup>SAC Consulting Veterinary Services, Scotland, UK <sup>3</sup>MSD Animal Health

### Background and Objectives

The recommended method for monitoring PRRSV stability in a pig herd consists of testing 30 piglets at weaning for PRRSV by PCR. This method requires testing a larger number of samples in breeding herds undergoing virus elimination, leading to a costly expense for small-to-medium size family-owned farms. The aim of this study was to find an efficient, practical, and non-expensive means of detecting PRRSV around farrowing to help monitor PRRSV stability in British pig farms. For this aim, two studies were carried out. Study 1 aimed to determine the most sensitive method when a small budget (one PCR test) was available for detecting PRRSV at batch level by comparing whole blood, blood swabs, tongue fluid and tail fluid. Study 2 aimed to compare the most sensitive method (obtained from study 1) with blood samples collected from individual animals.

### **Material and Methods**

A total of 16 herds with a history of PRRS participated in the study. For study 1, whole blood (n=5), blood swabs (n=5), tongue fluid from dead piglets and tail fluid after processing were collected in each farm from piglets belonging to the same farrowing batch. Samples were pooled by sample type for PRRS PCR (Virotype PRRS RT PCR test, Indical). McNemar's test was used for comparing collection methods. For study 2, blood samples and tongue fluid from the same individual dead piglets were collected and PRRS PCR test performed. Cycle thresholds (Ct-values) were compared between both sample types for each pig using paired t-test.

#### Results

Tongue fluid obtained from dead piglets was found to be the most sensitive sample to detect the presence of PRRSV at batch level. No significant differences were detected in Ct-values between blood and tongue fluid from the same individual pigs.

## **Discussion and Conclusion**

The results of this study indicate that tongue fluid can be a reliable and non-expensive means of testing for the presence of PRRSV and to help determine PRRSV stability in small-to-medium sized farms. This concession in test costs may increase the risk of losing detection power. However, targeting dead or ill piglets improves sensitivity by sampling a subpopulation of animals that are more likely to harbour PRRSV.

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# DETECTION OF INFLUENZA D VIRUS IN PIGS IN ENGLAND

H.E. Everett <sup>1</sup>, <u>P.M. Van Diemen</u><sup>1</sup>, E. Fullick <sup>2</sup>, S. Williamson <sup>3</sup>, A. Ramsay <sup>1</sup>, B. Mollett <sup>1</sup>, A. Byrne <sup>1</sup>, M. Khatri <sup>1</sup>, A. Banyard <sup>1</sup>, J. James <sup>1</sup>, I. Brown <sup>1</sup>

<sup>1</sup>Animal and Plant Health Agency (APHA), Virology, New Haw, United Kingdom <sup>2</sup>Animal and Plant Health Agency, Station Road, Thirsk, North Yorkshire, England, UK <sup>3</sup>Animal and Plant Health Agency, Rougham Hill, Bury St Edmunds, Suffolk, England, UK

## **Background and Objectives**

Influenza D virus (IDV), a member of the influenzavirus D genus within the Orthomyxoviridae family, was initially detected in 2011 in pigs and later in cattle, a possible reservoir. IDV likely also circulates in other mammals. IDV has been found worldwide including several countries in Europe. Here we describe the recent isolation of IDV from pigs in England.

# **Material and Methods**

Nasal swabs or respiratory tissue pools (trachea, tonsil, lung) from pigs with respiratory disease submitted through the APHA diagnostic network since November 2022 were tested for IDV and influenza A RNA by real-time RT-qPCRs (RRT-qPCRs). IDV isolates were obtained by inoculation of swine testis cell line and whole genome sequencing was performed.

## Results

Four of 479 pig samples tested positive for IDV and 40 were positive for influenza A. Positives were from three unrelated farms sampled in April, June and October 2023. The detections were in a 15-week-old pig with complex respiratory disease in which PRRSV and Streptococcus suis were also present, nasal swabs from postweaned pigs with respiratory disease (influenza A negative) and a two-week-old piglet found dead due to Klebsiella pneumoniae septicaemia without evidence of respiratory pathology. One cell line culture was positive for IDV at first passage and an isolate was obtained (D/swine/England/126471/2023). WGS analysis revealed close phylogenetic relationship with other European IDV strains.

## **Discussion and Conclusion**

IDV was detected in respiratory samples from pigs in England although at a lower rate than influenza A. It was also detected in cattle. Whether it contributes significantly to porcine respiratory disease needs to be explored, it appeared to be incidental in preweaned piglets, however its role in the postweaned pigs was harder to determine. It is unclear whether IDV is zoonotic; IDV infection has not yet been detected in humans. There is no indication that IDV will infect avian species. IDV is not notifiable or reportable. It is structurally similar to influenza C virus (ICV) which infects humans and causes influenza-like illness. Importantly, both ICV and IDV are distinct from influenza A and B viruses and do not have pandemic potential. IDV cannot recombine with influenza A, B or C viruses.

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# DEVELOPMENT OF A NOVEL AFRICAN SWINE FEVER VIRUS PCR TO ADDRESS CURRENT MARKET REQUIREMENTS.

<u>K. Valentine</u><sup>1</sup>, V. Geurts<sup>1</sup>, F. Priller<sup>2</sup> <sup>1</sup>BioChek BV, Smart Veterinary Diagnostics <sup>2</sup>Hygiena Diagnostics GmbH

## **Background and Objectives**

African Swine Fever increasingly threatens swine populations globally. Fast, accurate, robust diagnostic methods (including pooling) are required for detection, mass-screening, surveillance-and-monitoring. RT-PCR, the "gold standard" method for ASFV Detection, has limitations including false negative results (inhibition), temperature sensitive reagents and time-consuming protocols. The vetproof® African Swine Fever Virus qPCR LyoKit, a triplex qPCR with precoated lyophilized plates, a double control system (endogenous and exogenous Internal Control) and UNG enzyme to prevent false positives by PCR amplicon contamination. This study evaluates the performance and robustness of this new lyophilized kit against a reference assay.

# Material and Methods

Evaluation of the assay requires internal- and external validation. Analytical sensitivity, robustness and reproducibility assessed by Hygiena Diagnostics GmbH on spiked-, negative- and reference material sets provided by CISA-INIA(Spain) and Państwowy Instytut Weterynaryjny(Poland) according to the protocol provided. Applicability on a range of thermal cyclers demonstrated. External validation performed by The Pirbright Institute (OIE ASF Reference Laboratory). DSe and DSp assessed by 322 defined samples and compared to the OIE reference method by King et al. Samples included ETDA Blood, porcine serum, cell cultured virus, swab samples and homogenized tissue.

#### Results

Internal validation shows highly reproducible detection (<2%CV) and a clear dose/response relationship between 1 million to 10 viral genome copies/reaction. The LOD95% is approximately 10 copies/reaction. Inclusivity shown for a diverse range of ASFV genotypes and exclusivity against CSFV and swine-negative matrices. The assay detects ASFV in pooled serum samples (n=10) with high sensitivity. The kit can be paired with PCR standards viral load quantification.

The external validation demonstrated agreement (97,2%) between the newly developed assay and the King et al. PCR with no obvious difference in Dse (>97%) and Dsp (100%). ASFV was detected in samples ranging from highly to weakly positive for ASFV.

# **Discussion and Conclusion**

The vetproof® African Swine Fever Virus qPCR LyoKit demonstrated perfect suitability for the detection of ASFV genetic material for all relevant sample types. The lyophilized format offers improved storage and handling for the diagnostic laboratory. Possibilities of extending this lyophilized assay to Point-of-Care applications should be further investigated.

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# FORECASTING VIRAL DISEASE OUTBREAKS AT THE FARM-LEVEL FOR COMMERCIAL SOW FARMS IN THE U.S

I.A. Paploski 1, R. Bhojwani 2, J.M. Sanhueza 3, C. Corzo 3, K. Vanderwaal 3

<sup>1</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

<sup>2</sup>University of Minnesota

<sup>3</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

## **Background and Objectives**

Porcine epidemic diarrhea virus (PEDv) was introduced to the U.S. in 2013 and is now considered to be endemic. Like many endemic diseases, it is challenging for producers to estimate and respond to spatial and temporal variation in risk. Our objective was to leverage data that is already collected by systems and use it to predict PEDv occurrence on a farm-level and a weekly scale.

# **Material and Methods**

Participating swine production companies in a swine-dense region of the U.S. share weekly information on a) PEDv status of farms and b) animal movements for the past week and scheduled movements for the upcoming week. Environmental (average temperature, humidity, among others) and land use characteristics (hog density, proportion of area with different land uses) in a 5 km radius around each farm were summarized. Using the Extreme Gradient Boosting (XGBoost) machine learning model with Synthetic Minority Over-sampling Technique (SMOTE), we developed a near real-time tool that generates weekly PEDv predictions (pertaining to two-weeks in advance) to farms of participating stakeholders.

#### Results

Based on retrospective data collected between 2014 and 2017, the sensitivity, specificity, positive and negative predictive values of our model were 19.9, 99.9, 70.5 and 99.4 %, respectively. Predictions for more than 200 weeks, each week for more than 250 farms, have been sent since the project real-time implementation.

### **Discussion and Conclusion**

This platform has been used to deliver weekly real-time forecasts since December 2019. The forecast platform has a builtin feature to re-train the predictive model in order to remain as relevant as possible to current epidemiological situations, or to expand to a different disease. These dynamic forecasts, which account for recent animal movements, present disease distribution, and environmental factors, help promote data-informed and targeted disease management and prevention within the U.S. swine industry.

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## IS PRRSV REALLY ABLE TO CAUSE CONJUNCTIVITIS?

<u>C. Unterweger</u><sup>1</sup>, T. Schobersberger<sup>1</sup>, N. Leitner<sup>2</sup>, G. Balka<sup>3</sup>, A. Ladinig<sup>1</sup> <sup>1</sup>University Clinic for Swine, University of Veterinary Medicine Vienna, Austria <sup>2</sup>Institute for pathology and forensic, University of Veterinary Medicine, Austria <sup>3</sup>Department of Pathology, University of Veterinary Medicine, Budapest, Hungary

## **Background and Objectives**

In the scientific literature, conjunctivitis in pigs is commonly associated with an infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), although there is not a single verification of causality. The objective of this study was to investigate the occurrence of conjunctivitis during an experimental PRRSV infection trial in nursery pigs.

### **Material and Methods**

16 six-week-old PRRSV-free piglets were infected with a pathogenic PRRSV-1 isolate (AUT 15-33), while 16 other piglets of the same age acted as negative controls. All animals were clinically examined daily, including a detailed eye examination. Eight animals from each group were euthanised either 10 days or 28 days after infection. Conjunctival swabs were taken for virus detection and the lower right eyelid for histological examination and further testing using an RNA scope method.

#### Results

Compared to the control group, significantly more infected piglets showed clinical occurrence of eyelid edema (p=0.002) and chemosis (p=0.038); minor reddening of the conjunctiva was present to approximately the same extent in both groups (about 30%). There was a significant increase in PRRSV viral load in serum and nasal swabs starting three days after infection. PRRSV was not detected in any conjunctival swabs at any time. Histologically, low to medium grade conjunctivitis was seen 10 days after infection in 2 of 8 infected animals; 28 days after infection, different grading of conjunctivitis and edema was seen in 6 of 8 infected animals. Using RNA scope, one conjunctiva sample showed a positive result around a blood vessel. The involvement of chlamydia could be excluded.

## **Discussion and Conclusion**

After initial PRRSV infection, eyelid edema was present at least over a period of 28 days. Clinical conjunctivitis was not seen, but inflammation was shown histologically in infected animals 10 and 28 days after infection. PRRSV shedding does not seem to happen in lacrimal fluid, but PRRSV could be found by RNA scope within the conjunctiva of one pig during the viremic period. Severe cases of conjunctivitis, as often seen in pig herds with PRRSV-circulation, might therefore be due to a higher load of conjunctivitis causing bacteria, e.g. Chlamydia or Mycoplasma spp. which can take advantage of immunosuppression caused by PRRSV.

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## IS SAPOVIRUS AN EMERGING DISEASE IN CANADA?

<u>F. De Grau</u><sup>1</sup>, C. Malgarin <sup>1</sup>, S. Von Berg <sup>2</sup> <sup>1</sup>Merck Animal Health, Kirkland, QC, Canada <sup>2</sup>MSD Tiergesundheit, München, Germany

#### **Background and Objectives**

Sapovirus (PSaV)) has been detected worldwide in symptomatic and asymptomatic pigs or as part of mixed diarrheic infections with other viral, bacterial, or protozoal pathogens. However, an increased number of diarrhea cases in piglets ten days of age or older were reported in Iowa in 2019. Piglets were negative for significant pathogens, and Sapovirus was the only etiological agent found to cause disease (Shen et al., 2021).

The first Sapovirus case related to pigs born in Canada was a case of wean piglets exported to lowa in 2022; it was not until recently that a Sapovirus case was reported in Ontario affecting nursery pigs by the Animal Health Laboratory (AHL) in Guelph (DeLay and Ojkic, 2023).

## **Material and Methods**

In 2023, our Animal Health Canada team received 516 laboratory case reports from lactating piglets scouring; from tissue, fecal, and swabs collected, 221 viral sequences were obtained for Rotavirus VP7 and only 14 for Sapovirus VP1 (ORF1). The Sapovirus cases came from investigations from scouring piglets born to vaccinated sows against Rotavirus that were negative for other common enteric pathogens. Diarrhea occurs around ten days of age, causing mortality and weight loss. The PCR testing was performed at the AHL in Guelph, and VP1 sequencing was completed at the lowa State University Veterinary Diagnostic Laboratory. The only pathogen found in high viral concentration (CTs < 20) and histological lesions causing atrophic enteritis was Sapovirus.

#### Results

To this date, Sapovirus has only been found in four Canadian provinces. So far, the genogroups found in Canada are G III, SVP1 and SVP2. The homology among SVP1 strains is 96.87 % to the US strains and 94.93 % among Canadian strains, and for SVP2, 95.84 % to US strains and 97.76 % among Canadian sequences.

## **Discussion and Conclusion**

Since the first Canadian case was diagnosed during the outbreak in Iowa, PSaV has been in 14 cases of chronic diarrhea as the sole cause. Suggesting that the prevalence is high and that PSaV should be included in the diagnostic workout of pig diarrhea.

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# ONLINE MAP OF PRRS PREVALENCE IN DENMARK – AN INVALUABLE TOOL IN THE NATIONAL REDUCTION STRATEGY

<u>M. Fertner</u><sup>2</sup>, O. Helverskov <sup>1</sup>, S. Kjærgaard Boldsen <sup>2</sup>, B. Lorenzen <sup>3</sup>, N. Rosager Weber <sup>3</sup> <sup>1</sup>SEGES Innovation P/S, Digital, Agro Food Park 15, DK-8200 Aarhus, Denmark <sup>2</sup>SEGES Innovation P/S, Datamanagement, Agro Food Park 15, DK-8200 Aarhus, Denmark <sup>3</sup>Danish Agriculture and Food Council, Axeltorv 3, Copenhagen, Denmark

## **Background and Objectives**

In Denmark, a national reduction strategy targeting Porcine Reproductive and Respiratory Syndrome (PRRS) was launched in May 2022. PRRS may spread by local transmission and movement of pigs. Therefore, the identification of geographic location, PRRS status, density of pig herds and the movement of pigs, is crucial. To initiate and support local reduction initiatives, a regional approach was suggested based on the location of pig herds in Denmark.

## **Material and Methods**

A real-time interactive online map was established in Power BI. All herds enrolled in the PRRS reduction program (herds with >10 sows or >100 pigs in total) were included based on register data from the Central Husbandry Register (herd location, herd size and movement of pigs) and SPF register (serological PRRS status). Further, a regionalization of Denmark into regions of around 100 pig herds/region was suggested based on an algorithm developed in R.

#### Results

A transparent illustration of herd location, serological PRRS status, herd size and production type of all Danish pig herds enrolled in the national PRRS reduction program has been made available online for all producers and professionals in the Danish pig industry since November 2022. Regions were initially based on the algorithm-suggested regions, and thereafter manually adjusted according to local expectations.

At the time present, the PRRS map holds 4,425 pig herds, split into 43 regions with an average of 102 [6;476]min.max pig herds/region. In addition, the movement of pigs and their PRRS declared status is illustrated on the map in a timely scale.

## **Discussion and Conclusion**

The Danish PRRS map is an invaluable tool in the national PRRS reduction strategy. It is widely used by producers, coordinators, and veterinarians in the planning and implementation of local eradication strategies. It has only been made possible due to the transparent sharing of data.

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# PHYLOGENETIC ANALYSIS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN SOME PROVINCES IN VIETNAM FROM 2021 TO 2023

T.T.H. Le<sup>1</sup>, T.B.N. Nguyen <sup>2</sup>, N.H. Nguyen <sup>3</sup>, X.P. Dinh <sup>4</sup>, T.D.T. Nguyen <sup>5</sup>

<sup>1</sup>PhD student -1 Vetlatech Joint Stock Company 2 Faculty of Biological Sciences, Nong Lam University Hochiminh City, Vietnam

<sup>2</sup>Vetlatech Joint Stock Company, Vietnam

<sup>3</sup>Faculty of Animal Sciences and Veterinary Medicine, Nong Lam University HoChiMinh City Vietnam <sup>4</sup>Faculty of Biological Sciences, Nong Lam University, HoChiMinh City, Vietnam

<sup>5</sup>Institute of Biotechnology, Vietnam Academy of Science and Technology

### **Background and Objectives**

Porcine respiratory and reproductive syndrome virus (PRRSV) causes significant economic losses in the swine industry worldwide. PRRS-2 and PRRS-HP are currently predominant in Vietnamese swine herds. However, PRRS-1 isolates are gradually increasing over recent years. Due to the high incidence of PRRSV mutation and recombination, PRRSV infection is difficult to prevent and control in Vietnam and worldwide. This study aimed to investigate the genetic diversity of PRRS-1 to assist in evaluating and providing a basis for further development of prevention and control measures for PRRS-1 the effectiveness of vaccines and control of PRRS in Vietnam.

## **Material and Methods**

RT-PCR confirmed a total of 13 PRRS-positive sera or organs (lung, lymph node) samples. All samples of PRRS-1 strains were from the provinces of Dong Nai, HaTinh, Ninh Binh, Thai Nguyen, and Hanoi during 2021- 2023. Full-length PRRSV ORF5 genes from the 13 samples were amplified, sequenced, and compared to the corresponding sequences of referenced PRRS strains from Genbank. The phylogenetic tree was inferred using the (MEGA 7.0) software.

#### Results

Based on the phylogenetic analysis of ORF5 and the whole genome of PRRS-1. According to the phylogenetic tree, all new Vietnamese PRRS-1 strains are four subgroups namely, AMERVAC-like, PORCILIS-like, Subtype New, and BJEU06-1-like. Sequence comparison of ORF5 genes showed that the nucleotide sequence identities of the ORF5 genes among the thirteen isolates ranged from 71 to 99% nucleotides, and the amino acid similarities of the proteins ranged from 72 to 99%. Four isolates are similar (86 -98%), nine isolates are similar (73-98%), and also similarities with the AMERVAC (73 - 95% with the PORCILIS vaccine (71 -92%).

# **Discussion and Conclusion**

Phylogenetic analysis based on the ORF5 full sequence indicated the diversity and the genetic relationships of the newly identified PRRS-1 strains of PRRSV in Vietnam. All strains are more similar to the EU strains in Vietnam (2016-2022), Thailand strain (2008-2010), China strains (2020 -2022), and different groups of the BJEU06-1. This study expands existing knowledge on the genetic diversity of PRRSV in Vietnam and assists the effective strategies for PRRS vaccine and control of PRRS in Vietnam.

VVD – Virology and Viral Diseases

# PREVALENCE AND DIVERSITY OF PORCINE ROTAVIRUS GROUPS A, B, C AND H IN DANISH PIGS

<u>N.B. Goecke 1</u>, M.V. Agerlin 1, K. Skadborg 2, E.O. Nielsen 2, S. Haugegaard 3, N.R. Weber 3, L.E. Larsen 1 <sup>1</sup>University of Copenhagen, Institute for Veterinary and Animal Sciences, Stigbøjlen 4, DK-1870 Frederiksberg C, Denmark <sup>2</sup>SEGES Innovation P/S, Livestock, Axeltorv 3, DK-1609 Copenhagen V, Denmark <sup>3</sup>Veterinary and Quality Services, Danish Agriculture and Food Council, F.m.b.A., Denmark

#### **Background and Objectives**

Rotaviruses (RVs) are one of the main infectious causes of acute gastroenteritis and are widespread among pigs with enteric disease. Rotaviruses are classified into ten groups (RVA-RVJ) and, to date, five of these (RVA, RVB, RVC, RVD, RVE and RVH) have been identified in pigs, with RVA being the most frequent and characterized species. In Danish pigs, RVA is frequently detected, whereas the prevalence of the other RVs have only been investigated in few small-scale field studies. The aim of the present study was to investigate the presence of RVA, RVB, RVC and RVH in diarrheic pigs.

## **Material and Methods**

Ninety (90) pigs from 22 Danish herds (2-8 pigs per herd) were included in the study. The pigs were 1-35 days of age and the majority had a history of diarrhea and macroscopic signs of enteritis. Rectal swab samples were analyzed for the presence of RVA, RVB, RVC and RVH using real-time RT-PCR. Furthermore, a portion of the positive samples were selected for sequencing, and phylogenetic analysis were made.

## Results

Overall, RVs were detected in 81.1% of the pigs with RVA (37.8%) and RVB (38.9%) being predominant, followed by RVC (28.9%) and RVH (1.1%). Co-infections with two or three different RV species were observed in 10.0% and 7.8% of the pigs, respectively. On herd level, RVs were detected in 95.5% of the investigated herds with the following distribution: RVA (59.1%), RVB (54.5%), RVC (45.5%) and RVH (4.5%). In 40.9% of the herds, more than one RV species were present. Looking at the distribution of the RV findings in three defined age groups (1-3 days, 4-20 days and 21-35 days) across the herds, showed that RVA, RVB and RVC were detected in all three age groups, while RVH was only detected in the age group 4-20 days.

## **Discussion and Conclusion**

It is known that RVA is frequently detected in Danish pigs, but this study shows that there is a rich RV diversity in Danish pigs and herds. The frequent detection of RVB and RVC in diarrheic pigs could indicate that especially these two RVs also could act as causative agents of diarrhea, however, this should be investigated further.

VVD – Virology and Viral Diseases

## PREVALENCE OF ROTAVIRUS SEROGROUPS AND VP7 SEQUENCES ACROSS HERDS IN CANADA

<u>J. Buchan</u><sup>1</sup>, C. Werth<sup>1</sup>, B. Cregan<sup>1</sup>, K. Vilaca<sup>1</sup> <sup>1</sup>South West Ontario Veterinary Services, Stratford, Ontario, Canada

#### **Background and Objectives**

Porcine rotaviruses (RVs) significantly impact global swine production, with infections causing malabsorptive diarrhea leading to significant growth retardation and increased fallbacks. Globally, the RVA serogroup has been described to have the highest prevalence, followed by RVC then RVB. Furthermore, analysis of VP7 sequencing revealed that RVCg06 was the most prevalent RV genotype in Canada. The objective of this study was to expand the current understanding of RV prevalence in Canada and the distribution of genotypes observed.

# Material and Methods

As part of standard veterinary practice, fecal and/or tissue samples collected between January 2017 to June 2023 for clinical scour cases were submitted for RV PCR testing, with a subset of severe clinical cases submitted for VP7 sequencing. Data were exported from a veterinary clinic database representing a large proportion of the Ontario, Canada swine herd. Data will be analyzed until December 2023.

## Results

The clinic's database included 1,598 samples from 554 cases; 91% of cases were RV positive. Overall serogroup prevalence was 80.4% RVA, 35.4% RVB, and 66.5% RVC with positive mean Ct values of 24.2, 31.0, and 27.2, respectively. The prevalence of the RVA serogroup was significantly different across years (p<0.001). Of the suckling pig cases, 64% were single RV type infections, while 89% of weaned pig cases were mixed infections. Although each serogroup had 5 different VP7 genotypes identified by sequencing across the sampling timeframe, the RVAg09 and RVCg06 genotypes were the most prevalent (31% and 22%). Percent homology to reference strains ranged from 89.6-97.4% for the RVAg09 genotype and 89.6-99.4% for the RVCg06 genotype. RVB genotypes were rarely sequenced, with the most common genotype, RVBg20, representing only 4% of all sequenced samples. Data will continue to be analyzed until December 2023.

## **Discussion and Conclusion**

Our data confirms that porcine RV continues to be an evolving and diverse virus making treatment decisions challenging. Sequencing of samples from clinically severe cases identified the RVAg09 and RVCg06 genotypes as most prevalent; however, the virus was genetically diverse within genotypes and across years. Further investigation into assigning clinical severity to dominant genotypes would inform treatment approaches and strategic vaccine development.

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# SELECTION OF EFFECTIVE PORCINE EPIDEMIC DIARRHEA VIRUS VACCINE CANDIDATES

D. Kim<sup>1</sup>, S.H. Moon<sup>2</sup>, S. Kim<sup>2</sup>, Y. Oh<sup>3</sup>, H.S. Cho<sup>2</sup>, D. Tark<sup>4</sup>

<sup>1</sup>1. Laboratory for Infectious Disease Prevention, Korea Zoonosis Research Institute, Jeonbuk National University, Iksan 54531, Republic of Korea

<sup>2</sup>2. College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan 54596, Republic of Korea

<sup>3</sup>3. College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Republic of Korea

<sup>4</sup>1. Laboratory for Infectious Disease Prevention, Korea Zoonosis Research Institute, Jeonbuk National University, Iksan 54531, Republic of Korea.

#### **Background and Objectives**

Since the occurrence of the first porcine epidemic diarrhea (PED) outbreaks, it was annually reported from several countries including Europe and Asia. PED is caused by porcine epidemic diarrhea virus (PEDV) and is characterized by symptoms such as watery diarrhea, vomiting, anorexia, and dehydration. Despite the existence of commercial vaccines, their efficacy remains controversial, leading to economic losses for many swine industries. Consequently, there is a need for more effective PED vaccines to control these outbreaks.

#### **Material and Methods**

In this study, a highly virulent PEDV was isolated from the Republic of Korea and subsequently serially passaged under six different culture conditions. The biological characteristics of the virus were analyzed in terms of cytopathic effects and virus growth kinetics. The virulence of the strains was evaluated using five-day-old piglets, and alteration of genome was determined by next generation sequencing according to culture conditions. Subsequently, immunogenicity assessments were conducted based on the vaccination route (oral, oronasal, intramuscular) and the adjuvant used (CABIGEN, IMS1313, aluminum gel).

#### Results

A new strain was successfully isolated, and this strain was passaged under six different culture conditions up to 180 times. The biological characteristics of the strains were observed, including typical and atypical aspects such as syncytium formation, cell lysis, and detachment. Among the strains, the highest titer was recorded at  $8.67 \pm 0.29 \log_{10} TCID_{50}/ml$ . Virulent tests indicated that the strain was attenuated, as no observable symptoms were present. Furthermore, immunogenicity assessments were performed with the attenuated strain, revealing the formation of higher neutralizing antibodies in the intramuscular injection group compare to other routes.

## **Discussion and Conclusion**

Collectively, live attenuated strains were developed under six different culture conditions, evaluated for virulence, and subjected to various approaches to enhance immunogenicity. These studies provide insights into more efficient PED live attenuated vaccine candidates. Further research will be conducted to determine efficacy through sow testing.

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# STRUCTURAL PROTEIN VP4 OF FMDV INHIBITS IRF3 NUCLEAR TRANSLOCATION TO ABATE HOST TYPE-I INTERFERON PATHWAY

A. Subasinghe <sup>1</sup>, <u>L. Jong-Soo</u><sup>1</sup> <sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

#### **Background and Objectives**

Foot-and-mouth disease (FMD) is a highly infectious virus that causes acute vesicular diseases in hoofed mammals. In this study, we evaluated the specific mechanism by which FMDV VP4, a known capsid protein of FMD virus, aids in evading the host immune response.

#### **Material and Methods**

In vitro virus replication assays were performed to demonstrate the surrogate virus replication in the presence of FMDV VP4. ELISA and qT-PCR were performed to evaluate the cytokine secretion and ISG transcription level. Mass spectrometric analysis was performed to identify a potential binding partner par with the luciferase assay results. Immunoprecipitation assays were conducted to confirm the interaction. A competition assay was staged to evaluate the binding affinity of FMDV VP4 to the binding partners. A confocal microscopy was prepared to check the IRF3 nuclear localization in the presence of FMDV VP4, followed by a fractionation assay.

#### Results

RNA and DNA virus replication was enhanced with reduced cytokine secretion and ISG transcription levels of cells overexpressing FMDV VP4. Luciferase assay results indicate that a potential binding partner would be downstream of IRF3, and, as par, KPNA molecules were selected in the mass spectrometric result analysis. Immuno-precipitation assay confirmed the interaction between KPNA2 and 4 with FMDV VP4, and competition assay indicated that FMDV VP4 has an elevated binding affinity to both KPNA molecules compared to IRF3. Hence, IRF3-KPNA interaction leading to IRF3 nuclear translocation was assumed to be hindered and confirmed with confocal microscopy and fractionation assay results.

#### **Discussion and Conclusion**

These results collectively suggest that FMDV-VP4 negatively regulates the type-I IFN pathway and leads to severe FMDV infection by inhibiting the IRF3 nuclear translocation. IRF3 is a common molecule in both DNA and RNA virus-mediated IFN pathways. The results showed that the DNA virus replication was also elevated, and cytokine levels examined to be diminished in the presence of VP4. [National Research Foundation of Korea (2021R1A6A1A03045495)]

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# CLASSICAL SWINE FEVER VIRUS (CSFV) ERNS ANTIBODY ALPHALISA FOR RAPID DETECTION AND CAPABLE OF DIFFERENTIATING INFECTED FROM CSFV-VACCINATED ANIMALS (DIVA)

Y. Panyasing <sup>2</sup>, L. Gimenez-Lirola <sup>1</sup>, R. Thanawongnuwech <sup>2</sup>, P. Prakobsuk <sup>3</sup>, Y. Kawilaphan <sup>3</sup>, A. Kittawornrat <sup>3</sup>, T. Cheng <sup>4</sup>, J. Zimmerman <sup>1</sup>

<sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>2</sup>Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand <sup>3</sup>CPF (Thailand) Public Company Limited, Bangkok, Thailand

<sup>4</sup>Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA

### Background and Objectives

Classical swine fever (CSF) is a highly contagious viral disease causing significant economic losses to the swine industry. Highly effective diagnostic tools are required for detection, control, and/or elimination of CSFV. The bead-based AlphaLISA® (Amplified Luminescent Proximity Homogenous Assay) antibody format reportedly achieves both high analytical sensitivity and rapid turn-around because of short incubation times and no wash steps. The aim of this study was to develop and evaluate a CSFV Erns AlphaLISA for serum and oral fluids that would complement the current CSFV E2 DIVA vaccines.

#### **Material and Methods**

The CSFV Erns AlphaLISA was designed to detect IgG isotype-specific antibody in serum and oral fluids. Diagnostic performances of the assay were evaluated using a panel of well-characterized serum (n = 760) and individual (n = 528) or pen-based (n = 30) oral fluid samples from 4 groups of animals: (1) unvaccinated and uninoculated; (2) unvaccinated and inoculated with wild-type CSFV (ALD strain); (3) uninoculated and vaccinated with a live CSFV (LOM strain) vaccine; and (4) uninoculated and vaccinated with live CSFV marker vaccine. The presence of CSFV antibody in samples from inoculated or vaccinated pigs was confirmed by the serum-virus neutralization test (VNT). The diagnostic performance of the CSFV AlphaLISA was determined by receiver operating characteristic (ROC) analyses of the serum and oral fluid testing data. Associated 95% CIs for diagnostic sensitivity and specificity were estimated using a procedure for normally distributed correlated data.

## Results

At a cutoff of  $S/P \ge 0.7$ , the aggregate estimated diagnostic sensitivity and specificity of the assay was, respectively, 97.4% (95% CI 95.9, 98.3) and 100% for serum and 95.4% (95% CI 92.9, 97.0) and 100% for oral fluid.

## **Discussion and Conclusion**

We developed and optimized an AlphaLISA system for detecting serum and oral fluid CSFV Erns IgG antibody in swine. The assay provided excellent diagnostic performance in terms of detecting Erns antibody in CSFV-infected or whole virus-vaccinated pigs and demonstrated the ability to differentiate pigs vaccinated with CSF E2-based DIVA vaccines.

VVD – Virology and Viral Diseases

# COMPARING PRRSV NUCLEIC ACID DETECTION BY RT-QPCR IN SERUM AND SWABS OF WEANING-AGE PIGS

<u>O.H. Osemeke</u><sup>1</sup>, G. Trevisan <sup>1</sup>, G. De Souza E Silva <sup>1</sup>, P. Gauger <sup>1</sup>, D. Correia Lima Linhares <sup>1</sup> <sup>1</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

#### **Background and Objectives**

The porcine reproductive and respiratory syndrome virus (PRRSV) continues to pose a significant challenge to global swine production. Due to its ability to sustain very low prevalence, effective PRRSV surveillance can be challenging. Weaning-age pigs are a crucial subpopulation for surveilling PRRSV in breeding herds, yet limited sample types have been validated for this purpose. This study aimed to compare PRRSV RNA reverse transcription-quantitative polymerase chain reaction (RT-qPCR) detection rates in serum, oral swabs (OS), nasal swabs (NS), ear-vein blood swabs (ES), and family oral fluids (FOF) from weaning-age pigs. The impact of pooling samples at the litter level on RT-qPCR detection of PRRSV RNA was also assessed.

# Material and Methods

Three eligible PRRSV-positive herds in the Midwestern USA were selected for this study. Six hundred sixty-six piglets across 55 litters were sampled for serum, NS, ES, OS, and FOF. RT-qPCR tests were conducted on both individual samples and litter-level pools of the swabs. Litter-level pooling involved combining equal volumes of swabs collected from pigs within the same litter.

### Results

The sensitivity and specificity estimates of ES, NS, and OS relative to serum were (0.83, ~1.00), (0.83, 0.99), and (0.75, 0.99), respectively. Comparative analyses demonstrated strong agreement among all paired ES, OS, NS, and serum sample comparisons. Serum RT-qPCR cycle threshold values (Ct) were strongly correlated with PRRSV detection in swab samples. The study established a  $\geq$  95% probability of detecting PRRSV in pooled ES-, OS-, and NS samples when the proportion of positive swab samples within pools were  $\geq$  23%,  $\geq$  27%, and  $\geq$  26%, respectively<sup>10.5emeke, O. H. et al. (2023). The study established a  $\geq$  95% probability of detecting PRRSV in pooled ES-, OS-, and NS samples when the proportion of positive 303.1903/fb/ll</sup>

## **Discussion and Conclusion**

Results indicate that ES, NS, and OS are effective surveillance samples for detecting PRRSV RNA via RT-qPCR in weaning-age pigs. The minimum sample sizes required for a 95% confidence level in detecting at least one infected piglet using serum, ES, OS, and NS are 30, 36, 36, and 40 piglets, respectively, when PRRSV prevalence is  $\ge$  10%.

VVD – Virology and Viral Diseases

#### DETECTION OF RESPIRATORY AND SYSTEMIC PATHOGENS IN CASES OF PNEUMONIA IN WILD BOAR IN SPAIN

A. Kouznetsova <sup>1</sup>, <u>M. Sagrera</u><sup>2</sup>, J. Estruch <sup>1</sup>, M. Sibila <sup>3</sup>, M.L. Abarca <sup>4</sup>, R. Rosell <sup>5</sup>, F. Correa-Fiz <sup>3</sup>, M. Escobar-González <sup>1</sup>, E. Serrano <sup>1</sup>, J. Segalés <sup>3</sup>, R. Velarde <sup>1</sup>

<sup>1</sup>Wildlife Ecology & Health group (WE&H) and Servei d'Ecopatologia de Fauna Salvatge (SEFaS), Departament de Medicina i Cirurgia Animals. Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Spain

<sup>2</sup>IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), 08193 Bellaterra, Spain / Ceva Salud Animal, Barcelona, Spain

<sup>3</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain.

<sup>4</sup>Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, UAB, 08193 Bellaterra, Barcelona, Spain

<sup>5</sup>IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), 08193 Bellaterra, Spain / Departament d'Acció Climàtica, Alimentació i Agenda Rural, Generalitat de Catalunya, Barcelona, Spain

# **Background and Objectives**

Pneumonia and their causative agents have been poorly characterized in wild boar. This gap of knowledge is particularly concerning, given their role as pathogen reservoirs for various animal populations, including domestic pigs. This study aimed at characterizing pulmonary lesions in wild boar and identifying the associated bacterial and viral etiological agents through polymerase chain reaction (PCR) detection and bacterial isolation.

#### **Material and Methods**

Between 2018 and 2022, wild boar with gross pulmonary lesions (n=30) were selected. Lung samples were tested by PCR for African swine fever virus (ASFV), Aujeszky's disease virus (ADV), classical swine fever virus (CSFV), porcine circovirus 2 (PCV-2), PCV-3 and PCV-4, porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), Mycoplasma hyopneumoniae (Mhp), Mycoplasma hyorhinis (Mhr), Glaesserella parasuis (Gp, distinguishing virulent and non-virulent strains), and Actinobacillus pleuropneumoniae (App). PCV-2 positive samples (Ct<30) underwent genotyping. Conventional bacterial isolation was also attempted from all lungs.

## Results

All examined animals displayed either suppurative bronchopneumonia or fibrinous pleuropneumonia. Lungs tested negative for CSFV, ASFV, ADV, PCV-4 and non-virulent Gp. Mhp was detected in 96.7% (n=29) of the animals, virulent Gp in 70% (n=21), PCV-3 in 26.7% (n=8), PCV-2 and Mhr in 23.3% (n=7), and App in 10% (n=3). PRRSV and SIV were detected in only one animal (3.3%). Four wild boar (13.3%) had co-infections with up to 5 of these pathogens, 3 (10%) with 4, 8 (26.7%) with 3, 9 (30%) with 2, and only 6 (20%) were positive to a single agent. PCV-2 genotyped samples (n=4) were PCV-2d. Bacterial isolation yielded Streptococcus spp. (Sspp, n=10), Pasteurella multocida (Pm, n=8), Trueperella pyogenes (n=2) and Salmonella spp. (n=1).

#### **Discussion and Conclusion**

Studied cases of suppurative bronchopneumonia and fibrinous pleuropneumonia in wild boar revealed a polymicrobial etiology, with Mhp being predominantly detected. Co-infection combinations closely resembled those observed in clinical cases of porcine respiratory disease complex (PRDC) in domestic pigs, including viral (PCV-2, PRRSV and SIV) and bacterial agents (Pm, Mhp, Mhr, Gp, App and Sspp). The detection of PCV-2d in the sequenced cases probably reflects the epidemiologically most prevalent genotype currently seen in domestic pig.

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# DIAGNOSIS OF PRRSV IN BREEDING HERDS USING TONGUE TIPS FROM STILLBORN PIGLETS AND PIGLETS DEAD DURING LACTATION: THE FRENCH EXPERIENCE

<u>S. Turci</u><sup>1</sup>, P. Hourcq<sup>2</sup>, M. Abed<sup>3</sup>, M. Baudouard<sup>3</sup>, I. Messager<sup>4</sup> <sup>1</sup>BREIZHPIG SCOP SAS, 1 rue Georges Guynemer 22190 Plérin, France <sup>2</sup>BREIZHPIG SCOP SAS, 63 Rue Ar Men, 29800 Plouedern, France <sup>3</sup>Labocea Ploufragan - Service Immunologie Virologie PCR. 7 rue du Sabot, Ploufragan, 22440. France <sup>4</sup>Labocea Ploufragan - 7 rue du Sabot, Ploufragan, 22440. France

### **Background and Objectives**

In recent years, alternative approaches have been developped to diagnose PRRSv, such as the use of oral fluids and "processing fluids". However, more and more farms are raising entire males and, in these cases, processing fluids are no longer available. Recently, fluids obtained from tongue tips (TTF) from dead animals have been described as a relevant alternative for the diagnosis of PRRSv. The aim of this study was to evaluate this type of sampling in France.

#### **Material and Methods**

Firstly, a "blank" test was performed to evaluate the feasibility of the fluids collection coming from tongue tips. Then, the detection capacity of PRRSv in TTF of dead animals (stillborn and piglets dead during lactation) was evaluated in two French breeding herds: a "control" negative herd (N) and a positive herd (P) with recent clinical signs suggestive of a PRRSv infection in late gestation confirmed by diagnostics tests on sick sows. For each farm, PRRSv PCR were carried-out on TTF from stillborn and on piglets dead during lactation (3 bags for N and 2 bags for P). They were compared to PCRs performed on 30 blood samples (six pools of five for N and 8 pools of four or two for P) collected from piglets of the same batch as tongue tips.

#### Results

At the laboratory, the collection of TTF was feasible. In this study, the consistency between PCR results on TTF and on piglets' sera confirmed the suitability of the method. All PCRs performed for (N) were PRRS negatives (sera and TTF). For the positive farm, 2/2 PCRs on TTF were positives versus 5/8 sera positive pools. The PRRS viral load on TTF and sera enabled ORF5 and ORF7 sequencing, and results were similar.

#### **Discussion and Conclusion**

This study confirms the value of TTF from stillborn piglets for the evaluation of PRRSv vertical transmission and the additional information from piglets dead during lactation. This approach is easy to implement and non-invasive and can especially be used on farms which have stopped castration. The quality of the samples allows ORF5 and ORF7 sequencing, which is useful for epidemiological monitoring.

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VVD – Virology and Viral Diseases

# ENHANCING FIELD SAMPLE QUALITY FOR MORE ACCURATE PRRSV MOLECULAR DIAGNOSTICS

<u>O.H. Osemeke</u><sup>1</sup>, J. Torrison <sup>2</sup>, G. Trevisan <sup>3</sup>, G. Allison <sup>4</sup>, C. Lefevre <sup>6</sup>, P. Gauger <sup>6</sup>, D. Correia Lima Linhares <sup>7</sup> <sup>1</sup>College of Veterinary Medicine, Iowa State University <sup>2</sup>Longhorn Vaccines and Diagnostics LLC, USA <sup>3</sup>Iowa State University, Ames. USA

<sup>4</sup>Walcott Veterinary Clinic, Walcott, Iowa, USA

<sup>5</sup>Carthage Veterinary Service Ltd, USA 4Walcott Veterinary Clinic, USA \*Corresponding author: oosemeke@iastate.edu <sup>6</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

<sup>7</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

#### **Background and Objectives**

Processing fluids (PF) and oral fluids (OF) currently dominate submissions (about 70%) to major US veterinary laboratories for PRRSV RNA testing via RT-qPCR. Despite their heightened herd-sensitivity for detecting PRRSV RNA, these samples contain fewer RNA copies and degrade faster than individual-piglet samples, like serum. Consequently, late cycle threshold (Ct) RT-qPCR test results are rarely repeatable, while Next Generation Sequencing (NGS) seldom furnishes complete sequences. The above challenges necessitate the exploration of methods to better preserve- or enhance the diagnostic quality of the mentioned sample types. Such efforts aim to provide superior value to practitioner investments in PRRSV surveillance. In addition, enhancing the precision of PRRSV RT-qPCR helps avoid false laboratory results and fosters a timely implementation of effective control measures.PrimeStore MTM (PS-MTM), an inexpensive and commercially available reagent, is formulated to preserve nucleic acids in field samples from collection to testing. This study aimed to compare the outcomes of PRRSV RT-qPCR and NGS between untreated and PS-MTM-treated PF samples.

#### **Material and Methods**

PF was aggregated at the level of the room (n = 5 rooms) across 2 PRRSV-infected farms. For each room, the PF was homogenized and divided into the Control group (Untreated PF, 5 replicates), Treatment A (PF with PS-MTM mixed in the ratio 1:3, 5 replicates), Treatment B (swab stick soaked in PF and put in 1.5 ml PS-MTM, 5 replicates). Thereafter, only Control group samples were put in cold storage. All samples were submitted to a NAHLN-approved veterinary diagnostic laboratory for PRRSV RT-qPCR and NGS.

#### Results

Across all sampled rooms, the least-square mean Ct in the Control group was higher than the Treatment A group by 1.58 and the Treatment B group by 1.32. PF from one room was negative for the Control group (0/5) but was positive for the Treatment A (3/5) and Treatment B groups (2/5). (NGS results will be presented at the conference)

#### **Discussion and Conclusion**

PS-MTM-treated PF had better diagnostic quality and performance. The use of PS-MTM in swine samples submitted for PRRSV RT-qPCR and NGS significantly improves the accuracy of results from these tests.
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# EVALUATION OF THE USEFULNESS OF PROCESSING FLUID TO DETECT ANTIBODIES AGAINST PRRSV AND PCV2 AND THE CONSEQUENCES OF SAMPLE POOLING

<u>H. Turlewicz-Podbielska</u><sup>1</sup>, A. Augustyniak<sup>1</sup>, A. Dors<sup>1</sup>, I. Kucinska<sup>1</sup>, M. Pomorska-Mól<sup>1</sup> <sup>1</sup>Department of Preclinical Sciences and Infectious Diseases, Poznan University of Life Sciences, Poland

#### **Background and Objectives**

Detection of antibodies against porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) in noninvasive specimens provides significant advantages for herd surveillance. The aim of the study was to evaluate the utility of processing fluid (PF) to detect these antibodies and assess the impact of pooling PF samples on the ability of ELISAs to correctly classify the sample.

# **Material and Methods**

In total, 252 paired serum and PF samples collected from piglets were tested with commercial ELISAs. The sensitivity and specificity of ELISAs for PF samples and agreement between the results of PF and sera were tested. Samples were classified as low, moderate, and high positives using S/P or OD ranges. Four samples of each range were diluted in a negative-control sample of PF to simulate pools of 10, 20, 40, and 80 samples containing 1 positive and 9, 19, 39, and 79 negative samples, respectively.

### Results

Cohen's Kappa testing showed a moderate agreement between the PF and sera results regarding PRRSV ( $\kappa = 0,59$ ) and almost perfect agreement regarding PCV2 ( $\kappa = 0,81$ ). Regarding PRRS, the sensitivity and specificity of the ELISA used for PF samples were 69,8% and 96,8%, respectively, while for PCV2 95,1% and 100%, respectively. At dilution 1:10, anti-PRRSV antibodies were not detected in low positives but were found in 75% of the moderate, and in 100% of the high positives. Anti-PCV2 antibodies were detected in 50% of low and in 100% of the moderate and high positives. At dilution 1:20, anti-PRRSV antibodies were detected only in 50% of the high positives, while anti-PCV2 antibodies were detected in 75% and 100% of the moderate and high positives, respectively. At dilutions 1:40 and 1:80, antibodies were detected in 25% of the moderate-positives. In the case of high-positives, they were detected in each case in both dilutions.

## **Discussion and Conclusion**

PF may represent a noninvasive, economical tool to monitor PRRSV and PCV2; however, when testing pooled PF samples, the results may not reflect the actual herd epidemiological situation and give false negative results. The research was financed by NCN project UMO-2020/37/N/NZ7/00084.

#### VVD – Virology and Viral Diseases

# MAPPING THE GEOGRAPHICAL SPREAD OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN THE NETHERLANDS

K. Eenink <sup>1</sup>, <u>H. Kreutzmann</u>, C. Vermeulen <sup>1</sup>, K. Koenders <sup>2</sup>, V. Manders <sup>2</sup>, M. Houben <sup>1</sup> <sup>1</sup>Royal GD, Deventer, The Netherlands <sup>2</sup>Lintjeshof Veterinary Practice, Nederweert, The Netherlands

## **Background and Objectives**

The Porcine Reproductive and Respiratory Syndrome virus (PRRSv) poses a significant threat to the swine industry worldwide. Currently, there is a lack of data on the relative contribution of direct and indirect routes to the transmission of PRRSv. In this analysis, we wanted to align the prevalence of Dutch PRRSv-positive farms in defined pig-dense and pig-sparse areas, respectively. Additionally, the relative contribution of direct contacts to PRRSv transmission should be investigated.

# Material and Methods

Forty farms, equally distributed across four areas (two pig-dense and two pig-sparse), and their supplying gilt- and pigletproducing farms (n=11) were incorporated. Collected data encompassed spacing, animal flow, and one-time sampling for PRRSv-PCR and ORF5 sequencing. Blood samples were taken from 30 piglets at the end of nursery, or, in case if only fatteners were present, six oral fluid samples were obtained. Phylogenetic analysis of ORF5 aimed to identify close matches (>98% similarity) and trace them back to potential direct or indirect contacts between farms. Chi-squared tests were applied to detect differences between areas/farms.

### Results

The prevalence of the virus in pig-sparse areas was 44% and 75%, while in pig-dense areas, it was 80% and 91%. The prevalence of the wild-type virus ranged from 30% to 38% across all four areas. 55% of the suppling farms were PCR-positive, and in 5/6 cases wild-type virus could be detected. Farms with external animal supply showed higher PRRSv prevalence (84%) compared to those without (63%). The wild-type virus was less often detected on farms without animal supply (5%) and more prevalent on those with animal supply (63%; p=0.002). Analysis of 58 ORF5 sequences identified four close matches, three linked to direct contact between farms.

#### **Discussion and Conclusion**

In conclusion, our study revealed no significant difference in wild-type virus prevalence between pig-dense and pig-sparse areas within the study group. However, farms with external animal supply exhibited a significantly higher prevalence of wild-type virus positive samples. While it is essential to acknowledge the limited subset of suppliers included in this study and the potential for false negatives due to one-time sampling, the identification of three closely matched sequences from connected farms highlights the effectiveness of our approach.

#### VVD – Virology and Viral Diseases

MATERNALLY DERIVED ANTIBODIES AND WEANING STRESS PLAY PIVOTAL ROLES IN PIGLET SUSCEPTIBILITY TO HUMAN H3N2 INFLUENZA A VIRUS

<u>G. Ciacci Zanella</u><sup>1</sup>, M. I. Cardenas<sup>2</sup>, C. Snyder<sup>1</sup>, C. R. Hutter<sup>1</sup>, B. Arruda<sup>1</sup>, D. Perez<sup>2</sup>, T. K. Anderson<sup>1</sup>, D. S. Rajão<sup>2</sup>, A. L. Baker<sup>1</sup>

<sup>1</sup>Virus and Prion Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA

<sup>2</sup>Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

## **Background and Objectives**

The diversity of influenza A virus (IAV) in swine in the USA has been shaped by regular interspecies transmission of human seasonal IAV. A subset of these IAV incursions establish in swine, evolve, and become endemic lineages that are genetically and phenotypically discrete. Vaccination control strategies are routinely used, with most vaccines applied to sows which transfer maternal antibodies to piglets. Stress can affect immune function and susceptibility to infectious diseases, and weaning imposes a significant stress event on piglets. Our work assessed the effect of matched and mismatched antibodies acquired from vaccinated sows and the stress of weaning on the susceptibility of piglets to a human-origin IAV.

# **Material and Methods**

To recreate a human IAV introduction to pigs, a virus was generated by reverse genetics to contain the HA and NA gene segments from a human seasonal H3N2, and internal gene segments from endemic swine viruses. Challenged seeder piglets were divided by immune- and wean-status groups: matched maternal antibodies, mismatched maternal antibodies, or no maternal antibodies; then further sub-divided into weaned or not weaned. Nasal swabs were collected daily. At 2 days post inoculation naïve direct contact pigs were placed with seeders to evaluate transmission. Seeder piglets were humanely euthanized at 5 days post-infection and contact pigs at 9 days post-contact to collect bronchoalveolar lavage fluids. IAV qRT-PCR was performed on all samples to evaluate viral shedding and transmission kinetics.

#### Results

Matched maternal antibodies effectively reduced shedding in challenged pigs and minimized transmission of the humanlike H3N2 to contacts. There was an increase in viral shedding and transmission from weaned pigs compared to suckling piglets to their contacts. Additional analyses, such as cortisol measurements to evaluate the response to stress are in progress.

### **Discussion and Conclusion**

These results identify control points in swine production where changing practices can mitigate human-to-swine and swineswine transmission to prevent establishment of novel lineages and onward transmission of IAV. Improving how swine are handled by on-farm personnel and biosecurity during the pre- and post-weaning time-periods may reduce stress and IAV transmission. These data inform vaccination recommendations for sows to minimize the risk of human-origin viruses establishing in the U.S. swine herd.

#### VVD – Virology and Viral Diseases

# MONITORING EMERGING PATHOGENS USING NEGATIVE NUCLEIC ACID TEST RESULTS FROM ENDEMIC PATHOGENS IN PIG POPULATIONS

A.P. Serafini Poeta Silva<sup>1</sup>, G. Cezar<sup>1</sup>, E. Magalhães<sup>1</sup>, G. De-Sousa-E-Silva<sup>2</sup>, R. Main<sup>1</sup>, A. Roivira<sup>3</sup>, F. Matias-Ferreyra<sup>4</sup>, A. Pillatzki<sup>5</sup>, M. Prarat<sup>6</sup>, A. Arruda<sup>7</sup>, C. Bowen<sup>8</sup>, D. Linhares<sup>1</sup>, G. Trevisan<sup>1</sup>

<sup>1</sup>lowa State University

<sup>2</sup>Iowa State University College of Veterinary Medicine

<sup>3</sup>University of Minnesota

4Kansas State University

<sup>5</sup>South Dakota State University, Brookings, USA

<sup>6</sup>Ohio Animal Disease and Diagnostic Laboratory, Reynoldsburg, USA

<sup>7</sup>The Ohio State University

<sup>8</sup>Purdue Animal Disease and Diagnostic Laboratory, West Lafayette, USA

# **Background and Objectives**

This study aimed to evaluate the potential of PCR-negative-based monitoring to address the potential issue of delayed detection of emerging pathogens due to the investigation of endemic pathogens with similar clinical diseases. During the porcine epidemic diarrhea (PEDV) emergence (USA 2013), there was an increase in porcine diagnostic submissions for transmissible gastroenteritis virus (TGEV) at veterinary diagnostic laboratories (VDL) due to the alike enteric clinical disease (Swine Disease Reporting System, SDRS, www.fieldepi.org/SDRS).

#### Material and Methods

This retrospective study used porcine submissions, including only PCR-negative results from tested samples submitted to six swine-centric US VDLs in the SDRS. To prove the concept, the database was searched for TGEV-negative submissions from January 2010 - May 2013, when the first PEDV case was diagnosed in the USA. Time series (weekly TGEV-negative counts) and Seasonal Autoregressive-Integrated Moving-Average (SARIMA) were used to control outliers, trends, and seasonality in the baselines. The SARIMA's fitted and residual values were then subjected to anomaly detection algorithms (EARS, EWMA, CUSUM, Farrington) to identify alarms, e.g., weeks of increased TGEV-negativity preceding the PEDV emergence. The best-performing detection algorithms were the one(s) with the lowest false alarms (number of alarms detected in the baselines) and highest timing (number of weeks between the first alarm and PEDV emergence). The same methodology was applied to TGEV-negative cases to detect the porcine delta coronavirus (PDCoV) emergence in the USA in 2014.

# Results

The best-performing detection algorithms were Farrington using SARIMA fitted values, alongside CUSUM and EWMA using SARIMA fitted and residuals. These algorithms resulted in 0% false alarms and identified alarms in TGEV-negative cases at 5 to 4 weeks before PEDV and PDCOV emergences. Thus, these 3 detection algorithms were selected to prospective monitor negative cases in 2023.

#### **Discussion and Conclusion**

The negative-based monitoring system will function in case of a propagating epidemic (like PEDV) and secondary emerging pathogen (PDCoV) in the presence of a concurrent propagating epidemic. External events and variations on the baseline will demand periodic revision of model parameters. Currently, SDRS incorporates an ongoing collection of PCR results of 7 pathogens, thus, the monitoring system can be improved as SDRS adds more pathogens.

VVD – Virology and Viral Diseases

# PCV2 VIRAL LOADS IN PIGS ARE CORRELATED WITH CONCURRENT PORCINE PARVOVIRUS INFECTION(S).

<u>C. Bonckaert</u><sup>1</sup>, C. Rigauts<sup>1</sup>, C. Brossé<sup>1</sup>, T. Vandersmissen<sup>1</sup>, S. Theuns<sup>2</sup>, H. Nauwynck<sup>3</sup> <sup>1</sup>DGZ Vlaanderen, Hagenbroeksesteenweg 167, 2500 Lier, Belgium <sup>2</sup>PathoSense BV, Lier, Belgium <sup>3</sup>Laboratory of Virology, Faculty of Veterinary Sciences, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.

### Background and Objectives

PCV2 causes several syndromes, commonly known as porcine circovirus-associated disease (PCAD). Despite this vaccination, veterinarians have reported an increase in PCV2 viral loads over the years. The clinically most important and best described porcine parvovirus (PPV) is PPV1. However, little is known about various other PPV types (PPV2-7) circulating on farms in Belgium. Furthermore, research data show that co-infection(s) with porcine parvovirus(es) (PPV) may underlie the rise in PCV2 load. Therefore, this study aims to investigate if increasing PCV2 viral loads in pigs on Flemish farms can be correlated with systemic PPV infections.

#### **Material and Methods**

Pig serum samples submitted to DGZ Vlaanderen between July 2022 and June 2023 were pooled up to five samples and analyzed by qPCR for PCV2. PCV2 positive samples were selected and categorized based on the viral PCV2 load into different groups for further viral & bacterial metagenomic sequencing at PathoSense: Group 1 consisted of PCV2 samples with a viral load of <10<sup>4</sup> genome copies/ml; group 2 had a viral load of 10<sup>5-7</sup>; group 3 had a viral load of >10<sup>8</sup>. In this way, the PCV2 loads were linked to the detection of PPV co-infections in a semi-quantitative manner.

#### Results

Samples with a viral PCV2 load of <10<sup>3</sup> genome copies/ml with PCR (group 1) were tested negative for PPV. Sequencing by PathoSense confirmed the presence of PCV2 in the samples in group 2 (10<sup>5-7</sup> PCV2 genome copies) and 3 (10<sup>8-10</sup> PCV2 genome copies) and also at least one PPV type in those groups. In total, five different types of parvoviruses (not PPV1) were detected by PathoSense: PPV2, 3, 4, 5 and 7. Importantly, an increase of total PPV in samples with a high PCV2 load was observed.

# **Discussion and Conclusion**

The suspicion of field veterinarians that PCV2 is more present has been confirmed in this study. In addition, we found evidence that different PPVs can be found in association with high PCV2 loads. This is logical from a pathogenetic point of view, as both viruses rely on proliferating lymphoblasts, which is the result of an activated immunity during co-infections.

VVD – Virology and Viral Diseases

# PERFORMANCE OF A NEWLY DESIGNED QPCR FOR MULTIPLEX DETECTION OF PRRSV-1 AND PRRSV-2: FIELD STUDY EVALUATION

M. Angelichio <sup>1</sup>, L. Plourde <sup>1</sup>, L. Gow <sup>1</sup>, H. Webber <sup>1</sup>, <u>A. Hidalgo</u> <sup>1</sup> <sup>1</sup>IDEXX Laboratories

#### **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) continues to be one of the most economically significant diseases affecting the swine industry worldwide. Effective management strategies for controlling PRRSV rely on the use of diagnostic assays, with ELISA and qPCR being the most common. This study investigates the performance of a newly designed multiplex qPCR assay for detection and differentiation of PRRSV-1 and PRRSV-2 and compares this to a reference qPCR.

# Material and Methods

A total of 370 swine samples, including serum, oral fluids, lung lavage, processing fluids and tongue tip exudates, were obtained from several farms in the US. Samples were extracted and independently tested using two different qPCR diagnostic assays using the same qPCR instrument. qPCR-A refers to the RealPCR PRRSV Type 1 and Type 2 Multiplex RNA Test, IDEXX Laboratories while qPCR-B refers to another commercially available qPCR test for PRRSV-1 and PRRSV-2.

#### Results

PRRSV-1 results: 101 samples were PRRSV-1 positive by one or both qPCRs. 269 samples were negative by both PCR assays. The percentage of agreement was 95.1%. The average Ct values for positive samples was 30.8 for qPCR-A and 32.1 for qPCR-B. qPCR-A identified six more positive samples (confirmed by an alternate method) than qPCR-B. PRRSV-2 results: 170 samples were positive by one or both qPCRs. 200 samples were negative by both assays. The percentage of agreement between qPCR-A and qPCR-B was 95.1%. The average Ct values for positive samples was 29.8 for both assays. Discrepant results corresponded to high Ct values (> 36).

#### **Discussion and Conclusion**

The comparison of two qPCR assays for detection of circulating strains of PRRSV-1 and PRRSV-2 in North America showed that both tests identified the same number of PRRSV-2 RNA positive samples with the same average Ct value. However, qPCR-A identified more PRRSV-1 positive samples compared to qPCR-B and had a lower average Ct value. According to this evaluation, qPCR-A showed high performance for detection of PRRSV-1 and PRRSV-2 strains circulating in North America.

VVD – Virology and Viral Diseases

# POLYMERASE COMPLEX ACTIVITY OF SWINE H1 INFLUENZA A VIRUS WITH DIFFERENT GENOME PATTERNS AND PHENOTYPES

C. Kunzler Souza <sup>1</sup>, <u>J. Ciacci Zanella</u><sup>2</sup>, T.K. Anderson <sup>1</sup>, G. Janzen <sup>1</sup>, P. Gauger <sup>3</sup>, A.L. Baker <sup>1</sup> <sup>1</sup>Virus and Prion Research Unit, National Animal Disease Center, USDA – ARS, Ames, IA, USA <sup>2</sup>Brazilian Agricultural Research Corporation (EMBRAPA), Concórdia, Brazil <sup>3</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

# **Background and Objectives**

Influenza A viruses (IAV) have considerable genetic diversity. We assessed the pathogenesis and transmission of five representative swine lineage H1 viruses in pigs. Viruses were assigned a genome constellation using HA and NA clade, with internal gene lineage (TRIG=T or Pandemic=P) in the order of PB2, PB1, PA, NP, M, and NS. The 5 viruses replicated in the nose and lungs, but 1A.3.3.3/N1c-TTPPPT presented significantly higher lung lesions while 1A.1.1.3/N2-02-TTPTPT had higher nasal shedding. We evaluated whether the polymerase complex activity (PCA) of the different genomes influenced in vivo phenotypes.

# **Material and Methods**

PCA using a pHW-SP reporter plasmid containing Gaussia luciferase (GLuc) in a minigenome assay was assessed. The PB2, PB1, PA, and NP segments of the five viruses (1A.1.1.3/N2-02-TTPTPT, 1B.2.2.1/N2-02-TTTTPT, 1B.2.2.2/N2-02-TTTPPT, 1B.2.1/N2-02-TTTTPT, and 1A.3.3.3/N1c-TTPPPT) were cloned into the pHW-SP reporter plasmid (gift of D.Perez, UGA, USA), transfected into MDCK-SIAT1 cells, and incubated at 37°C and 39°C. Cell supernatant were collected at 12, 24, 48, and 72 hours post-transfection (hpt). Reporter gene expression with GLuc activity was used to measure PCA.

#### Results

At 37°C, 1A.1.1.3/N2-02-TTPTPT had significantly higher PCA at 24 and 72hpt than others, consistent with the early peak of virus replication in the nose. By 48hpt, all five minigenomes had PCA at 37°C and remained active until 72hpt. At 39°C, the PCA of 1A.1.1.3/N2-02-TTPTPT was significantly higher at 48hpt than others. 1A.3.3.3/N1c-TTPPPT did not show PCA that was significantly different from others at any timepoint at 39°C.

# **Discussion and Conclusion**

1A.1.1.3/N2-02-TTPTPT demonstrated PCA at both temperatures that matched the high viral replication in the nose and lungs. The 1A.3.3.3/N1c-TTPPPT showed lower PCA at both temperatures. Host factors and specific viral gene combinations, like HA and NA pairing with an efficient polymerase complex, are likely involved in increased replication of swine adapted viruses rather than PCA alone. These data demonstrate that diverse internal IAV genes affect phenotype, and specific combinations may have an advantage due to PCA in the swine host.

VVD – Virology and Viral Diseases

## PORCINE CIRCOVIRUS 3 (PCV-3) REPRODUCTIVE DISEASE IN IBERIAN GILTS IN SPAIN

A. Cobos <sup>1</sup>, M. Sibila <sup>2</sup>, E. Huerta <sup>1</sup>, M. Perez <sup>3</sup>, M. Marcos <sup>4</sup>, R. Menjon <sup>4</sup>, M. Jiménez <sup>4</sup>, L. Gálvez-Pérez <sup>5</sup>, <u>J. Segales <sup>1</sup></u> <sup>1</sup>*IRTA, Centre de Recerca e Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona,* 08193 Bellaterra, Spain

<sup>2</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain.

<sup>3</sup>IRTA, Centre de Recerca e Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

⁴MSD Animal Health ⁵MONTESANO

## **Background and Objectives**

Several clinical-pathological disorders have been related to PCV-3 infection in pigs, most notably reproductive (PCV-3-RD) and post-natal diseases. However, their diagnosis is largely limited and their real prevalence and impact on swine production are unknown. Although mainly reported in intensively reared pigs, PCV-3-RD in extensive reared pigs has not described. Therefore, the aim of this study was to characterize a case of PCV-3-RD in an Iberian semi-outdoors sow farm.

# **Material and Methods**

A farm of Iberian sows (n=420) displaying reproductive problems such as increase of abortions, stillborn, weak born and mummified piglets was studied. Five piglets from two gilts were submitted to the Servei de Diagnòstic en Patologia Veterinària (SDPV) at the Universitat Autònoma de Barcelona (UAB). A complete post-mortem examination was performed, including gross and histopathological assessment. A pool of fresh tissues per animal (spleen, lung, brain, mesenterium and heart) was tested for PCV-3 detection by a quantitative PCR (qPCR). Moreover, viral genome distribution within lesions was assessed using in situ hybridization (ISH). Infection by PCV-2 and PRRSV was ruled out by immunohistochemistry.

## Results

Examined animals included 2 weakborn piglets, 2 mummies and 1 stillborn. Only the stillborn piglet had gross changes (anasarca, hydrothorax, ascites, and cardiomegaly). Histologically, systemic lymphohistiocytic arteritis and periarteritis was observed in 4/5 animals, as well as lymphohistiocytic myocarditis and endocarditis in the piglet with cardiomegaly. PCV-3 was detected by qPCR in all 5 animals, yielding high (Ct<20) and intermediate (Ct between 20 and 30) viral loads in 2 and 3 of them, respectively. Abundant PCV-3 genome within lesions was confirmed by ISH in all animals, mainly within vascular lesions and myocardium.

# **Discussion and Conclusion**

The present case fulfilled the proposed diagnostic criteria for PCV-3-RD, comprising compatible clinical signs, characteristic histological lesions, and high viral load within tissues. Although one fetus had gross lesions, they are seldom present in PCV-3-RD cases. Therefore, based on clinical suspicion, laboratory diagnostic investigation should be considered mandatory (histology and qPCR and/or ISH). This is the first description of PCV-3-RD in Iberian semi-outdoor reared sows, which mainly affected gilts.

VVD – Virology and Viral Diseases

# RESILIENCE EFFECTS OF SGK1 AND CD163 MARKERS DURING A HIGH VIRULENCE PRRSV STRAIN CHALLENGE IN NURSERY PIGS

<u>A.M. Stoian</u><sup>1</sup>, P. Curto<sup>1</sup>, A. Pey<sup>2</sup>, A. Trabal<sup>2</sup>, E. Aulinas<sup>2</sup>, R. Pena<sup>1</sup>, L. Fraile<sup>1</sup> <sup>1</sup>University of Lleida, Lleida, Spain <sup>2</sup>AM Animalia, Bianya SL, Spain

## **Background and Objectives**

Pig resilience is critical for the sustainability of the pig production system. GBP5 has been validated as a genetic marker for pig resilience after infection with porcine reproductive and respiratory syndrome virus (PRRSV)-1 and -2. In addition, the influence of other reported regions on chromosome 1 (SGK1) and 5 (CD163) in reproductive performance and immune response during PRRSV outbreaks in sows has been identified. However, the effects of these markers remain unknown when rearing pigs are exposed to high virulence PRRSV strains.

# **Material and Methods**

A challenge study was carried out using a high-virulence PRRSV strain on ten 8-weeks-old PRRSV-negative pigs, administered intranasally at a dose of 10<sup>s</sup> TCID<sub>50</sub>/ml. Daily clinical evaluations considered body condition, dyspnea, fever, cough, nasal discharge, digestive signs and behavior over the 63-day trial. A resilient animal was defined as an animal surviving until the end of study, with a clinical score below the group average, whereas a susceptible animal either died during the study or had a clinical score above the group average. DNA from these animals was genotyped for SGK1 gene (rs338508371, C>A) and CD163 (rs1107556229, G<A) using high resolution melting. Genetic variability within GPB5 was absent in this population. The effect of the different markers on resilience or susceptibility was described, while recognizing the low statistical potency of this assay.

#### Results

After the challenge, the percentage of resilient or susceptible animals varied among the studied genotypes. Specifically, 71.4% and 33.3% of resilient animals had AG or AA genotype for SGK1, respectively. For CD163, the percentages of resilient animals were 75%, 60% and 0% for GG, GA or AA genotype, respectively.

# **Discussion and Conclusion**

Our findings highlight SGK1 and CD163 as potential markers for resilience in animals infected with high-virulence PRRSV during the growing period. These markers had been previously identified by our group in PRRSV naturally infected sows exposed to low-virulence PRRSV strains and high-virulence strains in rearing pigs under field conditions, albeit only for SGK1. Finally, these results must be validated in follow-up studies involving other pig populations.

VVD – Virology and Viral Diseases

# SURVEILLANCE FOR DETECTION OF ASFV IN PIG FARM: INVASIVE VERSUS NON-INVASIVE SAMPLING

<u>S. Hong</u>, K. Cho<sup>1</sup>, H. Kang<sup>1</sup>, Y. Kim<sup>1</sup> <sup>1</sup>Foreign animal disease division, Animal and Plant Quarantine Agency, South Korea

#### **Background and Objectives**

African swine fever (ASF) remains one of the most devastating swine viral disease and poses a severe threat to the global pig industry. Alternative methods to reduce the difficulty of sampling and cost from sick pigs have been discussed. Better insights into non-invasive, field-friendly sampling of ASF by surveillance could help control virus detection. However, field-friendly sampling methods for detection of African swine fever virus (ASFV) from South Korea have not been established. Therefore, we investigated and explored alternative sampling methods for detection of ASFV.

# **Material and Methods**

In this study, we analyzed data from positive samples collected from 20 domestic pigs challenged with ASFV isolated from South Korea under different routes fo infection. The samples including environmental samples (rope, floor and feed bin swab) were applied for ASFV using WOAH TaqMan quantitative polymerase chain reaction (qPCR) to evaluate the sensitivity and efficiency of diagnosis.

# Results

Detection of ASFV was occurred earlier in invasive samples than non-invasive samples and was confirmed continuosly until death. Furthermore, analyses revealed that at relevant sampling timepoints, PCR-positive blood samples especially with syringe collection were detected more earlier and reached higher Ct values than other samples, ensuring a higher probability of ASFV detection. In environmental samples results showed intermittently occasional detections of rope, floor and feed bin swab, with later detection than invasive samples. Moreover, nucleic acids of ASFV was not found in some matrices of environmental samples.

## **Discussion and Conclusion**

Therefore, this study showed that during ASFV surveillance, blood sampling for qPCR by syringe is essential for the accurate diagnosis of ASF and provides the highest probability of detection of the disease.

VVD – Virology and Viral Diseases

# THE RISK OF INFLUENZA VIRUS TRANSMISSION BETWEEN PIGLETS AND NURSE SOWS

F. Thorup 1, L. Erik Larsen 3, N.B. Goecke 2

<sup>1</sup>SEGES, Danish Pig Research Centre

<sup>2</sup>University of Copenhagen, Dpt. of Veterinary and Animal Sciences, Grønnegårdsvej 2, DK-1870 Frederiksberg C, Denmark

<sup>3</sup> Department of Veterinary and Animal Sciences, Grønnegårdsvej 2, DK-1870 Frederiksberg C, University of Copenhagen, Denmark

# **Background and Objectives**

One of the highest risks of transmission of viruses in the farrowing unit is mixing of animals. Transfer of piglets between litters is an essential part of optimal management of hyper-prolific sows, where collection of super numerous piglets with nurse sows is recommended. The risk of transmitting virus between different age groups and litters was investigated in this study.

#### **Material and Methods**

The full study will include data from three herds (A to C). At present, the study has been completed in one herd (A). In herd A, three farrowing sections were emptied, cleaned, and disinfected before sows were introduced one week before expected farrowing. Five pens per section were reserved for nurse sows. Movement of piglets to nurse sows were registered and nasal swabs were collected from five piglets per litter on day 10 and day 21 after farrowing. In addition, udder swaps were collected from all nurse sows entering the section. All swabs were tested for 16 different porcine bacteria and viruses by qPCR using a high-throughput qPCR platform (Biomark HD). Swine influenza A virus (SwIAV) was selected as the primary index pathogen in this preliminary report.

#### Results

In the three batches in herd A, 3/30, 6/36 and 29/30 of the litters were positive for swIAV on day 21. A total of 15 nurse sows came from sections with older piglets, and of these sows three tested positive for swIAV in udder swabs. None of these three sows nursed litters that were infected with swIAV on day 21. In the first batch, the sows were stalled in crates in five rows of six pens. All three infected litters were found side by side in one row. In the second batch, where loose lactating sows were stalled in six rows of six pens, three and two infected litters, respectively, were in neighboring pens, while one infected litter did not have infected neighbors.

# **Discussion and Conclusion**

The initial data does not indicate that nurse sows with swIAV on the udder caused swIAV infected litters due to transmission between age groups. However, there seems to be transmission of swIAV between neighboring pens.

VVD – Virology and Viral Diseases

# WHAT IS "NORMALIZATION" AND WHY SHOULD WE NORMALIZE DIAGNOSTIC PCR RESULTS?

B. Armenta-Leyva <sup>1</sup>, B. Munguia-Ramirez <sup>1</sup>, T. Cheng <sup>2</sup>, F. Ye <sup>3</sup>, A. Henao-Diaz <sup>4</sup>, K. Doolittle <sup>5</sup>, S. Zimmerman <sup>5</sup>, L. Gimenez-Lirola <sup>1</sup>, J. Zimmerman <sup>1</sup>

<sup>1</sup>Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.

<sup>2</sup>Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA

<sup>3</sup>Department of Statistics, Iowa State University, Ames, IA, USA. <sup>4</sup>PIC Latinoamérica, México.

5IDEXX Laboratories, Inc., USA

# **Background and Objectives**

"Normalization" is expressing a test result relative to a reference standard (RS) tested under the same conditions to account for run-to-run variation (sample handling, pipetting, etc.). Unique to PCR, amplification efficiency (E) is also an important source of variation because E affects the final number of PCR copies. Results reported as raw Cqs assume that E is 100%, but nothing is  $\ge$  100% efficient. In this study, we normalized PRRSV PCRs by converting Cqs to "Efficiency standardized Cqs" (ECq = E<sup>- $\Delta$ Cq}) where E = amplification efficiency and  $\Delta$ Cq = (Sample Cq - RS Cq).</sup>

# **Material and Methods**

A sample set including serum (n = 132) and individual OF (n = 130) samples (-7 to 42 DPV) from 12 pigs vaccinated with a PRRSV MLV was tested for PRRSV RNA using commercial reagents. RS were created by rehydrating and diluting (1  $\times$  10<sup>-4</sup>) a PRRSV MLV (Ingelvac) with serum or oral fluid (OF) to match the sample matrix being tested. Sample ECqs were calculated using the mean E and mean RS Cq calculated from 4 RS run on each plate.

#### Results

ECqs express target concentration relative to the RS: the larger the ECq, the more nucleic acid in the sample relative to the RS. In this study, PRRSV RT-qPCR positives were detected on DPV 3 in serum ( $\bar{x}$  ECq 0.90) and OF ( $\bar{x}$  ECq 1.07), i.e., the concentration of virus was about the same as the RS. Thereafter, virus concentration peaked on DPV 7 in serum ( $\bar{x}$  ECq 2.24) and on DPV 9 in OF ( $\bar{x}$  ECq 2.00). Since all results have a numeric value, including "indeterminate" results, diagnostic sensitivity and specificity and cutoffs can be estimated for ECqs. For serum, diagnostic sensitivity and specificity was estimated as 97.9% and 100% at an ECq cutoff of  $\geq$  0.20 and, for OF, 82.6% and 100%, respectively, at ECq  $\geq$  0.45.

#### **Discussion and Conclusion**

The benefits of normalizing PCRs are: 1) labs using the same RS have comparable results (Cqs are not comparable); 2) results are easily interpreted: the larger the ECq, the more nucleic acid in the sample relative to the RS; 3) diagnostic sensitivities and specificities can be calculated for normalized PCRs.

VVD – Virology and Viral Diseases

# A MULTIPLEX IMMUNOFLUORESCENCE APPROACH TO STUDY THE PATHOGENESIS OF PORCINE RESPIRATORY CORONAVIRUS (PRCV)

<u>I. Ruedas Torres</u><sup>1</sup>, F.J. Salguero<sup>1</sup>, B. Paudyal<sup>2</sup>, E.Z. Tchilian<sup>2</sup> <sup>1</sup>United Kingdom Health Security Agency (UKHSA), Porton Down <sup>2</sup>Pirbright Institute, Woking (United Kingdom)

# **Background and Objectives**

Respiratory coronaviruses (CoV) are a major threat to global health, including the ongoing Covid-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Porcine respiratory CoV (PRCV) are globally endemic in pigs, causing substantial economic losses. PRCV infection induces interstitial pneumonia and immunological impairment. Digital pathology constitutes a basic tool for the study of the immune response against infections, facilitating the study of cellular co-localization, phenotyping pathology and interaction among different cell populations. The aim of this study was to analyse the local pulmonary immune response of pigs infected with PRCV.

#### **Material and Methods**

17 commercial breed piglets were used. 2 animals were used as negative controls (mock) and 15 were challenged intranasally with PRCV/swine/Belgium/PS-071/2020. Animals were humanely culled at 1-, 5- and 12-days post challenge (dpc) (n=5). Samples from the right lung (cranial, medial and caudal lobes) were fixed in neutral buffered formalin. Haemoatoxilin and eosin and multiplex immunofluorescence stainings (IBA-1, panCK, and anti-PCRV) (opal 4-color automation IHC kit, AKOYA biosciences) were performed. Slides were scanned (Akoya PhemoImager) and processed through digital analysis (Inform software, AKOYA biosciences).

#### Results

Histopathological lesions consisted of mild to severe broncho-interstitial pneumonia, necrosis/attenuation of the bronchiolar epithelium and lympho-plasmocytic perivascular/peribronchiolar cuffing. Animals culled at 1dpc showed minimal changes, while animals culled at 5dpc showed the highest severity of lesions. At 12dpc, mild to moderate pneumocyte type II hyperplasia was observed. Viral antigen was detected in macrophages (PRCV+IBA1+) and epithelial cells (PRCV+panCK+) from the parenchyma and bronchioles, mostly at 5dpc. Clusters of IBA1+panCK+ cells were observed in the lung from these animals. Fewer number of PRCV+IBA1+ cells were observed at 1 or 12dpc.

### **Discussion and Conclusion**

Knowing the pathogenesis of PRCV infection is vital for the pig industry but also serves as a model for human coronavirus infection. Lesions induced in the lung are minimal at 1dpc, while other respiratory viruses can induce more severe pathology at the first hours after infection. However, lesions are quite severe by 5dpc and associated to the presence of the virus in airway epithelial cells and macrophages.

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# AFRICAN SWINE FEVER VIRUS B175L INHIBITS THE TYPE I INTERFERON PATHWAY BY TARGETING STING AND 2'3'-CGAMP

#### L. Ranathunga <sup>1</sup>, <u>L. Jong-Soo</u><sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

### **Background and Objectives**

The host's early defense against viral infections relies on type I interferon (IFN-I)-mediated responses. The DNA-sensing cGAS-STING signaling pathway is a central component of the IFN-mediated antiviral response and is often targeted by viruses to evade the host immune system. Our objective was to identify how African swine fever virus (ASFV) B175L disrupts IFN signaling, with a specific focus on its influence on IFN- $\beta$  production and subsequent IFN-mediated signaling responses.

# **Material and Methods**

In our analysis, we identified B175L as an IFN-I antagonist in STING-mediated IFN-β luciferase activity. We assessed the antiviral role of B175L during GFP-tagged DNA virus infections in PK15, stable PAM, PIB, and MA-104 cells, utilizing fluorescence microscopy and cytokine ELISA kits. Phosphorylation of IFN-I molecules was examined through immunoblotting. Confirmation of the STING and B175L interaction involved luciferase assays, mass spectrometry, and various binding assays in HEK293T, PK15, and PAM-stable cells. Co-localization of STING and B175L in HeLa and PK-15 cells was visualized using confocal imaging. Immunoprecipitation of STING and B175L domain structures aimed to identify the interaction interface. The SDD-AGE assay assessed STING polymerization, and in vitro binding assays utilized 2',3'-cGAMP-Biotin or Cy5 conjugates with purified STING/B175L proteins. Mutation analysis identified crucial binding residues on STING.

#### Results

B175L significantly inhibited DNA virus-induced IFN-β production and IFN-mediated signaling responses. The conserved zf-FCS motif of ASFV B175L competitively interacted with cGAMP and the cyclic dinucleotide binding domain (CBD) of STING. The interaction hinged on the crucial R238 and Y240 amino acids, inhibiting the cGAMP-STING interaction and downstream signaling, including STING polymerization and phosphorylation of TBK1 and IRF3.

# **Discussion and Conclusion**

These findings underscore the pivotal role of ASFV B175L in suppressing IFN responses, providing a novel target for potential interventions such as live-attenuated ASFV vaccines. Understanding viral mechanisms to evade the host immune system is crucial for effective antiviral strategies. The identified immune evasion mechanism, involving B175L's interference with the STING-mediated IFN signaling pathway, contributes valuable insights to viral pathogenesis and offers a promising avenue for future ASFV vaccine development efforts. [National Research Foundation (2021R1A6A1A03045495) and Ministry of Environment (NIWDC-2021-SP-02)]

VVD – Virology and Viral Diseases

# CHARACTERISATION OF LUNG CELL INFILTRATION AFTER THE INOCULATION OF PRRSV-1 STRAINS WITH DIFFERENT VIRULENCE

<u>K. Fristiková</u>, J.M. Sánchez-Carvajal <sup>1</sup>, F. Larenas-Muñoz <sup>1</sup>, M. Rodríguez-Ruiz <sup>1</sup>, F.J. Salguero <sup>2</sup>, F.J. Pallarés <sup>1</sup>, L. Carrasco <sup>1</sup>, I.M. Rodríguez-Gómez <sup>1</sup>, J. Gómez-Laguna <sup>1</sup> <sup>1</sup>Universidad de Córdoba <sup>2</sup>UKHSA

# Background and Objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) is considered one of the major diseases in the pig sector. From the last decade, new virulent strains with different clinical signs and lesions are continuously emerging. Thus, this study focuses on comparing and characterising lung lesions induced by strains of PRRSV-1 of different virulence to better understand their immunopathogenesis.

# **Material and Methods**

To perform this study piglets were intranasally inoculated with PRRSV-1 strains of different virulence: 215-06, SU1-Bel, LV, 3249 or Lena strains. The animals were divided into groups of 4 to 7 piglets depending on the strain. At 7-10 dpi the animals were euthanised and lung samples (cranial lobe) were taken for histopathological and immunohistochemical study to evaluate antigen expression of CD3, CD20, CD163 and calprotectin.

### Results

Piglets infected with the virulent strains SU1-Bel and Lena showed the highest microscopic score characterised by a severe interstitial pneumonia with areas of bronchopneumonia, more intense in the case of Lena. In addition, perivascular and peribronchiolar infiltrate was observed in animals infected by virulent strains, although also in a lesser extent in the other animals. Interstitial pneumonia was characterised by the infiltration of mainly CD3<sup>+</sup> (T cells) and calprotectin<sup>+</sup> (macrophages and neutrophils) cells in the alveolar septa, without significant differences among strains although variability was observed in CD3 counts according to the strain. The alveolar septa also presented infiltrate of CD20<sup>+</sup> cells (B cells), although in a lesser extent than previous ones, displaying a trend to a higher frequency in 3249 strain. A decreasing count in the frequency of CD163<sup>+</sup> cells was observed in agreement with the virulence of PRRSV-1 strain.

#### **Discussion and Conclusion**

Virulent PRRSV-1 strains induced a severe interstitial pneumonia, in some cases (specially for Lena strain) accompanying by a secondary broncopneumonia. Interstitial pneumonia mainly consisted of infiltrate of T cells and myeloid cells, as well as in a lesser extent B cells. However, no significant differences were observed in the frequency of T cells, B cells or myeloid cells according to the virulence of the strain or the histopathological lesions. Virulent strains induced a substantial drop of CD163<sup>+</sup> cells, possibly associated with a higher replication of these strains.

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# CHARACTERIZATION OF CASES OF SWINE INFLUENZA VIRUS IN THE STATE OF JALISCO, MEXICO

<u>J.F. Rivera-Benitez</u><sup>1</sup>, J. De La Luz-Armendáriz<sup>2</sup>, M. Macías-García<sup>3</sup>, R. Martínez-Bautista<sup>4</sup>, H. Pérez<sup>4</sup>, M.J. Martínez-Mercado<sup>4</sup>, A. Galindo-Barboza<sup>5</sup> *INIFAP* <sup>2</sup>*Facultad de Medicina Veterinaria y Zootecnia. UNAM.* 

Private Practice Veterinarian
Private Practice Veterinarian
ZOETIS, MEXICO
National Institute of Forestry, Agricultural and Livestock Research (INIFAP)

# **Background and Objectives**

In Mexico, the main challenges with swine flu are prevention and control. An important negative effect on pig production has been identified, considerably impacting the producer's economy. The lack of updated diagnostic tests adapted to the different regions prevents us from facing the disease in a more adequate way. The objective of the study was to identify pig farms positive for the swine influenza virus or antibodies against the virus in the State of Jalisco.

# Material and Methods

A convenience-directed study was conducted in swine farms. Nasal swab samples and oral fluids were collected from pigs in 13 farms with a clinical history of respiratory illness associated with swine influenza virus. Five individual serum samples were collected from the production line (3-21 weeks of age).

#### Results

4 positive farms were identified in nasal swab samples (5/33.15%), two farms in oral fluid samples (2/7.28%) by real-time RT-PCR. Sequencing allowed the identification of two main subtypes, H3N1 and H3N2. In all cases, the sequences obtained from HA correspond to subtypes of porcine origin, identified in North America or the USA; for NA, four sequences obtained are of porcine origin and two from viruses identified as human influenza; the samples correspond to Mexican strains or from USA. In relation to the detection of antibodies, positivity was identified in all the analyzed farms, however, there were negative individuals or individuals with low titers.

# **Discussion and Conclusion**

The circulation of the different subtypes associated with H3N1 reflect the need to carry out exhaustive studies regarding their pathological characteristics and immune response. The emergence of understudied subtypes has not been an isolated event in national pig farming. In 2018, Saavedra et al. identified the presence of subtypes H1N2 and H5N2, the latter of avian origin, in lung samples from pigs with clinical disease. The identification of influenza genetic segments of diverse origins is of high importance because rearrangements can lead to strains with high pandemic potential in the swine or human population (Shaikh et al., 2021). Financing FONSEC SADER-CONACYT 2017-06-292826 and INIFAP. SIGI No. 7285536076.

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# CO-INFECTED MYCOPLASMA SUIS AND PORCINE CIRCOVIRUS TYPE 3 CAUSING REPRODUCTIVE FAILURE IN A LARGE FARM

T.K.T. Nguyen <sup>1</sup>, T.N.T. Ngo <sup>1</sup>, T.K.D. Le <sup>1</sup>, M. Kaewthamasorn <sup>2</sup>, M.N. Nguyen <sup>3</sup>, R. Thanawongnuwech <sup>2</sup>, <u>T.D. Do</u> <sup>1</sup> <sup>1</sup>Faculty of Animal Science and Veterinary Medicine, Nong Lam University, HCMC, Vietnam <sup>2</sup>Faculty of Veterinary Science, Chulalongkorn University, Thailand <sup>3</sup>Center for Genetics and Reproductive Health (CGRH), School of Medicine, National University HCMC, Vietnam

# **Background and Objectives**

Porcine Circovirus Type 3 (PCV3) and Mycoplasma suis (M. suis) pose significant challenges to swine reproductive health. The primary objective was to identify the incidence of PCV3, M. suis and other common pathogens; understand the clinical and pathological characteristics and pathogenicity of the pathogens on swine reproductive performance.

# **Material and Methods**

The study involved an intensive examination of 6750 sows in a one-site production system, with a focus on 85 sows and their offspring exhibiting reproductive failures, with the condition lasting more than 1 year. Clinical and pathological manifestations were monitored, and samples, including serum, colostrum, vulva, oropharyngeal fluid, and blood and visceral tissue from piglets, were collected. PCR/RT-PCR and Realtime-PCR techniques were employed for the detection of PCV3, M. suis and other pathogens. Whole-genome sequencing was conducted for PCV3, and the partial 16S rRNA gene was analyzed for M. suis.

# Results

The study revealed a high prevalence of PCV3 and M. suis in both sows and their offspring, with co-infection occurring prominently in neonatal weak-born piglets. Notably, other major swine pathogens were absent or detected at low rates. Detection rates of PCV3 in piglets decreased with age, while M. suis persisted at high rates from 1 to 4 weeks of ages. Viral load (Ct-value Realtime PCR) was highest in neonatal weak-born piglets (30.32±7.5), then in whole blood in sows (35.57±1.88), stillborn (34.42±7.92), mummified (35.3±0), colostrum (34.91±2.55), oropharyngeal fluid (35.67±1.57), and vulva fluid (30.9±5.47). The clinical and pathological findings of the cases supported evidence of the pathogenic role of PCV3 and M. suis. PCV3b was identified as the prevalent genotype and the partial 16S rRNA gen of M. suis showed high identity with isolates from China and Germany at 99.8% (KF740480, FN391018), Brazil at 97.39% (MK287839), Japan and USA at 96.94% (AB610847, CP002525).

#### **Discussion and Conclusion**

The findings underscore the unique co-infection of PCV3 and M. suis in a reproductive disorder setting, highlighting their potential combined impact on swine reproductive health. The absence of other pathogens suggests a distinctive role for PCV3 and M. suis in the observed reproductive failures. The study prompts the need for further research to fully elucidate the pathogenic roles of these agents.

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# DETECTION AND ISOLATION OF ROTAVIRUS A IN FAECAL SAMPLES OF DIARRHOEA OUTBREAKS ON SPANISH SWINE FARMS

<u>H. Puente</u><sup>1</sup>, J.A. Tezza<sup>1</sup>, L. Pérez-Pérez<sup>1</sup>, S. Gómez-Martínez<sup>1</sup>, H. Argüello<sup>1</sup>, A. Carvajal<sup>1</sup> <sup>1</sup>Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, León, Spain

#### **Background and Objectives**

Rotaviruses (RVs) are one of the main aetiological agents of enteric diseases in humans and animals worldwide. RVA, RVB, RVC and RVH have been identified in pigs being associated with diarrhoeas. Their environmental resistance favours an endemic presence on pig farms and makes their elimination difficult, having to focus efforts on control measures that reduce infection pressure and enhance the immune response. The main objective of this work was to investigate the relevance of RVA as aetiological agent of diarrhoea outbreaks in pigs of different ages and to develop and evaluate a cell culture isolation protocol for RVA in pig faecal samples.

# **Material and Methods**

The study was performed in 15 Spanish swine farms with diarrhoea outbreaks during suckling (5), postweaning (2) and fattening periods (8). Total RNA was extracted from a pooled sample per farm and RT-qPCRs with the primers and probes targeting the VP6 gene of RVA described by Masuda et al. (2016) was used for detection. An attempt to isolate RVA in MA-104 cells was carried out in all positive samples (Wang et al., 2018) and adaptation to in vitro conditions was monitored during the first five passages using RT-qPCR.

#### Results

RVA was detected in 33% of the investigated outbreaks being this prevalence higher in the nursing and postweaning stages (60 and 50%, respectively) compared to the fattening period (12.5%). The success rate of the isolation method reached 60%, being closely associated with the viral load in the faecal sample.

# **Discussion and Conclusion**

RVA infections are prevalent on pig farms with diarrhoea outbreaks in Spain, particularly in nursing and post-weaning stages and RT-qPCR is a very useful tool for its detection providing a quantitative measure of the viral load present in faeces and, consequently, an indication of the degree of intestinal replication. The inoculation of MA-104 cells allows the isolation of RVA present in stool samples, particularly when the viral load is high. This result could be of interest for the development of control strategies such as autovaccines, as well as for studies to characterize the pathogenicity of different isolates of RVA in the porcine host.

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# DETECTION OF PORCINE CIRCOVIRUS TYPE 3 IN NURSERY AND FINISHER PIGS IN TAIWAN

H. Kuo<sup>1</sup>, Y. Chen<sup>1</sup>, T. Liang<sup>1</sup>, C. Wang<sup>1</sup>, C. Wu<sup>1</sup>, <u>S. Huang<sup>1</sup></u> <sup>1</sup>Department of Veterinary Medicine, National Chiayi University, Chiayi, Taiwan

#### **Background and Objectives**

To date, four types of porcine circoviruses (PCVs) have been recognized. Among them, PCV2 is the best well-known pathogen that causes significant economic losses in the swine industry. As PCV2, newly emerging PCV3 may be involved in a broad range of diseases and has been detected in sows, mummified fetuses, stillborn piglets, and even in invertebrates. Because there are only few researches on PCV3 in Taiwan, the main purpose of this study, therefore, is to investigate the prevalence of PCV3 in nursery and finisher pigs in Taiwan.

### **Material and Methods**

A total of 52 oral fluids and 1245 fecal samples were collected from pig farms in Taiwan. 152 clinical tissue samples were submitted to the Yunlin-Chiayi-Tainan Animal Disease Diagnostic Center in Chiayi City, Taiwan, from January 2022 to January 2023. PCV3 was detected using quantitative polymerase chain reaction (qPCR) targeting Cap gene. Full-length Cap genes of PCV3-positive samples (Ct values under 25) were amplified for sequencing and analysis. Categorization of all PCV3 strains was based on two amino-acid mutations (A24V and R27K) in the Cap protein.

#### Results

The results of PCV3 detection of the farm samples from different regions showed that 17.3% (9/52) of the oral fluid samples, 10.5% (131/1245) of the fecal samples, and 32.9% (50/152) of the tissue samples were positive. All the PCV3 isolates were identified as PCV3c strain. Co-infection with either PRRSV or PCV2 was noted in 43 tissue samples and 2 tissue samples, respectively.

# **Discussion and Conclusion**

The highest PCV3-positivity rate was found in the tissue samples and the co-infection of PCV3 with other pathogens was quite common. However, the pathogenesis of PCV3 is still unclear and we will do further investigation using molecular biology tools to determine the pathology of PCV3.

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# EFFICACY OF ATTENUATED PORCINE EPIDEMIC DIARRHEA G2B LIVE VACCINE : INTRAMUSCULAR AND ORAL ADMINISTRATION ROUTES COMPARISON STUDY

M.A. Jeong <sup>1</sup>, B. Seo <sup>1</sup>, S. Lee <sup>1</sup>, G. Park <sup>1</sup>, C. Kim <sup>1</sup> <sup>1</sup>Woogene B&G Co., LTD

## **Background and Objectives**

Porcine epidemic diarrhea virus (PEDV) is a significant cause of enteric disease, characterized by symptoms such as watery diarrhea, vomiting, dehydration, and mortality in nursing piglets. Recently prevalent G2b strains of PEDV differ from commercial vaccines (G1), being efforts to develop G2b-based PEDV vaccines, considering the antigen type, vaccine type (live or killed), or the route of administration. The live attenuated PED vaccine (IMMUNIS® PED-M Live, WOOGENE B&G, South Korea) has confirmed high safety and efficacy, utilizing the safe attenuated PEDV G2b (HSGP-strain) antigen. In this study, we evaluated efficacy through different administration routes [intramuscular (IM) or oral (PO)].

# **Material and Methods**

Twenty-one, pregnant sows were vaccinated IM or PO with attenuated PEDV live vaccines at three different antigen concentrations (-high: 6.5, -medium: 5.5, -low: 4.5 log<sub>10</sub>, TCID<sub>50</sub>/dose, respectively). Serum and colostrum were collected at designated time points, to evaluate neutralizing abilities and maternal immunity. All neonates were orally challenged with QIA1401 strain (10<sup>30</sup> TCID<sub>50</sub>/dose). The blood was collected for viral neutralization at the challenge and necropsy. To identify vaccine effectiveness, the piglets were monitored for survival rates, clinical significance scores, and body weight until the end of the experiment.

# Results

Regardless of the doses or route of administration, piglets observed significant (p-0.05) differences in neutralizing and IgA antibodies when compared to control groups. In addition, there was a significant (p-0.05) difference in neutralizing antibodies and clinical symptom scores among doses (Medium, Low), not based on the route. The significance (p-0.05) was confirmed in survival rates, with vaccination groups over the medium dose representing upper 70 % survival.

# **Discussion and Conclusion**

This study evaluated the efficacy of an attenuated PEDV live vaccine in piglets from vaccinated sows based on serumneutralizing antibodies, clinical symptom scores, survival rates, and average weight gain ratio. Taken together, regardless of the route of administration, the vaccine (IMMUNIS® PED-M Live) has good safety and efficacy against PEDV infection.

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# EVALUATION OF DIFFERENT RT-QPCR TESTS TO DETECT ROTAVIRUS A AND ROTAVIRUS C IN FECAL SAMPLES.

M. Marcos-Cienfuegos <sup>1</sup>, M. Jiménez <sup>2</sup>, R. Menjon <sup>2</sup>, J.J. Castillo-Pérez <sup>4</sup>, J. Martinez-Lobo <sup>3</sup>, C. Prieto <sup>4</sup> <sup>1</sup>MSD AH

<sup>a</sup>MSD Animal Health <sup>3</sup>University of Lleida <sup>4</sup>Universidad Complutense de Madrid

## **Background and Objectives**

Among the nine species of Rotavirus (RV) described, RVA and RVC have been identified as enteric pathogens in swine. Quantitative RT-PCR (RT-qPCR) has been described as the most sensitive technique for RV diagnosis. The aim of this study was to compare the performance of different RT-qPCRs techniques for the detection of RVs in fecal samples.

# **Material and Methods**

A set of 61 fecal samples of known RV status, 50 of pig and 11 of human origin, were assayed using seven commercial kits and a combination of two in-house RT-qPCRs. Two commercial kits detected RV generically, three were species specific for RVA and two for RVC. Nucleic acids were extracted using a commercial kit and the RT-qPCR were set following the manufacturer's instructions. AgPath-ID<sup>™</sup> One-Step RT-PCR Reagents (Applied Biosystems<sup>™</sup>), was used for the in-house RT-qPCRs. Reactions were run in a 7500 Fast Real Time PCR System. Kappa Coefficient was calculated to determine the agreement between the techniques compared using a public access software (Working in Epidemiology, 2023).

## Results

RVA was detected by most techniques, with detection range between 94.9% and 100%. Notably, all human samples were positive by all techniques. In the case of RVC, only the two specific kits detected all RVC-positive samples. The in-house RT-qPCR detected 83.3% and the two generic kits 72.2% and 44.4%, respectively. Finally, all negative control samples were negative with all systems, except for one, which had a Ct-value of 36.2 with one of the kits specific for RVA. The best agreement was observed between the two kits specific for RVC (100%, kappa coefficient = 1), followed by the agreement observed between two kits specific for RVA (97.9%, kappa coefficient = 0.951). On the contrary, the lowest agreement was obtained between one of the generic kits and the kits specific for RVA and RVC (79.2%, kappa coefficient = 0.362).

#### **Discussion and Conclusion**

Under the conditions of this study, the detection capacity of RVA and RVC in fecal samples differed between the different RT-qPCR detection systems available. In general, the species-specific systems were the most accurate. However, for the detection of RVC, generic kits had a poor sensitivity.

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# EVALUATION OF THE INFECTIVITY OF THREE WILD-TYPE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) VARIANTS

<u>M. Melini</u>, M. Kikuti<sup>4</sup>, M. Torremorell<sup>2</sup>, K. Vanderwaal<sup>4</sup>, S. Rossow<sup>3</sup>, J. Torrison<sup>3</sup>, C. Corzo<sup>4</sup> <sup>7</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN,

USA

<sup>2</sup>College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

<sup>3</sup>Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA

<sup>4</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

# **Background and Objectives**

The United States of America swine industry has seen the emergence of several porcine reproductive and respiratory syndrome virus (PRRSV) variants that have been described as more virulent. In fall 2020, a newly emerged variant classified as L1C 1-4-4 rapidly disseminated and was reported to result in an increased wean to market mortality. The objectives of this study were to compare the infectivity of three PRRSV variants (i.e., L9 1-4-2, L1A 1-7-4, and L1C 1-4-4) using an estimation of the median infectious dose (ID<sub>50</sub>), characterize the nasal and rectal shedding patterns, and describe the generated histologic lesions to understand why this variant was rapidly disseminating.

# **Material and Methods**

Objectives were met by obtaining five (e.g., 10°, 10', 10², 10³, 104 TCID<sub>50</sub>/ml) 10-fold dilutions from an inoculum of each variant and intranasally challenging six individually housed pigs per group. Blood, rectal, and nasal swabs samples were collected throughout different time points. Additionally, two animals per group were selected for euthanasia and tissue sample collection at 11 days post-inoculation (DPI). PRRSV detection was assessed through RT-PCR and tissue lesions were blindly assessed by a pathologist. ID<sub>50</sub> was calculated from titer results from each dilution dose and the serum RT-PCR positive pigs at 4 DPI using a probit general linear model.

### Results

The ID<sub>50</sub> estimates for L9 1-4-2, L1A 1-7-4, and L1C 1-4-4 variants were  $10^{26}$  TCID<sub>50</sub>/ml (95% CI  $10^{2.0}$ ,  $10^{3.2}$ ),  $10^{2.3}$  TCID<sub>50</sub>/ml (95% CI  $10^{1.6}$ ,  $10^{3.0}$ ), and  $10^{1.3}$  TCID<sub>50</sub>/ml (95% CI  $10^{0.4}$ ,  $10^{2.2}$ ), respectively. Histopathology lesions were more frequently observed in the lung, heart, and brain, and some in different lymphoid tissues in the L1C group. Similar shedding patterns were seen among variants.

# **Discussion and Conclusion**

Our results suggest that the 2020 L1C 1-4-4 variant needs less virus to infect 50% of the challenged pigs and have more severe lesions during histopathology assessment than other studied variants. These findings partially explain why this variant disseminated rapidly and caused important mortality rates. Given the level of infectiousness of these new variants, biosecurity protocols need to be constantly assessed to ensure effectiveness and compliance.

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# FEATURES OF CASES IN WHICH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VACCINE-LIKE STRAINS DETECTED IN ENGLAND BETWEEN 2018 AND 2023.

L. Pittalis <sup>1</sup>, J. Frossard <sup>1</sup>, E. Fullick <sup>1</sup>, S. Clive <sup>1</sup>, T. Floyd <sup>1</sup>, S. Williamson <sup>1</sup> <sup>1</sup>Animal and Plant Health Agency, England, UK

### **Background and Objectives**

Four live-attenuated vaccines are licensed in the United Kingdom against porcine reproductive and respiratory syndrome (PRRS). This study describes analysis of vaccine-like PRRSV PCR-positive submissions and strains together with background surveillance data from sampled pigs.

### **Material and Methods**

ORF5 gene sequences of PRRSV PCR-positive submissions between April 2018 and August 2023 were analysed to assess if they were vaccine-like (>98.5% homology to one of the vaccine viruses). Vaccine-like strains were further analysed by partial nsp2 sequencing to identify possible recombinants. Lung histopathology and PRRSV immunohistochemistry were undertaken. Sequence, pathology and surveillance data were combined for analysis.

# Results

PRRSV PCR-positive samples were sequenced and 24% of 578 were vaccine-like. Data was available for 106 vaccine-like detections in diseased pigs. Eighty-eight% were from pigs known be vaccinated with the same vaccine as detected; 5.8% had no vaccination information and 1.9% were detected in unvaccinated pigs. Vaccine-like detections from a few days to 17 weeks of age were linked to vaccine A in 45%, B 31%, C 16% and D 8%. Most detections (54%) were in pigs aged 5-8 weeks. Where vaccine timing was known, 56% of detections were within 4 weeks, 36% between 5 and 8 weeks and 7% over 8 weeks after vaccination, with differences between vaccines. Lung histopathology consistent with PRRS was present in 13-38% of the cases, varying between vaccines and these lesions were present over four weeks after vaccination in 15% of cases. Immunohistochemistry detected PRRSV antigen in 25-43% of lungs varying with the vaccine-like PRRSV detected.

## **Discussion and Conclusion**

Vaccine-like PRRSV classified based on ORF5 homology are not always vaccine viruses, but likely derive from them. They represented a minor but significant proportion of strains sequenced and, in this study, none represented likely recombinants. Detection of vaccine-like strains in a few unvaccinated pigs, and for extended periods after vaccination, emphasises the potential for their transmission and recombination with field strains. Finding lung pathology and PRRSV antigen in association with the presence of vaccine-like strains indicates the value of full diagnostic investigation to confirm the involvement of PRRS in clinical disease, and the possibility that more than one PRRSV strain may be infecting pigs.

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# HOW MANY PRRS OUTBREAKS ARE THERE IN MY REGION? AN EARLY WARNING TOOL FOR DISEASE OCCURRENCE IN THE U.S. BREEDING HERDS

X. Yue 1, M. Kikuti 2, M. Melini 1, C. Corzo 2 1 University of Minnesota 2 Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

## **Background and Objectives**

Understanding the regional disease risk is critical for disease prevention and control in swine herds, thus tools to understand real-time risk are needed. Since 2011, the Morrison Swine Health Monitoring Project (MSHMP) has built capacity to create such tools through strong collaborations of practitioners and producers as they communicate health events (e.g., PRRS breaks) at the U.S. national level. Using MSHMP data, we developed "The Early Reginal Occurrence Warning Tool (TEROW)" to generate an automated near-real-time report to alert the current regional PRRS occurrences of participating herds.

#### Material and Methods

MSHMP-participating production systems were invited to participate in this study. Upon voluntary weekly reporting, an algorithm was developed to calculate the number of PRRS-positive sites near each enrolled herd. To balance the epidemiological relevance and confidentiality, an individual reporting radius for each herd was determined in 3 steps. First, initial radius was set, 25 miles for herds in pig-dense states and 50 miles for herds in other states. Secondly, four variables were generated to count the herds within the initial radius: A) Number of herds that are PRRS positive unstable (AASV PRRS status 1); B) Number of positive herds from other production systems; C) Number of herds participating in this study, and D) Number of MSHMP-monitored herds. Subsequently, the reporting radius is automatically increased when there is a confidentiality concern, thus preventing participants from knowing which specific herds from other production systems are PRRS positive. The results were compiled into system-specific reports and shared individually with each participant on a weekly basis.

#### Results

Currently, 187 breeding herds (approximately 554K sows and boars) participate in this study. TEROW has been shared with participants since May 9, 2023. Examples of how participants use TEROW are to adjust biosecurity programs, frequency of supply introduction and transportation route.

# **Discussion and Conclusion**

The early PRRS occurrence warning tool developed in this study enhances producers' ability to effectively communicate and to quickly respond to health threats to mitigate regional PRRS transmission, which ultimately can result in lower regional disease pressure. Together with MSHMP, TEROW can be particularly useful in the emergence of health threats allowing stakeholders to timely tailor prevention and control programs.

VVD – Virology and Viral Diseases

# INTRODUCTION AND EFFICACY OF A NEW COMMERCIAL PCV2D AND MHP COMPLEX VACCINE

<u>G. Park 1</u>, S. Lee 1, B. Seo 1, M.A. Jeong 1, C. Kim 1 Woogene B&G Co., LTD

### **Background and Objectives**

Porcine circovirus type 2 (PCV2) has been one of the most economically important viral pathogens in the global swine industry and is also linked to a wide variety of diseases and syndromes, which are known as porcine circovirus-associated diseases (PCVADs). Currently, most commercial PCV2 vaccines are made using the PCV2a or PCV2b genotypes as an active ingredient, however, these vaccines have limitations such as neutralizing abilities or cross-protection. In this study, we developed the PCV2d and Mycoplasma hyopneumoniae (Mhp) complex vaccine and then evaluated its effectiveness.

### **Material and Methods**

Forty-nine, 3-week-old piglets were purchased from PRRS-, PCV2-, and Mhp free farm and randomly distributed. To confirm the vaccine safety, two groups (Vaccine overdose, and Negative control) were evaluated. The protective efficacy of the vaccine was evaluated by a dual challenge of PCV2d and Mhp. 35 piglets were randomly divided into seven groups and vaccine groups were intramuscularly vaccinated. After 3 weeks post-vaccination, all groups were challenged with PCV2d, Mhp, PCV2d, and Mhp co-infection, respectively, and the negative control group remained unchallenged, The titer of challenge virus and bacteria were 10<sup>5.0</sup> TCID<sub>50</sub>/ml, and 1x10<sup>7</sup> CCU/ml, respectively. The clinical signs and body weight were measured, The blood was collected on designated days to evaluate the serum antibodies and viral-neutralizing abilities. For pathological evaluations, all pigs were humanly euthanized and collected tissues. The statistical significance was determined with a one-way or two-way analysis of variance (ANOVA)

#### Results

In the case of the vaccine safety test, clinical symptoms such as sudden death, high fever, and abnormal behavior were not observed. After the challenge, the vaccinated groups showed significant (p<0.05) increased ADWG, PCV2- and Mhp-specific antibodies, and interferon-gamma (IFN- $\gamma$ ) secreting cells at 14 days post-challenge. Under histopathological evaluations, the vaccinated groups observed significantly (p<0.05) lower levels of antigens than the positive control.

# **Discussion and Conclusion**

The PCV2d and Mhp complex vaccine (IMMUNIS-DMVAC) has been developed and observed good safety and efficacy. Taken together our complex vaccine might contribute to preventing PCVADs and growing the domestic swine industry in South Korea.

VVD – Virology and Viral Diseases

# PORCINE ROTAVIRUSES: DIVERSITY AND GENOTYPE DISTRIBUTION IN EUROPEAN COUNTRIES

L. Joudou <sup>1</sup>, F. Beilvert <sup>1</sup>, J. Eclercy <sup>1</sup>, E. Thibault <sup>2</sup>, <u>D. Sperling</u> <sup>3</sup>, A. Sigognault Flochlay <sup>1</sup> <sup>1</sup>*R&D department, FILAVIE, Sèvremoine, France* <sup>2</sup>*Autogenous vaccines, Ceva Santé Animale, Beaucouzé, France* <sup>3</sup>*Swine corporate, Ceva Santé Animale, Libourne, France* 

# **Background and Objectives**

Rotavirus (RV) infections pose a significant threat to human and animal health worldwide. Porcine rotaviruses (pRVs) are a prevalent cause of severe diarrhoea in nurseries, considerably affecting welfare of suckling piglets, and leading to substantial economic losses to the pig industry.

RVs are classified into 10 groups (A–J) based on antigenic relationships of their VP6 proteins and the most common groups that infect pigs are groups A, B and C (RVA, RVB and RVC), with a highest prevalence for RVA strains. The outer capsid proteins, VP7 and VP4, induce neutralizing antibodies and form the basis for the G and P dual typing system. This study was conducted to better understand the diversity and the distribution of porcine rotavirus genotypes in different

# European countries.

# Material and Methods

From October 2021 to October 2023, a total of 181 intestine samples were collected from 50 pig nurseries suffering from endemic neonatal diarrhoea in Spain, the United Kingdom, France, Denmark, Germany and the Netherlands. The presence of rotavirus was investigated by RT-PCR targeting VP6 gene. The VP7 and VP4 genes from 53 positive samples were sequenced.

### Results

176 samples (97.2%) were pRV RT-PCR positive, with 76.1% pRVA and 23.9% pRVC.

Twenty of the 43 pRVA isolates sequenced belonged to four genotype combinations: G4P[32] (8/43), G3P[32] (4/43), G5P[32] (4/43) and G9P[23] (4/43). Only 1 sample displayed the genotype combination of the strain included in European commercial vaccines (G5P[7]).

Four of the 10 pRVC isolates sequenced belonged to the genotype combination G6P[5].

Multiple other combinations were identified.

# **Discussion and Conclusion**

These results confirm the high diversity of genotypes combinations of RVA circulating in pig herds in Europe.

The control of rotavirus infections through vaccination suffers from the limited number of vaccines approved for swine and from the co-circulation of multiple rotavirus genotypes other than the reference vaccine strains.

The detected genotypes provide a basis for prospective rotavirus surveillance and supporting data for effective selection of genotypes for new pRVA vaccine candidates. Further investigations are needed to understand the degree of cross-protection between pRVA genotype combinations.

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# PRODUCTION OF PORCINE ALVEOLAR MACROPHAGE-DERIVED IMMORTALIZED CELL SUSCEPTIBLE TO KOREAN AFRICAN SWINE FEVER VIRUS, PAJU STRAIN

H. Gu<sup>1</sup>, E. Lee<sup>2</sup>, Y. Oh<sup>3</sup>, H.S. Cho<sup>4</sup>, <u>D. Tark<sup>2</sup></u>

1. Laboratory for Infectious Disease Prevention, Korea Zoonosis Research Institute, Jeonbuk National University, Iksan 54531, Republic of Korea.

<sup>2</sup>1. Laboratory for Infectious Disease Prevention, Korea Zoonosis Research Institute, Jeonbuk National University, Iksan 54531, Republic of Korea

<sup>3</sup>2. College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Republic of Korea

<sup>4</sup>3. College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan 54596, Republic of Korea

#### **Background and Objectives**

In Korea, as of November 2023, ASF has been continuously occurring in 38 farms and 3,365 wild boars. Since there is no available vaccine or treatment to date, it is important to block the spread through rapid diagnosis, and isolating and characterizing viruses using susceptible cells should be followed. The use of porcine alveolar macrophages (PAMs) for ASFV studies provides distinct advantages, such as host-viral interaction and mimicking conditions in vivo. However, there are several drawbacks to difficult primary culture and not maintenance. Here we report the development of a novel cell line, derived from PAMs, susceptible to ASFV infection.

#### Material and Methods

To produce the immortalized PAM cell lines, the PAMs were infected with pre-generated lentiviruses including genes of porcine telomerase reverse transcriptase (pTERT), SV40 Large T antigen (SV40LT), and HOX. The immortalized cells were produced using serial passages and selected using real-time PCR, FA, and HAD tests after infection with the Korean Paju isolate strain.

#### Results

We produced the immortalized cells, termed porcine kidney macrophage (PoKMc), which similarly expressed the macrophage markers as SLA, CD14, and CD68 on the cell surface, but CD163 was not expressed. Additionally, It was confirmed that PoKMc cells are susceptible to ASFV infection and can proliferate through qRT-PCR and immunofluorescence assay. Futhermore, a highly susceptible cell clone was obtained through cell cloning.

# **Discussion and Conclusion**

The PoKMc cells can be actively used in future ASFV research. Subsequent studies will explore the potential use of these cells for research on viral genetic mutations, the production of vaccine viruses, and their application as diagnostic cells.

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# SWINE INFLUENZA VIRUS EVOLUTION IN SPAIN FROM 2018 TO 2023

C. Casanovas <sup>1</sup>, S. Oliver <sup>1</sup>, S. Cárceles <sup>1</sup>, L. Garza <sup>1</sup>, K. Lillie-Jaschniski <sup>2</sup>, <u>D. Espigares <sup>1</sup></u> <sup>1</sup>Ceva Salud Animal, Barcelona, Spain <sup>2</sup>Ceva Santé Animale, Libourne, France

#### **Background and Objectives**

Hemagglutinin (HA) and Neuraminidase (NA) are used to classify the 3 subtypes of Swine Influenza A Virus (swIAV) that affect swine (H1N1, H1N2 and H3N2). HA type 1 (H1) can be of avian, human, or pandemic origin (H1av, H1hu and H1pdm). NA type 1 (N1) can be avian or pandemic (N1av, N1pdm). This range of different HA and NA, added to the high reassortment capacity of swIAV, generates diverse combinations of HA and NA that add diversity to the virus. The objective of this article is to review the evolution of swIAV subtypes and lineages in Spain.

# **Material and Methods**

Results from clinical respiratory cases in 457 swIAV positive farms between 2018 and June 2023 were analysed. Most samples derived from lactating and nursery piglets, using nasal swabs, but also tracheobronchial lavages, lungs, and oral fluids have been used. Analyses have been performed in Spanish laboratories capable of differentiating the type of HA and NA by PCR.

### Results

H1av variants (H1avN1 and H1avN2) were detected in around 60% of the cases in 2018, dropping to 25% in 2023. H1hu variants (H1huN1 and H1huN2) are detected between 25-34% over the years with ups and downs. Pandemic variants (H1pdmN1 and H1pdmN2) are constantly progressing, from 6% of cases in 2018 to 25% in 2023. The same for H3 variants (H3N2 and H3N1), growing from 2% in 2018 to 26% in 2023.

# **Discussion and Conclusion**

SwIAV shows a constant evolution in the Iberian Peninsula. The situation is changing year after year, with a significant increase in H1pdm and H3 subtypes in Spain in recent years. These results reflect the importance of continuous monitoring of farms over time to know the evolution of the subtypes present and thus be able to adapt vaccination programs to be more efficient.

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# CLINICAL PARAMETERS AND LESIONS AT SLAUGHTER IN SWINE INFLUENZA VIRUS (SWIAV)-VACCINATED ANIMALS

<u>G. Ramis</u><sup>1</sup>, F. Murciano<sup>2</sup>, E. Goyena<sup>3</sup>, B. González-Guijarro<sup>2</sup>, A. Cuellar-Flores<sup>2</sup>, D. Espigares<sup>4</sup>, K. Lillie-Jaschniski<sup>5</sup> <sup>1</sup>Departamento de Producción Animal. Universidad de Murcia <sup>2</sup>Dpto. Producción Animal., Facultad de Veterinaria., Universidad de Murcia, Spain <sup>3</sup>DEPARTAMENTO DE SANIDAD ANIMAL, FACULTAD DE VETERINARIA, UNIVERSIDAD DE MURCIA, SPAIN <sup>4</sup>Ceva Salud Animal, Barcelona, Spain <sup>5</sup>Ceva Santé Animale, Libourne, France

# **Background and Objectives**

SwIAV remains an underestimated disease, at least in Spain, where the virus is systematically found in swine populations. However, it is often difficult to estimate the impact on the health and production of fattening animals. The aim of this study was to determine the impact on clinical signs and lung lesions at slaughter of vaccination with RESPIPORC FLUpan H1N1 and RESPIPORC FLU3.

# Material and Methods

In a Spanish farrow-finish-farm, two batches of 300 piglets were vaccinated at beginning of fattening simultaneously with both vaccines (VAC1 and VAC2) and two batches were taken as unvaccinated controls (CON1 and CON2). In three of them (CON2, VAC1 and VAC2) cough index (CI) and sneeze index (SI) were measured every two weeks (W1 to W13) during finishing, both at rest (CI1 and SI1) and after movement (CI2 and SI2). At slaughter, an assessment of lung lesions was carried out using the CLP system including assessment of dorsocaudal pleurisy (DCP). Results

## Results

Significant differences were observed between the 3 groups studied in the Cl1 (p=0.03), Cl2(p=0.009), Sl1 (p=0.016) and Sl2 (p=0.012). Sorted by measurements, differences between groups were observed in W3 for Cl2 (p=0.004) with differences between the CON group with the two vaccinated groups and trend in Cl1 (p=0.075), and in W5 for Cl1 (p=0.064) between the CON and VAC2 groups.Regarding Madec's index for lung lesions there were significant differences (p=0.001) between the two control groups (CON1=1.83 and CON2=1.79) compared to the vaccinated groups (V1=1.01, V2=0.3). V1demonstrated reduced App-index (CON1=1.1, CON2=0.94, V1=0.66 and V2=0.92, p=0.002).Discussion & ConclusionsIn this trial

#### **Discussion and Conclusion**

In this trial, even in absence of obvious swIAV related clinical signs, an improvement in respiratory disease and lung lesions at slaughter was observed. A decrease in coughing and sneezing rates was observed in both vaccinated groups. Although V1 suffered an outbreak of pleuropneumonia linked to App, the DCP rate was lower compared to the two control groups. SwIAV efficient protection might have reduced the severity of Enzootic pneumonia and pleuropneumonia. In conclusion the vaccination against swIAV resulted in a general improvement of health indicators measured.

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# COMPARISON OF THE PROTECTION OF MATERNALLY DERIVED ANTIBODIES IN THE PIGLETS BORN TO SOWS VACCINATED WITH EITHER E2 SUBUNIT VACCINE OR ATTENUATED LIVE VACCINE OF CLASSICAL SWINE FEVER VIRUS

Y. Huang <sup>1</sup>, C. Chang <sup>2</sup>, <u>K. Tsai</u>, H. Liu <sup>1</sup>, S. Tsai <sup>1</sup>, M. Deng <sup>3</sup>, N. Lin <sup>4</sup> <sup>1</sup>Veterinary Research Institute, Ministry of Agriculture, Taiwan <sup>2</sup>School of Veterinary Medicine, National Taiwan University, Taiwan <sup>3</sup>Veterinary Research Institute, Ministry of Agriculture, Taiwan. <sup>4</sup>Animal and Plant Health Inspection Agency, Ministry of Agriculture, Taiwan

# **Background and Objectives**

Classical swine fever (CSF) is an important transboundary swine disease that causes systemic hemorrhage and high mortality in domestic pigs and wild boars. Generally, vaccination is employed for CSF control and prevention in endemic areas. However, the efficacy of CSFV vaccine is decreased by maternally derived antibodies (MDA). The best vaccinated stage of CSFV vaccine is at MDA with lower than 16 folds anti-CSFV neutralizing antibody (NA). The protection and antibody level of MDA in the piglets are correlated with vaccination programs of sows but its effectiveness against CSFV inoculation remains unclear. In this study, we compared the protection of MDA in piglets born to sows vaccinated with either E2 subunit vaccine or attenuated live vaccine (ALV) against CSFV inoculation.

# **Material and Methods**

Thirty-two piglets, including 24 piglets born to ALV-vaccinated sows and 8 piglets born to E2 subunit vaccine-vaccinated sows, were separately inoculated with high, medium, or low viral loads of CSFV TD/96 strain (genotype 2.1).

## Results

The results revealed that 8-week-old piglets born to E2 subunit vaccine-vaccinated sows exhibited higher anti-CSFV NA and better protection than piglets born to ALV-vaccinated sows. However, the high anti-CSFV of MDA did not provide complete protection in piglets against CSFV inoculation, as those with high anti-CSFV NA of MDA experienced short period of viremia after inoculation with high CSFV loads. Piglets with low anti-CSFV NA of MDA displayed severe CSF-associated clinical signs and high mortality after inoculation with low CSFV loads. MDA is passive immunity and only provides humoral immune protection, and the anti-CSFV NA of MDA gradually decreases with age.

# **Discussion and Conclusion**

In conclusion, MDA alone cannot offer sufficient protection against the inoculation of high CSFV loads. The risk of CSFV infection is still existence in the piglets with anti-CSFV NA of MDA.

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# DETECTION OF PRRSV AND SIV INFECTIONS IN DUTCH NURSERIES

<u>J. Beek</u><sup>1</sup>, T. Nunes<sup>4</sup>, A. Schuttert<sup>2</sup>, E. Van Esch<sup>3</sup>, D. Giralt<sup>4</sup>, L. Nodar<sup>4</sup>, J. Miranda<sup>4</sup> <sup>1</sup>HIPRA Benelux <sup>2</sup>De Oosthof, The Netherlands <sup>3</sup>Technical Services Manager Swine, HIPRA Benelux <sup>4</sup>HIPRA, Amer (Girona), Spain

# **Background and Objectives**

Coinfection with the Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and swine Influenza virus (SIV) is associated with enhanced clinical presentation of PRDC, a higher incidence of secondary infections and increased postweaning mortality. Infection in the early stages of production is particularly costly and concerning, leading to the objective of keeping nurseries PRRSV-free even on virus-positive farms. Oral fluids are an easy-to-use tool to check for PRRSV/SIV infection, allowing a large number of individuals to be sampled quickly. The aim of this study was to assess the incidence of these viruses in Dutch nurseries, including nurseries on organic swine farms (farms producing under the european regulation for biological farming).

# **Material and Methods**

Oral fluid samples were collected from 8-week-old nursery piglets in 110 farms (including 11 organic farms) using cotton ropes (3 ropes/farm, 12-24 pigs per rope), conditioned in sterile flasks, and sent refrigerated to the laboratory within 24 hours. PRRSV and SIV presence was assessed by RT-PCR on all samples and each farm was classified as positive or negative for the viruses. A descriptive analysis of these 110 farms was performed using R software v.4.3. and the results were presented the results as percentages and frequencies.

#### Results

In total, 40% (44/110) of the nurseries were positive for PRRSV and 45.5% (50/110) were positive for SIV. The simultaneous presence of the 2 viruses was identified on 21.8% (24/110) of the farms. The incidence was higher on conventional farms than on organic farms, both for PRRSV (42.4% versus 18.2%) and for SIV (46.5% versus 36.4%), with more cases of simultaneous circulation of both viruses on the conventional farms (23.2% versus 9.1%).

#### **Discussion and Conclusion**

The results showed a relevant presence of PRRSV and SIV in the nurseries, with a substantial number of farms having simultaneous circulation of both viruses. The real incidence might be underestimated due to the limited sampling strategy (only 1 age group). As the incidence of PRRSV was considerably lower on organic farms, studying the differences in disease control programmes between organic and conventional farms may lead to new insights into how to implement control measures against PRRSV and coinfecting pathogens.

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## EVALUATING CONTROL MEASURES FOR SENECAVIRUS A IN THE MARKETING CHAIN

A. Buckley <sup>1</sup>, <u>E. Zanella</u>, N. Humphrey <sup>2</sup>, K. Lager <sup>1</sup> <sup>1</sup>Agricultural Research Service United States Department of Agriculture <sup>2</sup>Animal and Plant Health Inspection Service United States Department of Agriculture

#### **Background and Objectives**

Senecavirus A (SVA) infection in swine can lead to the development of vesicular lesions indistinguishable from foot-andmouth disease (FMD). FMD is a reportable disease; therefore, in FMD free countries, FMD must be ruled out when a vesicular lesion is observed. In the United States, many of these investigations have occurred at sow slaughter plants. The objectives of this study were to understand the environmental burden of SVA in slaughter plant lairage and evaluate the efficacy of SVA controls measures including disinfection and an autogenous vaccine.

# **Material and Methods**

Swab samples were collected from the environment of four sow slaughter plants including flooring, waterers, and gating on a regular basis between June and December of 2020. Samples were tested for SVA nucleic acid by PCR and live virus by virus isolation (VI). An isolate was selected for autogenous vaccine production and challenge virus. Weaned pigs were split into two groups: autogenous vaccine + challenge (Group 1, n=12) and sham vaccine + challenge (Group 2, n=12). Pigs were observed daily for the development of vesicular lesions. Blood and rectal swab samples collected regularly for a serum virus neutralization (VN) assay and SVA PCR.

#### Results

Three slaughter plants had positive environmental swabs with August having the highest percentage of PCR positive samples. Floor samples were the most common positive sample type. Environmental samples collected after cleaning protocols were negative for SVA. Group 1 did not have measurable neutralizing antibody titers at the time of challenge; however, only 2/12 animals in Group 1 developed vesicular lesions, while 5/12 animals in Group 2 developed lesions. Less animals in Group 1 had PCR positive rectal swabs and serum samples compared to Group 2.

# **Discussion and Conclusion**

This study demonstrated that SVA can be readily found in the environment of slaughter plants; however, current cleaning protocols were able to eliminate the virus. Two doses of an inactivated autogenous SVA vaccine did not generate a robust neutralizing antibody response, though may have reduced clinical disease. Enforcement of strict biosecurity and regular cleaning in slaughter plant lairage as well as continued investigation into efficacious vaccine candidates could help control SVA spread in marketing channels.

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# EVALUATION OF IMPACT ON PRODUCTIVITY DATA FOLLOWING PRRSV1 ('HORSENS') INFECTION IN DANISH SOW HERDS.

M. Qvist Pawlowski <sup>1</sup>, G. Blach Nielsen <sup>1</sup>, <u>S.L. Musse</u><sup>2</sup> <sup>1</sup>MSD Animal Health <sup>2</sup>MSD Animal Health, Nordic, Denmark

## **Background and Objectives**

In July 2019, a recombinant PRRSV1 variant ('Horsens') was isolated in Denmark derived from 2 different MLV-PRRS1vaccines. The herd of origin was located near a boar station (supplying semen to Danish sow herds) that subsequently became infected. Consequently, PRRSV sperm transmission occurred, and several sow herds were infected. The aim of this case study was to evaluate the impact on productivity data following PRRSV1 ('Horsens') introduction via semen in Danish sow herds.

# **Material and Methods**

Retrospectively, productivity data before and after infection with PRRSV1 ('Horsens') were compared for four sow herds (number of sows: average 1706; range: 1000-2500) with known time of infection and the virus strain confirmed by sequencing. All four herds were PRRSV-negative before the outbreak. Immediately after the outbreak, all herds implemented the same control strategy (e.g., sow and piglet vaccination using MLV-PRRS1 vaccine (Porcilis® PRRS Vet.) and strict McRebel procedures). Farrowing rate % (FR%), number of live born piglets (LB) and pre-weaning mortality % (PM%) extracted from herd databases were compared for 6- and 12-months periods before and after PRRSV introduction. Unfortunately, only 2 herds had data concerning PM%. For each parameter, the corresponding economic consequence for the year in question was estimated based on calculations provided by Danish Agriculture & Food Council/SEGES.

## Results

When comparing 6-month periods before and after PRRSV-introduction, average change (min; max) was: -4.01 (-1.24; -6.35) for FR%; -2.01 (-2.97; -0.53) for LB; and +9.26 (8.88; 9.63) for PM%. When comparing 12-month periods, average change was -3.35 (-0.41; -7.91) for FR%; -0.17 (-0.92; 1,53) for LB; and +4.27 (2.38;6.13) for PM%. The average economic impact (EUR/sow/year) for the 12-month periods comparison was -10.30 (-1.26; -24.26) for FR%; 9.20 (-50.54; 84.05) for LB; and -40.62 (-22.70; -58.50) for PM%.

# **Discussion and Conclusion**

This study found acute and severe impact on the evaluated productivity parameters following PRRSV1 outbreak. With the chosen management strategy, the parameters returned to the level before the outbreak after 6-12 months although having caused a clear financial loss. However, large variations existed between herds both in the impact and duration of infection.

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# FIRST EVIDENCE OF PORCINE CIRCOVIRUS 4 (PCV-4) INFECTION IN EUROPE

<u>J.L. Arnal Bernal</u><sup>1</sup>, R. Holgado-Martín<sup>2</sup>, M. Sibila<sup>3</sup>, G. Franzo<sup>4</sup>, D. Martín<sup>1</sup>, D. Risco<sup>2</sup>, L. Gómez<sup>2</sup>, J. Segalés<sup>5</sup> <sup>1</sup>EXOPOL, San Mateo Gállego (Zaragoza), Spain <sup>2</sup>Universidad de Extremadura

<sup>3</sup>IRTA-CReSA

<sup>4</sup>Dept. of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy.

<sup>5</sup>Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), and Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona,

# **Background and Objectives**

Porcine Circovirus 4 (PCV-4) was initially documented in 2019 among domestic swine in China. Subsequently, its presence has been confirmed in South Korea and Thailand. Nonetheless, so far has not been described outside of Asia. Intensively reared pigs and wild boar are susceptible to infection. In Spain, swine farming predominantly adheres to intensive practices; however, the Iberian breed is raised in a semi-extensive system, fostering potential interactions with wildlife. This study aims to report the first-ever detection of PCV-4 in Europe.

# Material and Methods

A retrospective study was conducted in Spain and Italy using samples collected between 1998 and 2022 from domestic swine, including both commercial (n=1297) and Iberian breed (n=170), as well as wild boar (n=256). Wild boar samples encompassed lymph nodes, lungs and sera, while samples from pigs were from cases of specific clinical diseases, including digestive, respiratory, or reproductive disorders. Molecular detection was carried out through conventional or real-time PCR, employing various protocols. Samples yielding positive results were subsequently confirmed by Sanger sequencing.

### Results

PCV-4 was exclusively detected in Spanish samples from wild boar and Iberian pigs. Among wild boar, 33.7% of lymph nodes and 8.9% of sera samples analyzed tested positive. Additionally, 7% of digestive samples and 3.5% of lung samples from Iberian pigs yielded positive results. Notably, PCV-4 was not identified in samples from commercial breed pigs in Spain or Italy. The presence of PCV-4 was confirmed through the partial sequencing of Rep or Cap genes in selected samples.

# **Discussion and Conclusion**

This study represents the first documented occurrence of PCV-4 infection outside of Asia. Furthermore, the virus was identified within a specific epidemiological context. All positive samples originated from a limited geographic area: the south-mid-west of the Iberian Peninsula. In this region, Iberian pigs and wild boar share a common habitat, wherein wild boar may act as reservoirs, and Iberian pigs may be sporadically infected. The findings indicate a localized spread of the virus with no apparent impact on commercial farms. While the pathogenic potential of PCV-4 remains unestablished, its detection underlines the importance of maintaining rigorous biosecurity measures on farms.

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# HISTORICAL EVOLUTION OF THE PREVALENCE OF SWINE INFLUENZA VIRUS SUBTYPES IN THE NETHERLANDS

<u>B. Cornelis</u><sup>1</sup>, P. De Backer <sup>2</sup>, J. Schumans <sup>3</sup>, S. Van Colen <sup>3</sup> <sup>1</sup>DVM Ceva Santé Animale <sup>2</sup>Ceva Santé Animale Benelux <sup>3</sup>Ceva Santé Animale. Naaldwiik. the Netherlands

## **Background and Objectives**

Swine influenza A virus (swIAV) is a relevant pathogen, causing respiratory disease and reproductive losses. Furthermore, swIAV is on the agenda as a potential zoonotic disease. By facilitating diagnostic services, data regarding prevalence of subtypes of swIAV become available. An overview of the prevalence of different swIAV subtypes isolated on Dutch farms between January 2018 and September 2023 is presented in this report.

# **Material and Methods**

Nasal swabs, lungs, oral -fluids, BALF or udder wipes were collected by veterinarians in Dutch pig herds suspected of swIAV infection. Samples were investigated by PCR (VetMAX-Gold In-fluenza SWIAV-PCR swine) for presence of swIAV. Subtyping was performed by PCR (swIAV Influenza Subtyping Multiplex-PCR) on samples with a Ct-value £ 30. In the period January 2018 to September 2023, 437 swIAV strains were subtyped.

#### Results

From January 2018 to September 2023, the classical subtype H1avN1 was the most prevalent (41.0%), followed by the pandemic subtype H1pdmN2 (29.1%) and further followed by the subtypes H1huN2 (16.0%), H1avN2 (6.9%), H1pdmN1pdm (3.9%), H1huN1 (1.8%) and H3N2 (1.2%). The pandemic subtypes H1pdmN2 and H1pdmN1pdm account together for 33.0%. Comparing subtypes found in the period 2018-2019 (n=58), 2020 (n=116), 2021 (n=108), 2022 (n=120) and Jan.-Sept. 2023 (n=35), in these respective periods, the H1avN1 subtype accounted for 43.1%, 46.6%, 38.0%, 37.5% and 40.0%, H1pdmN2: 19.0%, 32.8%, 26.9%, 32.5% and 28.6%; H1huN2: 25.9%, 12.1%, 20.4%, 10.8%, 17.1%; H1avN2: 8.6%, 5.2%, 2.8%, 10.8%, 8.6%; H1pdmN1pdm: 0.0%, 0.9%, 6.5%, 5.8%, 5.7%; H1huN1 was only found in 2020, 2021 and 2022, in 2.6%, 1.9% and 2.5% of the samples respectively; the H3N2 subtype only in 2018-2019 and 2021, in 3.4% and 3.7% of the cases.

### **Discussion and Conclusion**

The classical swIAV subtype H1avN1 is still the most prevalent in the Dutch swine population, although its proportion has reduced over the years. The pandemic subtype H1pdmN2 is the second most prevalent and together with the other pandemic subtype H1pdmN1pdm, they account for 1 out of the 3 subtypes found in Dutch farms.

#### VVD – Virology and Viral Diseases

# MUSCULAR LESIONS IN PIGLETS WITH PCV3-ASSOCIATED DISEASE

F.A. Molossi <sup>1</sup>, I.R. Dos Santos <sup>2</sup>, B.A. De Almeida <sup>2</sup>, L.T. De Castro <sup>2</sup>, C. Pissetii <sup>3</sup>, L. Brandalise <sup>4</sup>, <u>G.M.R. Simão <sup>5</sup></u>, F. Vanucci <sup>6</sup>, D. Driemeier <sup>2</sup> <sup>1</sup>Universidade do Oeste de Santa Catarina (UNOESC) <sup>2</sup>Universidade Federal do Rio grande do Sul <sup>3</sup>CEDISA - Centro de Diagnóstico de Sanidade Animal, Concórdia, Santa Catarina, Brazil <sup>4</sup>Agroceres PIC, Rio Claro, SP, Brazil <sup>5</sup>Agroceres PIC <sup>6</sup>University of Minnesota

# **Background and Objectives**

PCV3 is linked to multiple clinical diseases. However, the clinical manifestation of weakness and throwback ears requires further clarification. We aim to describe systemic lesions of skeletal and cardiac muscles in PCV3-associated disease in piglets, investigating their potential relationship with weakness and throwback ears.

# Material and Methods

Forty-four piglets were split into two groups based on the presence of caudally rotated ears: "Throwback ears" – TE and control group - CG). The piglets ranged in age from late gestation abortion, stillbirths, to those up to 1-day-old. CG were sourced from the same farms/origin as the "sick" piglets. Clinical signs and the macroscopic evaluations were recorded. Fragments of the main organs and several muscles, including those around the ears, were systematic collected for histological evaluation. Tissues were submitted to PCR analysis for PCV3, PCV2, and PPV. In each muscle across all cases, including the heart, inflammatory cell counts were semi-quantitatively scored in 2.37mm<sup>2</sup> (affected perivascular and parenchymal areas).

# Results

Late gestation abortions and throwback ears were observed. Weakness was also noted in surviving piglets up to 1-dayold. All TE group piglets displayed caudally rotated ears and 12/22 showed non-collapsing lungs. Microscopically, TE group piglets exhibited a multissystemic lymphohistiocytic inflammatory infiltrate disrupting and surrounding vessel walls in most tissues. Interstitial pneumonia, gliosis, myocarditis and myositis were frequently observed, often with intralesional fibrosis. Piglets from CG did not exhibit microscopic lesions. Both groups tested positive in PCV3 qPCR; however, TE group piglets displayed microscopic lesions and significantly lower Ct compared to the CG. Furthermore, the inflammatory infiltrates in lesioned perivascular and parenchymal areas of skeletal and cardiac muscle were significantly higher in the TE group than in the CG.

# **Discussion and Conclusion**

The absence of clinical signs and lesions may be attributed to the low viral load in the analyzed piglets. PCV3-associated disease induces muscular lesions and we hypothesized that the clinical manifestation of throwback ears and weakness in the evaluated piglets could be linked to the presence and severity of muscular lesions associated with PCV3 infection. However, further studies are necessary to provide clearer insights into this matter.
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# NASAL MICROBIAL DIVERSITY AS A PREDICTOR OF SURVIVAL IN PIGLETS INFECTED BY A HIGHLY VIRULENT PRRSV-1 STRAIN

P. Obregon-Gutierrez <sup>1</sup>, M. Cortey <sup>2</sup>, G. Martin-Valls <sup>2</sup>, H. Clilverd <sup>2</sup>, F. Correa-Fiz <sup>1</sup>, V. Aragón <sup>1</sup>, E. Mateu <sup>2</sup>

<sup>1</sup>Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Spain.

<sup>2</sup>Department de Sanitat i Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain

#### **Background and Objectives**

The recent introduction of a highly virulent PRRSV-1 strain in Spain is causing a huge impact on mortality and productive parameters in the affected farms. Several studies have tackled the impact of PRSSV infection on gastrointestinal microbiota, while the role of the nasal microbiota has been barely assessed. Here, we aimed to gain insight on the role of the diversity and composition of nasal microbiota in relation with the survival and mortality in piglets during an outbreak caused by the highly virulent PRRSV-1 strain.

# Material and Methods

A cohort of piglets was followed up from birth to 12 weeks off age during an outbreak of highly virulent PRRSV-1. Detection of PRRSV genome was done weekly from nasal swabs. At the end of the follow-up period, the nasal microbiota at three weeks of age (weaning) in animals that died was compared to that of survivors. Available nasal swabs from age-matched piglets taken before the outbreak were also analyzed. The microbiota composition was inferred by 16S rRNA gene sequencing and the bioinformatic analysis was done with Qiime2. Detection of PRRSV genome was done by means of a commercial RT-qPCR.

#### Results

Significant differences in microbiota diversity, composition and functionality were detected when comparing animals that survived versus animals that died. The surviving piglets showed richer and differentiated nasal microbial communities compared to piglets that died, both in qualitative and quantitative beta diversity analyses. Accordingly, different metabolic capabilities were inferred from the microbiota of both groups. Interestingly, these differences were also related with the litter since there were litters where either all animals died or survived. Also, we were able to associate a higher representation of Bergeyella, Neisseria, Glaesserella, Moraxella strains among others with survival after PRRSV infection; while increased abundances of Escherichia and other Moraxella strains were associated with a fatal outcome. Moreover, similar tendencies were detected in the nasal microbiota of piglets before the PRSSV-1 outbreak.

## **Discussion and Conclusion**

Altogether, these results suggest that nasal microbiota composition may correlate with the outcome of highly virulent PRSSV-1 infections, emphasizing the need of a better understanding on the role of the nasal microbiome in PRRSV infection.

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# AFRICAN SWINE FEVER VIRUS B354L TARGETS CYCLIC GMP-AMP TO SUPPRESS CGAS-STING SIGNALING PATHWAY

# S. Abesinghe 1, L. Jong-Soo 1

<sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

#### **Background and Objectives**

African Swine Fever Virus (ASFV) is a highly contagious DNA arbovirus, that causes African Swine Fever (ASF) in domestic pigs and wild boars with 100% mortality. Many of the ASFV-encoded proteins have not been studied experimentally. In contrast, some proteins have been characterized for their diversified strategies to hijack host antiviral innate immune responses. ASFV B354L is one such protein with A32 ATPase homology. However, its immune suppression mechanism remained undetermined. Our objective of this study was to disclose the host immune suppression mechanism of ASFV B354L.

# Material and Methods

To assess the effect of ASFV B354L on DNA virus replication, we checked fluorescence absorbances, virus titer, and levels of secreted cytokines with three GFP-tagged DNA viruses (ADV, VACV, and HSV). To specifically determine the target for ASFV B354L, IFN- $\beta$  luciferase assay was performed, and in vitro binding assays were used to confirm the interaction. Intracellular and extracellular cGAMP levels suppression was evaluated by 2',3'-cGAMP ELISA and HPLC with purified ASFV B354L proteins. To assess ASFV B354L ATPase activity, an ATPase assay was performed with synthetic ATP as the substrate. Sodium vanadate was used as the ATPase inhibitor while checking the interaction with 2',3'-cGAMP.

#### Results

Here, we have reported that ASFV B354L inhibits interferon (IFN)-mediated immune responses by its unique interaction with 2',3'-cGAMP and utilizes ATPase activity to cleave 2',3'-cGAMP and disrupt its structural integrity. Importantly, ASFV B354L encodes functional ATPase motifs, which are largely conserved in the nucleocytoplasmic large DNA viruses (NCLDV) family A32 ATPases. Unlike the wildtype B354L, the enzymatic activity mutants failed to cleave and downregulate 2',3'-cGAMP levels and subsequent enhancement of virus replication. Similarly, three other viral A32 ATPase proteins (Vaccinia virus A32L, Molluscum contagiosam MC140L, and fowlpox virus FPV197) also showed impaired 2',3'-cGAMP levels with enhanced ATPase activity upon DNA virus infection.

#### **Discussion and Conclusion**

Collectively, these results revealed a specific molecular mechanism used by ASFV B354L to inhibit the IFN responses and provide a theoretical basis for the development of protective vaccines against ASFV. [National Research Foundation (2021R1A6A1A03045495) and Ministry of Environment (NIWDC-2021-SP-02)]

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# AFRICAN SWINE FEVER VIRUS DP71L INHIBITS TYPE I INTERFERON SIGNALING BY TARGETING THE STING-TBK INTERACTION

#### L. Ranathunga 1, L. Jong-Soo 1

<sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

#### **Background and Objectives**

African swine fever virus (ASFV) is a lethal virus that infects pigs. This large double-stranded DNA virus encodes 150 to 167 proteins, the functions of most of which remain unknown. Hence, the primary aim of this study was to elucidate the role of the ASFV DP71L protein in antiviral immunity, specifically focusing on its impact on type I interferon (IFN-I)-mediated responses. These responses play a critical role in the early defense against viral infections, and the cGAS-STING signaling pathway, often targeted by DNA viruses for immune evasion, is a key player in this process.

# **Material and Methods**

In this study, DP71L was identified as an IFN-I antagonist in STING-mediated IFN-β luciferase activity. We explored the antiviral role of DP71L in PK15, stable PAM, PIB, and MA-104 cells during GFP-tagged DNA virus infections, employing fluorescence microscopy and cytokine ELISA kits. The phosphorylation status of IFN-I molecules was evaluated through immunoblotting. Confirmation of the STING and DP71L interaction encompassed luciferase assays, mass spectrometry, and various binding assays in HEK293T, PK15, and PAM-stable cells. Confocal imaging enabled the visualization of co-localization and STING/TBK1 puncta formation in HeLa and PK-15 cells. Immunoprecipitation of STING and DP71L domain structures was conducted to pinpoint the interaction interface.

#### Results

Results revealed that DP71L specifically impeded the production of IFN-I induced by DNA viruses and the subsequent downstream signaling. The conserved protein phosphatase 1 (PP1) motif of DP71L competed with the C-terminal tail (CTT) of STING for interaction. This interaction was contingent on specific amino acids, namely P371Q, L374, and R375. Consequently, this interaction disrupted the binding of STING and TBK1, thereby inhibiting downstream antiviral signaling events involving IRF3.

## **Discussion and Conclusion**

The study underscores the importance of early antiviral defense through IFN-I-mediated responses and the cGAS-STING signaling pathway. Targeted by DNA viruses like ASFV, this pathway is crucial for immune evasion. ASFV DP71L inhibits IFN-I signaling via STING, revealing its role in suppressing IFN-I responses and providing insights for developing live-attenuated ASFV vaccines. [National Research Foundation (2021R1A6A1A03045495), Ministry for Food, Agriculture, Forestry and Fisheries (119081-5) and Ministry of Environment (NIWDC-2021-SP-02)]

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# CASE REPORT ABOUT EARLY PIGLET VACCINATION WITH RESPIPORC FLUPAN H1N1 ON THE PERFORMANCE IN NURSERY ON A SOW FARM IN GERMANY

T. Voss <sup>1</sup>, <u>K. Lillie-Jaschniski</u><sup>2</sup>, M. Köchling <sup>3</sup> <sup>1</sup>Vet-Team-Sögel, Sögel, Germany <sup>2</sup>Ceva Santé Animale, Libourne, France <sup>3</sup>Ceva Tiergesundheit GmbH, Düsseldorf, Germany

## **Background and Objectives**

Influenza A virus in swine (swIAV) plays an important role within the porcine respiratory disease complex (PRDC), and a lot of studies show the interaction between Influenza and other PRDC associated pathogens like Glaesserella parasuis (GPS) or Mesomycoplasma hypopneumoniae. Still its impact on health and performance of nursery pigs and fatteners is often underestimated. This data evaluation aims to better assess the impact of swIAV vaccination of piglets on health parameters and performance in a sow farm in Germany.

#### **Material and Methods**

In February 2023 a 500-sow farm using 3-week batch management in Germany experienced increased respiratory distress in 6- and 9-week-old piglets, with suspicion of swIAV involvement. Nasal swabs of diseased pigs were negative by swIAV-PCR. Piglets sampled at the end of nursery were slightly indicative for a recent contact to H1pdmN2 by Hemagglutination inhibition test. Treatment with Amoxicillin against Streptococcus suis involved, did not solve the situation in nursey. Sows have been vaccinated against swIAV. Maternally derived antibodies can only clinically protect piglets, but do not prevent from infection. Thus, piglets were vaccinated with Respiporc FLUpanH1N1 (twice at 1 and 4 weeks of age) respecting the cascade rules (regulation (EU) 2019/06 Art. 112-114), to be able to protect the piglets before expected timepoint of infection at around 5 weeks of age, 1 week before obvious clinical signs. Number of losses, average selling weight (ASW), average daily weight gain (ADWG) and antimicrobial use were monitored and compared in 4 groups before start of vaccination (NV) and 4 groups vaccinated (V).

#### Results

The average number of losses was reduced from 4% (NV) to 1.8% (V). The ADWG increased from 370 g/day to 440 g/day, leading to 25.5kg (NV) to 29.5kg (V) selling weight after 50 days in nursery. In vaccinated group only 5 days antimicrobial treatment was needed compared to 21 days antimicrobial treatment in non-vaccinated group before. Another observation was a reduction in frequency of ear necrosis and tail biting.

# **Discussion and Conclusion**

On this farm being monitored, early piglet vaccination against pandemic swIAV helped to improve performance parameters and health status of nursery piglets.

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# DEVELOPMENT OF REAL-TIME PCR REAGENTS AND CONTROLS TO GENETICALLY DIFFERENTIATE PIGS INFECTED WITH THE EURASIAN STRAIN OF AFRICAN SWINE FEVER VIRUS (ASFV) FROM VACCINATED PIGS.

E. Martin <sup>1</sup>, A. Blanc <sup>1</sup>, S. Moine <sup>1</sup>, I. Leyva Baca <sup>2</sup>, <u>Q. Anne <sup>3</sup></u> <sup>1</sup>Thermo Fisher Scientific, Lissieu, France <sup>2</sup>Thermo Fisher Scientific, Austin, Texas <sup>3</sup>LSI - Thermo Fisher Scientific

#### Background and Objectives

African Swine Fever (ASF) is a highly contagious and deadly viral disease affecting domestic and wild pigs of all ages. ASF is among the most dangerous diseases impairing pig production worldwide.

After decades of failed attempts due to the complexity of the virus, significant advances have been made recently in the development of vaccine candidates to protect pigs against this virus.

Vaccines against African swine fever being tested in Vietnam are showing promising results and are close to approval. It would represent a breakthrough to tackle the deadly animal disease that ravages pig farms.

With the anticipated availability of vaccines in the market, and with the aim to complete our broad range of products related to ASFV monitoring, we developed accompanying qPCR reagents to discriminate between infected and vaccinated animals (DIVA), a necessity during an ASFV vaccination campaign.

#### **Material and Methods**

VetMAX<sup>™</sup> African Swine Fever Virus I177L DIVA Reagents enable the differentiation of current ASFV circulating strains from vaccinated animals by real-time PCR on porcine.

It contains primers, probes, enzymes, and buffer optimized for qPCR identification of ASFV-Gal177I strain, in a ready-touse, premixed format. It was used on qPCR thermal cyclers compatible with FAM dye, on standard and express amplification programs.

355 ASFV negative blood and serum, 71 ASFV positive blood samples coming from vaccinated animals, and 74 ASFV positive samples coming from reference panels were evaluated.

## Results

71 pigs inoculated with one dose of ASFVG-△I177L strain were differentiated from the 429 other samples that were not vaccinated.

The qPCR characteristics of the reagents fulfils high standard validation criteria : 99% of qPCR efficiency over a 7-log dynamic range with an analytical sensitivity down to 16 copies/PCR.

This robust product remains unaffected by variations in critical qPCR parameters and provides repeatable and reproducible results with CV < 1.1%

### **Discussion and Conclusion**

This study demonstrate the potential of this ready-to-use qPCR product to be used as genetic DIVA reagents, supporting vaccination campaigns associated.

This product extends the broad range of Thermo Fisher Scientific products related to the accurate research of ASFV.

"For Laboratory Use Only. Regulatory requirements vary by country; products may not be available in your geographic area."

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# EVALUATION OF SANGER SEQUENCING LIMITATIONS ON THE DETECTION OF PRRS CO-INFECTIONS

<u>J. Alvarez-Norambuena</u><sup>1</sup>, M. Kikuti<sup>2</sup>, A. Rovira<sup>3</sup>, C. Corzo<sup>4</sup> <sup>1</sup>University of Minnesota <sup>2</sup>College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA <sup>3</sup>University of Minnesota College of Veterinary Medicine <sup>4</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

## **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) virus is known to cause great economic losses. Modified-live vaccines (MLV) have been used in the field to control and mitigate the impact of wild-type (WT) infections. The main diagnostic test for PRRS is RT-PCR together with ORF5 sequencing. Interpretation of these results can be complex when two or more variants are present in the same sample. Therefore, the aim of this study is to assess the in-vitro sequencing detection of wild-type or vaccine-like viruses co-infected samples at different concentrations.

#### **Material and Methods**

A wild-type PRRS variant 1-7-4 L1A and a vaccine virus 2-5-2 L5 (Ingelvac PRRS MLV Boehringer Ingelheim) were conveniently selected for this study. The 1-7-4L1A variant was grown in MARC-145 cells to a 10<sup>6.5</sup> titer. Then, 500 µL of each virus was subjected to quantitative PCR (qPCR) and subsequently diluted to obtain three different concentrations of RNA copies per mL (10<sup>6</sup>, 10<sup>5</sup>, 10<sup>4</sup>). Samples comprised a mix of all possible concentration combinations of WT and MLV to create 9 different groups with 3 replicates each. Samples were then RT-PCR tested and ORF5 sequenced.

#### Results

An MLV-like sequence was most frequently obtained when the vaccine had a concentration equal or higher to the wildtype virus (in 5 out of 9 groups, 55.6%). A WT sequence was most frequently obtained in 1 out of 9 (11.1%) groups, when concentration of wild-type was 2 logs higher than the concentration of vaccine. In vaccine at 10<sup>5</sup> and wild-type at 10<sup>6</sup>, one sequence was MLV-like, and 2 sequences were wild-type (RFLP 1-10-4). Only one sequence was generated in vaccine at 10<sup>4</sup> and wild-type at 10<sup>5</sup>, which was classified as wild-type, and none in group 9 yielded a sequence. One to three nucleotide differences from either the original MLV or WT sequences were observed in 8 samples.

# **Discussion and Conclusion**

Vaccine was more frequently sequenced in presence of the same or higher concentrations than wild-type in the same sample. When vaccinated herds are undergoing WT elimination, it is important to consider that the vaccine may be masking wild-type infections. Further studies are needed with different vaccines and variants to better understand this phenomenon.

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# FIRST DETECTION OF PCV4 IN THE UNITED STATES AND ITS ASSOCIATION WITH PCV2 AND PCV3 COINFECTION

<u>M. Kroeger</u><sup>1</sup>, D.S. Vargas-Bermudez <sup>2</sup>, J. Jaime <sup>2</sup>, J. Parada <sup>3</sup>, P. Gauger <sup>1</sup>, P. Pineyro <sup>4</sup> <sup>1</sup>Iowa State University

<sup>2</sup>Universidad Nacional de Colombia, Sede Bogotá. Facultad de Medicina Veterinaria y de Zootecnia. Departamento de Salud Animal. Centro de Investigación en Infectología e Inmunología Veterinaria (CI3V). Carrera 30 No. 45-03 Bogotá. Colombia. CP 111321.

<sup>3</sup>3CONICET- Departamento de Patología Animal. Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto. Argentina

<sup>4</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

# **Background and Objectives**

PCV4 was first reported in China in 2019 pigs displaying porcine dermatitis and nephropathy syndrome, respiratory, and enteric disease. However, its clinical significance remains not fully elucidated. Since its first description, PCV4 has been reported in South Korea, Thailand, and Spain. Currently, no studies have determined the presence of PCV4 in the United States. Therefore, the objective of this study was to determine the detection rate of PCV4 from varying sample matrices and its codetection rate with PCV2 and PCV3.

#### Material and Methods

A total of 512 clinical samples were obtained from the Iowa State University Veterinary Diagnostic Laboratory from August to October 2023 representing a variety of sample matrices including: lung (n=100), feces (n=100), spleen (n=100), serum (n=100), lymphoid tissue (lymph node and tonsil, n=64), and fetus (n=48). Nucleic acid extracts were tested by a PCV4 gPCR singleplex and PCV2/3 gPCR multiplex.

#### Results

PCV4 was detected in 44 of the 512 samples (8.6%) occurring in lung (9/100, 9%), feces (5/100, 5%), spleen (9/100, 9%), serum (10/100, 10%), and lymphoid tissue (11/64, 17.2%). The overall average PCV4 Ct was 33 ranging from 21.3 to 36.3, and the average Ct between different samples types was not significantly different (p>0.05). A total of 23/44 (52.3%) were PCV4 single positive. However, 14/44 samples (31.8%) had PCV4/2 codetection, 3/44 samples (6.8%) had PCV4/3 codetection, and 4/44 samples (9.1%) had PCV4/2/3 codetection. In samples with codetection, the average PCV2 and PCV3 Ct was 27.4 and 32, respectively. Reported clinical signs in cases where PCV4 was detected most commonly included enteric and respiratory disease in 2-17-week-old pigs. PCV4 was found incidentally in 33/44 (75%) cases and the most common main diagnoses where PRRSV, Lawsonia intracellularis, Streptococcus suis, and PCV2.

### **Discussion and Conclusion**

PCV4 was detected in several sample matrices for the first time in the United States. Given the relatively high detection rate in cases with PCV2, PCV3, and PRRSV coinfections, future studies should address the role of PCV4 in a coinfection model to determine the effect on clinical disease. Additionally, in situ analysis is needed to confirm the lesions associated with PCV4 infection.

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# GENOMIC DIVERSIFICATION OF SWINE INFLUENZA A VIRUS BY HUMAN-TO-SWINE SPILLOVER OF THE 2009 PANDEMIC VIRUS IN KOREA

H. Cho<sup>1</sup>, S. Kim<sup>1</sup>, Y. Oh<sup>2</sup>, D. Tark<sup>3</sup>, D. Yoo<sup>4</sup>, W. Kim<sup>1</sup>

<sup>1</sup>College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan, Republic of Korea <sup>2</sup>College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon, Republic of Korea

<sup>3</sup>Laboratory for Infectious Disease Prevention, Korea Zoonosis Research Institute, Jeonbuk National University, Iksan, Republic of Korea

\*College of Veterinary Medicine, Chonnam National University, Gwangju, Republic of Korea

## **Background and Objectives**

The 2009 influenza A H1N1 pandemic (pdm09) originated from the swine influenza A virus (swIAV) through multiple reassortment events with avian and human IAVs. The pdm09 reportedly reintroduced the virus to pigs, contributing to the evolution and diversity of swIAV through frequent reassortment and drifts.

# Material and Methods

To update current swIAV epidemiology, swIAV have been actively sampled and isolated from a total of 40 farms for one year (from 2021 to 2022). Phylogenetic analysis has been conducted with retrieved whole genome sequences of isolated viruses, and additional bioinformatic tools have been utilized to identify antigenic shifts and drifts among Korean swIAVs.

#### Results

The genetic diversity of H1 and H3 swIAV was continuously enriched after human-to-swine spillover of pdm09 viruses with long-term maintenance, persistence, and reassortment of virus lineages. Evidence of additional human-to-swine spillover of viruses that are different from the 2009 virus but close to that of the recent H1N1pdm09 human vaccine, was also identified in this study. The identification of swine-adapted pdm09 viruses, which have accumulated amino acid mutations with potentially altered antigenicity and a unique potential N-glycosylation site within the haemagglutinin, suggests the distinctive evolution of spillover pdm09 viruses in swine. The genetic constellation of the recently emerging Eurasian avian-like swine lineage and the preexisting classical swine lineage H1 viruses in Korea has been expanded through reassortment with cocirculating pdm09 viruses and/or H3N2 IAV-S harbouring the pdm09 M gene (H3N2pM).

#### **Discussion and Conclusion**

Collectively, After the major shift of Korean swIAV from the classical swine lineage to the pdm09 lineage in 2009, the frequent spillover of pdm09 viruses and the circulation of swIAV harbouring pdm09 gene segments led to the continuous diversification of swIAV through antigenic drift and shift, raising concerns about the potential reintroduction of these viruses to humans.

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## IMMUNE ANTAGONISM-REPROGRAMMED PRRS VIRUS IS CLINICALLY ATTENUATED AND DEVOID OF OVEREXPRESSION OF PROINFLAMMATORY CYTOKINES DURING COINFECTION WITH A SECONDARY PATHOGEN

<u>D. Yoo</u><sup>1</sup>, C. Su<sup>1</sup>, J. Kim<sup>1</sup>, J. Tang<sup>1</sup>, Y.F. Hung<sup>1</sup>, F. Zuckermann<sup>1</sup>, R. Husmann<sup>1</sup>, P. Roady<sup>1</sup>, J. Kim<sup>2</sup>, Y. Lee<sup>2</sup> <sup>1</sup>Department of Pathobiology, University of Illinois at Urbana-Champaign, USA <sup>2</sup>Department of Animal, Dairy, and Veterinary Sciences, Utah State University, USA

### **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) is known to suppress type I interferon (IFNs- $\alpha/\beta$ ) response for innate immune evasion and also activates the NF- $\kappa$ B signaling, leading to the production of inflammatory cytokines during infection. In swine farms, coinfection of PRRSV and a secondary bacterial pathogen is common and exacerbates the production of proinflammatory cytokines, contributing to the porcine respiratory disease complex (PRDC) which is clinically a severe disease. Previously, we identified the viral non-structural protein 1<sub>β</sub> (nsp1<sub>β</sub>) as an IFN antagonist and the nucleocapsid (N) protein as the NF- $\kappa$ B activator.

# **Material and Methods**

The leucine 126 of nsp1ß was the catalytic residue for IFN suppression and this residue was mutated to alanine to generate IFN suppression-negative. For NF-kB activation, the region spanning the nuclear localization signal (NLS) of N was deleted to generate NF-kB activation-negative. A double-mutant PRRSV was constructed by reverse genetics to carry the L126A mutation in the nsp1ß gene and the NLS mutation in the N gene. The immunological phenotype of this double-mutant virus was examined in macrophages in vitro and in young pigs in vivo by coinfection with Streptococcus suis.

#### Results

In macrophages, the double-mutant virus did not suppress IFN-β expression and decreased the NF-kB-directed proinflammatory cytokine productions compared to those for wild-type PRRSV. Co-infection of macrophages with the mutant PRRSV and S. suis also reduced the production of NF-kB-directed inflammatory cytokines. For studies in natural host animals, 6 groups of pigs, 7 animals per group, were used for coinfection with the mutant PRRSV and S. suis. The double-mutant PRRSV was clinically attenuated, and the expressions of proinflammatory cytokines and chemokines were significantly reduced in pigs after bacterial coinfection. Compared to the wild-type PRRSV and S. suis coinfection control, pigs coinfected with the double-mutant PRRSV exhibited milder clinical signs, lower titers and shorter duration of viremia, and lower level expression of proinflammatory cytokines.

# **Discussion and Conclusion**

Our study demonstrates that the reprogramming of viral immune antagonism and inflammatory cytokine productions allows us to design a novel vaccine candidate to alleviate the clinical severity of PRRS and PRDC during bacterial coinfection.

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# IMPACT OF ROTAVIRUS TYPE A SOW VACCINATION ON TECHNICAL PERFORMANCES AND ANTIMICROBIAL TREATMENTS IN PIGLETS DURING SUCKLING PERIOD

A. Lebret <sup>1</sup>, T. Nicolazo <sup>1</sup>, C. Chevance <sup>1</sup>, P. Berton <sup>2</sup>, E. Bousquet <sup>3</sup>, J. Jeusselin <sup>1</sup>, C. Teixeira-Costa <sup>1</sup>, V. Normand <sup>1</sup>, G. Boulbria 1

<sup>1</sup>Rezoolution Pig Consulting Services, Noval-Pontivy, France

<sup>2</sup>Virbac France, Espace Azur Mercantour, 3ème Rue, 06510 Carros, France <sup>3</sup>Virbac SA,13ème rue, 06510 Carros, France

# **Background and Objectives**

Neonatal diarrhoea is a frequent issue in pig farms and can lead to dramatic costs. In France, Rotavirus type A (RVA) is identified in approximately 50% of clinical cases; until now, prevention only dealt with risk factors and coinfections management. The objective of this study is to ensure field efficacy of a new vaccine SUIGEN® ROTA COLI on technical performances of a RVA positive farm, and to estimate the evolution of antimicrobial treatments of piglets during lactation.

#### **Material and Methods**

The study took place in a 600-sow-farrow-to-finish farm, with good biosecurity management and health status, implementing correct pig and human flows. Sows are routinely vaccinated with an enterotoxinogenic Escherichia coli (ETEC)-Clostridium perfringens type C (CpC) vaccine. Before the study, RVA has been regularly identified since 2020 in 2 to 7 day-old-piglets suffering from diarrhoea, in association with evocative microscopic lesions. Thirty to 50% of litters per batch were impacted and treated with antimicrobials. Ten batches have been included in the study between February and April 2023 and divided into 2 groups: group A (5 batches vaccinated with ETEC-CpC vaccine) and group B (5 batches vaccinated with ETEC-RVA vaccine). Technical results (mortality rate during lactation - MRL, number of weaned piglets/sow - WP, piglet weight at weaning - PWW, litter weight at weaning - LWW) and litters antimicrobial treatment rate (AMTR) in both groups have been compared. Statistical analysis included a comparison between 2 groups according to Wilcoxon test (MRL, WP, PWW, LWW) and Chi2 (AMTR).

#### Results

Between groupA and groupB, average MRL decreased (19.3% to 15.3% respectively), PWW and WP increased (average 5.3kg to 5.4kg and 13.7 to 14.7 piglet/sow respectively). LWW increased (average 72.2kg to 79.8kg) with a statistical tendency (p=0.09). AMTR was 10-fold decreased (average 32.6% to 3.3%; p<0.05).

## **Discussion and Conclusion**

SUIGEN® ROTA COLI helped reducing clinical signs due to RVA. There was also a numerical reduction of mortality rate. This was characterized by a significant drop of AMTR. Even if the evolution of the parameters is not significant, the numerical increase of LWW and PWW (considering that WP also increased) suggest an improvement of the guality of piglets at weaning.

VVD – Virology and Viral Diseases

# INFLUENZA A VIRUSES SUBTYPES IN NURSERY AND FINISHING PIGS IN THE SOUTH OF BRAZIL

M.S. Goslar <sup>1</sup>, A.C. De Lara <sup>1</sup>, F. Aguiar <sup>1</sup>, B. Fornari <sup>1</sup>, <u>J.R. Ciacci Zanella</u> <sup>2</sup> <sup>1</sup>Animal Health laboratory, JBS Foods, Seara, Brazil <sup>2</sup>Brazilian Agricultural Research Corporation (EMBRAPA), Concórdia, Brazil

#### **Background and Objectives**

Influenza A virus (IAV) is a zoonotic RNA virus that causes respiratory diseases in commercial pig farming in Brazil, with a prevalence estimated at 70%. Swine can be infected with various subtypes, including H1N1, H1N2, H3N2, and reassortment, including human IAV. This study aimed to understand IAV subtypes in swine herds of an agroindustry in South Brazil through subtyping and genomic sequencing.

## Material and Methods

Nasal swabs and swine lungs were collected from nursery and finishing pigs in Santa Catarina and Rio Grande do Sul (RS) States between April and December 2021. A total of 1988 samples were screened for IAV using RT-qPCR, and positive samples were selected for subtyped by multiplex RT-PCR viral, SPF eggs isolation and sequenced. IAV genes (8 segments) sequenced by Illumina MiSeq System<sup>™</sup> had their complete genome analyzed.

## Results

IAV was detected in 16.65% of 331 samples, with Zone A having 27.68% of positive samples. All farms from each zone were significantly different for the presence of IAV. Lungs from clinical cases resulted in 84.21% positivity for IAV RNA, compared to nasal swabs (16%). Multiplex RT-qPCR subtyped 110 IAV isolates, and 57.27% HA or NA were identified: H1N1pan (3.51%) and one H1huN1pan subtype samples. H3hu was the most frequent (27 samples), followed by N1pan, H1hu, H1pan, and N2hu. Zone A was the most diverse and the only one where the N2hu subtype was detected. Eleven samples were completed sequenced and in 5/11 there were co-infection with at least 2 different IAV. The results demonstrated several introductions of human H3N2 subtypes and at least two different clusters of H1N1 subtypes (Brazil and USA).

#### **Discussion and Conclusion**

The study showed IAV infection detection in the studied pig farms in all four zones. The H3hu subtype gene presented the highest predominance, found mostly in RS. However, the N1pan glycoprotein gene was well distributed in both states. These results provide important information on IAV circulation in swine herds, helping to better interpret its epidemiology and provide control tools for each region. Coinfection of different or genetically distant subtypes was confirmed by sequencing, demonstrating the interaction of human and swine origin IAV in the evaluated farms.

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# MULTINUCLEATED GIANT CELLS IN ASSOCIATION WITH THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

<u>R. Renzhammer</u><sup>1</sup>, R. Brunthaler<sup>2</sup>, G. Balka<sup>3</sup>, J. Reif<sup>4</sup>, G. Schoder<sup>5</sup>, A. Auer<sup>6</sup>, T. Rümenapf<sup>6</sup>, A. Ladinig<sup>1</sup> <sup>1</sup>University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria <sup>2</sup>Institute of Pathology, University of Veterinary Medicine Vienna, Austria <sup>3</sup>Department of Pathology, University of Veterinary Medicine Budapest, Hungary <sup>4</sup>Dein Landtierarzt, St. Agatha, Austria <sup>5</sup>Oberösterreichischer Tiergesundheitsdienst, Linz, Austria <sup>6</sup>Institute of Virology, University of Veterinary Medicine Vienna, Austria

#### Background and Objectives

Microscopic lung lesions being frequently associated with the porcine reproductive and respiratory syndrome virus (PRRSV) include hyperplasia of type 2 pneumocytes, infiltration of the alveolar septa with macrophages, lymphocytes and plasma cells, interlobular oedema and haemorrhages. However, intra-alveolar multinucleated giant cells in the lungs have not been associated with PRRSV infections so far.

#### **Material and Methods**

The case herd is a fattening farm located in Upper Austria regularly purchasing pigs from two different PRRS-unsuspicious piglet-producing farms. Since the farmer observed an increased number of pigs with cyanotic ears as well as an increased mortality rate (3%) in finishers in March 2023, the veterinarian decided to take tissue samples from four pigs for the further diagnostic workup.

#### Results

While no bacteria associated with septicaemia were detected in investigated spleens and nucleotides of African swine fever virus, classical swine fever virus, pseudorabies virus, influenza A virus, porcine circovirus type 2, porcine respiratory corona virus, Mycoplasma hyopneumoniae, Mycoplasma hyorhinis, Actinobacillus pleuropneumoniae and Glaesserella parasuis were not detected in investigated lung samples, PRRSV RNA was detected in the lung tissue from all four sampled animals (ct-values: 16.7 – 21.9). Sequencing of the open reading frames 2 – 7 resulted in a 94.6% homology to a PRRSV-1 field strain (AUT 15-33), commonly detected in Austrian farms. While infiltrations with macrophages and interstitial pneumonia were observed in the lungs of all four pigs, a high number of intra-alveolar multinucleated giant cells was detected in the lung of the pig with the lowest ct-value. Therefore, paraffin embedded lung tissue was further investigated by RNA scope. PRRSV-1 RNA could be visualized by RNA scope inside the macrophages and multinucleated giant cells in the lung tissue.

### **Discussion and Conclusion**

Although multinucleated giant cells are formed by macrophages, which are the primary target cells of PRRSV, so far multinucleated giant cells have only been detected in seminiferous tubes of PRRSV infected boars. While PRRSV could be visualized in the multinucleated giant cells, we cannot exclude the involvement of other less abundant viruses in the formation of the intra-alveolar multinucleated giant cells.

#### VVD – Virology and Viral Diseases

## STABILIZING PRRSV RNA IN ORAL FLUIDS

B. Munguia-Ramirez <sup>1</sup>, B. Armenta-Leyva <sup>1</sup>, L. Gimenez-Lirola <sup>1</sup>, J. Zimmerman <sup>2</sup>

<sup>1</sup>Veterinary Diagnostic and Production Animal Medicine. Iowa State University, Ames, IA, USA.

<sup>2</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA

## **Background and Objectives**

Oral fluids (OFs) are widely used in swine surveillance for essentially all pathogens of economic importance. Preserving PRRSV RNA in OFs during storage is crucial to avoid false negative results at PCR testing. Commercial products sold to preserve nucleic acids in diagnostic specimens are expensive, and, in truth, there is little peer-reviewed evidence of their efficacy. Alternatively, we tested readily available proteins and carbohydrates for their capacity to stabilize PRRSV RNA in OFs stored under adverse conditions.

## **Material and Methods**

A stock solution (30 ml) of field PRRSV-negative OFs was centrifuged (3,300× g for 3 hr) and then inoculated with Ingelvac<sup>®</sup> PRRS MLV (1 × 10<sup>-3</sup>). Aliquots (1 ml) were assigned to one of 7 treatments: Three proteins (5 w/v%) and four carbohydrates (20 w/v%). All samples (n = 21) were stored at 30°C for 24 hr and tested for PRRSV RNA by RT-qPCR (IDEXX Laboratories, Inc.). For comparison, two untreated controls were included: one held at -80°C, and one held at 30°C for 24 hr. RT-qPCR Cqs were re-expressed as a function of the PCR efficiency as efficiency standardized Cqs (ECqs) and analyzed in terms of percent (%) recovery relative to the -80°C untreated control.

#### Results

PRRSV RNA (%) recoveries were: Untreated 30°C control (68.5%), protein 1 (83.7%), protein 2 (31.4%), protein 3 (48.5%), carbohydrate 4 (92.5%), carbohydrate 5 (91.1%), carbohydrate 6 (91.6%), carbohydrate 7 (90.0%).

# **Discussion and Conclusion**

Overall, samples receiving treatments 4 to 7 and held at 30°C for 24 hr retained ~90% of the concentration of PRRSV RNA detected in the matched sample held at -80°C. This preliminary data demonstrated that it is possible to stabilize PRRSV RNA in OFs under adverse storage conditions using cost-effective, readily available agents.

Although sample handling recommendations include prompt chilling or freezing samples after collection to preserve viral nucleic acid, it is not always logistically possible to meet this requirement. It follows that protecting viral RNA from degradation in OFs is crucial. Future work will focus on combining chemical agents and concentrations that optimize the stability of PRRSV RNA and other pathogens of economic importance in swine oral fluids.

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# PATHOGENESIS AND TRANSMISSION OF PERSISTENT CLADES OF HUMAN SEASONAL H1N1PDM09 INFLUENZA A VIRUSES IN PIGS

G. Ciacci Zanella<sup>1</sup>, A. Markin<sup>1</sup>, C. Snyder<sup>1</sup>, M. Neveau Thomas<sup>1</sup>, C. K. Souza<sup>1</sup>, B. Arruda<sup>1</sup>, T. K. Anderson<sup>1</sup>, A. L. Baker

<sup>1</sup>Virus and Prion Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA

#### **Background and Objectives**

The 1A.3.3.2 H1N1 pandemic (pdm09) lineage of influenza A viruses (IAV) emerged in North America in 2009 and quickly developed into a human influenza pandemic. It spread rapidly due to its efficient transmission and limited human immunity, replacing the previous human seasonal H1. Human-to-swine transmission of pdm09 IAV has since contributed to genetic diversity in pigs. While most spillover events did not sustain transmission, there have been ~160 instances where human-origin H1N1pdm09 persisted in pigs for at least one year and, in most cases, reassorted with other endemic swine IAVs. Persistent clades of pdm09 accumulated substitutions that led to genetically and antigenically distinct viruses that can potentially evade pre-existing human population immunity. There is a need to identify how ongoing transmission, evolution and reassortment with endemic IAV viruses in swine impacts viral traits and zoonotic risk.

#### **Material and Methods**

We selected four swine H1N1pdm09 viruses that were introduced during different human influenza seasons, persisted in swine for different lengths of time and acquired different gene segment combinations. The strains were used in a swine pathogenesis and transmission study. Nasal swabs, serum, bronchoalveolar lavage fluid and formalin-fixed lower respiratory tract tissues were collected to assess viral infection, replication, and shedding.

## Results

All swine H1N1pdm09 strains in the study infected and transmitted but with variable kinetics. Each animal group presented distinct lower respiratory tract gross and microscopic lesion scores. Persistent human-origin pdm09 viruses isolated from swine retained antigenic similarities with human vaccine strains of the same season of incursion but cross-reacted variably with antisera derived from vaccine strains of other seasons.

## **Discussion and Conclusion**

Reverse zoonosis of human seasonal H1N1pdm09 impacts swine health by increasing diversity of endemic IAV. H1N1pdm09 from different human influenza seasons reassort with swine IAV and persist over varying periods of time. This ongoing circulation results in reassorted viruses with variable virulence, shedding, and transmission kinetics. Swine H1N1pdm09 strains displayed antigenic diversity and a tendency to drift away from contemporary human seasonal H1N1pdm09 vaccine strains. Control of H1N1pdm09 at the human-swine interface is critical for reducing IAV burden in swine and subsequent risk of swine to human zoonosis.

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## PCV3 INFECTIONS IN STILLBORN AND MUMMIFIED PIGLETS; A CASE SERIES OF TWO

S. Klarenbeek <sup>1</sup>, R. Dijkman <sup>1</sup>, E. Gijsen <sup>2</sup>, C. Gielen <sup>3</sup>, R. Brunthaler <sup>4</sup>, <u>T. Tobias <sup>1</sup></u> <sup>1</sup>Royal GD, Deventer, The Netherlands <sup>2</sup>Veterinary practice "VarkensArtsenZuid", Panningen, The Netherlands <sup>3</sup>De Varkenspraktijk, the Netherlands <sup>4</sup>Institute of Pathology, University of Veterinary Medicine Vienna, Austria

### **Background and Objectives**

Causal inference for PCV3 to induce reproductive problems in sows is debated. Recently, in two cases for which diagnostics was requested, PCV3 was found as the sole probable cause. This case series describes the clinical and diagnostic work up and aims to contribute to the scientific debate about the relevance and impact of PCV3 in swine.

# **Material and Methods**

Farm 1: a 1,300 head multiplier farm with self-rearing of gilts reported an increased of a terme born litters with higher numbers of mummified foetuses and still born pigs in gilts over an 8 week period.

Farm 2: a 450 head multiplier farm experienced incidental premature farrowing (D108 post insemination) in ~10 gilts over a 3 week period with increased stillborn and mummified foetuses.

In both farms, besides anorexia, there was no evidence of respiratory or gastro-intestinal disease, nor of systemic disease in sows or gilts. In both farms, gilt rearing and housing was strictly separated from the sows and adaptation procedures before service was limited in time.

#### Results

For both cases, two foetuses were submitted for pathological and microbiological investigations. No macroscopic abnormalities were found. Bacterial culture of gastric content was negative. PCR tests on foetal tissues for parvovirus, PCV2 and PRRSV had negative results as well.

In case 1, a lymphocytic and neutrophilic perivascular inflammation was observed in the myocardium via histopathology. In case 2, myocarditis was not observed.

Subsequently, myocardial tissue was submitted for PCV3 testing by PCR and returned positive with low Ct values (~14 and ~21 resp). In Situ Hybridisation (ISH) for PCV3 revealed evidence for multifocal presence of PCV3 DNA in the nuclei of cardiomyocytes and in the tunica media of myocardial arterioles.

#### **Discussion and Conclusion**

PCV3 was present in foetal tissues and found as sole probable cause for the preterm farrowing and abortions in both cases. Case 2 illustrates that PCV3 DNA may be detected in the myocardium in the absence of myocarditis. Also, absence of PCV3 in one of the investigated foetuses indicates that multiple foetuses may need to be investigated for diagnosing PCV3 infection in cases of stillborn or mummified foetuses.

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# PORCINE CIRCOVIRUS TYPE 2 (PCV-2) GENOTYPING IN VACCINATED COMMERCIAL FARMS IN BRAZIL FROM 2020 TO 2022

<u>R. T Lippke</u><sup>1</sup>, E. R De Conti<sup>1</sup>, L. F Hernig<sup>1</sup>, F. De Quadros<sup>1</sup>, A. L Fiúza<sup>1</sup>, A. P Teixeira<sup>1</sup>, C. Pisseti<sup>2</sup> <sup>1</sup>Boehringer Ingelheim Brazil <sup>2</sup>Centro de Diagnóstico de Saúde Animal (CEDISA)

#### **Background and Objectives**

Circovirus type 2 (PCV-2) is the causative agent of the group of diseases known as porcine circovirus-associated diseases (PCVD). In the early 1990s, the most prevalent genotype was PCV-2a, which was replaced ten years later by PCV-2b. This study aimed to describe the PCV-2 genotypes in serum and fetus samples from pig farms in Brazil without clinical signs between 2020 and 2022.

## **Material and Methods**

A total of 96 samples from 14 farms in Brazil were collected between 2020 and 2022. All farms used a PCV-2a vaccine only in piglets and did not show clinical signs characteristic of PCVD. The DNA extracted by the IndiMag Pathogen kit (Indical Bioscience) was submitted to the PCV-2 genotyping assay by the Kylt® PCV-2 Typing kit (AniCon Labor GmbH) for differentiation between genotypes.

#### Results

The most prevalent genotype was b (PCV-2b), with 55.2%; 39.6% belonged to genotype d (PCV-2d), and 5.3% had genotypes b and d (PCV-2b and 2d). None of the samples were positive for genotype a (PCV-2a).

## **Discussion and Conclusion**

Taking into consideration that all samples were from farms with no clinical signs of PCVD and vaccinated the piglets with PCV-2a, it can be concluded that even though the virus has evolute to PCV-2b and PCV2-d, the efficacy of the PCV-2a vaccines continues to work. Thus, it is observed that vaccines based on PCV-2a antigen present cross-protection against PCV-2b and PCV-2d. The results suggest that the detection of these genotypes in vaccinated farms probably simply reflects the evolution of the virus over time, at the epidemiological level, without escaping PCV-2-specific vaccine immunity.

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# PREVALENCE OF INFLUENZA A IN SWINE IN KOREAN PIG FARMS, 2021-2022

Y. Oh 1, G. Kim 1, D. Tark 2, D. Yoo 3, S. Lee 4, C. Kim 4, H. Cho 5, B. Seo 4

<sup>1</sup>College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon, Republic of Korea

<sup>2</sup>Laboratory for Infectious Disease Prevention, Korea Zoonosis Research Institute, Jeonbuk National University, Iksan, Republic of Korea

<sup>3</sup>College of Veterinary Medicine, Chonnam National University, Gwangju, Republic of Korea <sup>4</sup>WOOGENE B&G CO., LTD., Seoul, Republic of Korea

<sup>5</sup>College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan, Republic of Korea

# **Background and Objectives**

Swine influenza is a highly prevalent disease affecting swine populations worldwide. It is caused by the influenza A virus of swine (IAV-S), leading to symptoms like fever, dullness, loss of appetite, and respiratory issues. In this research, we conducted a comprehensive serological and virological surveillance of IAV-S in pig farms across Korea

# Material and Methods

We collected nasopharyngeal and environmental swabs, along with blood samples, from 40 pig farms representing different age groups (weaned piglets, growers, finishers, sows, gilts and runt piglets). These samples were then tested using ELISA to detect IAV-S antibodies and RT-PCR to identify viral antigens.

#### Results

The findings revealed positive confirmation of IAV-S antibodies or antigens in 39 out of 40 farms (97.5%). In 38 farms (95%), both antigens and antibodies were detected. A total of 32 viruses were isolated, H1N1 (6 cases), H1N2 (11 cases), and H3N2 (15 cases).

## **Discussion and Conclusion**

In conclusion, our study identified various patterns of IAV-S infection in domestic pig farms, emphasizing the need for continuous monitoring to implement effective IAV-S eradication program. Additionally, further investigations are warranted to enhance the application of commercially available vaccines through the characterization of the isolated IAV-S strains. AcknowledgementThis work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Animal Disease Management Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (321008-1).

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# REFINING GENETIC CLASSIFICATION OF GLOBAL TYPE 1 PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS AND CHARACTERIZATION OF THEIR GEOGRAPHIC DISTRIBUTIONS

W. Yim-Im <sup>1</sup>, T. Anderson <sup>2</sup>, J. Bohmer <sup>3</sup>, J. Baliellas <sup>4</sup>, T. Stadejek <sup>5</sup>, P. Gauger <sup>1</sup>, K. Krueger <sup>1</sup>, C. Vermeulen <sup>6</sup>, R. Buter <sup>6</sup>, A. Kazlouski <sup>7</sup>, <u>J. Zhang</u> <sup>1</sup>

<sup>1</sup>Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, United States

<sup>2</sup>Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA, United States

<sup>3</sup>IVD Gesellschaft für Innovative Veterinaerdiagnostik mbH, Seelzer-Letter, Germany

<sup>4</sup>Grup de Sanejament Porci (GSP), Lleida, Spain

<sup>5</sup>Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Science, Warsaw, Poland

<sup>6</sup>Royal GD (GD Animal Health), P.O. Box 9, 7400 AA Deventer, The Netherlands

7Vitebsk State Academy of Veterinary Medicine, Viciebsk, Belarus

## **Background and Objectives**

PRRSV-1 is predominantly prevalent in Europe, yet it is also detected in North America and Asia. Previous investigations, utilizing ORF5 sequences, categorized PRRSV-1 into four subtypes. Subtype 1 further underwent classification into 12 clades (A-L) or three lineages, with lineage 1 encompassing clades 1A-1G and lineage 3 encompassing clades 3A-3G. However, neither system has been widely accepted for use.

## Material and Methods

Here, we proposed a statistically supported PRRSV-1 genetic classification system based on 10,400 global PRRSV-1 ORF5 sequences spanning the years 1991-2023.

## Results

We replaced "subtype" with "lineage", and, subsequently, PRRSV-1 was classified into four lineages (L1-L4) with L1 including 18 sublineages (L1.1 to L1.18). This classification system is flexible for expansion if additional lineages, sublineages, or more granular classifications are needed. Geographic distributions of global PRRSV-1 at lineage and sublineage levels were investigated. A small number of PRRSV-1 sequences were classified in lineages L2, L3, and L4 and were exclusively found in Eastern Europe. In contrast, lineage L1 exhibited widespread distribution, circulating in North America, Asia, and across Europe. In Europe, 17 out of 18 sublineages (excluding L1.13) within L1 were identified. In North America, only sublineage L1.1 was detected. In Asia, sublineages L1.1, L1.2, L1.6, L1.10, L1.11, L1.13 (confined to China), and L1.17 were identified. This study also determined the classification and ORF5 nucleotide identity of six commercial PRRSV-1 vaccines (Porcilis L1.1, Unistrain L1.2, Pyrsvac-183 L1.2, Ingelvac PRRSFlex EU L1.3, ReproCyc PRRS EU L1.3, and Suvaxyn L1.11) against each lineage and sublineage and the detection frequency of vaccine-like viruses. The phylogeny based on whole-genome sequences demonstrated a slightly different tree topology compared to that based on ORF5 sequences due to recombination. Recombination of PRRSV-1 was observed at intra-sublineage and intersublineage levels. A set of PRRSV-1 ORF5 reference sequences representing global diversity and these refined classifications are available for future diagnostic and epidemiological applications.

## **Discussion and Conclusion**

This study provides a benchmark, delineating the current genetic diversity of PRRSV-1, and the revised ORF5-based classification system can be used to characterize the genetic and phenotypic evolution of PRRSV-1.

VVD – Virology and Viral Diseases

# REGION-SPECIFIC PORCINE ENTEROID CULTURE FROM SMALL INTESTINES DEMONSTRATES HIGHER SUSCEPTIBILITY TO PORCINE EPIDEMIC DIARRHEA VIRUS INFECTION IN JEJUNUM-DERIVED ENTEROIDS

L. Yen <sup>1</sup>, R.K. Nelli <sup>1</sup>, J.C. Mora-Diaz <sup>1</sup>, N.C. Twu <sup>1</sup>, <u>L. Gimenez-Lirola</u> <sup>1</sup> <sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames. IA. USA

## **Background and Objectives**

The development of 3D enteroids has emerged as a promising alternative to traditional cell culture and pig bioassay, offering unprecedented control in establishing a strict in vitro environment that recapitulates the proxy intestine epithelial cell composition and structure. This study describes the development and characterization of duodenum-, jejunum-, and ileum-derived porcine enteroid cultures in matrigel (PEMCs) and transwells (PETCs) and its use as infection model to assess region-specific susceptibility to porcine epidemic diarrhea virus (PEDV).

## **Material and Methods**

Intestinal crypt cells isolated from duodenum, jejunum and ileum of three 3-week-old CDCD pigs were cultured in L-WRN conditioned media supplemented with growth factors in Matrigel for differentiation into PEMCs. After characterization, PEMCs were enzymatically dissociated and subcultured on transwell inserts (PETCs), which allow apical side exposure of the enteroids for infection studies. Region-specific PEMCs and PETCs were characterized based on morphology, proliferation and viability (microscopy, OrganoSeg software, and 3D cell viability assay), and cellular phenotyping (presence of goblet, enterocytes, Paneth, enteroendocrine, and epithelial cells, using IHC and gene expression). PETCs were inoculated with 10<sup>5</sup> TCID50/mL of high pathogenic PEDV non-S INDEL strain and incubated for 24 h at 37°C with 5% CO<sub>2</sub>. The infection outcome was evaluated via microscopic assessment of cytopathic effect, PEDV N protein expression by IFA, and PEDV RNA detection by RT-qPCR.

#### Results

Gene expression and IHC analysis showed no differences in morphology and cell composition between PEMCs and PETCs regardless intestinal region. The growth rate of ileum-derived PEMCs was comparatively higher than the duodenum- and jejunum-derived PEMCs. PEDV infection results from three biological and technical replicates showed that all PETCs, regardless the region of the small intestine, were susceptible to PEDV. Infection led to rounding, detaching, vacuolation, and cytoplasmic stranding of cells. The infection was confirmed by IFA and RT-qPCR; quantitative analysis of the PETC cell fraction showed higher PEDV replication in jejunum-derived PETCs.

# **Discussion and Conclusion**

This study further demonstrates the potential of small intestine-derived porcine organoids as alternative infection model for enteric pathogens at real time under control conditions. For the purpose of this study, jejunum-derived PETCs showed higher susceptibility to PEDV, which needs to be further investigated.

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# AFRICAN SWINE FEVER VIRUS EP364R AND C129R ABROGATE THE CGAS-STING SIGNALING BY DEGRADING 2'3'CGAMP

N. Dodantenna 1, L. Jong-Soo 1

<sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

#### **Background and Objectives**

Live attenuated vaccines stand out as the most effective among the various types of African Swine Fever Virus (ASFV) vaccines. In developing live attenuated vaccines against ASFV, a crucial consideration involves targeting genes for deletion that act as interferon inhibitors. Therefore, this research seeks to elucidate the significance of EP364R and C129R in the innate immune regulatory mechanisms associated with innate immunity.

## **Material and Methods**

The effect of ASFV genes on DNA virus replication was evaluated by infecting GFP-tagged ADV, HSV, and VACV viruses into ASFV EP364R and C129R proteins expressing PK-15 cells, PAMs, and MA104 cells. To determine the target of ASFV genes, IFN- $\beta$  and NF- $\kappa$ B luciferase assays were conducted. cGAMP degradation was evaluated by cGAMP ELISA and HPLC. IBMX, a pan phosphodiesterase inhibitor, was used to inhibit ASFV gene-induced cGAMP degradation to check the ASFV genes-cGAMP interaction. STING aggregation assay was performed to check the STING activation by cGAMP.

#### Results

EP364R and C129R proteins increased DNA virus replication and reduced IFN-β and IL-6 production, inhibiting type I IFNinduced ISG gene transcription. Luciferase assays found that both ASFV genes target mammalian second messenger 2'3'cGAMP. ASFV genes inhibited the type I IFN production, degrading 2'3'cGAMP and exerting their phosphodiesterase activity whereas IBMX treatment induced the immune responses. Moreover, both genes declined bystander cell activation induced by 2'3'cGAMP. HPLC data showed that cGAMP-degraded by-products were neither AMP nor GMP. Sequence analysis of the EP364R gene found a cGAMP binding motif similar to STING that competes with STING for cGAMP interaction. In vitro, mutagenesis of ASFV-EP364R indicated that the Y76 and N78 amino acids interact with 2'3'cGAMP, and amino acid mutations restored subsequent antiviral responses. Finally, both ASFV genes antagonized STING aggregation induced by the cGAMP confirming that both genes reduced IFN production by declining cGAMP-mediated STING activation.

# Discussion and Conclusion

The findings underscore the crucial involvement of EP364R and C129R in inhibiting IFN responses, suggesting their potential utility in developing live attenuated vaccines against ASFV. [National Research Foundation (2021R1A6A1A03045495) and Ministry of Environment (NIWDC-2021-SP-02)]

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# AFRICAN SWINE FEVER VIRUS L11L TARGETS IRF3 AND PKR TO ESCAPE THE HOST'S INNATE IMMUNE RESPONSES

## D.K. Haluwana <sup>1</sup>, <u>L. Jong-Soo <sup>1</sup></u>

<sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

#### **Background and Objectives**

The African Swine Fever Virus (ASFV) is a large, complex DNA virus that infects wild boars and domestic pigs. It causes African swine fever (ASF), a serious and often fatal disease that has a 100% fatality rate. After infection, ASFV employs specific viral proteins to enhance its pathogenicity. However, the functions of many of these proteins remain unknown. ASFV L11L is one of those ASFV proteins the exact function is unknown. In this study, we investigated the molecular mechanism by which ASFV L11L evades the type-I interferon (IFN-I) pathway and protein kinase R (PKR)-mediated antiviral responses.

## **Material and Methods**

First, the full-length of L11L gene was cloned into Flag-tagged pIRES vector and GST-tagged pEGB vector. Using constructed LIIL plasmid, A large scale mass spectrometry analysis, interferon  $\beta$  (IFN- $\beta$ ) assay were conducted to determine the target of ASFV L11L protein. Immunoprecipitation and immunofluorescence assays confirmed the ASFV L11L protein interaction with IRF3 and PKR. DNA virus replication was evaluated by infecting GFP tagged ADV, HSV and VACV viruses into ASFV L11L stably expressing porcine macrophages, MA104 cells and transiently transfected PK-15 cells. IFN- $\beta$ , pro-inflammatory cytokines and chemokines secretion were assessed by ELISA.

#### Results

ASFV L11L enhancing DNA and RNA virus replication and reduced cGAS-STING signaling and anti-viral gene transcription, ASFV L11L interacted with and inhibited the IRF3 phosphorylation. Most notably, the ASFV L11L associated specifically with the S396 and S398 amino acid in the autoinhibitory domain (AIE) of IRF3. Besides, ASFV L11L specifically interacted with and inhibited the dimerization of PKR, hampering PKR autophosphorylation and phosphorylation of eIF2 $\alpha$ . Remarkably, the K196 residue of PKR played a pivotal role in its interaction with ASFV L11L. In particular, we discovered that ASFV L11L is crucial in suppressing IFNs, pro-inflammatory cytokines production and promotes viral replication.

## **Discussion and Conclusion**

African Swine Fever Virus L11L targets major two immune modulators, IRF3 and PKR. These novel finding conclude that ASFV L11L suppresses antiviral immunity and promotes viral replication, implying the identification of a novel target for directing the advancement of live-attenuated vaccines against ASFV. [National Research Foundation (2021R1A6A1A03045495) and Ministry of Environment (NIWDC-2021-SP-02)]

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# CORRELATION BETWEEN MAJOR PATHOGENS AND SELECTED BIOMARKERS IN ORAL FLUIDS FROM ANIMALS VACCINATED AGAINST SWIAV

E. Llamas-Amor <sup>1</sup>, F. Murciano <sup>1</sup>, E. Goyena <sup>2</sup>, B. Gonzalez-Guijarro <sup>3</sup>, A. Cuellar-Flores <sup>4</sup>, A. Muñoz-Prieto <sup>5</sup>, D. Espigares <sup>6</sup>, K. Lillie-Jaschniski <sup>7</sup>, J.J. Cerón <sup>8</sup>, G. Ramis <sup>4</sup>

<sup>1</sup>Facultad de Veterinaria, Universidad de Murcia, Spain

<sup>2</sup>DEPARTAMENTO DE SANIDAD ANIMAL, FACÚLTAD DE VETERINARIA, UNIVERSIDAD DE MURCIA, SPAIN <sup>3</sup>Departamento de Producción Animal. Universidad de Murcia

<sup>4</sup>Dpto. Producción Animal, Facultad de Veterinaria., Universidad de Murcia, Spain

<sup>5</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Campus de Espinardo s/n, 30100 Murcia, Spain <sup>6</sup>Ceva Salud Animal, Barcelona, Spain

<sup>7</sup>Ceva Santé Animale, Libourne, France

<sup>s</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Campus de Espinardo no16, 30100 Espinardo, Murcia, Spain

## **Background and Objectives**

In pig health, there is a continuous search for biomarkers that can indicate the health status of the animals. In addition, the aim is to use non-invasive samples. In this work, we have compared the values of biomarkers found in oral fluids with the frequency of observation of major pathogens in oral fluids in pigs vaccinated against swIAV.

#### **Material and Methods**

Two batches of 300 piglets (VAC1 and VAC2) were vaccinated at 56 and 77 days of age simultaneously with two vaccines: RESPIPORC FLUpan H1N1 and RESPIPORCFLU 3 (Ceva Santé Animale, France) and one batch was taken as unvaccinated controls (CON1). All piglets were vaccinated against M. hyopneumoniae, PCV2 and Aujeszky's disease virus. The three groups were followed up by OF starting beginning of fattening, one day before vaccination. The OF was taken from 6 pens every two weeks (7 samplings) and swIAV, PRRSv, M. hyopneumoniae, M. hyorhinis and PCV2 were quantified by PCR. In addition, the amount of Adenosine deaminase (ADA), Creatine Kinase (CK) and Myeloperoxidase (MPO) was determined in the OF.

### Results

Taking all samplings together, increases in MPO in positive samples to SwIAV (694.8±43.3) were obtained compared to negative samples (558.9±25.7). SwIAV was found in CON1 in the three first samplings, while in VAC1 was found in the two first sampling and in VAC was not found.In addition, when segmented by sampling, Increases in ADA were found between SwIAV positive and negative for ADA in W1 (POS=925.9±114.9 VS NEG=1275.5±61.7, P=0.009, W3 (POS=1280.0±66.4 VS NEG=1012.5±61.1, P=0.011), and there was a trend for increase MPO in W5 (POS=822.6±101.3 VS NEG=533.4±60.2, P=0.061).

## **Discussion and Conclusion**

SwAIV seems to have an influence on ADA and MPO, both related to the immune system, being increased in positive animals in the second sampling, while MPO does not show differences until the third sampling. CK does not seem to vary with the presence of any virus.

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# EVALUATION OF HAND WASHING HYGIENE PROCEDURES TO MITIGATE RISK OF INFLUENZA A VIRUS TRANSMISSION

M. Torremorell <sup>1</sup>, L. Davis <sup>2</sup>, <u>J. Alvarez-Norambuena</u> <sup>2</sup>, C. Li <sup>2</sup>, M. Culhane <sup>3</sup>, Y. My <sup>1</sup> <sup>1</sup>University of Minnesota College of Veterinary Medicine <sup>2</sup>University of Minnesota <sup>3</sup>University of Minnesota. College of Veterinary Medicine. St. Paul. MN

<sup>3</sup>University of Minnesota, College of Veterinary Medicine, St. Paul

# Background and Objectives

Influenza A virus (IAV) is an important respiratory pathogen that is endemic in swine and people. Proper hand hygiene is important to mitigate the risk of IAV transmission. In people, IAV has been cultured from hands of infected people and there are recommended practices on how to wash hands. In pig farms, hands of farmworkers that handle IAV infected pigs, are contaminated with IAV. However, there is limited information on how to properly wash hands contaminated with IAV secretions of pigs. Furthermore, there is limited information on the viability of IAV on hands of farmworkers. Thus, we assessed four hand hygiene protocols for their effectiveness at reducing IAV from contaminated hands and evaluated IAV viability on hands for up to 120 minutes after handling IAV-infected pigs.

# **Material and Methods**

Pigs were inoculated with an H1N1 IAV. Consented participants that handled IAV-infected pigs for 10 min were sampled before and after that. Participants carried out to one of the procedures: 1) water only; 2) water and soap; 3) alcohol-based sanitizer, and 4) use of disposable gloves. Each procedure was evaluated in triplicate. A subset of participants had their hands sampled for up to 120 minutes. Hands were tested by IAV RT-PCR and virus isolation to evaluate the effectiveness of hygiene procedures and IAV viability. All procedures were approved by the University of Minnesota IACUC, IBC and IRB.

## Results

Upon handling infected pigs, the hands of all participants became readily contaminated with IAV. All hand treatments resulted in reduction of IAV RT-PCR. However, reduction was limited in hands washed with water only, and soap and water. In contrast, samples from hands treated with an alcohol-based sanitizer and hands with gloves had larger reduction or IAV was not detected at all. Notwithstanding, the virus was isolated only after pig handling and for up to 10 min after handling infected pigs. There was evidence of IAV RNA degradation from hands as time when by.

## **Discussion and Conclusion**

Hand hygiene procedures are necessary to reduce IAV from contaminated hands. Wearing disposable gloves and using an alcohol-based hand sanitizer were the most effective treatments at decreasing or preventing IAV contamination from the hands.

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# EXPERIMENTAL SENECAVIRUS A INFECTION OF BOVINE CELL LINES AND COLOSTRUM-DEPRIVED CALVES

A. Buckley <sup>1</sup>, K. Hoffman <sup>1</sup>, K. Lager <sup>1</sup>, S. Falkenberg <sup>2</sup>, <u>E. Zanella</u> <sup>1</sup> <sup>1</sup>Agricultural Research Service United States Department of Agriculture <sup>2</sup>College of Veterinary Medicine, Auburn University, Auburn, USA

#### **Background and Objectives**

Senecavirus A (SVA) is a causative agent for vesicular disease in swine that is clinically indistinguishable from foot-andmouth disease (FMD). FMD is a highly contagious reportable disease that can infect cloven hoofed animals. A research article from China reported a buffalo with mouth ulcers and lameness that tested positive for SVA. It is important to understand potential host range of SVA, thus the objective of this study was to assess the susceptibility of cattle (Bos taurus) both in vitro and in vivo to experimental infection with SVA.

# **Material and Methods**

Swine testicular (ST) cells, porcine kidney (PK-15) cells, Madin-Darby bovine kidney (MDBK) cells, bovine turbinate (BTu) cells, swine peripheral blood mononuclear cells (PBMCs) and bovine PBMCs were each inoculated with a 2020 SVA isolate. Cells were used in the PrimeFlow assay with a VP1 probe to detect percentage of cell infected with SVA. Six colostrum-deprived (CD) Holstein calves were intranasally challenged with SVA with four sham challenged animals as controls. Animals were observed daily for vesicular disease and lameness and sampled regularly for PCR and neutralizing antibody testing.

## Results

One hundred percent of ST, PK-15, and Btu cells were positive for SVA VP1 protein and 99.8% of MDBK cells were positive by flow cytometry. Less PBMCs were infected with an average of 10% of bovine PBMCs and 8% of swine PBMCs positive for SVA. No clinical signs of vesicular disease were observed in the six challenged CD calves. Four calves had a single PCR positive nasal swab on either 2 or 3 days post infection; however the Ct values were high, thus not suggesting there was any productive replication of SVA. There was no evidence of neutralizing antibodies against SVA in serum.

## **Discussion and Conclusion**

This work reinforces that in vitro cell culture susceptibility does not always reflect susceptibility of animals in vivo. Based on the isolate used in this study, bovine cell lines were susceptible to SVA infection whereas calves were not susceptible. Thus, SVA not a significant concern for the cattle industry currently; however, as new strains emerge, it is important to monitor for variants that may have a broader host range.

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# GENETIC DETERMINATION OF SURVIVAL UPON PRRSV OUTBREAKS

M. Ballester <sup>1</sup>, J. Tarrés <sup>1</sup>, T. Jové-Juncà <sup>1</sup>, C. Hernández-Banqué <sup>1</sup>, L. Ganges <sup>2</sup>, S. Gol <sup>3</sup>, M. Diaz <sup>3</sup>, <u>L. Fraile</u> <sup>4</sup>, R. Pena <sup>4</sup> <sup>1</sup>Animal Breeding and Genetics Program, Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Spain <sup>2</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain. <sup>3</sup>Selección Batalle SA, Riudarenes, Spain <sup>4</sup>University of Lleida, Lleida, Spain

### **Background and Objectives**

The identification of genetic markers associated to resilient responses could aid in the selection of animals with more robust phenotypes facing external challenges such as infectious diseases in animals sharing the same environment. A major infectious challenge worldwide for pigs is the porcine reproductive and respiratory syndrome virus (PRRSV). In this study, we have explored the ability of five genetic markers to predict the survival rates during a PRRSV outbreak.

#### **Material and Methods**

Ten-week-old female Duroc pigs were naturally infected with a highly pathogenic PRRSV genotype 1 strain (rosalia strain). PRRSV infection was confirmed through serology and RT-qPCR laboratory analysis. Genomic DNA was isolated from blood samples of 49 dead pigs and 80 surviving animals. The 129 pigs were screened for the following genetic markers using qPCR-HRM or end-point PCR protocols: GBP5 (rs340943904), CD163 (rs1107556229), MX1\_c.-547ins+250, SGK1 (rs338508371) and MMRN1 (rs695254451). The risk of dying was analysed with survival analysis using a proportional hazard model including all markers as explanatory variables.

#### Results

The genotype of the animal for the CD163 and GBP5 markers was significantly associated with the number of surviving offsprings. In both markers, the GG genotypes had over two times more risk of dying than genotypes AA and GT, respectively. Animals AA for CD163 had significantly higher survival up to 20 weeks than animals GG (76% vs 47%). Animals with the GT genotype for GBP5 also had significantly higher survival than animals with the GG genotype (72% vs 55%). The interaction of both markers is even more significant, animals AA for CD163 and GT for GBP5 had a 90 % survival rate. Animals with the GG genotype for both markers had 16 times more risk of dying than those with other genotypes and only survived 40% of the animals in this category during the outbreak.

## **Discussion and Conclusion**

Our results indicate that the mortality in growing pigs affected by highly pathogenic PRRSV strains could be improved through GBP5 and CD163 marker selection. The interaction between both genotypes could be explained by the role of these genes in virus entry and the subsequent innate immune response.

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# IMPACT OF NON-COMPLIANCE OF RECALL PRRSV VACCINATION IN SOWS ON PRRSV INCIDENCE AND GENETIC DIVERSITY IN NURSERIES.

#### H. Clilverd <sup>1</sup>, G. Martin-Valls <sup>1</sup>, Y. Li <sup>1</sup>, M. Martin <sup>1</sup>, M. Cortey <sup>1</sup>, E. Mateu <sup>1</sup>

<sup>1</sup>Department de Sanitat i Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain

#### **Background and Objectives**

PRRSV-1 exhibits considerable genetic diversity, yet the precise mechanisms governing its generation remain incompletely elucidated. The main objective of this study was to examine the evolution and persistence of PRRSV-1 over time within an endemically infected and vaccinated farm.

## Material and Methods

An 8-month observational study was conducted on a farrow-to-fattening farm (1,700 sows, farrowing batches every three weeks), where sows received PRRSV vaccination quarterly. The study monitored three farrowing batches of the same year, during which piglets were followed from birth to 9 weeks of age (umbilical cords and blood samples analysed by RTqPCR). An incident leading to improper vaccine storage occurred during the last blanket vaccination before the sampling of the third batch. Fifty-five samples were whole genome sequenced (Illumina Miseq), while 213 samples underwent ORF5 Sanger sequencing. Anti-PRRSV-1 ELISA antibodies were assessed at 3 weeks of age.

#### Results

Viral circulation was primarily confined to the nursery facilities, with vertical transmission occasionally detected. A notable surge in circulation was observed in the third batch restricted to 6- and 9-week-old pigs with vertical transmission unaffected. Thus at 6 weeks of age the cumulative incidence of PRRSV for batches 1 to 3 was 20%, 6% and 99%, respectively Interestingly, the percentage of seropositive piglets at 3 weeks of age dropped significantly compared to earlier batches (83% and 88% in batches 1 and 2 vs. 37% in batch 3). This decline was most likely attributed to a vaccination failure during summer months. Phylogenetic analyses based on complete genome and ORF5 revealed the persistence of the same viral strain over the study period. Notably, the viral variant from the third batch exhibited an increased genetic diversity compared to the same strain detected in earlier batches. The reestablishment of the vaccination program ultimately led the farm to achieve a PRRSV-negative status.

#### **Discussion and Conclusion**

The results highlight that a disruption in the vaccination protocol led to a surge of both incidence and genetic diversity of a low-level circulating PRRSV-1 strain in weaners, linked to a decline in maternally derived antibody levels and emphasizing the importance of strict compliance to the vaccination schemes.

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# PCV2 PREVALENCE AND SUBTYPING IN THE NETHERLANDS

<u>J. Kok</u><sup>1</sup>, K. Koenders - Van Gog<sup>2</sup>, T. Van Berlo<sup>3</sup>, V. An<sup>3</sup>, T. Meyns<sup>3</sup> <sup>1</sup>Lintjeshof Farm Health Solutions <sup>2</sup>Lintjeshof Veterinary Practice and Merefelt Livestock Diagnostics <sup>3</sup>ZOETIS Benelux, Mercuriusstraat 20, 1930 Zaventem, Belgium

## **Background and Objectives**

PCV2 is a small, nonenveloped single stranded DNA virus and is associated with a high mutation rate. Recent research suggests that the genotype of PCV2 on farms is changing to PCV2d. To monitor the changes of PCV2 types present in the Netherlands, pigs of various ages were sampled on 50 farms.

## **Material and Methods**

Between February 2023 and June 2023, 50 farms were selected based on the potential presence of (sub)clinical signs of PCV2, such as reduced growth, poor performance and wasting syndrome. In each of the 50 farms, oral fluids were collected from pigs in the finisher barn from start, middle and end of the fattening period. Samples were investigated with PCV2 Q-PCR and when the viral load was above 10<sup>4</sup> genetic copies per mL, subtyping for PCV2a, PCV2b and PCV2d was performed by PCR (Kylt® PCV-2 Typing kit). Additionally, information from sampled farms was collected by a survey.

#### Results

On 74% of farms, PCV2 was found in oral fluids and in 48% of samples PCV2 subtyping was performed. With 54%, PCV2d was the most detected genotype, followed by PCV2a (28%) and PCV2b (18%). Farms not vaccinating piglets for PCV2 were more often positive and had on average 100 times higher PCV2 load. No relation was found between PCV2 load and (sub)clinical signs, nor was there a relation between PCV2 load and age of positive sampling.

# **Discussion and Conclusion**

The results of the PCV2 prevalence project revealed the presence of PCV2 in many of the sampled farms. PCV2d was found in more than half of the samples eligible for subtyping. This could indicate that on farms where (sub)clinical signs of PCV2 are present, changes in PCV2 genotype should be taken into consideration.

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# PERFORMANCES IN SWIAV-VACCINATED PIGS AND THE CORRELATION WITH THE QUANTIFICATION OF PATHOGENS

<u>G. Ramis</u><sup>1</sup>, F. Murciano<sup>2</sup>, E. Goyena<sup>3</sup>, B. Gonzalez-Guijarro<sup>4</sup>, A. Cuellar-Flores<sup>1</sup>, D. Espigares<sup>5</sup>, K. Lillie-Jaschniski<sup>6</sup> <sup>1</sup>Dpto. Producción Animal, Facultad de Veterinaria., Universidad de Murcia, Spain <sup>2</sup>Facultad de Veterinaria. Universidad de Murcia. Spain

<sup>3</sup>DEPARTAMENTO DE SANIDAD ANIMAL, FACÚLTAD DE VETERINARIA, UNIVERSIDAD DE MURCIA, SPAIN <sup>4</sup>Departamento de Producción Animal. Universidad de Murcia

<sup>5</sup>Ceva Salud Animal, Barcelona, Spain

<sup>e</sup>Ceva Santé Animale SA, 10 Avenue de la Ballastière, Libourne, France

# **Background and Objectives**

The prevalence of Swine Influenza Virus (swIAV) is very high in most pig producing countries. In many farms the infection is considered subclinical and often its effect on the animals is systematically underestimated. However, as a primary virus, its presence in pig herds can be expected to have impact on the productive performance. The objective of this study was to compare the productive performances of fattening pigs vaccinated with two swIAV vaccines (RESPIPORC FLUpan H1N1 and RESPIPORC FLU 3) simultaneously, in a case-control study.

#### **Material and Methods**

Two batches of 300 piglets (VAC1 and VAC2) were vaccinated at the beginning of fattening with both vaccines simultaneously and one batch was taken as unvaccinated controls (CON1). In every batch 200 animals were individually weighed at start finishing period (W1) and at 100 days of finishing period (W2). The weight at slaughter (WS) and the FCR were recoded recorded and calculated as an average for all batches. To compare the average data, raw data were normalized using the formulas proposed by INRA (2001). Moreover, swIAV, PRRSv, Mesomycoplasma hyopneumoniae, Mesomycoplasma hyorhinis and PCV2 were quantified in oral fluids from 6 pens each group every two weeks (7 samplings). The Ct value for the pen was assigned to each individual animal.

#### Results

The average weight (kg.) at finishing start was CON1=22.86, VAC1=22.16 and VAC2=26.17 (p<0.001). The W2 was CON1=79.9, VAC1=96.4 and VAC2=105.6 (P<0.001). The average normalized WS was CON1=110.1, VAC1=107.6 and VAC2=115, ADGN was CON1=0.748, VAC1=0.742 and VAC2=0.794 (p<0.001) and FCRN was CON1=2.431, VAC1=2.418 and VAC2=2.576. There were positive correlations between the Ct values of swIAV with W1 at weeks 1, 3, and 5 and among Ct values of PRRSv at weeks 1, 3, 5, 7 and 9 with W1 and W2.

## **Discussion and Conclusion**

The VAC2 group showed a significant difference in the weights obtained and in the ADG compared to CON. However, this difference was not observed in FCR since it was introduced with higher initial weight. The positive correlations between the Ct of the two viruses and the weight parameters are interesting, indicating that the detected viral load influences the growth parameters significantly.

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# PRODUCTION OF PSEUDORABIES-FREE FINISHING PIGS AND GILTS ON A PSEUDORABIES VIRUS INFECTED FARM WITH FARROW-TO-FINISH PATTERN IN CHINA

J. Zhang <sup>1</sup>, <u>P. Mortensen</u><sup>2</sup>, N. Guerra <sup>2</sup>, D. Niu <sup>1</sup> <sup>1</sup>Ceva Animal Health China <sup>2</sup>Ceva Animal Health France

## **Background and Objectives**

Pseudorabies (PR) (Aujeszky's disease) is one of the main pig viral diseases and the cause of considerable economic losses in the pork production industry. It was widely reported that PR had reoccurred in many swine farms across China since late 2011. The aim of this study was to produce Pseudorabies free pigs and gilts by optimizing the vaccination and biosecurity management on a PRV infected farm in China.

# **Material and Methods**

The study was performed in a 3000-sow and 116-boar farrow-to-finish farm located in northern China. Before April 2022, vaccine-A (PRV MLV) was used. Boars and sows were mass vaccinated intramuscularly (IM) every 3 months. Piglets were vaccinated by intranasal spray the first day of life, and IM at 7 and 10 weeks of age (WOA) with vaccine A, one more shot for young gilts and boars at 25 WOA before mated. Following internal evaluation (data not shared), vaccine-A was replaced with vaccine-B (Auphyl® plus; Ceva, China) starting June 2022. Piglets were vaccinated at 10 and 13 WOA while other program was implemented as previous except the vaccine replacement. PR status before (30 sera samples for sows and 15 sample/period for pigs 90 and 150 days old) and during the study (ongoing) was determined by serology of gE antibodies using a commercial ELISA kit (IDEXX-PRV gI). Boars would be culled if detected gE positive or suspect from then. Farm adopted strict age-segregated (all-in-all-out) production during the period. Breeding herds' renewal rate kept 30% as normal.

## Results

Before implementation of vaccine-B and procedures, gE positive rate of sows, 90-day-old growers, and 150-day-old finishers, reached 43%, 40% and 100%, respectively. After 2 months, 20 randomly selected 90-day-old young gilts from the herds demonstrated gE negative. All finishers and gilts were tested as gE negative in March-23 (104, 120 and 150 day-old) and again in September-23 (118, 143, 160, 175, 200, 220, and 230 day-old).

## **Discussion and Conclusion**

The combined implementation of recommended vaccination schedule of vaccine-B and recommended basic management procedures demonstrated full PRV-viral control with a potential of eradication.

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# RETROSPECTIVE STUDY OF THE MOLECULAR IDENTIFICATION OF PRRS, PCV2 AND PCV3 IN MEXICO

<u>J.F. Rivera-Benitez</u><sup>1</sup>, J. De La Luz-Armendáriz<sup>2</sup>, F. Diosdado-Vargas<sup>1</sup>, L. Gómez<sup>1</sup>, M. Macías-García<sup>3</sup> *INIFAP* 

<sup>2</sup>Facultad de Medicina Veterinaria y Zootecnia. UNAM. <sup>3</sup>Private Practice Veterinarian

# **Background and Objectives**

Among the main factors that cause a negative impact on swine production is the presence of viral agents such as the porcine reproductive and respiratory syndrome virus (PRRS) and porcine circovirus type 2 and 3 (PCV2 and PCV3). In Mexico, prevalence of 78 to 84% have been demonstrated for PRRS, for PCV2 a prevalence of between 12 and 87% has been described depending on the circulating genogroup and PCV3 has not been widely described in Mexico. The objective of this study was to identify the presence of PRRS, PCV2 and PCV3 in swine production units in the states of Guanajuato and Jalisco, Mexico.

## **Material and Methods**

Serum samples were collected from 144 production units in the state of Guanajuato (n=2898 sera), they were collected between 2012 and 2013; and samples from 717 production units in the state of Jalisco (n=10,799 sera) from 2013 to 2015. The samples were used for the extraction of RNA and DNA and their subsequent analysis with real-time PCR tests.

## Results

For PRRS, in this study a greater number of cases was recorded in 2012, with a frequency of between 5 and 6%. The frequency identified for PCV2 was 5 to 42%, identifying the greatest number of cases in the 2013. Finally, a frequency of between 2 and 31% was recorded for PCV3. With these results, the presence of PRRS, PCV2 and PCV3 was confirmed from 2012 to 2015, in the Guanajuato and Jalisco states and coinfections were recorded between these three viruses.

## **Discussion and Conclusion**

Chen et al, in 2019 describe that among the main viral agents that are co-infected and that cause economic and productive losses in pig production are PRRS, PCV2 and PCV3, which when interacting worsen the clinical symptoms. In the present study it was identified that, of the pigs infected with the three viruses, in Guanajuato 22% were co-infected. With PCV2 - PCV3, 4% with PCV2- PRRS and 3% with PCV3- PRRS. With respect to Jalisco, PCV2-PCV3 co-infection was recorded in 6% and PCV2-PRRS in 9%. Financing FONSEC SADER-CONACYT 2017-06-292826.

VVD – Virology and Viral Diseases

# ROTAVIRUS INFECTIONS IN NEONATAL PIGLETS IN TWO POLISH SOW HERDS

<u>W. Rybkowska</u><sup>1</sup>, N. Bakkegård Goecke<sup>3</sup>, P. Cybulski<sup>2</sup>, A. Woźniak<sup>1</sup>, L.E. Larsen<sup>3</sup>, T. Stadejek<sup>1</sup> <sup>1</sup>Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Science, Warsaw, Poland

<sup>2</sup>Goodvalley Agro S.A., Przechlewo, Poland

<sup>3</sup>Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark

## **Background and Objectives**

Rotaviruses (RV) are a major cause of diarrhea in humans and animals, including pigs. They cause coinfections with enteric bacteria increasing severity of clinical signs. In pigs RVA, RVB, RVE, and RVH were identified. RVA was considered the most prevalent and pathogenic in pigs but recently IVC was identified as an important factor of neonatal diarrhea, while RVB is more common in older animals. The prevalence of RV groups in Polish pig herds is unknown. The study was launched in order to determine the incidence of RV infections in cases of neonatal diarrhea in two Polish sow herds (3300 and 4000 sows) operating in a large production system.

# Material and Methods

Fecal and intestinal samples were obtained from several live piglets and carcasses from 5 consecutive technological groups. The samples were tested with real time PCR for RVA, RVB, and RVC. Selected samples were subjected to nucleotide sequencing and genotyping.

#### Results

In herd A RVC was detected in diarrheic piglets, from every weekly group. RVA was only detected in 1 of 8 samples of ileum content from two weekly groups. RVB was found in 1 of 5 fecal samples in one group. In herd B the most prevalent was also RVC. RVB was the second most prevalent virus, detected in 4 of 5 weekly groups. RVA was detected in only 1 of 4 ileum content samples from one group. DNA sequencing of VP6 coding inner capsid protein was performed on RVC samples and the sequence analysis showed that the viruses present in each farm were highly similar and the identity of the viruses between the herds was about 93%.

## **Discussion and Conclusion**

The study provided the first evidence of the presence of RVB and RVC in cases of neonatal diarrhea in Polish sow herds. Moreover, RVC may be considered the most important viral pathogen causing neonatal diarrhea in described herds. The results of the study may be used to design a diarrhea control program.

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# THE AFRICAN SWINE FEVER VIRUS DP96R TARGETS IRF3 AND IKK ${\mbox{\tiny \alpha}}$ SUPPRESSING INTERFERON AND CYTOKINE PRODUCTION

N. Dodantenna <sup>1</sup>, <u>L. Jong-Soo</u><sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

#### **Background and Objectives**

DP96R of African swine fever virus (ASFV), also known as uridine kinase (UK), encodes a molecularly uncharacterized protein. Researchers have been examining DP96R alongside other genes to create live attenuated vaccines. While experiments in pigs have explored the impact of DP96R on the pathogenicity of ASFV, the precise molecular mechanism underlying this phenomenon remains unknown. Here, we describe a novel molecular mechanism by which DP96R suppresses IRF3- and IKK $\alpha$ -mediated antiviral responses.

## **Material and Methods**

A large-scale mass spectrometry analysis, interferon  $\beta$  (IFN- $\beta$ ), and NF- $\kappa$ B luciferase assays were conducted to determine the target of ASFV DP96R protein. Immunoprecipitation and immunofluorescence assays confirmed the ASFV DP96R protein interaction with IRF3 and IKK $\alpha$ . DNA virus replication was evaluated by infecting GFP-tagged ADV, HSV, and VACV viruses into DP96R stably expressing porcine macrophages (PAMs and PIBs), MA104 cells, and transiently transfected PK-15 cells. IFN- $\beta$ , pro-inflammatory cytokines and chemokines secretion were assessed by ELISA. A mutation assay kit was used to construct different IRF3 mutant constructs.

#### Results

DP96R reduced cGAS-STING signaling and anti-viral gene transcription, enhancing DNA and RNA virus replication. Most notably, the ASFV DP96R is associated with the major karyopherin alpha (KPNA) binding residues of IRF3, inhibiting KPNA-activated IRF3 interaction and leading to inhibition of activated IRF3 nuclear translocation, which in turn reduces IFN production. Besides, DP96R specifically interacted with and inhibited the phosphorylation of IKK $\alpha$ , hampering the activation downstream of NF- $\kappa$ B. HSV-GFP induced transcription and translation of pro-inflammatory cytokines and the chemokine was significantly declined by DP96R protein. In particular, we discovered that the ASFV DP96R central region is crucial in suppressing IFNs and pro-inflammatory cytokines production.

# **Discussion and Conclusion**

Our findings emphasize the essential role played by the highly conserved ASFV DP96R in infiltrating the innate immune system. [National Research Foundation (2021R1A6A1A03045495), Ministry for Food, Agriculture, Forestry and Fisheries (119081-5) and Ministry of Environment (NIWDC-2021-SP-02)]

VVD – Virology and Viral Diseases

# THE USE OF AN IMMUNOPEROXIDASE MONOLAYER ASSAY (IPMA) FOR THE DETECTION OF ROTAVIRUS A ANTIBODIES IN SERUM, COLOSTRUM AND MILK OF VACCINATED AND UNVACCINATED GILTS

K. Visscher <sup>1</sup>, <u>A. Dereu</u><sup>2</sup>, E. Seesing <sup>2</sup>, H. Van Gisteren <sup>2</sup>, H. Nauwynck <sup>3</sup>

<sup>1</sup>Coöperatie VARKENSARTSEN

<sup>2</sup>Kernfarm B.V., Breukelen, The Netherlands

<sup>3</sup>Laboratory of Virology, Faculty of Veterinary Sciences, Ghent University. Salisburylaan 133, 9820 Merelbeke, Belgium.

## Background and Objectives

Rotavirus A is known to cause diarrhea in suckling piglets especially from gilts (Theuns et al., 2016). Its role is frequently underestimated. Here we describe a case where rotavirus A was diagnosed through a virus whole genome sequencing technique (Pathosense). It was the purpose to examine the effect of a double vaccination of gilts with FIXR® Rota Coli vaccine at the end of gestation on the anti-rotavirus A antibody titer in the serum, colostrum and milk of the gilts. Case description:

A 550 sow farm in the Netherlands had a history of diarrhea in newborn piglets from gilts. Although the piglet mortality was rather limited before weaning, the body condition at weaning was very variable. A vaccination strategy was planned to solve this problem.

## Material and Methods

Two groups of each 10 gilts were used in this experiment. Half of the gilts were vaccinated twice at 75-79 days and 96-100 days of gestation (swine registrated Rota Coli vaccine); half of the gilts remained unvaccinated. Blood was collected from the gilts at 75 days and 112 days of gestation. In addition, colostrum/milk was collected at 1, 4 and 7 days after birth. Anti-rotavirus A antibodies were determined in the collected sera and colostrum/milk. For this purpose, an IPMA-test was developed based on MA104 cells infected with rotavirus A 12R050 (Belgian isolate) and used to detect anti-rotavirus A antibodies and to determine the antibody titer in 2 groups of each 10 gilts.

#### Results

In the two groups, the vaccinated gilts had higher antibody titers (2,560-40,960) than the unvaccinated gilts (160-2,560) after vaccination. The antibodies in the colostrum/milk of vaccinated gilts decreased from 40,960-163,840 at 1 day after birth to 160-10,240 at 7 days after birth. The antibodies in the colostrum/milk of the unvaccinated gilts dropped from 2,560-163,840 at 1 day after birth to 40-160 at 7 days after birth.

## **Discussion and Conclusion**

A double vaccination of gilts with FIXR® Rota Coli vaccine at the end of gestation resulted in a higher concentration of anti-rotavirus A antibodies in serum, colostrum and milk. These higher antibody titers in colostrum and milk will give a better protection of piglets during the first weeks after birth.

VVD – Virology and Viral Diseases

# CHARACTERISTICS OF SWINE INFLUENZA A VIRUS SURVEILLANCE AND MONITORING SYSTEMS IN EUROPE IN 2022

G.A. Puspitarani <sup>1</sup>, <u>K. Pedersen <sup>2</sup></u>, G. Simon <sup>3</sup>, G. Dauphin <sup>4</sup>, L.E. Larsen <sup>2</sup>, T. Tobias <sup>5</sup>

<sup>1</sup>Unit of Veterinary Public Health and Epidemiology, University of Veterinary Medicine Vienna, Veterinaerplatz 1, 1210 Vienna, Austria & Complexity Science Hub Vienna, Josefstaedter Strasse 39, 1080 Vienna, Austria

<sup>2</sup>Department of Veterinary and Animal Sciences, Stigbøjlen 4, DK 1870 Frederiksberg C, University of Copenhagen, Denmark

<sup>3</sup>Anses, Ploufragan-Plouzané-Niort Laboratory, Swine Virology Immunology Unit, Ploufragan, France,

<sup>4</sup>Ceva Santé Animale, Scientific Support and Investigation Unit, Libourne, France

<sup>5</sup>Royal GD, Deventer, The Netherlands & Utrecht University, Faculty of Veterinary Medicine, Dept. Population Health Sciences

# **Background and Objectives**

Attention from researchers and One Health institutes is directed towards swine Influenza A Viruses (swIAV) because of their impact on animal health and productivity. Expanding genetic diversity increases the risk of the emergence of strains with zoonotic potential. Thus, there is a shared need for surveillance of swIAV and its genetic diversity.

There is currently no standardized and cooperative scheme for efficient surveillance of swIAV in pig herds across Europe. As a preparation for improved guidelines on swIAV surveillance, Working Group 3 of the European Swine Influenza Network (ESFLU) conducted an electronic survey to characterize existing swIAV surveillance/monitoring systems in Europe in the year 2022.

#### **Material and Methods**

An e-survey (using Qualtrix<sup>™</sup>) was distributed to 76 ESFLU participants representing 25 countries in July 2023 and remained open for approximately three months. Descriptive analyses of results were performed using R programming language.

## Results

The survey gathered responses from 44 entries representing 39 institutions from 25 countries. Of these, 17 respondents described 26 swIAV surveillance/monitoring systems. Their focus is on detecting emerging pig-specific strains as a primary or secondary objective (8 and 9, respectively). Other objectives are detecting novel zoonotic strains (9) or unknown variants. Nine of 16 national programs are permanently funded. Four of these nine share genetic sequences with OFFLU, and eleven consistently perform comparative genetic analysis. No aligned approach between schemes could be identified. Sampling during clinical outbreaks is the approach in fourteen systems, and sampling of clinically affected pigs is performed in eighteen systems.

#### **Discussion and Conclusion**

Given the targeted sampling approach, we conclude that swIAV passive surveillance is performed throughout Europe but with variety in intensity. The most common aim is to detect emerging pig-specific strains. We conclude that there is difference in the design and objectives of surveillance and monitoring systems for swIAV in Europe, and no harmonized approach exists. Further research of entries to this e-survey will form the basis for establishing optimized guidelines for future swIAV surveillance in pigs.

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# COMPARISON OF A NOVEL RAPID TONSIL-ORAL-SCRAPING (TOSC) SAMPLING METHOD TO SERUM, ORAL FLUID, AND TONSIL SCRAPING TO DETECT PRRSV IN SOWS OVER TIME

P. Li<sup>2</sup>, T. Petznick<sup>1</sup>, E. Pratt<sup>1</sup>, G. Cezar<sup>2</sup>, K. Will<sup>2</sup>, M. Mil-Homens<sup>2</sup>, H. Tong<sup>2</sup>, I. Machado<sup>2</sup>, D. Moraes<sup>2</sup>, R. C. Paiva<sup>2</sup>, A. Berte<sup>2</sup>, H. Osemeke<sup>2</sup>, C. Mainquist<sup>3</sup>, P. Yeske<sup>4</sup>, G. S. Silva<sup>2</sup>, <u>D. Linhares<sup>2</sup></u>

<sup>1</sup>ArkCare, Omaha, USA <sup>2</sup>Iowa State University, Ames, USA <sup>3</sup>Pillen Family Farms, Columbus, USA <sup>4</sup>Swine Vet Center, St Peter, USA

#### **Background and Objectives**

Our team developed a novel sow Tonsil-Oral-Scraping (TOSc) collector, which collects fluids within seconds from sow oral/tonsillar area with/without snaring and showed comparable PRRSV RNA detection rate with tonsil scraping and higher detection rate than serum in acutely infected sows. As PRRSV detection rate varies among different sample types over time, this study aimed to compare sow PRRSV detection rate by RT-qPCR between TOSc, serum, oral fluid (OF), and tonsil scraping at three time points post live virus inoculation (LVI).

#### **Material and Methods**

A breeding-herd seeking PRRSV elimination after outbreak was selected. Four different sample types were collected from same 60 sows at 1, 2, and 3 months after LVI, respectively. OF were collected by hanging a rope in front of each sow. Afterwards, other samples were collected in the order of TOSc, tonsil scraping, and serum when the sows were snared. All samples were tested individually for PRRSV by RT-qPCR. A logistic regression model was built. Tukey-Kramer test was used for pairwise comparisons of detection rates.

#### Results

In the 1<sup>st</sup> month, there was no significant difference in detection rate between TOSc (66.3%, [50.9-78.7%]) and tonsil scraping (85.3%, [71.2-93.2%]), but TOSc had significantly higher positivity than OF (8.8%, [3.3-21.9%]) and serum (13.2%, [5.9-27.1%]). In the 2<sup>rd</sup> month, there was a significant difference between TOSc (40.9%, [29.8-53.1%]) and tonsil scraping (74.2%, [62.4-83.3%]), and a significant difference between TOSc and OF (8.0%, [3.0-19.5%]) and serum (12.1%, [6.2-22.4%]). In the 3<sup>rd</sup> month, there was a significant difference between TOSc (3.28%, [0.5-16.9%]) and tonsil scraping (29.5%, [17.2-45.7%]), and no significant difference between TOSc and OF (17.86%, [5.9-42.6%]) or serum (9.84%, [3.6-24.1%]).

#### **Discussion and Conclusion**

PRRSV detection rate for TOSc and tonsil scraping decreased over time, while that for OF and serum remained relatively constant, suggesting a similar PRRSV detection pattern between TOSc and tonsil scraping and potential large-scale use of TOSc for sow PRRSV detection. However, in the 2<sup>nd</sup> and 3<sup>rd</sup> month after LVI, a significant difference between TOSc and tonsil scraping was probably due to decreased tonsillar material collected when the sows were restrained and mouth held open, reducing abrasion between TOSc and tonsils, which requires further research.

VVD – Virology and Viral Diseases

# COMPARISON OF LUNG LESIONS PRE AND POST PIG VACCINATION WITH RESPIPORC FLU3 IN A FARM IN NORTHERN IRELAND

J. Borobia Belsue <sup>1</sup> <sup>1</sup>Mossvet Ltd

#### **Background and Objectives**

Swine Influenza virus (swIAV) is commonly detected on pig farms in Europe and can result in respiratory disease and a reduction in reproductive performance. It is a highly important pathogen both in terms of economic impact as well as its zoonotic potential. The objective of this study was to determine whether vaccination of piglets against swIAV had an effect on the prevalence of lung lesions in a farm affected with the virus.

## **Material and Methods**

A farrow to finish farm located in Northern Ireland presented with general respiratory symptoms, predominantly coughing. A high level of bronchopneumonia was observed during lung assessment at the abattoir, despite good control of Mycoplasma hyopneumoniae through vaccination. Six lung tissue samples were submitted for PCR analysis with all testing negative for PCV2, PRRS and Mycoplasma hyopneumoniae. However, Influenza type A was identified in all samples, with CT values ranging from 19.5 to 36.7. Pasteurella multocida was also cultured from the samples. As a result, piglet vaccination with Respiporc Flu3 was implemented at 4 and 7 weeks old, prescribed in accordance with the Cascade.

## Results

Pre and post vaccination comparison was based on lung assessments performed at the abattoir using the Goodwin scoring method. Pre swIAV piglet vaccination, the Pneumonia Index (which is based on the percentage of lungs affected and the severity of those lesions) was 5.4 and 50% of the pigs were affected. Post swIAV piglet vaccination, significant reductions were recorded in both the Pneumonia Index and the percentage of pigs affected, with values of 2.8 (P<0.05) and 23.3% (P<0.05) respectively.

#### **Discussion and Conclusion**

The results obtained in this study demonstrate that vaccinating pigs against swIAV at an early stage can help to control respiratory disease in the growing and finishing stages, by reducing both clinical signs and the severity of lung lesions, evaluated with the Goodwin score, in farms infected with swIAV.
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### COMPARISON OF THREE DIFFERENT COMMERCIALLY AVAILABLE ENZYME-LINKED-IMMUNOSORBENT-ASSAYS (ELISAS) AND HEMAGGLUTINATION INHIBITION TEST (HI) FOR THE DETECTION OF ANTIBODIES AGAINST SWINE INFLUENZA VIRUSES (SWIAV)

<u>J. Stadler</u><sup>1</sup>, K. Grau <sup>1</sup>, K. Lillie-Jaschniski <sup>2</sup>, S. Pesch <sup>3</sup>, A. Graaf-Rau <sup>4</sup>, T. Harder <sup>4</sup>, M. Ritzmann <sup>1</sup> <sup>1</sup>Clinic for Swine at the Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-Universität München, Oberschleißheim, Germany

<sup>2</sup>Ceva Tiergesundheit GmbH

<sup>3</sup> Ceva Innovation Center, Dessau, Germany

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut (FLI), Suedufer 10, 17493 Greifswald-Insel Riems, Germany

## **Background and Objectives**

The combination of indirect and direct detection methods can be beneficial for swIAV diagnosis, particularly in herds with low incidence scenarios. Studies on the performance of different serological tests, used for routine diagnosis, are scarce. Therefore, the aim of the present study was to evaluate the use of different serological detection methods as screening tools for identification of herds enzootically infected with swIAV.

### **Material and Methods**

Blood samples were available from 25 German sow farms with proven enzootic swIAV infection. Blood samples were collected from 30 sows per farm of different parities and production stages and tested for swIAV antibodies by hemagglutination inhibition (HI) test (H1avN1, H1huN2, H1pdmN1, H1pdmN2, cut-off titer ≥20). Subsequently, all 749 serum samples were analyzed for swIAV antibodies with three different commercial ELISA Kits comprising an indirect ELISA (ELISA 1: ID Screen® Influenza A Nucleoprotein Swine Indirect; ID.vet, Grabels, France), and two competitive ELISAs (ELISA 2: ID Screen® Influenza A Antibody Competition; ID.vet, Grabels, France, and ELISA 3: IDEXX Influenza A Ab Test; IDEXX, Maine, United States). ELISAs were performed according to the protocol recommended by the manufacturers.

#### Results

Out of the 749 samples 700 were positive by ELISA 1, 614 by ELISA 2 and 530 by ELISA 3. HI positivity to at least one antigen was detected in 703/749 sera. Of a total of 46 HI negative samples 24 were positive by ELISA 1, 17 by ELISA 2 and 11 by ELISA 3 respectively. Overall, the agreement between the ELISAs and the HI was fair ( $\kappa$ =0.40). The indirect ELISA 1 showed highest agreement with the HI ( $\kappa$ =0.43), whereas agreement between ELISA 2 ( $\kappa$ =0.25) and HI was fair and slight between ELISA 3 and HI. ( $\kappa$ =0.18).

## **Discussion and Conclusion**

The results underline the usefulness of ELISAs as screening tests for seroepidemiological investigations of swIAV. However, as the performance varied between the different ELISAs, caution should be applied when selecting the appropriate purpose for the different test kits. With regard to HI testing, the extension of the antigen panel used might help to reduce the occurrence of false negative results but may also grossly increase labour, time and costs.

VVD – Virology and Viral Diseases

# FINE-SCALE ANALYSIS WITHIN OWNERS OF INFLUENZA A VIRUS IN SWINE REVEALS PERSISTENT CLADE CIRCULATION AND LIMITED CLADE DIVERSITY

<u>B. Arruda</u><sup>1</sup>, M. Thomas<sup>2</sup>, A. Vincent Baker<sup>1</sup>, T. Anderson<sup>1</sup>, G. De-Sousa-E-Silva<sup>2</sup>, P. Gauger<sup>2</sup> <sup>1</sup>Agricultural Research Service United States Department of Agriculture <sup>2</sup>Iowa State University College of Veterinary Medicine

## **Background and Objectives**

Influenza A virus (IAV) remains a dominant cause of morbidity in swine with continuous expansion of genetic and antigenic diversity due to incursions of human IAV and evolution in swine. The aggregated national USDA IAV swine surveillance system data documents significant numbers of co-circulating genetic clades; yet, less is known about the diversity present at finer spatial scales or within epidemiologically linked populations of swine within an owner.

## **Material and Methods**

To identify the amount and duration of diversity shared among sites with the same owner, we conducted a fine-scale analysis of IAV at the owner level using large scale data mining of nearly 8,000 hemagglutinin (HA) sequences collected and submitted between January 2013 and June 2021 to a veterinary diagnostic laboratory.

## Results

Twenty-five HA clades were detected in the data set, and fourteen of these clades were detected more than twenty times. Of the top twenty-three contributing owners, clinical clade count ranged from 2/25 (8%) to 9/25 (36%). Within the top 6 contributing owners, the average length of time a clade was detected was 3.6 years, most clades were detected for longer than 2 years, and some clades were detected for at least 8 years. A clade was commonly detected within an owner across multiple farm types, including growing, breeding, and isolation stock. Evaluation of H3 antigenic motifs identified four motifs made up nearly 60% of the detections.

## **Discussion and Conclusion**

Fine-scale analysis demonstrated that the number of detected HA genetic clades and H3 predicted antigenic diversity at the owner level is much less than the national level, supporting the use of autogenous and custom vaccines for control at the owner level. For the owners we evaluated, the duration of circulation of a specific clade detected indicated that a vaccine update every 3 to 4 years may be sufficient to facilitate control and preventative measures unless a lateral introduction of a new clade is detected. Continuous introduction of susceptible animals including gilts and newborn piglets may contribute to persistent clades that circulate for multiple years within an owner, suggesting vaccination of incoming gilts and sows may achieve a more homogenous and less susceptible population.

VVD – Virology and Viral Diseases

# INVESTIGATION OF PORCINE CIRCOVIRUS TYPE 2, TYPE 3 AND TYPE 4 IN SWINE CLINICAL SAMPLES IN BRAZIL

R. Miotto <sup>1</sup>, C. Pissetti <sup>2</sup>, C. Reck <sup>3</sup>, M. Martelo <sup>3</sup>, á. Menin <sup>4</sup>, L.C. Bordin <sup>5</sup>, <u>J. Ciacci Zanella</u> <sup>5</sup>

<sup>1</sup>Federal Institute of Education, Science and Technology, Concórdia, SC, Brazil

<sup>2</sup>Center for Animal Health Diagnoses (CEDISA) BR 153, km 110, SC, 89715-899, Distrito de Tamanduá, Concórdia, SC, Brazil

<sup>3</sup>Department of Research and Innovation, VERTÁ Laboratórios, Curitibanos, SC , Brazil <sup>4</sup>Department of Biosciences and Unified Health - Federal University of Santa Catarina, Curitibanos, SC, Brazil <sup>5</sup>Brazilian Agricultural Research Corporation (EMBRAPA), Concórdia, Brazil

## **Background and Objectives**

Porcine circovirus (PCV), includes PCV1, PCV2, PCV3, and PCV4 that can infect pigs. PCV2 causes PCV disease (PCVD) which can be controlled by vaccination and management practices. However, new genotypes and co-infections with other PCVs can aggravate PCVD. This work studied the etiology of PCVD in PCV2 vaccinated herds by characterizing PCV2 genotypes, and PCVs coinfections. Also, verified the presence of PCV3 and PCV4 in samples from PCV2 positive herds.

## **Material and Methods**

PCVD clinical cases samples diagnosed with PCV2 in 2021 and 2022 were used. DNA from 333 samples (serum, uterus, kidney, lung, lymph node, intestine, fetuses, brain) from 333 PCV2 vaccinated pigs, 191 farms (all production phases) and 8 Brazilian states was extracted. PCV2, PCV3 and PCV4 genome detection was carried out using real-time PCR (qPCR) assay as previously described. For PCV2 genotyping, a PCR aimed the PCV2 ORF2 region, having each sample tested for PCV2a, PCV2b and PCV2d.

#### Results

PCV2b and PCV2d genotypes were the most prevalent genotypes from all Brazilian states examined. PCV2b was the most prevalent genotype, detected in 151 out of 266 genotyped samples. PCV2b was most common genotype in nurseries, but PCV2d was more frequent in finishing and sow's herd. Finishing phase had the largest number of PCV2 positive samples, possibly due to decreased vaccine protection. Since the majority of vaccines used in Brazil are for PCV2a genotype, PCV2a was not found in any clinical samples. Co-infections between genotypes (PCV2b and PCV2d) was detected in 27 samples and between PCV3 and PCV2 in 26 samples from our study. In 320 samples analyzed, none was positive in qPCR tests for PCV4.

## **Discussion and Conclusion**

PCVD is an important disease for Brazilian and global pig farming. Challenges include the control of known infectious agents, but also the identification new viruses, mainly in mixed infections in vaccinated herds. This study found that PCV2b is prevalent in Brazilian swine herds, with an increase in PCV2d detection. Coinfections between PCV2 genotypes and PCV3 are observed. PCV4 or PCV2a DNA were not identified in tested herds. Monitoring vaccine effectiveness and the emergence of clinical circovirus is crucial.

#### VVD – Virology and Viral Diseases

# MONITORING OF ROTAVIRUS FECAL EXCRETION BY SUCKLING PIGLETS ISSUED FROM VACCINATED SOWS IN AN ENZOOTIC CONTEXT OF NEONATAL DIARRHOEA DUE TO ROTAVIRUS

E. Bousquet <sup>1</sup>, A. Lebret <sup>2</sup>, T. Nicolazo <sup>2</sup>, C. Chevance <sup>2</sup>, J. Jeusselin <sup>2</sup>, C. Teixeira-Costa <sup>2</sup>, V. Normand <sup>2</sup>, P. Berton <sup>3</sup>, A. Morillo Alujas <sup>4</sup>, G. Boulbria <sup>2</sup>

<sup>1</sup>Virbac SA,13ème rue, 06510 Carros, France

<sup>2</sup>Rezoolution Pig Consulting Services, Noyal-Pontivy, France

<sup>3</sup>Virbac France, Espace Azur Mercantour, 3ème Rue, 06510 Carros, France

<sup>4</sup>Get in Touch, 22400 Monzon, Huesca, Spain

## **Background and Objectives**

Rotavirus type A (RVA) is often isolated in piglet neonatal diarrhoea cases. This study was performed to assess the effect of a sow vaccine (SUIGEN® ROTA COLI, combining E. coli F4/F5/F6/F41 and RVA) on RVA fecal excretion in a context of diarrhoea due to RVA.

#### Material and Methods

Twenty-three pregnant sows from the same batch were randomly allocated to T or C group according to parity. Tested vaccine was administered twice at 6 and 3 weeks before farrowing in all sows of the T group. In C group, farm vaccine (combining E. coli F4/F5/F6 and Clostridium perfringens type C toxoid) was administered once at 3 weeks before farrowing on sows and twice on gilts (6 and 3 weeks before farrowing). Fecal swabs were taken on all piglets on D2, D5 and D21 (D0: birth, D2: diarrhoea start, D5: diarrhoea peak, D21: weaning). Parallely, at all stages, fecal consistency and general health scoring were recorded. Swabs were scrubbed on FTA® spots being stored at +4°C pending shipment to the laboratory (Anicon, Germany). RVA RT-PCR was done on each spot. Result was expressed as the Ct value or not detectable. Sequencing of VP4 and VP7 genes was done for one positive sample per date. Groups were compared by Fisher exact test. Correlation between Ct and clinical scores was done by linear regression.

#### Results

The percentage of PCR positive fecal samples was lower in T than in C group on D2 (respectively 51.9 and 64.4%, p < 0.05). This percentage was not different between groups on D5 (96.2%) and D21 (73.7%). The correlation between Ct and fecal or general score was significant on D2 (p < 0.0005). The three sequenced strains belonged to genotype G9P32.

## **Discussion and Conclusion**

Fecal shedding started early, concerned most of piglets at diarrhoea peak and was still present at weaning, whatever the group. Yet tested vaccine induced a significant decrease of RVA positive fecal samples on D2. As both groups were housed in the same rooms, RVA may have circulated between crates. Though PCR was qualitative, Ct correlated significantly with clinical score on D2.

#### VVD – Virology and Viral Diseases

## MONITORING THE EMERGENCE OF PRRSV VARIANTS

M. Kikuti <sup>1</sup>, M. Melini <sup>1</sup>, X. Yue <sup>1</sup>, I.A. Paploski <sup>2</sup>, N. Pamornchainavakul <sup>1</sup>, J. Baker <sup>1</sup>, D. Makau <sup>1</sup>, K. Vanderwaal <sup>3</sup>, <u>C. Corzo</u>

<sup>1</sup>University of Minnesota

<sup>2</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

<sup>3</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

### **Background and Objectives**

New PRRSV variants are constantly emerging in the population and monitoring them is crucial to quickly identify if there is potential for rapid spread and high impact due to disease severity. Here, we describe the emergence of a new PRRSV variant and provide considerations on how to monitor emerging PRRSV variants.

## **Material and Methods**

A sequence originating from one sow herd undergoing what they consider a mild PRRS outbreak in June 2022 was used as a reference. Additional cases were identified by a nucleotide identity of ≥98% between samples. Cases were described in space and time and time-to-stability (TTS) was calculated for breeding herds.

#### Results

A total of 382 case sequences were identified in sixteen production systems up to October 2023. Most (58.4%, 223) sequences originated from breeding sites while 33.3% (127) from grow-finishing sites. Notably, these originated from 115 individual sites (72 grow-finishing sites, 36 breeding sites, and 7 had no farm type information). No space-time clusters were found when accounting for system. However, two clusters in the Midwest were detected when system was not accounted for. Breeding herd stability was reached in 61.3% (19/31) of the outbreaks, within a median TTS of 88 weeks. However, outbreaks in which this was the only variant detected had a median TTS of 59 weeks, while outbreaks with multiple variants had a median TTS of 95 weeks. The average and finishing mortality of growing pig sites affected by this variant were not statistically different than the one found in L1C144 variant affected sites.

#### **Discussion and Conclusion**

To this point, no sufficient evidence was found that this variant is of higher concern, given its limited prevalence and slow geographic expansion. However, median time to stability was higher than previously reported in the literature and growing pig site mortality was higher than observed in other PRRSV variant affected sites, suggesting this may be a variant of interest for continued surveillance. Prospective monitoring of emerging variants should acknowledge the complex relationship between data limitations, multi-variant outbreaks, amongst other factors when considering if a variant is of concern.

#### VVD – Virology and Viral Diseases

# THE COMPETITIVE FITNESS OF DIFFERENT GENOTYPES OF CLASSICAL SWINE FEVER VIRUS UNDER THE SELECTIVE PRESSURE OF ANTIBODIES IN VITRO

<u>H.M. Liu</u><sup>1</sup>, Y.L. Huang<sup>2</sup>, M.C. Deng<sup>2</sup>, H.W. Chang<sup>3</sup>, C.Y. Chang<sup>3</sup> <sup>1</sup>School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan; Veterinary Research Institute, Ministry of Agriculture, New Taipei City, Taiwan <sup>2</sup>Veterinary Research Institute, Ministry of Agriculture, New Taipei City, Taiwan <sup>3</sup>School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

## **Background and Objectives**

Classical swine fever virus (CSFV) belongs to the genus Pestivirus of the family Flaviviridae. In Taiwan, the CSFV genotype 2.1 (G2.1) first emerged in 1994 and had dominated the field since 1996, replacing the historical CSFV genotype 3.4 (G3.4). A shift in virus genotypes was also observed in several CSFV-endemic countries. The attenuated lapinized Philippine Coronel (LPC) vaccine of genotype 1.1 (G1.1) has been used in Taiwan to protect pigs against CSFV until 2023. Whether the selective pressure imposed by vaccination played a role in the genotype shift has not been investigated.

## Material and Methods

To investigate in vitro the viral adaption and competitive fitness of G2.1 and G3.4 CSFV under selective pressure, singleor co-infection of G2.1 and G3.4 was inoculated in PK-15 cells with the presence of either G1.1, G2.1 or G3.4-specific pig antisera or monoclonal antibodies (mAbs), and serially passaged for thirty rounds. The G2.1 and G3.4 CSFV viral loads were quantified by indirect immunofluorescence assay with G2.1 and G3.4-specific mAbs and RT–qPCR with G2.1 and G3.4-specific probes.

#### Results

In single- and co-infection CSFV, the neutralization of CSFV by antibodies was stronger when inoculated with the homologous genotype-specific pig antisera. In addition, G2.1-specific pig antisera neutralized G3.4 stronger in co-infection with G2.1 than the single-infected G3.4. In co-infection CSFV with pig antisera derived from LPC (G1.1), the G3.4 was completely neutralized at the earlier passage than that of G2.1, suggesting that under LPC vaccination policy, G2.1 is more capable of escaping the neutralization. Whereas there were no differences between single- or co-infection of CSFV when inoculated with the presence of mAbs.

## **Discussion and Conclusion**

The present study demonstrated a superior fitness of G2.1 CSFV to G3.4 CSFV in vitro. To study the evolutionary adaptation of CSFV under selective pressure, comparing the ratios of non-synonymous and synonymous mutations on the  $E^{ms}$ , E1, and E2 genes of G2.1 and G3.4 is under investigation. Understanding the mechanism responsible for genotype switching is crucial for controlling and eliminating the disease.

#### VVD – Virology and Viral Diseases

## THE SEQUENCING PUZZLE OF PRRSV

<u>K. Heenemann</u>, S. Galland <sup>2</sup>, F. Goetz <sup>3</sup>, M. Harzer <sup>1</sup>, B. Euring <sup>1</sup>, C.S. Kiesewetter <sup>1</sup>, T.W. Vahlenkamp <sup>1</sup> <sup>1</sup>Centre for Infectious Diseases, Institute of Virology, Faculty of Veterinary Medicine, University of Leipzig, Germany <sup>2</sup>Zoetis Deutschland GmbH, Germany <sup>3</sup>GVG Diagnostics GmbH, Germany

## **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) is a highly relevant disease in pig production. Infection with porcine reproductive and respiratory syndrome virus (PRRSV), causes late abortions in sows and respiratory diseases in weaned piglets and heifers, associated with significantly reduced performance and, thus, very high economic losses. The virus belongs to the family Arteriviridae and was described simultaneously in Europe and North America around 1990 in two different genotypes, which are now known as PRRSV-1 (betaarterivirus suid 1, European type) and PRRSV-2 (betaarterivirus suid 2, North American type). Both genotypes have spread worldwide and have also diversified rapidly through mutation and recombination, which has also led to the emergence of highly pathogenic variants. This disease has been notifiable in Germany since 2021. This study was aimed to optimise existing RT-PCR systems for the detection of PRRSV and validating various sequencing methods in order to differentiate between PRRSV field and vaccine strains and to draw conclusions about their origin.

#### **Material and Methods**

In totel, 40 PRRSV-positive samples, including various materials such as tissue, serum, bronchoalveolar lavage, processing fluid and chewing gauze, were analysed. For this purpose, various RT-PCRs targeting different open reading frames (ORF) were established. Subsequent Sanger sequencing of the RT-PCR products and Next Generation Sequencing (NGS) was used to distinguish between PRRSV field viruses and vaccine viruses and to conduct molecular and virus epidemiological studies.

#### Results

Different sample materials were analysed using RT-PCR in combination with sequencing as well as NGS. Diverse types of material are suited for RT-PCR. Tissue, followed by testicular juice, provides the best results with NGS. The viral load in the sample is critical for both methods.

## **Discussion and Conclusion**

Sequencing and in particular NGS are methods that enable molecular epidemiology and, thus, virus epidemiology. It is possible to create genome profiles and identify possible pathogen variants (quasispecies). Sample management is a critical factor in molecular biology, as errors in sample collection, but above all in storage and/or shipping, can impair sample quality and may affect diagnostic results.

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## ASSOCIATION BETWEEN LESIONS AND CO-INFECTION IN PIGS WITH PRRSV IN ITALY DURING 2022-2023

<u>M. Tonni</u>, N. Formenti <sup>1</sup>, C. Salogni <sup>1</sup>, M.B. Boniotti <sup>1</sup>, A. Donneschi <sup>1</sup>, M. Recchia <sup>1</sup>, G.L. Alborali <sup>1</sup> <sup>1</sup>*IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna), Brescia ITALY* 

#### **Background and Objectives**

PRRS is one of the main diseases involved in the PRDC. The etiological factors involved in the PRDC include also bacteria, other viruses and farm management. PRRSV plays a central role, and the consequences of coinfections with several microorganism are complex. While several studies have analysed coinfections, few have revealed significant results. Baseline studies focused on respiratory pathogens, especially regarding PRRSV coinfections, are very important for deeper understanding of dynamics of infection. The aim of this study was to detect the pneumonia lesions associated to PRDC cases and analyse co-infections associated to PRRSV.

## **Material and Methods**

In this study, we analysed systematically necropsy samples submitted to the Veterinary Diagnostic Laboratory (IZSLER, Brescia), using a standardized diagnostic protocol in suspected cases of PRDC. During necropsy, both the lungs and carcasses were analysed to determine the severity and extension of lesions. Gross lung lesions were classified according to a pre-established anatomo-pathological protocol adapted from existing literature. Lungs and pleural lesions were scored to determine their severity and extension. Additionally, the presence of infectious agents was investigated. Bacterial cultures were adopted to detect A. pleuropneumoniae, P. multocida, Streptococcus spp. and T. pyogenes. PCR tests were utilized for G. parasuis, Mycoplasmas and viruses.

#### Results

A total of 787 samples of lungs and carcasses with PRDC, collected from 443 farms between 2022 and 2023 were analysed. Overall, the most frequently observed lesion was the catarrhal bronchopneumonia. Higher lung and pleurisy scores were detected in the PRRSV positive samples, accounting for 68,49% of the positive samples. The presence of PRRSV correlates with G. Parasuis and PCV2 infections, while M. hyopneumoniae was more frequently detected in PRRSV negative samples.

## **Discussion and Conclusion**

This study shows a high rate of PRRSV positive samples within the necropsy material examined at the IZSLER laboratory. The presence of PRRSV should be considered both as a primary pathogen but also as misshape element in the diagnosis suspect. A more comprehensive analysis aiming to understand the infection dynamics in PRDC is necessary. It's important to note that the data collected over approximatively 20 months from 2022 to 2023, offer a snapshot of the recent situation. Further studies regarding PRRSV coinfection are currently underway to supplement these findings.

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# COMPREHENSIVE GENETIC ANALYSIS OF BETAARTERIVIRUS SUID 2 (PRRSV-2) IN AN INTEGRATED US SWINE PRODUCTION SYSTEM

<u>R. Pogranichniv</u><sup>1</sup>, J. Richt<sup>1</sup>, B. Feehan<sup>2</sup>, D. Marthaler<sup>1</sup>, S. Chaudhary<sup>1</sup>, Q. Ran<sup>2</sup>, W. Valdivia-Granda<sup>3</sup> <sup>1</sup>Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA <sup>2</sup>Division of Biology, Kansas State University, Manhattan, KS 66502

<sup>3</sup>Orion Integrated Biosciences, Inc.

## **Background and Objectives**

The detailed genetic information obtained using NGS allows for a comprehensive genetic analysis of the swine virome in symptomatic and asymptomatic animals, including PRRSV-2 infections, while also providing the opportunity to assemble the complete genome of PRRSV-2 and other viruses of interest. The objective of the study was to perform NGS analysis of PRRSV positive samples.

### Material and Methods

In this study, 91 serum samples were collected from US swine herds across three states: Oklahoma, Kansas, and Texas. Samples underwent processing, extraction and PRRSV-2 RT-qPCR where only samples with a Ct value ≤ 26 progressed into library preparation and Illumina NextSeq sequencing. Kraken was utilized to assign viral taxonomy to sequencing reads.

#### Results

The present study determined that current PRRSV-2 analytic NGS methods seem insufficient in successfully identifying PRRSV-2 reads and assembling PRRSV-2 in positive samples. Interestingly, PRRSV-2 was nearly always (89% of cases) identified as a viral co-infection, indicating that the majority of PRRSV-2 infections seem co-infections with other swine pathogens. PRRSV-2 was found most often associated with a porcine endogenous retrovirus (57%) and a novel porcine lentivirus (27%). This increased diversity outside of the PRRSV-2 GP5 gene indicated that other PRRSV-2 genes could be suitable candidates to demonstrate PRRSV-2 diversity, such information could be useful for epidemiologal and analyses.

#### **Discussion and Conclusion**

Interestingly, both the whole genome- and GP5-based phylogenetic trees lacked clustering patterns relative to temporal or geographical distribution. The use of PRSSV-2 gene/s exhibiting greater diversity than the GP5 gene for detailed genetic analysis of PRRSV-2. While NGS can provide insights into the PRRSV-2 genome, this study indicates that different screening methods and bioinformatic approaches should be evaluated for assembling complete PRRSV-2 genome. Future studies should evaluate PRRVS-2 genes beyond the GP5 gene, as variation found in different genomic regions would likely be associated with PRRSV-2 phenotype such as disease severity and transmissibility as well as temporal and spatial distribution. The NGS-based approach will also be crucial in future studies to determine the implications of viral co-infections in the pathogenesis of PRRS.

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# FEEDING BACILLUS VECTOR VACCINE CARRYING AFRICAN SWINE FEVER VIRUS (ASFV) IMMUNOGENS ON THE LEVEL OF SECRETORY IMMUNOGLOBULIN A IN ORAL FLUID OF GILTS

Y. Maprang Roshorm1<sup>1</sup>, K. Polyiam<sup>1</sup>, N. Butkhot<sup>1</sup>, J. Ekawatpanich<sup>2</sup>, S. Sukchai<sup>2</sup>, <u>K. Kaeoket<sup>3</sup></u> <sup>1</sup>Division of Biotechnology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand

<sup>2</sup>Triple A Biotech Co., Ltd., Bang Phli, Samutprakarn 10540, Thailand

<sup>3</sup>Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand

#### **Background and Objectives**

ASF is a devastating viral disease in pigs. Transmission via ingestion of ASFV-contaminated blood, feces, secretion is one of the main ASFV transmission routes; thus, establishing mucosal immunity against ASFV in the mucosal sites is important. Bacillus subtilis is widely used as a probiotic in pig farm as it regulates intestinal flora, improves feed efficiency, enhances mucosal immunity, has short fermentation cycle and able to form heat-tolerant spores. With DNA recombination technology, B. subtilis can be used as an immunogen expression and delivery system for the prevention of various infectious diseases. With the aim to provide mucosal immunity (secretory IgA, S-IgA) against ASFV in saliva/oral fluid of pigs, we therefore, developed a platform called "Sporulated-Immunogen-Probiotic (SIP)" by using B. subtilis spore for expressing the ASFV immunogens as an oral vaccine.

#### **Material and Methods**

The immunogen genes encoding a chimeric protein designed from multiple-ASFV-proteins (CD2v, P54, P72) was synthetically made and integrated into the genome of B. subtilis. Recombinant clones were grown in special sporulating medium. The recombinant B. subtilis is so called "SIP". SIP was mixed with other species of probiotics including Lactobacillus spp. and Pediococcus spp. The SIP-mixed probiotic contains 1.0 x 10<sup>10</sup> cfu of each species/kg. Experiment was performed in 90 gilts: Control (n=30) were daily fed with commercial probiotics 1 kg/1-ton feed for 4wk and treatment were fed with SIP product at 1 kg/1-ton feed for 4 weeks. Saliva samples (10 gilts/group) were collected by rope-assisted swab method at initial time point and 4 weeks post-feeding. CD2v, P54, P72 Specific S-IgA were analyzed using indirect ELISA.

## Results

Treatment-gilts showed an increased level of S-IgA in saliva/oral fluid while the control group did not. The level of saliva S-IgA at 4 weeks post-feeding in treatment was significantly higher than that at initial timepoint.

## **Discussion and Conclusion**

This result indicates that SIP is able to stimulate mucosal immunity against ASFV, which may mainly occur in the intestine (inductive site). The induced ASFV-specific immune response could then be homed to other mucosal tissues including oronasal site (effector site). ASFV-specific S-IgA in the oronasal tissues may contribute to prevention/reduction of infection by ASFV entering via oronasal route.

#### VVD – Virology and Viral Diseases

FREQUENCY OF PCV-2 VIREMIA IN NURSERY PIGLETS FROM A SPANISH SWINE INTEGRATION SYSTEM IN 2020 AND 2022 CONSIDERING PRRSV INFECTION STATUS

M. Sagrera <sup>1</sup>, L. Garza <sup>2</sup>, M. Sibila <sup>3</sup>, S. Cárceles <sup>2</sup>, S. Oliver <sup>2</sup>, C. Casanovas <sup>2</sup>, P. Prieto <sup>4</sup>, A. García-Flores <sup>4</sup>, D. Espigares <sup>2</sup>, J. Segalés <sup>5</sup>

<sup>1</sup>IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), 08193 Bellaterra, Spain / Ceva Salud Animal, Barcelona, Spain

<sup>2</sup>Ceva Salud Animal, Barcelona, Spain

<sup>3</sup>IRTA, Centre de Recerca e Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>4</sup>Inga Food S.A., Zaragoza, España.

<sup>5</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain.

## **Background and Objectives**

Porcine circovirus 2 (PCV2) poses an economic threat to the swine industry. Despite PCV2 vaccine effectiveness, coinfections with pathogens like porcine reproductive and respiratory syndrome virus (PRRSV) can exacerbate disease severity leading to PCV2 systemic disease. Monitoring PRRSV and PCV2 in farms is crucial for effective management and vaccination programs. This cross-sectional study assessed PCV2 antibody levels in weaned piglets and measured PCV2 and PRRSV viremia in pooled serum samples at 3-4 (weaning), 6 and 9 weeks of age.

#### Material and Methods

48 farms were tested in 2020 (n=1,860 samples), and 28 re-tested in 2022 (n=1,140 samples). It included 10-30 samples per farm at weaning (in function of farm size), and 10 at 6 and at 9 weeks. PCV2 antibody levels were assayed on weaning piglets. PCV2 q-PCR and PRRSV RT-qPCR were performed in pooled serum samples. Infection status evolution was followed up at weaning, 6 and 9 weeks. A farm was considered PCV2 or PRRSV positive when at least one pool was qPCR/RT-qPCR positive. Farms were grouped considering their PCV2 virological status in both years.

## Results

Between 2020 and 2022, positive farms for each pathogen increased, leading to a high number having co-infections, especially in 2022. PCV2 antibody levels at weaning were lower in PCV2 qPCR positive farms compared to the negative ones. Based on their PCV2 virological status, farms tested in both years were categorized into four epidemiological scenarios: 2020-/2022-, 2020-/2022+, 2020+/2022- and 2020+/2022+. The 2020-/2022+ farms displayed a statistically significant increase in PRRSV detection and a reduction in PCV2 antibody levels, not being observed in the other scenarios.

### **Discussion and Conclusion**

From 2020 to 2022, PCV2 detection frequencies increased, probably associated to the spread of PRRSV virulent strains across Spain. Low PCV2 antibody levels at weaning in PCV2 qPCR positive farms could be associated to a decreased infectious pressure over time due to continuous piglet vaccination, so, overall herd immunity decreased and could have facilitated such early infections. These findings may suggest the need to reconsider PCV2 vaccination practices (sow/gilt and piglet age vaccination) in light of early PCV2 and PRRSV co-infections.

VVD – Virology and Viral Diseases

# IDENTIFICATION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) IN SWINE BY REAL-TIME PCR IN VIETNAM DURING 2021 -2023

<u>H. Le Thi Thu</u><sup>1</sup>, T. Pham Thi Hai<sup>1</sup>, H. Do Thi<sup>1</sup>, N. Nguyen Thi Bich<sup>1</sup> <sup>7</sup>Vetlatech Joint Stock Company

#### **Background and Objectives**

Porcine circovirus-associated disease (PCVAD), caused by porcine circovirus type 2 (PCV2), has ravaged the pig industry, causing huge economic loss. At present, PCV2b and PCV2d are highly prevalent genotypes worldwide and in Vietnam. Until now, diagnostic tests based on ELISA, PCR, PCR-RFLP, and Sequencing have been developed to confirm the presence or absence of PCV2. Although it has the advantage of being able to detect PCV2 in a short time, the PCV2 genotypes cannot be distinguished simultaneously. The aim of this study is to evaluate the performance of a commercially available kit of real-time PCR PCV2 typing for the rapid detection of PCV2, and direct identification of PCV2 genotypes.

## **Material and Methods**

A total of 94 samples including organs (lung, lymph node, kidney), and blood samples were collected from eleven provinces in Vietnam. Real-time PCR-Pools were tested for PCV2 by Kylt® PCV-2 Typing (Art – No 31543) (Anicon, Germany), with the AriaMx 96 system for thermocycling and fluorescence Channels FAM, HEX, Cy5, and TXR detected PCV2 and distinguish between PCV2a, PCV2b, PCV2d genotypes.

#### Results

A total of 38 PCR pools (24 organs & 14 PCR blood pools) PCV2 positivity was confirmed by real-time PCR. The most common genotypes detected in this study were PCV2d (23/38, 60.53%), followed by PCV2b (5/38, 13.16%) and PCV2b/d co-infection (2/38,5.26%%). PCV2a was not detected in this study. Samples did not have CT (38/8, 21.05%), this is the limit of the real-time PCR test kit when the infectiously of PCV2 subtypes can not be detected if Ct>31.

### **Discussion and Conclusion**

Our result the most common genotype detected was PCV2d (60.53%), followed by PCV2b (13.16%), and PCV2b/d coinfection 5.26%). Our study is similar to previous studies showing that the most common PCV2 genotypes detected worldwide are PCV2b (53.1%) in Taiwan and PCV2d (45.3%) and PCV2b (41.1%). %) in China. PCV2b (50%), PCV2d (33.3%) in Vietnam. The real-time PCR test is an accurate, rapid, and convenient tool for PCV2 identification and can also differentiate between PCV2 genotypes and detect it directly. Our study believes that tests can reduce the effort and time to diagnose PCV2 in industrial livestock farming areas.

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# INFLUENCE OF SWAIV VACCINE ON BIOMARKERS IN SALIVA

E. Llamas-Amor <sup>1</sup>, F. Murciano <sup>2</sup>, E. Goyena <sup>3</sup>, B. Gonzalez-Guijarro <sup>4</sup>, A. Cuellar-Flores <sup>2</sup>, A. Muñoz-Prieto <sup>5</sup>, D. Espigares <sup>6</sup>, K. Lillie-Jaschniski <sup>7</sup>, J.J. Cerón <sup>8</sup>, G. Ramis <sup>2</sup>

<sup>1</sup>Facultad de Veterinaria, Universidad de Murcia, Spain

<sup>2</sup>Dpto. Producción Animal, Facultad de Veterinaria., Universidad de Murcia, Spain

<sup>3</sup>DEPARTAMENTO DE SANIDAD ANIMAL, FACULTAD DE VETERINARIA, UNIVERSIDAD DE MURCIA, SPAIN <sup>4</sup>Departamento de Producción Animal. Universidad de Murcia

<sup>s</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Campus de Espinardo s/n, 30100 Murcia, Spain <sup>e</sup>Ceva Salud Animal, Barcelona, Spain

<sup>7</sup>Ceva Santé Animale S.A., Libourne, France

<sup>®</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Campus de Espinardo no16, 30100 Espinardo, Murcia, Spain

## **Background and Objectives**

In pig health, there is a continuous search for biomarkers that can indicate the health status of the animals. In addition, the aim is to use non-invasive samples. In this work, we have compared the values of biomarkers found in oral fluids with the frequency of observation of major pathogens in oral fluids in pigs vaccinated against swIAV.

#### **Material and Methods**

Two batches of 300 piglets (VAC1 and VAC2) were vaccinated at the beginning of fattening simultaneously with two vaccines: RESPIPORC FLUpan H1N1 and RESPIPORCFLU 3 (Ceva Santé Animale, France) and one batch (300 animals) was taken as unvaccinated controls (CON1). All piglets were vaccinated against M. hyopneumoniae, PCV2 and Aujeszky's disease virus. The three groups were followed up by OF starting beginning of fattening, one day before vaccination. The OF was taken from 6 pens every two weeks (7 samplings) and swIAV, PRRSv, M. hyopneumoniae, M. hyorhinis and PCV2 were quantified by PCR. In addition, the amount of Adenosine deaminase (ADA) and Myeloperoxidase (MPO) to evaluate immune system, Creatine Kinase (CK) for tissue damage was determined in the OF. These biomarkers were chosen because they are not affected by dirtiness contained in OF

### Results

Taking all data together, no differences in markers are observed when comparing vaccinated vs. control groups. However, when segmenting the data by sample, differences were observed in the third sample for MPO (VAC=489.5 vs CON=766 U/L p=0.022) and CK (VAC=53 vs CON=93 U/L, p=0.023), in the fifth for ADA (VAC=1391 vs CON= 848, p=0.002).

### **Discussion and Conclusion**

Vaccination "per se" does not produce significant changes in the biomarkers studied. The higher values of MPO and CK in the control group at the third sampling could be related to a higher pathogen circulation. Whereas the difference in ADA in the fifth sampling is explained by an outbreak of pleuropneumonia in the vaccinated group. Therefore, the biomarkers studied in this report are not influenced directly by SwIAV vaccination, but it can help to gain information about the health status of vaccinated pigs being increased in the case of ADA in situations of disease.

#### VVD – Virology and Viral Diseases

# ISOLATION AND FULL GENOMIC CHARACTERIZATION OF G9 GROUP A ROTAVIRUS IN CHINA

<u>G. Ahmed H</u><sup>1</sup>, Y. Guangyuan <sup>2</sup>, L. Mingkai <sup>2</sup>, X. Dongqi <sup>1</sup>, M. Hailong <sup>1</sup>, W. Hao <sup>1</sup>, J. Yunbo <sup>1</sup>, L. Wentao <sup>1</sup>, H. Qigai <sup>1</sup> <sup>1</sup>State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China

<sup>2</sup> State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China

#### **Background and Objectives**

Acute gastroenteritis (AGE) in young humans and domestic animals worldwide is most commonly caused by Group A rotaviruses (RVA), which are members of the genus Rotavirus within the Reoviridae family. However, A lack of genomic data exists on the rotavirus A strains circulating in farm animals in China that may infect humans.

## **Material and Methods**

Diarrhea samples from pigs were tested for rotavirus by RT-PCR. Positive samples were passaged in MA104 cells. The virus was evaluated by TEM, and indirect immunofluorescence assay. The complete genome of virus was obtained by whole genome sequencing (Illumina). The genetic characteristics and evolutionary relationship were analyzed in comparison with epidemic rotavirus sequences obtained from GenBank.

#### Results

We successfully isolated 2 samples in MA104 cells with a high titer (10 5.5 PFU/mL). RVA replication was confirmed by IFA detection of VP7-specific epitopes. The two Chinese G9P[23] porcine RVA strains had an unique genome constellation G9-P[23]-I5-R1-C1-M1-A8-N1-T7-E1-H1, a known porcine genotype constellation. The VP7 genes clustered into lineage VI, which consisted of porcine and human G9 strains from mainly Asia. The VP7 gene showed the highest nucleotide (nt) identity with the Japanese human strain RVA/Human/CHN/t203/1997/G9 (94.88% nt identity) and porcine strain RVA/Pig-wt/JPN/Hokkaido-14/2001/G9P[23] (93.65% nt identity). The VP4 genes clustered with that of Chinese porcine RVA strain RVA/Pig/China/FJSH01/2021/G26P[23] (96.79% nt identity). The VP6 genes clustered within the I5 clade with porcine RVA strains isolated in China and Italy and an unusual human porcine-like RVA strain (RVA/Human-wt/BEL/BE2001/2008/G9P[6] isolated in Belgium.

#### **Discussion and Conclusion**

This study determines for the first time the near complete genomes of porcine G9P[23] RVA strain with this constellation in China. The full genomic characterization of this strain contributes to the growing knowledge of G9 rotavirus strains in both humans and pigs. Continued research and surveillance are essential for mitigating the impact of rotavirus infections in both pigs and humans. Also, the development of a vaccine specifically targeting porcine G9 rotaviruses is necessary.

VVD – Virology and Viral Diseases

## OCCURRENCE OF ANTIBODIES AGAINST DIFFERENT INFLUENZA SUBTYPES IN FINISHER PIGS IN SWEDEN

E. Breukers <sup>1</sup>, S. Zohari <sup>2</sup>, A. Comin <sup>3</sup>, E. Jansson <sup>1</sup>, M. Sjölund <sup>1</sup>

<sup>1</sup>National Veterinary Institute (SVA), Department of Animal Health and Antimicrobial Strategies, Uppsala, Sweden <sup>2</sup>National Veterinary Institute (SVA), Department of Microbiology, Uppsala, Sweden <sup>3</sup>National Veterinary Institute (SVA), Department of Epidemiology and Disease Control, Uppsala, Sweden

## **Background and Objectives**

Influenza is a significant respiratory disease in pigs, caused by different subtypes of influenza A viruses (IAV). Influenza A viruses can infect multiple animal species, including humans, and are prone to genetic changes. Spontaneous mutations can lead to new variants and change the properties of the virus, such as adaptation to new hosts. Pigs are considered an important intermediate host where influenza viruses from humans and birds can simultaneously infect the pig and form new genotypes. The study aimed to investigate the seroprevalence of IAV and which subtypes circulate on farms with finishers in Sweden.

#### **Material and Methods**

Random serum samples collected for the surveillance of porcine reproductive and respiratory syndrome during 2022 and 2023 were used to detect antibodies against IAV. Samples were collected at slaughter from 600 finishing pigs representing 465 different epidemiological units. In total 444 farms were sampled; 296 farms were sampled once, 140 twice and eight three times. If same farm was sampled more than once at different time points, it was considered as different epidemiological units. Serum samples were first analysed using the ELISA test. Positive samples were further analysed by hemagglutination inhibition (HI) test to differentiate the subtypes. For 135 farms, two pigs were sampled on the same day; the individual results were therefore aggregated at the farm level, considering it positive if at least one sample tested positive for IAV antibodies.

## Results

The majority of samples originated from the three most pig-dense counties Västra Götaland (121), Skåne (107) and Halland (54). Antibodies against IAV were detected in 86 epidemiological units, resulting in a seroprevalence of 15.7% (95%CI: 12.7 – 19.3). The proportion of positive samples was 16.5%, 15.0% and 20.4% in the three regions, respectively. Out of 86 positive samples, 62 samples were further analysed by HI-test. The most common subtypes were H1avian-like (4%), H1pdm-like (4%) and H3swine-H3N2-like (0.33%).

#### **Discussion and Conclusion**

Regular monitoring for IAV in pigs is essential for the early detection of new variants and provides information about the animal health situation. This way, preventive measures can be taken to reduce the risk of disease and prevent the spread of infection.

VVD – Virology and Viral Diseases

# **ROTAVIRUS C GENOTYPES IN PIGS - ON THEIR OCCURRENCE AND DIVERSITY**

<u>B. Euring</u><sup>1</sup>, M. Harzer<sup>1</sup>, K. Heenemann<sup>1</sup>, R. Renzhammer<sup>2</sup>, A. Auer<sup>3</sup>, D. Piehler<sup>4</sup>, B. Schwarz<sup>4</sup>, T.W. Vahlenkamp<sup>1</sup> <sup>1</sup>Centre for Infectious Diseases, Institute of Virology, Faculty of Veterinary Medicine, University of Leipzig, Germany <sup>2</sup>University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

<sup>3</sup>Institute of Virology, University of Veterinary Medicine Vienna, Austria <sup>4</sup>Vaxxinova Diagnostics GmbH, Leipzig, Germany

### **Background and Objectives**

Rotaviruses are common enteric pathogens which can cause considerable economic damage due to reduced growth rates and fatal infections in pigs. In addition to group A rotaviruses (RVA), group C rotaviruses (RVC) are quite capable of causing severe clinical symptoms. For successful disease prevention it is essential to be aware of the circulating rotavirus serotype at the farm, as cross-protection between serotypes is limited. At present, only vaccines against RVA are available, as cultivation of RVC is only possible with very few strains. This hampers the determination of RVC serotypes, so genotypes based on viral genome sequences must be used instead. As very little sequencing data for porcine RVC is available, the study aims to fill this gap of knowledge and to provide information about the occurrence and distribution of different RVC strains in the pig population in Central Europe.

#### **Material and Methods**

Faecal samples and swabs as well as gut samples from pigs tested positive for RVC were used. Two specific RT-PCR protocols for the RVC gene segments encoding viral protein 4 (VP4) and 7 (VP7) were developed, as these two proteins determine the viral G- and P-types. Sanger sequencing of the RT-PCR-products was performed, genetic distances were calculated and phylogenetic analyses were conducted.

#### Results

A total of 119 sequences of gene segments encoding RVC VP7 (G-type) and 80 sequences encoding RVC VP4 (P-type) could be analysed and successfully genotyped. Overall, the most prevalent RVC G-type was G6 (60%), followed by G5 (11%) and G1 (10%), whereas the most prevalent RVC P-type was represented by P[21] (58%), followed by P[6] (8%) and P[4] (6%). The predominant G/-P-combination was G6P[21] (57%). Additionally, at least four new G-types and 10 new P-types were discovered.

### **Discussion and Conclusion**

The results show a great diversity of porcine RVC in Central Europe, although a dominant genotype can be clearly identified in both the G- and the P-types, which is G6 and P[21]. A first approach was made to get an overview of current RVC genotypes circulating in the European pig population. Our data provide evidence that RVC diversity based on G- and P-type is at least as distinct as is described for European RVA strains.

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# WEATHERING THE STORM: EXTREME WEATHER EVENTS AND THEIR ASSOCIATION WITH PED AND PRRS OCCURRENCE

I.A. Paploski <sup>1</sup>, G. Kiesel <sup>2</sup>, D. Makau <sup>2</sup>, N. Pamornchainavakul <sup>2</sup>, J. Baker <sup>3</sup>, M. Kikuti <sup>3</sup>, C. Corzo <sup>3</sup>, <u>K. Vanderwaal <sup>3</sup></u> <sup>1</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

<sup>2</sup>University of Minnesota

<sup>3</sup>Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

## **Background and Objectives**

Weather risk factors historically received little attention to their association with the occurrence of both Porcine Epidemic Diarrhea (PED) and Porcine Reproductive and Respiratory Syndrome (PRRS). Our objective was to investigate the relationship between exposure to extreme weather events and PED or PRRS outbreaks.

## **Material and Methods**

Outbreaks of both PED and PRRS were captured by the Morrison Swine Health Monitoring Project. County-level extreme weather event data were obtained from the National Oceanic and Atmospheric Administration storm events database for the states in the SE US. We modelled the association between weather events and PED/PRRS occurrence using a 1:4 unmatched logistic regression. For each of the analyzed weather event (flood, heavy rain, high wind and tornadoes) ten different models were run, each including a different lag of time between exposure and outbreak. Analyses were run on three periods of time based on PEDV incidence: epidemic (2014-15); endemic (2016-19) and overall (2014-19).

#### Results

We found that certain extreme weather events are consistently associated with the occurrence of PED. During the endemic period, farms located in counties exposed to floods had three to four times higher odds of having a PED outbreak in the four to nine weeks prior to the flood compared to controls. We also saw increased odds of PED occurrence after heavy rain (endemic period) and high wind (epidemic period). Associations of weather events and PRRS were less clear.

### **Discussion and Conclusion**

Associations tended to be weak and with wide confidence intervals. The mechanism by which each weather event influences the risk of disease occurrence was not investigated, but weather events may potentially influence outbreak occurrence by disrupting farm management, increasing the likelihood of biosecurity breaches or changing truck routes and delivery schedules. Swine-producing companies should develop biosecurity protocols for assessing physical damages to farms, and more stringent biosecurity practices in the immediate weeks following an extreme weather event.

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# CORRELATION BETWEEN PORCINE CIRCOVIRUS 3 (PCV3) AND PRODUCTION PARAMETERS IN COMMERCIAL SWINE FLOWS: RESULTS FROM A CANADIAN LONGITUDINAL STUDY

<u>G. Greaves</u><sup>1</sup>, C. Werth <sup>1</sup>, C. Lichty <sup>1</sup>, B. Dewolf <sup>1</sup>, M. Misener <sup>1</sup> <sup>1</sup>South West Ontario Veterinary Services, Stratford, Ontario, Canada

### **Background and Objectives**

Porcine circovirus 3 (PCV3) has been reported in commercial swine herds globally since 2016. As a ubiquitous virus, PCV3 may be identified from the tissues of any pig; however, the clinical impact of PCV3 has not been consistently described, resulting in ambiguity regarding its pathogenic role. The study objective was to understand PCV3 viral levels from birth to finishing on commercial farms in Canada, along with their potential relationship with production parameters.

#### **Material and Methods**

Beginning 2021, a longitudinal study monitored PCV3 from birth to finishing across 14 Canadian commercial pig flows varying in size (400-2950 sows), genetics, vaccination status, PRRS status, and baseline production performance. Starting at the sow site, each cohort of pigs was defined as 4-weeks of production, ranging from 373-10125 piglets, and 1-week of pooled processing fluids (PF) were collected during that timeframe. When the cohort moved to the nursery, pooled oral fluids (OF) were collected at 2- and 6-weeks post-entry. If possible, when the cohort moved to a finishing site, pooled OF were collected at 2-weeks post-entry. Samples were submitted for PCV PCR testing (Ct value <37=positive). Production parameters were recorded from each cohort when available. Each farm enrolled a maximum of 13 cohorts.

#### Results

PCV3 was detected in at least one sample per flow (170 PF, 458 OF samples submitted). Overall, 79% PF samples, 67% nursery, and 65% finishing OF samples were PCR positive for PCV3 (mean Ct values 25.7, 30.9, and 30.37, respectively). Despite seemingly high viral loads, overt clinical signs were not reported by any farm, and sow reproductive parameters were within normal ranges. PCV3 Ct values were not significantly correlated with the percentage of stillborn, mummies, or abortions (p=0.19, p=0.55, p=0.71).

## **Discussion and Conclusion**

Results demonstrate PCV3 virus is prevalent in healthy swine herds across Canada, with the greatest amounts of virus circulating in piglets <21 days of age. At an aggregate level, viral load was not correlated with reproductive parameters; however future analyses will consider the impact of parity and gestation length. In addition, viral genetic diversity research in swine populations may further refine the definition of PCV3-associated disease in the future.

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# OVERVIEW ON SWINE INFLUENZA A SUBTYPES DETECTED IN PASSIVE SURVEILLANCE PERFORMED IN BELGIUM FROM 2018 TO 2023

<u>E. De Jonghe</u><sup>1</sup>, P. De Backer<sup>1</sup> <sup>1</sup>Ceva Santé Animale, Brussels, Belgium

#### **Background and Objectives**

Swine influenza A virus (swIAV) is a major pathogen of the porcine respiratory disease complex (PRDC) and can cause reproductive failure in sows. In Belgium, both pandemic and classical subtypes circulate. The first detection of a pandemic strain in Belgium occurred in 2018. It was shown in a study of 2020 that the prevalence of pandemic subtypes is rising. The aim of this study is to investigate the subtypes of swIAV in Belgian swine farms over the last 6 years.

#### **Material and Methods**

Swine vets could send in nasal swabs (NS), oral fluids (OF), trachea-bronchial swabs (TBS) or lungs from clinical cases of presumed swine influenza. This was a part of Ceva Santé Animale's ongoing diagnostic service since 2018. The samples were either sent to DGZ (Dierengezondheidszorg Vlaanderen, Belgium), Dialab (Belgium) or IVD GmbH (Innovative Veterinary Diagnostics, Germany) for detection of swIAV and typing by a real-time quantitative PCR (RT-qPCR) or a third generation viral and bacterial metagenomic nanopore sequencing (Oxford Nanopore Technologies). This study summarises the influenza subtypes detected between January 2018 and September 2023 on Belgian farms.

### Results

Overall, on 161 farms it was possible to identify a swIAV subtype (2018: n=12, 2019: n=8, 2020: n=44, 2021: n=49, 2022: n=30, 2023: n=18). On average H1avN1 was detected in 65,2% (n= 105/161) of the positive farms with highest percentage in 2020 (79,5%, n=35/44) ) and lowest in 2019 (37,5%, n=3/8). H1avN2 was positive in 3,1% of the farms (n= 5/161) and was only detected in the years 2020, 2021 and 2023. In 9,3% (n=15/161) H1huN2 was found. Pandemic subtypes (H1pdmN1 and H1pdmN2) were found in 21,1% (n= 34/161) of the cases, with the highest percentage in 2023 (33,3%, n= 6/18).

#### **Discussion and Conclusion**

Over the last 6 years, H1avN1 was the most detected subtype. H1huN2 is the second most found classical subtype. In the last three years, no farm was positive for H3N2 in this surveillance. The results show also that there is an increase in the detection of pandemic strains from the year 2018 to 2023.

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# FEVER EVOLUTION IN ENZOOTIC EAR TIPS NECROSIS AND THE POSSIBLE IMPLICATION OF PORCINE TESCHOVIRUS.

<u>J. Carnero 1</u> 1Agrocesa

#### **Background and Objectives**

Ear tip necrosis has been described as a clinical sign in the appearance of pathologic outbreaks where Teschovirus (PTV13) has been detected. Furthermore, the study of ear tip necrosis outbreaks has showed the existence of previous high fever and it was suggested the participation of an infectious agent with oral-faecal transmission. Additionally, arteriolar perivasculitis and vasoconstriction has been described in ear tips biopsies of affected animals.

The objective of this work is to analyse the evolution of ear tip necrosis symptoms to study the possible involvement of Porcine teschovirus in the disease, focusing on a long-affected sow farm in northwester Spain where common virus were rule out.

#### **Material and Methods**

80 random piglets were marked at weaning in a farm affected by ear tip necrosis. Rectal temperature and clinical status data were taken at the same hour periodically. Faecal samples and blood were taken from febrile piglets to be tested against Teschovirus by PCR. All these evidences were recorded and analysed to find possible correlations among fever, necrosis and Teschovirus over time.

#### Results

High fever cases were widespread found prior to necrosis appearance, affecting up to 69.9% of individuals, and persisted for 30 days. First affected piglet of ear tips necrosis was detected on day 21 and affected up to 32.9% of individuals. Teschovirus PCRs were positive in faeces from day 5 and prior to fever detection. Also, positive PCR results were found in the blood of febrile piglets in each analysis, and increased over time.

### **Discussion and Conclusion**

Exposure to Teschovirus in faeces precedes the fever and its recirculation in the blood was detected at the same time. Fever and recirculation of Teschovirus in the blood occur before and during the detection of ear tips necrosis. It seems, in this case, that Teschovirus could play a role in the origin of fever and in the development of the disease. Teschovirus could be also suspected of causing the perivasculitis and vasoconstriction lesions found. Furthermore, the formation of immune complexes and intravascular coagulation phenomena cannot be ruled out due to prolonged exposure to the virus and could also explain the arteriolar obstruction which could cause ear tip necrosis.

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# IDENTIFICATION AND GENETIC CHARACTERIZATION OF PORCINE CIRCOVIRUS TYPE 3 INFECTION IN NURSERY PIGS IN TAIWAN.

H. Kuo<sup>1</sup>, Y. Chen<sup>1</sup>, C. Wang<sup>1</sup>, C. Wu<sup>1</sup>, <u>S. Huang<sup>1</sup></u> <sup>1</sup>Department of Veterinary Medicine, National Chiayi University, Chiayi, Taiwan

#### **Background and Objectives**

To date, four types of porcine circoviruses (PCVs) have been recognized. Among them, PCV2 is the best well-known pathogen that causes significant economic losses in the swine industry. As PCV2, newly emerging PCV3 has been detected in sows, mummified fetuses, stillborn piglets, and even in invertebrates. However, the pathogenesis and clinical and pathological features of PCV3 are still unknown. This study aimed to clarify PCV3 infection alone or in combination with other pathogens in nursery pigs in the central-southern region of Taiwan.

## **Material and Methods**

A total of 152 clinical tissue samples were submitted to the Yunlin-Chiayi-Tainan Animal Disease Diagnostic Center in Chiayi City, Taiwan, from January 2022 to January 2023. PCV3 was detected using quantitative polymerase chain reaction (qPCR) targeting Cap gene. Full-length Cap genes of PCV3-positive samples (Ct values under 25) were amplified for sequencing and analysis. Categorization of all PCV3 strains was based on two amino-acid mutations (A24V and R27K) in the Cap protein.

#### Results

The PCV3 positive rate of the clinical samples was 32.9% (50/152). All the PCV3 isolates were identified as PCV3c strain. The positive samples were found to be co-infected with other pathogens such as Porcine Reproductive and Respiratory Syndrome virus (PRRSV), Porcine Epidemic Diarrhea virus (PEDV), Rotavirus, PCV2, Glaeserella parasuis, Mycoplasma hyorhinis, Escherichia coli, Pasteurella multocida, Streptococcus suis, Staphylococcus hyicus, Salmonella spp., Trueperella pyogenes, and Balantidium coli.

#### **Discussion and Conclusion**

Although a high PCV3-positivity rate was detected in the nursery pigs, no obvious relationship between PCV3 infection and pathological changes was noted. Further studies will be needed to investigate the etiology of PCV3.

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# OVERVIEW OF PRRS PCR RESULTS IN PIGLETS DURING ONE YEAR IN THE NETHERLANDS 2022-2023

<u>M. Steenaert</u>, H. Prüst <sup>1</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Netherlands bv

#### **Background and Objectives**

Pig husbandry in the Netherlands suffers endemic PRRSv-1. We analyzed all PRRS PCR results from piglets in our Boehringer Ingelheim AH Netherlands database over one year.

## **Material and Methods**

PRRS PCR results of piglets sampled from birth to end of nursery, tested in different labs, were processed to percentages of positive batches per quarter of the year (Excel). The results are categorized as: 0-1 woa (weeks of age) (processing fluids or tongue tissue fluid from stillborn and dead piglets until processing), 2-4 woa (serum pooled 1:5, sampled before weaning or just before PRRS MLV vaccination at 2-3 weeks of age), 9-11 woa (serum pooled 1:5).

## Results

Per quarter of the year 20 to 34 farms participated (total 65). 1336 pools in 398 batches were tested. Per category the average number of pools per batch were: 0-1 woa 1.5 pools/ batch, 2-4 woa 6.1 pools/ batch, 9-11 woa 4.2 pools per batch.Per category the percentage of PRRS PCR positive results per quarter (yyyy-q) 2022-3 to 2023-2 were respectively: 0-1 woa 17%, 9%, 39%, 20%; 2-4 woa 43%, 39%, 71%, 40%; 9-11 woa 62%, 75%, 84%, 91%.

## **Discussion and Conclusion**

During the year farm characteristics that submitted did not differ significantly, so we considered changes over time to be relevant. Some PRRS PCR positivity may have been due to vaccine virus. In the 0-1 woa samples likely only PRRS wild type virus was found. In the 2-4 woa samples an effort was done to only sample piglets before PRRS MLV vaccination. In the 9-11 woa samples it was assumed that the post vaccination vaccine viremia had waned off. The high percentage of neonatal PRRS PCR positivity (0-1 woa) in 2023-1 reflects PRRS-infections in pregnant sows. This was predisposing PRRS positivity at 9-11 woa in 2023-2. With increasing piglet age, more batches were found PRRS PCR positive, suggesting spread of PRRSv between batches. The conclusions are that in the Netherlands during the winter of 2022-2023 a lot of PRRS outbreaks in sow herds occurred, and that PRRSv was able to spread in between batches of piglets.

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# PAPER SAMPLING FOR PASSIVE ENVIRONMENTAL SURVEILLANCE FOR SWINE PATHOGENS

B. Armenta-Leyva <sup>1</sup>, B. Munguia-Ramirez <sup>1</sup>, L. Gimenez-Lirola <sup>1</sup>, <u>J. Zimmerman</u> <sup>1</sup> <sup>1</sup>Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.

#### **Background and Objectives**

We continue to search for improvements in surveillance, i.e., efficient yet economical. Passive environmental surveillance (PES) detects pathogens in the population of interest by sampling their environment. Unlike point-in-time environmental sampling, PES samplers remain in place over time, thereby increasing the probability of detection. In laboratory animal colonies, PES was shown to be as sensitive as sampling the animals themselves. Our long-term objective is to adapt PES to swine production. For this application, sampling devices would be placed in production facilities, collected periodically, and tested for the pathogen(s) of interest. The first step in adapting PES to swine production is identifying the best material ("membrane") to use in PES samplers. In this pilot, we used PRRSV as the target.

## **Material and Methods**

Seven candidate membranes were evaluated: (1) Whatman filter paper; (2) Flinders Technology Associates (FTA) cards; (3) polyester filter paper; (4) SmartSolve cardstock (soluble in water); (5) dry surface polyester; (6) tacky surface polyester; (7) starch foam sheet (soluble in water). In brief, membranes were inoculated with 100 ul of four ten-fold dilutions (10<sup>-1</sup> – 10<sup>-4</sup>) of a PRRSV MLV (Ingelvac) that was rehydrated and diluted with PRRSV-free oral fluids. After overnight storage at 25°C, membranes were eluted with TE buffer and the eluate subjected to extraction and RT-qPCR using commercial reagents (IDEXX Laboratories).

### Results

Target recovery was expressed in terms of percent recovery relative to the inoculum for each dilution  $(1 \times 10^{-1}, 1 \times 10^{2}, 1 \times 10^{3}, 1 \times 10^{4})$ . Highest target recovery was obtained from candidate membrane (2), with recoveries of 30%, 36%, 28% and 19% for each dilution. Membrane (3) followed with 24%, 20%, 26%, 16%; and membrane (1) with 21%, 27%, 24% and 15%. The lowest target recovery was observed with membrane (5 - 1%, 2%, 3%, 0%) followed by membrane (7 - 10%, 11%, 8%, 7%).

## **Discussion and Conclusion**

These preliminary results indicated that PRRSV RNA can be recovered from various types of membranes, albeit more efficiently from some. Future experiments will focus on the optimization of target recovery from the membranes for swine pathogens and stability of pathogens on membranes by time and temperature.

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# PCV-2 DISEASE DIAGNOSTICS AND VIRUS EVOLUTION: PREDICTING VACCINE COVERAGE AGAINST EVOLVING PCV2 FIELD STRAINS

F.X. Bonto <sup>1</sup>, G. Velasco <sup>1</sup>, <u>M. Linatoc</u><sup>2</sup>, D. Umali <sup>3</sup>

<sup>1</sup>Zoetis Philippines Inc.

<sup>2</sup>Swine Consultant

<sup>3</sup>Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of the Philippines Los Banos, Laguna, Philippines

## **Background and Objectives**

The evolutionary rate for Porcine Circovirus Type 2 (PCV2) is unusually high for a DNA virus (Firth et al., 2009). Initial isolates were mainly PCV2a, which then shifted to PCV2b in the early 2000s (Xiao et al., 2015). More recently, a mutant PCV2b (now designated as PCV2d), and reported as a higher virulence virus emerged and became the most predominant genotype (Xiao et al., 2016; Foss et al., 2023). The authors aim to investigate if PCV2 has evolved in pigs from commercial swine farms in the Philippines. And based on the outcome of the study, predict the immunological coverage of commercial vaccines to field isolates using a genomic and bioinformatics tool.

## **Material and Methods**

Tissues samples from pigs from different regions manifesting PCVAD clinical signs were obtained and tested for PCV2 positivity, viremia and genotype using Polymerase Chain Reaction (PCR) test. The PCV2 PCR positive samples were then sequenced using ORF-2 gene. To determine the relatedness of T cell epitopes contained in a protein sequence of the isolated field virus to that of a PCV2a/PCV2b vaccine and 3 current PCV2a based vaccines the Epitope content Comparison test (EpiCC®, Epivax, USA) was used.

#### Results

Of the eight PCV2-positive samples, seven (87.5%) were PCV2d while one (12.5%) was PCV2a. Nucleotide sequencing was performed on the eight samples but only three were able to sequence. PCV2a/PCV2b vaccine showed 80.83% T-cell epitope coverage against the first PCV2d field strain, PCV2a based vaccines showed 60.47%, 60.63%, 66.81% coverage respectively. PCV2a/PCV2b vaccine showed 81.14% T-cell epitope coverage against the second PCV2d field strain, PCV2a based vaccines showed 60.7%, 63.87%, 67.07% coverage respectively.PCV2a/PCV2b vaccine showed 80.83% T-cell epitope coverage against the third PCV2d field strain, PCV2a based vaccines showed 60.47%, 60.63%, 66.81% coverage respectively.PCV2a/PCV2b vaccine showed 80.83% T-cell epitope coverage against the third PCV2d field strain, PCV2a based vaccines showed 60.47%, 60.63%, 66.81% coverage respectively.

## **Discussion and Conclusion**

The study was able to establish the presence of PCV2 in the Philippines and its subsequent evolution with the isolation of PCV2a and PCV2d genotypes. The local EpiCC® results provides Filipino swine farmers and Veterinarians better understanding of the current PCV2 genotypes for appropriate vaccination protocols at the farm level.

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# SEROPREVALENCE OF INFLUENZA VIRUS IN SWINE POPULATIONS IN TAIWAN, 2023

C. Tsai 1

<sup>1</sup>Agricultural Technology Research Institute

#### **Background and Objectives**

Since July 1998, an active surveillance program has monitored influenza activities in pig herds. This report aims to detect antibodies specific to representative influenza antigens collected from swine and avian populations in Taiwan.

### **Material and Methods**

In 2023, a total of 587 pig serum samples were collected from 53 active surveillance sampling events in Taiwan. These samples were tested using hemagglutination-inhibition assays to detect antibodies specific to various influenza antigens, including influenza A viruses (A/H1, A/H3, A/H5, A/H6, and A/H7) and swine influenza B virus.

## Results

The 142 serum samples from 31 events were positive for specific antibodies against at least one influenza antigen. The true prevalence rate of positive events for swine influenza A viruses was approximately 63.6% (95% CI: 48.9%-76.9%), and the true prevalence rate of serum positivity was approximately 26.3% (95% CI: 22.7%-30.2%). The true prevalence rate of A/H1 subtype antibody-positive events (59.5%; 95% CI: 44.9%-73.2%) was much higher than that of A/H3 subtype antibody-positive events (8.0%; 95% CI: 3.2%-19.0%). Regarding avian influenza, there was only one positive event observed for the A/H6 subtype, suggesting a low prevalence rate of approximately 2.1% (95% CI: 0.4%-10.7%). This event corresponded to a single positive serum sample, indicating a true prevalence rate of positive serum of approximately 0.2% (95% CI: 0.0%-1.0%). However, no antibodies against influenza B viruses or H5 and H7 subtype viruses were detected in the tested samples.

#### **Discussion and Conclusion**

Our research findings demonstrate that swine influenza A/H1 viruses were predominant in the majority of outbreaks. These viral strains were the result of reassortment, combining genes derived from the North American classical swine H1N1 (A/Swine/Iowa/1/1985-like) influenza virus, the human H3N2 (A/Hong Kong/1/1982-like) influenza virus from the 1980s, and the pandemic influenza H1N1 viruses that emerged since 2009. These variants co-existed in the pig population. Additionally, A/H3 viruses were observed in minor outbreaks. Due to the absence of approved influenza vaccines for pigs in Taiwan, the high prevalence of antibodies likely accurately reflects the current state of active outbreaks in pig herds.

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# VACCINATION WITH AN NADL-2 COMMERCIAL VACCINE CONFERS REPRODUCTIVE PROTECTION DESPITE THE CIRCULATION OF A PPV-27A-LIKE STRAIN.

<u>A. Puigredon 1</u>, A.M. Strunz 2, J. Miguel 1, I. Galé 1, M. Simon-Grifé 1, I. Ballarà-Rodriguez 1, M. Fontseca 1 1HIPRA, Amer (Girona), Spain 2Hipra Nordic Aps

#### **Background and Objectives**

Until approximately 20 years ago, Porcine Parvovirus (PPV) was considered to be very stable, both genetically and antigenically. However, more recent studies have revealed considerable genetic diversity among PPVs and a particular genotype called PPV-27a has become widespread. The aim of the present study was to evaluate whether PPV-27a is present on farms without reproductive problems relating to PPV where they vaccinate with a vaccine that contains a PPV strain that is genetically different to the PPV-27a.

## **Material and Methods**

Oral fluids and faecal socks were collected from 2 Danish farrow-to-finish pig farms without reproductive problems and within the national average productivity of Danish pig farms in 2022. On these farms, all the sows were vaccinated with ERYSENG® PARVO, a vaccine that contains an NADL-2 PPV strain. All the samples were analysed by PPV polymerase chain reaction (PCR). Positive samples were then sequenced to determine the genetic code of the part of ORF2 that encodes the VP1/2 proteins. A phylogenetic tree was then reconstructed using this and other available sequences. A pairwise nucleotide comparison was performed using an old Danish genotype 1 isolate (PPV\_Denmark\_isolate\_839) and a German genotype 2 (PPV\_Germany\_27a\_01) as reference strains.

### Results

PPV was detected in oral fluids on both pig farms, but not in faecal samples. The sequence analysis showed that on Farm 1 the PPV strain was a genotype 1 virus with an identity of 99.43% to the old Danish strain. On farm 2, the strain circulating belonged to genotype 2 and showed a 99.86% of identity with PPV-27a. Phylogenetic analysis showed that the strain isolated on farm 2 belonged to the same genetic phylogenetic cluster as the PPV-27a strain.

## **Discussion and Conclusion**

This study demonstrated that on farms where the PPV-27a-type strain was circulating, vaccination with ERYSENG®PARVO is effective in protecting against PPV disease in terms of reproductive performance. These results are in accordance with previous studies where the sera from animals vaccinated with a vaccine containing a PPV NADL-2 strain demonstrated virus neutralization to heterologous PPV such as 27a, 143a, and Kresse strains.

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# VETMAX™ AFRICAN SWINE FEVER VIRUS DETECTION KIT 2.0 - RAPID AND RELIABLE NEW MOLECULAR DIAGNOSIS KIT OF AFRICAN SWINE FEVER VIRUS (ASFV) BY REAL-TIME PCR

<u>A. Quijada 1,</u> C. Jean 1, S. Moine 1 <sup>1</sup>Thermo Fisher Scientific

#### **Background and Objectives**

Our existing ASF qPCR product (Screening kit, P/N A28809) has helped to control ASFV worldwide. The evolution of the virus and the needs on the field led us to develop a 2.0 version, improving the kit capabilities for high throughput management with fast time to results, consolidating diagnostic sensitivity, allowing environmental testing and complying with market needs of the European regions.

#### **Material and Methods**

The Applied Biosystems<sup>™</sup> VetMAX<sup>™</sup> African Swine Fever Virus Detection Kit 2.0 (P/N A57008) enables detection of current ASFV circulating strains by real-time PCR on porcine and wild species. This diagnosis tool is a triplex screening kit composed of a ready-to-use master mix with an upgraded ASFV design. It also includes dual Internal Positive Controls (exogenous and endogenous). The presence of two internal controls reduces the risk of false negative results and helps to validate purification and amplification steps.

#### Results

The diagnostic can be delivered within 1 hour using the Fast workflow. An extraction method was optimized using the Applied Biosystems<sup>™</sup> MagMAX<sup>™</sup> CORE Nucleic Acid Purification Kit with the KingFisher instrument: 96 samples can be extracted simultaneously in 22 minutes. A Fast qPCR amplification program was validated, running in 40 minutes. The A57008 Kit is a reliable and rapid qPCR tool for the detection of ASFV DNA from serum/blood, tissue, oral fluid and meat juice samples from pigs and wild species. Its performance fulfils validation criteria required by the French standard and Polish authorities. The kit detects 53 positive samples from ASF reference panels provided by the EURL ASFV (CISA-INIA). No cross-reactivity was detected on 25 various viruses, bacteria, and parasites. Pools up to 20 were evaluated on 12 bloods, 16 serum and 12 tissue samples. The validation was performed on 603 samples. 269 ASF-positive and 334 ASF-negative samples, including domestic pigs and wild species, were collected from Spain, Italy, Poland, Belgium, Germany and France.

## **Discussion and Conclusion**

The kit sensitivity and rapidity support the early detection of the virus to contribute to the free-of-disease status of pigs for trade. It provides a useful tool for control management of the spread of disease and permits the organization of an efficient monitoring in outbreak situation.

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# ALTERATIONS IN SALIVA AND SERUM ANALYTES IN DOMESTIC PIGS EXPERIMENTALLY INOCULATED WITH AFRICAN SWINE FEVER VIRUS

T. Carrau <sup>1</sup>, <u>A. Ortín Bustillo</u><sup>2</sup>, M.J. López Martínez <sup>2</sup>, C. Peres Rubio <sup>2</sup>, M. Botía <sup>2</sup>, E. García Manzanilla <sup>3</sup>, P. Deutschmann <sup>1</sup>, V. Friedrichs <sup>3</sup>, M. Beer <sup>3</sup>, A. Muñoz Prieto <sup>2</sup>, J.J. Cerón Madrigal <sup>2</sup>, S. Blome <sup>3</sup>, L. Franco Martínez <sup>4</sup>

11 Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Suedufer 10, 17493 Greifswald – Insel Riems, Germany

<sup>2</sup>2 Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, 30100 Murcia, Spain

<sup>3</sup>3 Moorepark Animal and Grassland Research Center, Teagasc, Irish Agriculture and Food Development Authority, P61 C996 Cork, Ireland

<sup>4</sup>2 Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, 30100 Murcia, Spain 3 Moorepark Animal and Grassland Research Center, Teagasc, Irish Agriculture an

## Background and Objectives

African Swine Fever (ASF) is a highly lethal viral infection that has attained an unprecedented global spread, posing a significant threat to domestic and wild pigs on all continents, necessitating a comprehensive understanding of its pathogenesis. This study is focused on assessing key biomarkers in saliva and serum to elucidate the disease's impact.

### Material and Methods

Seven crossbred domestic pigs, approximately five months old, were experimentally inoculated with 10<sup>4</sup> HAU<sub>50</sub> per ml of ASFV "Prenzlau 2022". This virus was isolated from a domestic pig outbreak in the German Federal State of Brandenburg in 2022. Clinical manifestations were monitoring daily, and animals were euthanized upon reaching a late humane endpoint.

Saliva and serum samples were collected on days 0 and 7 post-inoculation (d.p.i), and nine biomarkers that provide information about inflammation (haptoglobin, ITIH4, total protein, S100A8/A9 and S100A12), immune system (ADA), redox status (FRAP), and tissue damage (LDH and CK) were measured.

### Results

In saliva, significant increases in haptoglobin (2.66-fold), protein content (1.58-fold), S100A8/A9 (4-fold), S100A12 (11-fold), ADA (9.25-fold), and LDH (3.86-fold) were observed at 7 d.p.i. in comparison to 0 d.p.i.

In serum, increases at 7 d.p.i. were found for haptoglobin (6.3-fold), ITIH4 (2.8-fold), ADA (2.66-fold), LDH (10.3-fold), and CK (127.75-fold), while S100A12 (6.92-fold) decreased.

### **Discussion and Conclusion**

The increase of haptoglobin, S100A8/A9 and S100A12 in saliva, along with haptoglobin and ITIH4 in the serum, indicates the presence of inflammation in ASF. The elevated levels of ADA suggest activation of T lymphocytes, while alterations in LDH and CK could reflect damage in muscle tissues, that caused the release of these biomarkers into the bloodstream.In conclusion, African Swine Fever (ASF) induced alterations in selected saliva and serum analytes, highlighting changes in the inflammatory response, immune system activation, and tissular damage. Further studies should be performed to evaluate the potential of these analytes as biomarkers of ASF.

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# FREQUENCY OF PCV2 EARLY INFECTIONS AMONG A POPULATION OF SUSPICIOUS FARMS IN FRANCE

<u>A. Jardin</u><sup>1</sup>, P. Leneveu<sup>2</sup>, S. Brilland<sup>1</sup> <sup>1</sup>Ceva Santé Animale, 33 500 Libourne, France <sup>2</sup>Ceva Santé Animale, 10 Avenue de la Ballastière, 33500 Libourne, France

#### **Background and Objectives**

Porcine circovirus type 2 (PCV2) is an ubiquitous virus responsible for several PCV2-associated diseases such as PCV2 systemic disease (PCV2-SD). To prevent from clinical signs, technical and economic impacts due to PCV2, pigs are widely vaccinated worldwide and vaccines are considered as effective products. In 2022, Segales et Sibila have given several reasons to explain the occurrence of PCV2-SD on vaccinated animals. Among them, the existence of an early infection (before or closed to the PCV2 vaccination) is listed.

The aim of this study is to determine the frequency of PCV2 infections on young piglets (below 9 weeks of age) among a population of suspicious farms.

### **Material and Methods**

From 2019 to late 2023, samples from 61 groups of 3 to 8 weeks of age piglets were submitted to PCV2 RT qPCR (quantitative PCV2 VetMAX<sup>™</sup> Pig kit). These samples were coming from 56 different farms facing various symptoms like wasting, bad average daily gain or respiratory disorders. PCV2 was suspected to play a role in the observed troubles on weaners and/or fatteners despite a PCV2 piglets' vaccination in 50 of these farms. Samples consisted mainly of serums collected on sick or healthy pigs (1 to 30 samples collected per group of age and 1 to 3 groups of age collected per farm). PCV2 RT qPCR have been performed on pool of 3 to 5 serums.

#### Results

28% of the analysed groups had at least one pool PCV2 PCR positive. 16 out of 56 investigated farms (28.5%) were concerned by an early PCV2 infection. PCV2 vaccinal status of the reproductive animals was known in 10 of these qualified "early PCV2 infected farms": no regular PCV2 sows' vaccination was implemented in 70% of them.

## **Discussion and Conclusion**

In a population of farms with suspicious clinical symptoms, more than 1 out of 4 farms was concerned by a PCV2 infection occurring on piglets of less than 9 weeks of age. According to Segales and Sibila (2022), such an early PCV2 infection could compromised the quality of the vaccinal protection leading to the expression of PCV2-associated diseases in later age.

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## HOW TO REDUCE EARLY PCV2 PIGLET INFECTION?

<u>M.A. Larsen</u><sup>1</sup>, R. Krejci<sup>2</sup>, C.S. Christensen<sup>3</sup> <sup>1</sup>Ceva Animal Health, Denmark <sup>2</sup>Ceva Animal Health, France <sup>3</sup>Ceva Animal Health A/S, Denmark.

## **Background and Objectives**

The risk of Early PCV2 piglet infection is enhanced because the epidemiological picture of PCV2 has changed via the massive use of piglet vaccination, which has allowed a decrease in overall herd immunity. The objective of these field trials is to show how Circovac 2 ml PCV2 sow vaccination can reduce Early PCV2 piglet infection.

## **Material and Methods**

Early PCV2 piglet infection was examined by PCR-PCV2 (cutoff at < 3.0 Log 10) via saliva on 5 ropes among newly weaned pigs and via blood samples of 20 in 4 pools up to 8 weeks after weaning. If both are PCV2 positive, it's considered a case of Early PCV2 piglet infection. Sequence analysis was used to identify PCV2 type. Six herds with Early PCV2 piglet infection were identified. None of the herds were vaccinating neither gilts nor sows against PCV2. The following vaccination program with Circovac 2ml, which is registered to boost passive immunity of piglets via colostrum, were initiated for 5 months: All sows received 2 ml vaccine at 6 and 3 weeks before farrowing. Gilts receive forward in time 2 ml vaccine at 26 and 29 weeks of age. Then a new profile for detecting Early PCV2 piglet infection was performed.

## Results

All 6 nurseries before 2 x 2 ml PCV2 vaccine for gilts and sows were positive for PCV2 by PCR.

Saliva via 5 ropes in all 6 nurseries within the first week after weaning showed an average 6.0 Log 10. Blood samples of 20 in 4 pools in all 6 nurseries showed an average between 4.6 to 7.6 Log 10. The sequence analyzes, one from each nursery showed PCV2d.

The same 6 nurseries more than 20 weeks later: All samples of saliva within the first week after weaning and all pools of bloodsamples up till 8 weeks after weaning showed NO sample positive for PCV2 by PCR.

#### **Discussion and Conclusion**

After the vaccination program of sows was completed for five months, no PCV2 by PCR could be detected at the weaners in the nursery.

This could also be the result of the evolution of the sow herd immunity independantly from the vaccination.

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# IMPACT OF AN AGGLOMERATE OF SODIUM DIFORMATE AND MONOLAURATE ON THE REDUCTION OF PRRS VIRUS LOAD IN NURSERY PIGS

W. Chuang <sup>1</sup>, <u>C. Lückstädt</u><sup>2</sup> <sup>1</sup>Bienen Ltd., 630044 Yunlin County, Taiwan (R.O.C.) <sup>2</sup>ADDCON

## **Background and Objectives**

Monolaurin glycerol (GML) inhibitory effect on enveloped viruses has been discussed for several years. Monolaurin glycerol can inhibit viral activity by destroying the virus envelope and promoting the production of interferon-gamma. Furthermore, evidence suggests that GML is more effective in inhibiting pathogens in an acidic environment, but its impact on enveloped viruses remains unclear. Therefore, this study aims to investigate the effects of GML under acidic conditions on the expression of PRRS virus of nursery pigs.

## **Material and Methods**

In this experiment, 1520 nursery pigs (42 days old) were randomly assigned to a control group and a 0.5% 3G group (0.5% Formi 3G, ADDCON, which containing 18% GML and 74% sodium diformate), with 20 replicates (pens) per group. In the control group, 0.1% GML (>90%) was added to the basal diet, while the 0.5% 3G treatment group received 0.5% Formi 3G in the basal diet.

### Results

During the first four weeks (42-70 day-old), the PRRSv antibody levels in the 0.5% 3G treatment group were significantly higher than those in the control group. Moreover, the PRRS Elisa S/P ratio in the 0.5% 3G group remained stable within the range of 1.737 - 2.128, whereas 0.710 - 2.006 in the control group. Regarding the virus transcription levels (Ct value), the PRRSv exhibited lower Ct values in the control group compared to the 0.5% 3G treatment group, indicating a higher viral load. The PRRSv expression decreased continuously in 0.5% 3G group and after six weeks became undetectable.

#### **Discussion and Conclusion**

The co-addition of sodium diformate and GML is associated with reduced PRRSv expression. Its mechanism of action may involve reducing the production of pro-inflammatory cytokines and enhancing interferon- $\gamma$  release. The results also demonstrated that under the acid environment, GML has better effect on virus inhibition. Therefore, employing GML in an acidic environment appears to be a more reasonable strategy.

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# INFECTION AND COINFECTIONS OF NOVEL PORCINE PARVOVIRUSES 2 TO 7 (PPV2 TO PPV7), PPV1, PCV2, PCV3 AND PRRSV IN GILTS AND THEIR ASSOCIATION WITH REPRODUCTIVE PERFORMANCE IN SOWS

D.S. Vargas-Bermudez <sup>1</sup>, A. Diaz <sup>2</sup>, G. Polo <sup>3</sup>, J.D. Mogollon <sup>1</sup>, J. Jaime <sup>1</sup>

<sup>1</sup>Universidad Nacional de Colombia, Sede Bogotá. Facultad de Medicina Veterinaria y de Zootecnia. Departamento de Salud Animal. Centro de Investigación en Infectología e Inmunología Veterinaria (CI3V). Carrera 30 No. 45-03 Bogotá. Colombia. CP 111321.

<sup>2</sup>Pig Improvement Company, Hendersonville Tennessee, USA

<sup>3</sup>Fundación Universitaria San Martin. Facultad de Medicina Veterinaria y de Zootecnia. Bogotá DC, Colombia

#### **Background and Objectives**

In swine, eight porcine parvoviruses (PPV) have been reported; of these, PPV2 through PPV8 are called novel PPV (nPPV) and have been detected in different countries. Additionally, their pathogenesis is unknown, and they have been found in coinfection with other viruses.

#### **Material and Methods**

Gilt sera (n=234) were collected from 40 farms in Colombia and evaluated by Real-time PCR for PPV1 through PPV7, PCV2, PCV3, and PRRSV. In addition, it was recorded Reproductive Performance Parameters (RPP). To estimate the adjusted odds of performance (high vs. low) with the detection of each virus, a multivariate logistic regression model was applied, and multiple-correspondence analysis (MCA) was performed to describe relationships between the independent categorical variables.

## Results

In this study, all nPPVs (PPV2 through PPV7) were detected, with PPV3 being the most prevalent in both sera and farms. Comparing viral loads, these were, on average, higher (>10<sup>4</sup>) in nPPV and low (between 10<sup>2</sup> and 10<sup>3</sup>) for PCV2, PCV3, PPV1, and PRRSV. Regarding coinfections, they were in 83% (195/234) of the sera, the most frequent concurrences being PCV2/PPV3 or PPV6 and PRRSV/PPV3 or PPV6. Regarding viral associations, PPV3 was found in a higher proportion in PCV2 or PRRSV-negative gilts (p<0.01), PPV6 in PCV3-positive gilts (p<0.01), while PPV5 in PRRSV-positive gilts (p<0.05). The association between PCV3 and farrowing rate (FR) (p=0.0043) was significant regarding the productive variables. Furthermore, low FR tended to be associated with PPV4 (p=0.16). The odds of low RF were 96% lower in PCV3-positive gilts (OR:0.04, 95% CI: 0.002, 0.30). The probability of low RF was 9.6 times higher in farms with PPV4-positive gilts after adjusting for PCV3 status. The difference in coefficient estimates for PCV3 in the crude (-2.76) versus the adjusted model (-3.18) indicated that PPV4 confounded the association between PCV3 and RF.

## **Discussion and Conclusion**

The presence of the nPPVs in gilts indicates that they arrived infected to begin their reproductive cycle. Their effect could be associated with their participation in coinfections. The nPPVs circulate in herds of gilts (mainly PPV3), where they were found to be coinfected with PCV2, PCV3, and PRRSV. Likewise, some of these nPPVs can impact the RPP.

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# PRODUCTIVE AND ECONOMIC IMPACT OF TWO INCREASED VIRULENCE PRRS OUTBREAKS WITHIN A SWINE FARM IN SPAIN

A. García Flores <sup>1</sup>, J.I. Pérez Zapater <sup>1</sup>, <u>L. De Lucas</u><sup>2</sup>, L. Nodar <sup>2</sup> <sup>1</sup>Inga Food S.A., Spain <sup>2</sup>HIPRA, Amer (Girona), Spain

## **Background and Objectives**

Since 2020, a recombinant PRRSv strain has been circulating in Spain. Commonly known as "Rosalía", it has caused outbreaks of a severity never previously described that are characterized by high abortion rates and increased mortality rates. In the present study we assessed the productive and economic impact of this strain into a Spanish swine farm.

#### Material and Methods

From January 2022 to October 2023, PRRSV epidemiology on a PRRS-positive 1,780-sow farm was examined by PCR and ORF5 sequencing from serum and/or tongues from preweaning piglets (Together Programme, Hipra). For the productive analysis, these primary KPIs were visualized using CUSUM graphs: % abortions (AB), piglets born alive/litter (BA), weaned piglets/litter (WP), piglets born alive ratio (BAR), average stillbirths per litter (ST) and return to oestrus (RO). The onset of an outbreak was determined when one of these 6 KPIs deviated 3  $\sigma$  and was confirmed by diagnosis. The end of the outbreak occurred when all the KPIs returned to their pre-outbreak values (baseline production). Based on this, Time-to-Baseline Production (TTBP), productive and economic impact of the outbreak were calculated; the last of these based on the cost of lack of weaned piglets during the outbreak. The differences were tested by a t.test.

#### Results

An outbreak started in week 38 (2022). A Ct value of 20.7 from serum was detected with a highest similarity to the "Rosalia" strain of 97.03%. After 20 weeks and before production had fully recovered, a peak in abortions was observed (94.4% similarity to the Rosalia strain and 98.3% to the previous field strain). TTBP extended to 40 weeks, marked by two successive outbreaks. During this period there was a significant difference (p-val<0.05) in all the KPIs: +0.15% pts A, - 1.49 BA, -0.98 WP, -2.51 % pts BAR, +0.24 ST and 3.01 % pts RO. The total impact amounted to a reduction of 3,710 weaned piglets, resulting in an economic setback of  $\epsilon$  63,684.

## **Discussion and Conclusion**

This study revealed the high impact of an enhanced virulence PRRSv strain on a previously positive PRRSv farm and the difficulty in getting rid of it to achieve baseline productivity.

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# SAMPLING APPROACH FOR A LARGE-SCALE SOW FARM SUSPECTED OF SWINE INFLUENZA A VIRUS (SWIAV) INFECTION

<u>P. Cybulski</u><sup>1</sup>, R. Panek<sup>2</sup>, K. Lillie-Jaschniski<sup>3</sup> <sup>1</sup>Goodvalley Agro S.A., Przechlewo, Poland <sup>2</sup>Ceva Animal Health Poland, Warsaw, Poland <sup>3</sup>Ceva Santé Animale, Libourne, France

#### **Background and Objectives**

Since its emergence in 2009, pandemic H1N1 has spread globally and remains an economically important respiratory infection of swine. The objective of this evaluation is to describe a swIAV sampling approach to detect the responsible IAV subtype in clinically affected suckling piglets.

## **Material and Methods**

The investigation was performed in March 2022 in a PRRSV-negative 4000 sow farm using weekly batch system. Suckling piglets were affected by sneezing and retarded growing. The responsible veterinarian performed a cross-sectional sampling using nasal swabs (NS) from the clinically affected animals in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> week of life (15 samples in each group). For sampling a polyester-tipped swab was inserted into both nostrils and placed into a liquid universal transportation medium (Sigma-Virocult®). For investigation NS were pooled by 3 respecting the age of the animals. In the first step an Influenza A real-time RT-PCR was performed. Samples with a Ct-value <30 were further investigated by multiplex-PCR allowing determination of the subtypes.

#### Results

75.0 % (15/20) of all the tested pools were IAV positive. 80 % of pooled samples (4/5) collected from the youngest animals and all pooled samples (5/5) collected from 2-week-old piglets were IAV positive. The analysis of samples obtained from 3- and 4-week-old piglets revealed 80 % (4/5), and 40 % (2/5) positive pools, respectively. 66.7 % (10/15) were suitable for subtyping (Ct<30). All of them were positive for H1pdmN1pdm. The mean Ct-value in the NS pools from 1-, 2-, 3-, and 4-week-old piglets was 25.25 (SD: 5.56), 28.00 (SD: 6.28), 27.00 (SD: 2.31), and 36.50 (SD: 0.71), respectively.

## **Discussion and Conclusion**

Even though the sample size was calculated based on a low prevalence of 5 % in a population of 9800 piglets at 95% confidence level, and clinical signs could be observed in all the sampled age groups, nasal swabs collected from piglets prior to weaning were not suitable for subtyping. Our study highlights both the role of cross-sectional sampling of piglets in diagnostic investigation, and the constantly evolving dynamic of the disease, including early infection in suckling piglets. This is essential to be able to implement the right control strategy.

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# STABILIZING PRRS-POSITIVE HERDS THROUGH PRRSV VACCINATION AND GILT IMPROVEMENT

<u>Y. Woonwong</u><sup>1</sup>, P. Jirawattanapong<sup>1</sup>, S. Phuttapatimok<sup>1</sup>, W. Navasakuljinda<sup>2</sup>, A. Boonsoongnern<sup>1</sup> <sup>1</sup>Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, 10900 <sup>2</sup>Zoetis (Thailand) Limited, Bangkok, Thailand, 10500

### **Background and Objectives**

To control Porcine reproductive and respiratory syndrome (PRRS) virus circulation in the breeding herd, a strict biosecurity program, good gilt management, and effective immunization program must be employed. The aim of this study was to evaluate the protective efficacy of a modified live PRRS vaccine (MLV) to stabilize PRRS-positive sow herd and improved gilt replacement quality.

#### **Material and Methods**

The study was conducted at a one-site system farm having approximately 5,000 sows. All replacement gilts were internally replaced, lacking appropriate immunization and disease monitoring measures. This farm was a PRRSV endemic farm and suffering from PRRSV outbreak. Stabilization protocol was implemented with mass vaccination in the sow herd by a commercial modified live PRRS vaccine (Fostera® PRRS, Zoetis). Vaccination was then conducted on a 3 months interval. Gilt replacements were immunized with MLV PRRSV vaccine in acclimatization unit before introduction. Blood samples of replacement gilts, sows and umbilical cord of newborn piglets were collected and detected PRRSV by RT-PCR. The clinical signs and production parameters related to PRRSV infection were recorded using a computerized system (PigLive® software, Kasetsart University) throughout the period.

#### Results

At the beginning, the percentages of replacement gilts positive for PRRSV were 30-40%, The serum from abortion sows were detected PRRSV positive. Moreover, illness sows were detected PRRSV positive in umbilical cord samples (5 of 9 litters) and associated with the production losses in the farrowing units. After implement control procedures, no evidence of PRRSV viremia or shedding was found in all tested. Descriptive analysis of performances in farrowing unit had improved including the decline in abortions (from 4.5% to 1.2%), stillbirths (from 9.5% to 6.8%), mummies (from 7.3% to 3.4%) and sow mortality (from 7.7% to 2.8%).

## **Discussion and Conclusion**

It could be speculated that as a result of poor gilt management might result in PRRSV outbreak in the gestation and farrowing units, and affected their production performances. The data showed that overall performances in gilts and sows had improved compared to the previous situation, following vaccination with MLV PRRSV vaccine and adjusted gilt flow management. In conclusion, the proper vaccination procedure would be an important factor to stabilize PRRS-positive herds.

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## TEN YEARS OF PORCINE EPIDEMIC DIARRHEA VIRUS IN MEXICO

<u>J.F. Rivera-Benitez</u>, J. De La Luz-Armendáriz <sup>2</sup>, R. Martínez-Bautista <sup>3</sup>, M.J. Martínez-Mercado <sup>3</sup>, M. Macías-García <sup>4</sup>, R. González-Martínez <sup>5</sup> *1NIFAP* 

²Facultad de Medicina Veterinaria y Zootecnia. UNAM. ³ZOETIS, MEXICO ⁴Private Practice Veterinarian ⁵CARGILL, MEXICO

## **Background and Objectives**

The porcine epidemic diarrhea virus (PEDV) has significantly affected global pig farming. In Mexico, during cases of outbreaks, mortality in piglets has reached up to 100% (2013-2014). Severe diarrheal symptoms have occurred in weaning, growing and fattening pigs, despite the existence of previous immunity on farms acquired during a natural infection, vaccination or feedback in subsequent years (2016-2023). The characteristics of the complete genome of PEDV that has circulated in Mexico from the first outbreaks to the present has been poorly evaluated.

### **Material and Methods**

We chose samples of 51 outbreaks obtained from 2013 to 2023 and sequenced the spike gene of PEDV, which enabled us to identify the genetic variation and phylogeny in the virus in the ten years it has circulated in Mexico.

#### Results

A 99% identity was found among the analyzed pandemic strains; however, the 1% difference affected the structure of the S glycoprotein, which is essential for the binding of the virus to the cellular receptor. The S protein induces the most efficacious antibodies; hence, these changes in structure could be implicated in the clinical antecedents of the outbreaks. Antigenic changes could also help PEDV avoid neutralization, even in the presence of previous immunity.

#### **Discussion and Conclusion**

The phylogenetic analysis of the spike genes indicates that the first PEDV outbreaks in Mexico were caused by INDEL strains and pandemic strains related to USA strains; however, the possibility of the entry of European strains exists, which may have caused the 2015 and 2016 outbreaks.
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# VALIDATION OF THE PRIOCHECK (TM) AFRICAN SWINE FEVER VIRUS AB KIT

C. Emeric <sup>1</sup>, J. Roge <sup>1</sup>, I. Leyva Baca <sup>1</sup>, D. Donescu <sup>2</sup>, ž. šteingolde <sup>3</sup>, S. Moine <sup>1</sup>, <u>A. Quijada <sup>1</sup></u> <sup>1</sup>Thermo Fisher Scientific <sup>2</sup>Institute for Diagnosis and Animal Health (IDAH) <sup>3</sup>Institute of Food Safety, Animal Health and Environment (BIOR), Latvia

# **Background and Objectives**

African Swine Fever (ASF) is a highly contagious and mortal disease caused by the ASF virus (ASFV). The recent epidemic in Asia has demonstrated its capacity for a global spread and massive culling of pigs lead to a sharp rise in pork prices. Recent outbreaks and cases have been reported in eastern Europe, evidencing a high risk for the epidemic to spread. Hence, there is a continuous need for development of reliable, and cost-effective diagnostic tools. The current study contains diagnostic evaluation of an ELISA detecting antibodies from ASFV infected animals. A large panel of reference samples sourced from three independent European laboratories was used for this study to demonstrate that the assay validation met the standards defined by the World Organization for Animal Health (WOAH).

# Material and Methods

The immuno-assay is an Indirect ELISA designed to capture the antibodies directed against the p30 envelope protein of ASFV. Prediluted porcine serum is first incubated on the antigen-coated plated. Then a peroxidase labeled secondary antibody binds to the targeted antibodies. The complex is finally revealed by addition of a colorimetric substrate and the optical density (OD) is measured. Diagnostic performances were evaluated on 590 positive and 500 negative porcine serum samples. Statuses were determined by reference methods approved by WOAH. Three laboratories based in Latvia, Romania and France participated to this study.Diagnostic sensitivity and specificity were estimated with Wilson 95% interval of confidence. ROC analysis were used to assess the separation of the populations and the pertinence of the threshold set. Reproducibility between sites was evaluated with the use of 20 serum samples.

#### Results

Overall Diagnostic Sensitivity was 98.6% and Specificity 98.6%. There was no cross reactivity with other swine common viruses. There was no status shift between the three laboratories.

# **Discussion and Conclusion**

The PrioCHECK<sup>™</sup> African Swine Fever Virus Ab Kit demonstrated a high sensitivity and specificity on a large panel of samples tested by three European laboratories and constitute a highly performant tool for the screening and monitoring of potentially infected animals.

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# DESCRIPTION OF AN OUTBREAK OF PORCINE PARVOVIRUS ON A FULL-CYCLE FARM IN NORTHERN MEXICO

<u>J.D.L.L. Luevano-Adame</u><sup>1</sup>, C. Diaz-Rayo<sup>2</sup>, F.E. Martinez-Castañeda<sup>1</sup>, R.M. Molina-Barrios<sup>2</sup>, M.E. Trujillo-Ortega<sup>1</sup> <sup>1</sup>Universidad Nacional Autonoma de Mexico <sup>2</sup>Instituto Tecnologico de Sonora

#### **Background and Objectives**

Porcine parvovirus is caused by porcine parvovirus (PPV) infection, which is considered one of the main viral diseases that causes reproductive failure. The main finding in infected sows is the increase in mummified piglets at farrowing indicative of infection during gestation. The aim of this paper is to present an outbreak case of PPV in a farm from the north of Mexico.

#### **Material and Methods**

A 450-sow pig farm, located in the Northwest of Sonora, Mexico, during 2019 presented an increase in the percentage of piglets born mummified at farrowing, mainly in sows from first to third farrowing (which were vaccinated against PPV with a polyvalent inactivated vaccine). Presumptive diagnoses such as Porcine Reproductive and Respiratory Syndrome (PRRS), Swine Influenza (SIV), Porcine circovirus (PCV-2), Leptospira and porcine erysipelas were ruled out. Infection was confirmed by hemagglutination inhibition and hemagglutination, where hemagglutinating units against PPV were found. The presence of PPV-1 was verified by RT-PCR by the Iowa State University's veterinary diagnostic laboratory, from mummy tissue samples. Productive indicators of the farm during the outbreak were taken. These were acquired from the PigChamp® management program and captured in spreadsheets, where the mean and standard deviation were obtained for Total Born (NT), Live Born (NV), Stillborn (NM) and Mummy-born (NMO) Piglets.

#### Results

The clinical picture presented by the sows was mainly limited to the increase in the percentage of mummies. The indicators affected were mainly the increase in the percentage of NMO, the maximum percentage was 52%, the duration of this "explosive" presentation was 15 weeks.

# **Discussion and Conclusion**

The normal percentage of mummies ranges between 1-2%, while in this case the maximum was 52%. Other agents causing reproductive problems include erysipelas and leptospira, PRRS, SIV, and PCV-2. Mummies are usually homogeneous in size (affecting one third of gestation). On the other hand, PPV is usually a subclinical disease and the main finding is the presence of NMO of different sizes, indicative of different time damage and fetal death. Porcine parvovirus infection presents subclinically, when observing an increase in NMO the main diagnosis is focused on viral diseases, however, parvovirus tends to be omitted.

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# EVALUATING ONE-SHOT COMMERCIAL FMD VACCINES IN VIETNAM: A STUDY ON ANTIBODY TITER PERSISTENCE UNTIL SLAUGHTER IN PIGS

J. Membrebe <sup>1</sup>, D. Nguyen <sup>1</sup>, J. Filippi <sup>1</sup>, V.G. Nguyen <sup>2</sup>, F. Romero <sup>1</sup> <sup>1</sup>Biogenesis Bago <sup>2</sup>Vietnam National University of Agriculture

#### **Background and Objectives**

Foot-and-mouth disease (FMD) profoundly impacts livestock and carries severe economic consequences. Recent success in FMD management has led to the adoption of single-dose vaccination. Assessing the enduring efficacy of the available FMD vaccines is crucial to determine if immunity up to slaughter can be maintained using a "one-shot" approach. The objective of this study was to determine total antibody titers and their duration in piglets against FMD serotype O after vaccination with three different commercially available vaccines.

# **Material and Methods**

Ninety piglets of 8 weeks of age from a farm in Vietnam were enrolled in the study and allocated into 3 groups considering the following variables: MDA level against FMD serotype O, body weight, and sex. Each group allocated 30 piglets that received one dose of FMD serotype O vaccine, designated as Vaccine A (Aftogen Oleo), Vaccine B, Vaccine C. Serum samples were collected at -15; 29-, 64-, and 141-days post-vaccination (dpv) and analyzed for positivity rates and antibody titers using ID Screen FMD Type O Competition ELISA (ID Vet).

#### Results

Vaccine A showed a progressive increase in both positivity rates and mean antibody titers, peaking at 141dpv (100 % positivity). Vaccines B and C exhibited a notable decline in positivity and mean titers over time, with a remarkable increase in negative responses at 141dpv of 30% and 22%, respectively.

# **Discussion and Conclusion**

The data collected illustrates varied total antibody responses. Vaccine A shows consistent long-term antibody response, as the rising titers and high positivity indicate. Both Vaccines B and C show a decrease in antibody response along the study, which might imply that the animals might require a booster dose to maintain the antibody titers and positivity rates until 141dpv.Further studies should be conducted to validate these assumptions and determine the efficacy of the antibody response by virus neutralization tests (VNT).

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# EVALUATION OF PRRSV-2 PCR DETECTION USING COTTON ROPES, IN ORAL FLUID, IN ROOM-BASED MANURE PIT AND MANURE COLLECTION TANK, AS NURSERY PRRSV STATUS MONITORING

<u>P.H. Rathkjen</u><sup>1</sup>, L.M. Jensen<sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Denmark <sup>2</sup>PORCUS Oerbaekvej 276, DK-5220 Odense

# **Background and Objectives**

The objective of this study was to compare the detection of PRRSV in pooled oral fluid (OF) and manure samples using cotton ropes to establish a cost effective, non-invasive monitoring of PRRSV status.

# Material and Methods

A Danish nursery site with 4 barns/23 rooms was selected for sampling. Each of two consecutive weaning test batches (8 weeks) sourced from a PRRSV eliminated (PCR neg) breeding herd, were divided into two rooms (30 pigs/pen) and monitored for the entire nursery period. Twice/week manure from cooled room pits (10 - 15 °C) (RM) were pumped to an external tank (CMT) also emptied twice/week. Weekly OF was sampled by four ropes/room, each placed between 2 pens for 30 minutes. OFs were pooled to one PCR/room. Weekly, one CMT and one RM sample/room (same technique as OFs) were collected by dipping a rope in the manure and cooled and PCR-tested. Furthermore, all rooms containing pigs were sampled using OFs PCR at entry and exit of each test nursery batch.

#### Results

In the first batch, 57% OF, 64% RM and 100% CMT samples were positive from CT values ranging from 24.5 to 29.5 in OF, from 29.3 to 37.5 in RM and from 30.7 to 32.5 in CMT samples. In the second batch, 0% OF, 7% RM and 29% CMT samples were positive. The proportion of positive OF rooms at the beginning and end of batch 1 were 19% (3/16) and 38% (7/18) for batch 1 and (0/13) and 0% (0/17) second batch.

#### **Discussion and Conclusion**

Manure tank sampling is a cheap (1 time/week), non-invasive and accessible (from the outside of the farm) option for routine PRRS status monitoring on nursery sites. The positive CMT during the first batch reflected the status of RM and/or OF sampling. Manure cooling possibly helped to the conservation of the PRRSV RNA in RM and CMT. Positivity of CMT during the first sampling weeks of batch 2, without positivity in any tested rooms, might indicate left over from previous weeks. CMT testing offers cost effective monitoring of sites for PRRSV status.

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# OVER TWO HUNDRED THOUSAND PCR SUBMISSIONS IN THE U.S. VETERINARY DIAGNOSTIC LABORATORIES TESTED FOR PORCINE CIRCOVIRUSES: WHAT ALL THESE TEST RESULTS CAN TELL US ABOUT PCV2 AND PCV3?

G. Cezar <sup>1</sup>, R. Main <sup>1</sup>, A. Rovira <sup>2</sup>, F. Matias-Ferreyra <sup>3</sup>, A. Pillatzki <sup>4</sup>, M. Prarat <sup>5</sup>, C. Bowen <sup>6</sup>, D. Linhares <sup>1</sup>, <u>G. Trevisan <sup>1</sup></u> <sup>1</sup>lowa State University <sup>2</sup>University of Minnesota <sup>3</sup>Kansas State University <sup>4</sup>South Dakota State University, Brookings, USA <sup>5</sup>Ohio Animal Disease and Diagnostic Laboratory, Reynoldsburg, USA <sup>6</sup>Purdue Animal Disease and Diagnostic Laboratory, West Lafayette, USA

# **Background and Objectives**

Porcine circoviruses (PCVs) have been associated with diverse clinical syndromes in swine, resulting in economic losses for producers. Daily, thousands of polymerase chain reaction (PCR) tests are performed in veterinary diagnostic laboratories (VDLs) worldwide to detect DNA material of swine pathogens. This study aims to unravel epidemiological aspects of PCV2 and PCV3 PCR detection using the diagnostic data comprised of six VDLs in the United States.

# **Material and Methods**

Laboratory data underwent cleaning and standardization, with SAS scripts and C# application. The PCR data were compiled at a case level, using VDL-assigned accession IDs, excluding non-porcine and research samples. Cases with at least one PCR-positive sample were assigned as positive. Year, month, age categories, and specimen were assigned based on submission information for received dates, farm type/age, and sample. R algorithms and Microsoft Power BI enabled analysis and visualization of PCVs trends over time, region, specimen, and age category.

#### Results

The final PCR database comprised 161,788 PCV2 cases from 2002 to 2023, and, 51,822 cases from 2017 to 2023 for PCV3. The generated and aggregated information is publicly available online visualization platform at http://www.fieldepi.org/SDRS. Since 2019, the percentage of PCV3 positive cases from sow farms has been at least 10% higher than PCV2, with PCV3 reaching monthly levels above 56% of positive cases. From 2019 until 2023, 7,331 of 9,993 PCV3-positive specimens from the sow farm were processing fluids, followed by oral fluids (730 of 9,993), and serum (466 of 9,993). The number of cases tested for PCV2 and PCV3 simultaneously have been increased since 2018, and in 2023, 8,051 of 10,780 cases tested for PCV2 were tested for PCV3. Processing fluids, oral fluids, and lungs were the most frequently tested specimens for both pathogens. Processing fluid leads to the percentage of PCV2/PCV3 co-detection with 3,828 of 7,989 cases, followed by oral fluids (1,761 of 7,989), and lungs (1,053 of 7,989).

### **Discussion and Conclusion**

This study sheds light on the epidemiological trends of porcine circoviruses, demonstrating the increased trend of codetection of PCV2 and PCV3 by PCR, which may play a role in the detection dynamics of circoviruses.

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# COMPARISON OF CUTANEOUS INJURIES IN SMALLPOX IN THE NEONATAL AND PRE-WEANING STAGES OF MATERNITY

P. Sánchez-Giménez <sup>1</sup>, E. Ros Párraga <sup>2</sup>, L. Martínez-Alarcón <sup>3</sup>, F. Murciano <sup>5</sup>, R. Fernández-Rodríguez <sup>1</sup>, E. Rodríguez-Hernández <sup>5</sup>, J. Seva <sup>4</sup>, G. Ramis <sup>5</sup>

AGROPOR, Murcia, Spain

<sup>2</sup>Departamento de Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Murcia, Spain <sup>3</sup>Instituto Murciano de Investigación en Biomedicina (IMIB), Murcia, Spain

<sup>4</sup>Dpto. Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria de Murcia, Spain

<sup>5</sup>Dpto. Producción Animal,. Facultad de Veterinaria., Universidad de Murcia, Spain

# **Background and Objectives**

Swine pox is a viral disease that can affect animals of different ages. The most common presentation is in nursery animals, but it can also occur in a neonatal or congenital form in the first hours of life of piglets, although this is rare. The aim of this work was to compare the skin lesions in a case of neonatal smallpox with a pre-weaning case in the same farm.

# Material and Methods

On a 2,000-sow farm in SE Spain, formalin-fixed skin tissue samples were taken from two piglets with symptoms of neonatal swine pox (during the first week of life; CPV) and skin tissue samples from piglets with signs of piglet pox in the nursery (NPV) during the same period of time. The animals were necropsied and areas of skin showing the most representative lesions of the case were fixed in 4% formalin. Tissues were routinely processed in the histopathology laboratory, stained with HE and observed under the light microscope.

## Results

The first lesion observed in both groups of animals were subcorneal vesicles, sometimes extending into the stratum epidermis. In addition, vacuolated and degenerating keratinocytes were observed within the stratum epidermis leading to the formation of the vesicles. The main difference between CPV and NPV piglets was the size of the vesicles, which was much larger in the latter. The presence of virus was confirmed by observing acidophilic and intracytoplasmic inclusion bodies. In CPV piglets, the transition from a vesicular state, which is not very relevant and with little liquid, and how the vesicles evolved rapidly to form pustules with abundant degenerated neutrophils (pyocytes) and remains of keratin, amorphous eosinophilic material, mixed, could be observed. NPV piglets did not show this evolution.

# **Discussion and Conclusion**

In older animals there is no evolution of vesicular lesions characteristic of smallpox, whereas in neonatal piglets there is a rapid evolution. This evolution is probably due to the superinfection of the lesions due to the immune immaturity of the neonatal stage, and this occurs less frequently in the nursery.

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# FACING THE CHALLENGES OF ENDEMIC AFRICAN SWINE FEVER IN VIETNAM

#### C.D. Lai 1, T.N.T. Ngo 2, T.D. Do 2

<sup>1</sup>Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE 68583, USA <sup>2</sup>Faculty of Animal Science and Veterinary Medicine, Nong Lam University, HCMC, Thu Duc city, Ho Chi Minh city, Vietnam

#### **Background and Objectives**

The rapid change in the ASF epidemiological status to endemic is also clearly seen in recent years from the first introduction in Viet Nam. Thus, the challenge of endemic ASF and the futureof Vietnam's pig herd are of great concern. The objective of this paper is to review and evaluate the epidemiological picture of ASF from the typical epidemic to the endemic situation and confrontation with disease prevention and re-herding.

# **Material and Methods**

We searched academic databases such as Scopus, Web of Science, and Pubmed. We also extracted the findings of many studies that were published in Vietnamese journals and used the data of the General Statistics Office (GSO), Department of Animal Health (DAH) of Vietnam and Food and Agriculture Organization (FAO) to describe the status of ASF in Vietnam.

# Results

The first year of ASF introduction to Vietnam was 2019, it has had a great impact on pig industry. The spread of ASFV in infected farms in whole country was reported to be very rapid, which is due to the very special epidemiological characteristics of ASF in pigs raised in Vietnam. However, the Vietnamese pig herd has experienced rapid recovery and growth in the following two years. However, at current according to local reports from farms, the clinical signs of ASF outbreaks have been showing difference compare with previous reports. While previously characteristic signs were not evident, unusual clinical pathologies were noted in sow herds such as necrotizing mastitis, reproductive failure (abortion, stillborn and mummified), arthritis, necrotizing dermatitis, ect.

# **Discussion and Conclusion**

The adaptation strategies in Vietnam contribute experience and solutions to the pig production. In ASF endemic regions, a combination of strict biosecurity with new concept and rapid intervention in the outbreak to prevent spread and enable rapid restocking should be in place. Although some live attenuated vaccines received master-seed transfer from abroad are produced and used in the endemicASF context of Vietnam, they still need time and more rigorous research to be sure of their safetyand effectiveness on a large scale.

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# REDUCING PSEUDORABIES VIRUS CIRCULATION IN FATTENING PIGS BY PARTIAL-DEPOPULATION STRATEGY AND OPTIMIZING IMMUNIZATION PROTOCOL

<u>J. Wu</u><sup>1</sup>, K. Wang <sup>1</sup>, L. Yuan <sup>1</sup>, C. Liu <sup>2</sup> <sup>1</sup>Swine Business Unit, Zoetis China, Shanghai, P.R. China <sup>2</sup>Zoetis BioPharmaceutical Co., Ltd, Suzhou, P.R. China

# **Background and Objectives**

Pseudorabies (PR) is a highly infectious viral disease caused by pseudorabies virus (PRV). In this study, the seroprevalence of a PRV-infected herd was investigated, and then a partial-depopulation strategy and optimized immunization protocol were implemented to reduce the PRV infection pressure.

# **Material and Methods**

During November 2021 to May 2022, a total of 1874 serum samples were collected from a one-site farrow-to-finish farm (1,800 sows) located in Guangdong province. The first dose of PRV vaccine Bartha-K61 (Zoetis) was administered at 7-week-old of pigs, followed by a booster vaccination given 4 weeks later. The seroconverted PRV-gE antibodies in all pigs were detected by IDEXX PRV/ADV gE Ab Test. As part of the protocol, the sampling frequency and number of samples from sows were increased to detect seropositive sows with high parity (e.g., parity 5 or higher) and gradually remove them from the farm. For wean-to-finish pigs, maternal antibodies were determined by viral neutralization (VN) test, and the vaccination timing protocols were adjusted according to the results. In every six months, pigs in all phases of the farm were sampled to monitor the seroprevalence of PRV-gE.

# Results

At the beginning of the study, the seroprevalence of PRV-gE in sows, piglets, nursery pigs, fattening pigs were 54.1%, 71.7%, 43.3% and 57.1% respectively. During the fattening stage, seroconversion occurred significantly from 16-weeks of age. The mean VN titer of pigs with maternal immunity at 6-week-old was 1: 13.6. The immunization protocol was then adjusted to the prime immunization at 11-weeks of age and the booster immunization at 14-weeks of age. Meanwhile, a partial-depopulation strategy for sows was implemented. The seroprevalence of PRV-gE in sows and fattening pigs older than 12 weeks were decreased to 29.1% and 20.8% after 6 months of implementation, and 9.2% and 0% after 12 months of implementation, respectively.

# **Discussion and Conclusion**

The seroprevalence of PRV-gE in sows and fattening pigs was greatly reduced by partial-depopulation strategy and the adjustment of immunization protocol. Although the farm did not achieve PRV eradication, but since the seroprevalence of PRV-gE in sows has dropped below 10%, all the sows would be re-tested to confirm complete elimination of the PRV from the farm.

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# RESULTS OF A PASSIVE SURVEILLANCE ON INFLUENZA A VIRUSES DETECTED IN FRENCH PIG FARMS IN 2022 AND 2023

<u>A. Jardin</u><sup>1</sup>, S. Brilland<sup>1</sup>, P. Leneveu<sup>2</sup>, K. Lillie-Jaschniski<sup>3</sup> <sup>1</sup>Ceva Santé Animale, 33 500 Libourne, France <sup>2</sup>Ceva Santé Animale, 10 Avenue de la Ballastière, 33500 Libourne, France <sup>3</sup>Ceva Santé Animale, Libourne, France

#### **Background and Objectives**

Swine influenza A virus (swIAV) is one of the most important infectious diseases in pig farms for its financial and productive impact. The aim of this investigation is to share the results of a private hold passive surveillance on swIAV performed in France in 2022 and 2023.

# **Material and Methods**

From 2022 to late 2023, in 417 French farms with suspicion of swIAV infection,1 to 30 samples (nasal swabs, tracheal mucus, lungs or oral fluids) were taken by veterinary practitioners. Most of the sampled animals presented evocative clinical signs, mainly respiratory disorders and fever. Presence of IAV and subtyping were assessed by RT-qPCR (Real Time quantitative Polymerase Chain Reaction).

#### Results

Nasal swabs and broncho-tracheal mucus swabs (BTMS) were the preferred samples, chosen respectively in 40.1% and 37% of the diagnostic visits. The highest positivity rate (43%) was obtained on BTMS. In 39.3% of the investigated farms, swIAV was detected. Weaned piglets below 12 weeks of age represent the main investigated age category (55.5% of the sampled animals) with a positivity rate of 42.8 %. In 13.3% of the farms, samples were taken on breeders (gilts and sows). On sows, the swIAV detection rate was quite low (23.3%) compared to the others age categories. In 67.2% of the investigated farms with at least one sample positive for swIAV, virus subtyping was successful. The subtyping in 2022 and in 2023 provided very similar results: H1avN2 was detected in close to 50% (2022: 47.1%; 2023: 49.1%) followed by H1avN1 in around 40% (2022: 38.6%; 2023: 41.5%) and by all the viruses with a hemagglutinin or a neuraminidase coming from the H1N1pdm09 in about 11% (2022: 12.9%%; 2023: 9.4%) of the cases. H1huN2 was only detected once in 2022.

# **Discussion and Conclusion**

The data collected by this passive epidemiological surveillance allow to give an overview on the occurrence of different swIAV subtypes in French domestic swine through years. At farm level, this surveillance contributes to understand the dynamic of swIAV circulation and to identify the involved strains, helping to choose the best preventive strategies.

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# EVALUATION OF THE EFFECT OF TILMICOSIN (TILMOVET ORAL SOLUTION) ADMINISTERED IN DRINKING WATER IN PRRS-VIREMIC PIGS IN A FIELD STUDY

<u>U. Klein</u><sup>1</sup>, A. Garmon <sup>2</sup>, L. Claerhout <sup>1</sup>, W. Depondt <sup>1</sup> <sup>1</sup>Huvepharma NV Antwerp, Belgium <sup>2</sup>AM Animalia, Bianya SL, Spain

# **Background and Objectives**

The objective of the study was to assess the effect of tilmicosin administered in drinking water to PRRS viremic pigs to control PRRS infection.

# Material and Methods

The study was conducted in a Spanish farm with recent Rosalia PRRSV strain outbreak. The effect of tilmicosin water medication was examined in a 3-time medication programme (dosage 15mg/kg bw; treatment duration 5 consecutive days; pig age at medication period: 4, 6, 8 weeks) with 14 days treatment stop between the treatments. Assessed parameters: virus load and viremia (qPCR), PRRSV clinical signs, weight gain, health status, mortality rate. For evaluation of the medication effect three subgroups (PCR-negative: Ct value >40; PCR-positive low level viremia: Ct value 30-40; PCR-positive high level viremia: Ct value <30) were created. G.parasuis and M.hyopneumoniae were present during the study.

#### Results

High PRRS infection level at study start (3 weeks of age) was found with 51% and 46% PRRSV-positive pigs in the control and the tilmicosin medicated group, respectively.

PCR low level-positive pigs at study start show in the tilmicosin medicated group a higher infection reduction by 25% (6 weeks of age) and by 47% (8 weeks of age) versus the control group (viremia decrease by 15%/6 weeks of age; viremia decrease by 8%/8 weeks of age). The tilmicosin medicated group in PCR high level positive pigs at study start show a similar infection reduction by 25% (8 weeks of age) and by 40% (10 weeks of age) versus the control group (viremia decrease by 29%/8 weeks of age; viremia decrease by 40%/10 weeks of age). The PRRS infection suppression in medicated pigs resulted in higher survival rate (82.8%) versus the control group (77.5%). Concomitant treatments were more often required in non-medicated pigs (115 times) in comparison to medicated pigs (93 times). The average daily weight gain in medicated pigs (300g/day) was higher versus non-medicated pigs (270g/day).

#### **Discussion and Conclusion**

Tilmovet<sup>®</sup> water medication in PRRS viremic pigs post-weaning is an effectrive tool to reduce PRRS-viremia and stabilize PRRS-viremia situations. Tilmicosin use according registered treatment dose and administration time before high viremia is recommended.

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# SEROLOGICAL INVESTIGATION OF SWINE INFLUENZA A VIRUS IN POLISH FARROW-TO-FINISH PIG HERDS BETWEEN JANUARY 2018-JULY 2022

<u>K. Lillie-Jaschniski</u>, R. Panek<sup>2</sup>, R. Lewko<sup>2</sup>, K. Strutzberg-Minder<sup>3</sup> <sup>1</sup>Ceva Santé Animale, Libourne, France <sup>2</sup>Ceva Animal Health Poland, Warsaw, Poland <sup>3</sup> IVD GmbH, Innovative Veterinary Diagnostics, Seelze, Germany

## Background and Objectives

Swine influenza A virus (swIAV) plays a major role in the porcine respiratory disease complex (PRDC). Circulating in sow farms additionally huge impact on reproductive parameters have been demonstrated. The aim of this serological investigation was to study the distibution of subtypes in Polish farrow-finish herds and fattening farms.

# **Material and Methods**

From January 2018-July 2022 in total 2833 serum samples from 218 farms, producing pigs with Polish breed (no imported animals), were investigated for swIAV within routine diagnostic. The sera were tested for Influenza by Haemagglutination Inhibition (HI) test at IVD GmbH, Seelze, Germany. Only swIAV non vaccinated farms with a minimum of 10 samples/farm were included in this evaluation. A farm was considered positive if two or more samples were positive (HI titer ≥ 40) at least for one subtype. H1avN1 antigen (Ag) type1, H1avN1 antigen type2, H1huN2, H3N2, H1pdmN1 and HpdmN2 virus subtypes were used for HI.

#### Results

In total 74.8% (n=163) of the sampled farms were tested positive for swIAV. Among those farms 79.1% (n=129) tested positive for H1avN1 Ag type1, 75.5% (n=123) for H1avN1 Ag type2, 63.2% (n=103) for H1pdmN1, 66.3% (n=108) for H1pdmN2, 23.9% (n=39) for H1huN2, and 17.2% (n=28) for H3N2. Slight differences could be observed in the individual years. 19.3% (n=42) all farms were only positive for one subtype, 33.9% (n=74) for two, 16.1 (n=35) for three and 5.5% (n=12) for all 4 subtypes.

### **Discussion and Conclusion**

Our findings differ from the findings of Czyzewska-Dors et al. in 2017, following up 145 farrow-finish- farms in Poland in the period of March 2011- February 2015 by HI. There 59.3% of the herds were tested positive for H3N2, whereas in our study only 17.2% of the swIAV positive farms seroconverted to H3N2. The positivity rate for H1avN1 remained very similar with 77.2% compared to 79.1% in our study. The amount of farms positive for pandemic subtypes also was also higher compared to the afore mentioned study. Our data indicate that there is a change in the subtypes circulating in Poland. The detection rates of pandemic subtypes are rising over the years.

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# EVALUATION OF THE EFFECT OF TILMICOSIN (TILMOVET PREMIX) ADMINISTERED TO PRRS-VIREMIC PIGS POST-WEANING IN A FIELD STUDY

<u>U. Klein 1</u>, A. Garmon 2, L. Claerhout 1, W. Depondt 1 1Huvepharma NV Antwerp, Belgium 2AM Animalia, Bianya SL, Spain

# **Background and Objectives**

The objective of the study was to assess the effect of tilmicosin feed medication in PRRS viremic pigs to control PRRS infection.

# Material and Methods

The study was conducted in a Spanish farm with recent Rosalia PRRSV strain outbreak. The effect of tilmicosin feed medication was examined at 400ppm (appr. 16mg/kg bw) in nursery pigs (treatment duration 21 days; pig age at medication period: 4-6 weeks). Assessed parameters: virus load and viremia (qPCR), PRRSV clinical signs, weight gain, health status, mortality rate. For evaluation of the medication effect three subgroups (PCR-negative: Ct value>40; PCR-positive low level viremia: Ct value 30-40; PCR-positive high level viremia: Ct value <30) were created. On the farm G.parasuis and M.hyopneumoniae were present during the study.

#### Results

High PRRS infection level at study start (3 weeks of age) was found with 51% and 46% PRRSV-positive pigs in the control and the tilmicosin medicated group, respectively.

PCR low level-positive pigs at study start show in the tilmicosin medicated group a higher infection reduction by 30% (6 weeks of age) and by 40% (8 weeks of age) versus the control group (viremia decrease by 15%/6 weeks of age; viremia decrease by 8%/8 weeks of age).

The tilmicosin medicated group in PCR high level-positive pigs at study start show a higher infection reduction by 18% (6 weeks of age) and by 40% (8 weeks of age) versus the control group (viremia decrease by 8%/6 weeks of age; viremia decrease by 29%/8 weeks of age). The PRRS infection suppression in medicated pigs resulted in higher survival rate (82%) versus the control group (77.5%). Concomitant treatments were more often required in non-medicated pigs (115 times) in comparison to medicated pigs (97 times). The average daily weight gain in medicated pigs (280g/day) was higher versus non-medicated pigs (270g/day).

# Discussion and Conclusion

Tilmovet<sup>®</sup> feed medication in PRRS viremic pigs post-weaning is an effective tool to reduce PRRS-viremia and stabilize PRRS-viremia situations. Tilmicosin administration according to prudent use policies at registered treatment dose and administration time before high viremia is recommended.

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# IMPROVED DETECTION OF ANTIBODIES TO PRRSV-US STRAINS WITH THE ID SCREEN® PRRS INDIRECT ELISA

L. Comtet <sup>1</sup>, L. Olagnon <sup>1</sup>, A. Greatrex <sup>1</sup>, P. Pourquier <sup>1</sup> <sup>1</sup>IDvet – Innovative Diagnostics, Grabels, France

#### **Background and Objectives**

PRRS is a highly contagious and economically damaging viral disease in pigs, primarily leading to reproductive issues in sows and respiratory syndromes. The PRRS virus (PRRSV) has two distinct genotypes: European (EU-PRRSV, species 1) and American (US-PRRSV, species 2).

ELISA is widely used to detect antibodies directed toward PRRSV. This study summarizes the superior performance of ID Screen® PRRS Indirect ELISA kit (referred as kit A) and presents its correlation with the market leader (kit B).

# **Material and Methods**

Diagnostic specificity of kit A was evaluated on 1794 samples coming from PRRS-free herds. Sensitivity was evaluated with:

- 8 reference sera from the International PRRSV PTS organised in 2023 by the Dutch Animal Health Service (GD Deventer, The Netherlands);

- 113 and 440 swine sera respectively from European and North-American infected herds.

Four pigs were intradermally vaccinated with a PRRS Porcilis®-like strain, and their seroconversion kinetics were studied at 0/6/9/14/21/27 days post-vaccination (dpv).

Global correlation with kit B was studied on 2004 sera from negative and infected herds, and later on 590 samples from PRRS-free herds. All sera were tested in parallel with both kits A&B studied hereby.

#### Results

Measured specificity was 99.9 % Cl<sub>95%</sub> [99.8 % - 100.0]. Kit A efficiently detected indifferently both EU and US-type samples and detected seroconversion between 9 and 11 days post vaccination. The percentage of correlation with kit B was 98.6% (kappa = 0.97, [0.92 ; 1.00]) in the first run. In the second run (PRRS-free herds, n=590), 1 sample is falsely positive with kit A with a S/P% very close to the cut-off (S/P% = 42%), leading to a specificity of 99.8% [0.991;0.999], while 7 samples give false positive results with kit B, leading to a specificity of 98.8% [0.975;0.994].

# **Discussion and Conclusion**

Kit A demonstrates excellent specificity and high performance on a reference panel of sera. It efficiently detects positive animals in the field and correctly identified both PRRSV species indifferently (EU&US) and delivers similar results (Se, Sp, analytical Se) as kit B. Kit A demonstrate a high level of agreement with kit B while maintaining a very high specificity.

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# ISOLATION OF MAJOR PATHOGENS IN ORAL FLUIDS FROM ANIMALS VACCINATED AGAINST SWIAV

<u>G. Ramis</u><sup>1</sup>, F. Murciano <sup>1</sup>, E. Goyena <sup>2</sup>, B. Gonzalez-Guijarro <sup>3</sup>, A. Cuellar-Flores <sup>1</sup>, D. Espigares <sup>4</sup>, K. Lillie-Jaschniski <sup>5</sup> <sup>1</sup>Dpto. Producción Animal, Facultad de Veterinaria., Universidad de Murcia, Spain <sup>2</sup>DEPARTAMENTO DE SANIDAD ANIMAL, FACULTAD DE VETERINARIA, UNIVERSIDAD DE MURCIA, SPAIN <sup>3</sup>Departamento de Producción Animal. Universidad de Murcia <sup>4</sup>Ceva Salud Animal, Barcelona, Spain <sup>5</sup>Ceva Santé Animale, Libourne, France

# **Background and Objectives**

Porcine respiratory disease complex (PRDC) is a pathological entity in which there are synergies between viral, bacterial, and even parasitic pathogens. However, the interactions between the pathogens and the mechanisms marking the synergies often remain unclear. The objective of this work was to quantify the presence of pathogens in oral fluids (OF) in fattening pigs vaccinated against swIAV, PCV2 and Mesomycoplasma hypopneumoniae.

# **Material and Methods**

Two batches of 300 piglets (VAC1 and VAC2) were vaccinated at 56 and 77 days of age simultaneously with two vaccines: RESPIPORC FLUpan H1N1 and RESPIPORCFLU 3 (Ceva Santé Animale, France) and one batch was taken as unvaccinated controls (CON1). All piglets were vaccinated against M. hyopneumoniae, PCV2 and Aujeszky's disease virus. The three groups were followed up by OF starting beginning of fattening one day before vaccination. The OF were taken from 6 pens every two weeks (7 samplings) and swIAV, PRRSv, M. hyopneumoniae, M. hyorhinis and PCV2 were quantified by PCR.

## Results

None of the samples were positive for PCV2 and almost all were positive for M. hyorhinis. In CON1 in week 1, 2 and 3 (83.3%, 50% and 50%) and in VAC1 in weeks 1 and 2 (16.7 and 66.7%, respectively) swIAV could be detected, whereas in VAC2 all samples were negative for swIAV. PRRSv was detected in all samplings for CON1 and VAC1 groups and in 4 out of 7 samplings in VAC2. M. hyo was detected in 3 samplings from CON1, once in VAC1 and twice in VAC2. There were differences in frequencies for swIAV and PRRSv.There was a higher frequency of PRRSv positivity in swIAV positive pens. There was a correlation between Ct values for PRRSv and M. hyorhinis (r=0.368) and a trend between PRRSv and swIAV (r=0.493)

# **Discussion and Conclusion**

In the conditions of this study, a decrease of swIAV detection rates in vaccinated groups was found, resulting in modifications of the synergies among pathogens observed. The frequency of detection of swIAV, is related to the frequency of detection of PRRSv. Further research is needed to clarify if the vaccination against swIAV could influence the prevalence (or at least shedding) of PRRSv

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# PRRS VIRUS SURVEILLANCE: ROLE OF VIRUS SEQUENCING TO ADAPT AND UPDATE REAL-TIME RT-PCR DIAGNOSIS TOOL

<u>J. Charrot</u><sup>1</sup>, S. Daly <sup>1</sup>, A. Quijada <sup>1</sup>, M. Lelièvre <sup>1</sup>, S. Moine <sup>1</sup>, Y. Zein-Phillipson <sup>2</sup> <sup>1</sup>Thermo Fisher Scientific, Lissieu, France <sup>2</sup>Thermo Fisher Scientific, California - US

# **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases that have brought significant economic losses to the swine industry worldwide.

This disease is caused by a single stranded positive-sense RNA enveloped virus which leads to high heterogeneity of the nucleotide sequence between individual strains.

A key part of effective prevention, control, and elimination strategies for PRRS is the development and implementation of highly sensitive and specific diagnostic methods.

The high mutation rate of the virus is addressed through regular monitoring of circulating strains over Europe and USA using sequencing technologies.

The aim of this study was to monitor circulating PRRS Virus (PRRSV) strains throughout Europe using sequencing technologies to adapt and update our VetMAX<sup>™</sup> RT-PCR PRRSV detection kit.

#### **Material and Methods**

Since 2016, ThermoFisher has established different partnerships to collect positive samples for PRRSV detection, in 12 different EU countries.

The choice of the sequencing strategy was based on PRRS viral load and the quality of the sampling process: sample collection, storage, shipment.

For samples containing a high or medium PRRS viral load with a high quality sampling, RNA-Seq or Long Range protocols on Ion S5<sup>™</sup> system were developed to cover the whole PRRS genome.

For remaining samples containing a weak PRRS viral load or with a poor quality sampling, capillary electrophoresis protocol on Genetic Analyzer was performed to obtain targeted sequences of PRRS genome.

# Results

Our VetMAX<sup>™</sup> kit allows the detection of EU & NA circulating strains (tests performed on 500 field EU and NA samples). Diagnostic sensitivity and specificity was evaluated on a panel of 130 EU field samples previously characterized by routine labs.

Our full solution shows diagnostic sensitivity & specificity of 100%.

### **Discussion and Conclusion**

Our VetMAX<sup>™</sup> Kit has been updated in 2022 to keep high diagnostic sensitivity by identifying conserved regions for both EU & NA primers and probes assays.

The monitoring of circulating European PRRSV strains using sequencing technologies enabled the sequencing of viral RNA isolated directly from field samples.

The VetMAX<sup>™</sup> PRRSV EU & NA 3.0 Kit is designed strengthen the efficiency of PRRSV surveillance program in the field, with diagnostic sensitivity & specificity of 100%.

VVD – Virology and Viral Diseases

# SEROPREVALENCE OF VIRAL RESPIRATORY DISEASES IN SWINE BACKYARD FARMS IN SERBIA

<u>S. Solaja</u>, D. Glisic <sup>1</sup>, Z. Zurovac Sapundzic <sup>1</sup>, N. Jezdimirovic <sup>1</sup>, B. Milovanovic <sup>1</sup>, V. Milicevic <sup>1</sup> Scientific Institute of Veterinary Medicine of Serbia

#### **Background and Objectives**

Despite modern biotechnological measures, enzootic respiratory diseases, encompassing primarily the reproductive and respiratory syndrome (PRRS) and swine influenza (SIV) represent one of the main challenges in intensive pig production. Aujeszky's disease (AD) also has significant economic impact, especially in countries with no eradication programme. In Serbia, majority of homesteads implement an extensive farming system. Although recognized for environmental and animal welfare benefits, backyard farms represent high-risk infection sources for commercial farms because of low biosecurity measures, swill feeding, natural breeding and uncontrolled trade.

# **Material and Methods**

From 69 backyard holdings, 222 sera samples were collected. Commercial ELISA kits were used for specific antibody detection and chi-square test was used to evaluate the significance level between farm categories and disease seroprevalence.

# Results

Overall seroprevalence for AD was 17.1% with herd-level seroprevalence of 27.5%, found exclusively on 10.1% of farms. Within-herd seroprevalence for AD ranged from 33 to 100%. Anti-PRRSV antibodies were found in 2.7% animals, from two (2.9%) herds. Within-herd PPRS seroprevalence was 100%, while no seroconversion for the other two tested diseases was observed. No SIV antibodies were detected. There were 69,6% seronegative herds for all three diseases. Chi-square test showed no significant association between farm category and disease seroprevalence.

#### **Discussion and Conclusion**

Backyard farms are often unfenced with no supervision, thus enabling animals' contact with wild boars, and representing significant risk for mutual exchange of various pathogens and novel virus strains. Although generally disallowed, pig owners often work on commercial pig farms. Because of this connection, pig owners are considered a significant source of infection for commercial pig farms. In this study, AD was frequently present in backyard farms, seroprevalence of PRRS was very low, and SIV wasn't present. SIV and PPRS are common among densely populated farms and naïve animals, unlike backyard farms in which more common diseases are those transmissible from wild boar, such as AD. As Serbia lacks control and eradication plan for AD, one-quarter of backyard farms are AD seropositive, coinciding with average seroprevalence in wild boar populations across Europe. Education of backyard farmers and monitoring of their homesteads could be crucial for preventing disease outbreaks.

VVD – Virology and Viral Diseases

# **REDUCTION OF MUMMIFIED PIGLETS AFTER IMPLEMENTING A NOVEL VACCINE IN CHINA**

<u>W. Fei</u><sup>1</sup>, J. Miguel <sup>2</sup>, T. Yang <sup>1</sup>, B. Feng <sup>1</sup>, K. Zhou <sup>1</sup>, M. Wu <sup>2</sup>, D. Angelats <sup>2</sup> <sup>1</sup>HIPRA China, Beijing <sup>2</sup>HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

Porcine Parvovirus (PPV) is an endemic disease. In PPV-vaccinated herds, reproductive losses are typically low, going unnoticed because it does not significantly affect productive indexes. Farm monitoring data and statistical process control (SPC) can show the impact of changes in vaccination protocols related to reproductive vaccines. The aim of this study was to evaluate the impact of a novel vaccine in China, on a farm with no apparent reproductive problems related to PPV.

# **Material and Methods**

From July 2021 to July 2022, an 8,000 sow farm located in North China was using a local inactivated PPV-1, WH-1 strain vaccine (V1), with a mineral-oil adjuvant. From August 2022 to September 2023, V1 was switched by ERYSENG® PARVO (V2), an inactivated bivalent vaccine containing Swine Erysipelas and PPV antigens and adjuvanted with HIPRAMUNE® G. Both vaccines were administered twice at least 3 weeks before insemination in gilts, and in multiparous, every cycle in lactation, around 10 days after farrowing. The statistical analysis was performed using the R software. A Kruskal-Wallis test was employed to assess the indicators. A significance level of 0.05 (p < 0.05) was established to determine the statistical significance of the results.

#### Results

The percentage of mummified foetuses during the period using V1 was  $0.94 \pm 0.13$ . During that period, the percentage of farrows with  $\geq$ 1% of mummies was 30.77%. These parameters were significantly reduced (p < 0.05) to 0.65 ± 0.16 and 7.14% respectively after switching to V2.

# **Discussion and Conclusion**

Even though the percentage of mummified piglets was already below 2%, the implementation of ERYSENG® PARVO significantly reduced the percentage of mummified piglets per litter. In this case, PPV problems were not apparently detected on the farm, but it was causing clinical problems, as it is indicated by the significative reduction of litters with  $\geq$ 1% of mummies. These differences could be explained by better immune protection elicited by the PPV antigen and/or the adjuvant used. Performing a detailed data analysis is key to evaluate the potential performance improvement on sow farms.

VVD – Virology and Viral Diseases

# SAFETY ASSESSMENT OF THE PRRSV STRAIN ALL-183 ADMINISTRATED AS A MLV VACCINE IN PREGNANT SOWS UNDER LABORATORY CONDITIONS

L. Delgado <sup>1</sup>, A. Aguaron <sup>1</sup>, E. Roca <sup>1</sup>, M.L. De Arriba <sup>1</sup> <sup>1</sup>Laboratorios Syva, S.A.

#### **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome (PRRS) is still a great challenge and has one of the highest economic impact in the swine production system. Modified-live virus (MLV) vaccination is the principal strategy used in the field to control the impact of the infection, although safety can be a concern, especially in pregnant animals.

Laboratorios Syva is the Marketing Authorisation Holder of a PRRSV MLV vaccine intended to be used in piglets (Pyrsvac-183). The safety of its vaccine strain ALL-183, is assessed in this study in breeding pigs, for the potential development of a MLV adjuvanted vaccine.

# **Material and Methods**

Eleven pregnant sows were administered intramuscularly 10<sup>7.3</sup> TCID<sub>50</sub> of the vaccine strain at 80 days of pregnancy, followed by a single dose containing 10<sup>6.3</sup> TCID<sub>50</sub>, 14 days after. A control group of 8 sows was also included in parallel. General and local safety parameters were evaluated, as well as reproductive performance parameters, transplacental transmission, and general health and growing performances of the litters until weaning.

# Results

No significant systemic clinical signs were observed in the treated sows. The mean temperature increases were reached eight hours after each administration, with maximums of 1.61 and 1.77 ° C, respectively. Temperatures returned to normal in 24 to 48 hours. Redness, swelling and increase in local temperature at the injection sites were commonly observed after administration, and generally disappeared within 6-to-10 days.

Reproductive performance was not affected after vaccination; there were no abortions, and the pregnancy length was similar in both groups. No statistical differences were observed between control and treated groups in the number of total born, live born, stillborn or mummified piglets, or the weaned piglets. No significant differences were observed in ADWG from birth to weaning. PRRSV transplacental transmission was detected by PCR.

#### **Discussion and Conclusion**

Safety was demonstrated during pregnancy, and the vaccine strain, although vertically transmitted, had no consequences in the offspring.

VVD – Virology and Viral Diseases

# SOW DOUBLE-VACCINATION WITH AN SIV BIVALENT VACCINE IMPROVED WEANERS' ANTIBODY LEVELS AND POSTPONED THE TIME OF INFECTION

W. Van Herten <sup>1</sup>, J. Beek <sup>2</sup>, T. Nunes <sup>3</sup>, D. Giralt <sup>3</sup>, E. Van Esch <sup>4</sup> <sup>1</sup>DAC Zuid-Oost, Deurne, Netherlands <sup>2</sup>HIPRA Benelux <sup>3</sup>Hipra, Amer (Girona), Spain <sup>4</sup>Technical Services Manager Swine, HIPRA Benelux

### **Background and Objectives**

Different vaccination schemes are available to help control the Swine Influenza Virus (SIV) on swine farms. This case report describes the change in antibody levels and time of infection associated with different sow vaccination strategies used on a SIV-positive farm.

# **Material and Methods**

On a breeding farm where the sows were vaccinated with a trivalent vaccine (H1avN1, H1huN2, H3huN2) at 14 weeks of gestation (VS1), SIV infection was diagnosed in weaners with recurrent respiratory distress (PCR positive for H1avN1 and H1huN2). In May 2020, the sows additionally received a single dose of a monovalent vaccine (H1pdmN1) at 11 weeks of gestation and 2 doses of a bivalent vaccine (BiV: H1swN1, H3swN2) at 11 and 14 weeks of gestation (group VS2). A study was performed on VS1 and VS2 with the collection of sera from 10 piglets at 4, 6, 8, and 10 weeks of age. Also, four pools of oral fluids (OF) were collected from each piglet age group. The antibody level was assessed through ELISA testing of sera and the presence of SIV was assessed through PCR testing of OF.

#### Results

Piglets from VS2 showed significantly higher antibody titres at 4 weeks (114.8±25.4 versus 66.8±31, p=0.005) with a continuous decay until 10 weeks. The VS1 piglets had an increase in antibody titres after 8 weeks, with significantly higher values at 10 weeks (39.5±24.4 versus 16.1±7.8 in VS2, p=0.019). All OF pools from VS1 were PCR-positive for SIV from 4 weeks of age onwards, whilst the OF pools from VS2 only became positive at 8 weeks.

# **Discussion and Conclusion**

In this field case, heterologous vaccination, and the intense use of a BiV vaccine in sows corresponded to a significant increase in antibody levels in weaners, and a delay in the time of infection from 4 to 8 weeks of age. The rapid decay of antibody titres in VS2 piglets may have resulted from the high infection pressure on the farm and consequent consumption of these antibodies. Furthermore, the higher antibody titres in 10-week-old piglets in VS1 compared to VS2 could be explained by seroconversion after early infection.

# ONE HEALTH: VETERINARY PUBLIC HEALTH AND SUSTAINABLE PIG PRODUCTION

# VPH-PP-01

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

# ANTIMICROBIAL RESISTANCE LANDSCAPE IN FATTENING PIG FARMS: UNRAVELING THE IMPACT OF DIVERSE ANTIMICROBIAL USE PROGRAMS ON THE PREVALENCE AND CHARACTERISTICS OF ESBL-PRODUCING ESCHERICHIA COLI STRAINS

N. Supimon <sup>1</sup>, J. Yindee <sup>1</sup>, W. Niyomtham <sup>1</sup>, T. Pongpan <sup>2</sup>, <u>N. Prapasarakul</u> <sup>1</sup> <sup>1</sup>Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand <sup>2</sup>Charoen Pokphand Foods PCL, Bangkok, Thailand

# **Background and Objectives**

Multidrug-resistant Escherichia coli (E. coli) strains, particularly those carrying antimicrobial resistance genes relevant to human medicine, pose a severe global public health threat, especially when originating from food-producing animals. This study explores the prevalence, resistance profiles, and antimicrobial resistance gene landscapes of extended-spectrum beta-lactamase (ESBL)-producing E. coli strains isolated from pigs and their surrounding environments in a fattening pig farm with diverse antimicrobial use programs.

# **Material and Methods**

From September 2022 to June 2023, samples were collected from fattening farms across Thailand with varying antimicrobial use programs. Rectal swabs from both pigs and workers, as well as wastewater samples, were included. ESBL-producing E. coli strains were identified using specific agar and confirmed through combination disk tests. Antimicrobial profiles were elucidated using the broth microdilution technique, and the presence of antimicrobial resistance genes was assessed via polymerase chain reaction.

#### Results

Results revealed a significant variation in the prevalence of ESBL-producing E. coli among three distinct farm categories: 60.4% in conventional farms (CF) using antimicrobial classes in both animals and humans, 54.5% in animal drug use farms (AF) utilizing registered antimicrobial classes for animals only, and 41.1% in farms practicing pig raising without antibiotics (RF). Out of the 102 ESBL-producing E. coli isolates, a staggering 96% exhibited multidrug resistance (MDR), with none resistant to meropenem. Moreover, resistance phenotypes were observed across farm groups, emphasizing the impact of different antimicrobial use programs. CF farms displayed resistance to AMP-CTX-SUL-TET-TRI-CIP-CHL-NAL, AF farms exhibited resistance to AMP-CTX-SUL-TET-CIP-NAL, and RF farms demonstrated resistance to AMP-CTX-SUL-TET-TRI-GEN. Distinct patterns emerged in the prevalence of blactore groups among farm categories, with CF and AF farms predominantly harboring blactored in the prevalence of blactored group, while RF farms exhibited the prevalence of blactored group and blactored group. The blactored group was commonly found in pigs and workers in CF farms.

# **Discussion and Conclusion**

This study underscores the critical importance of understanding antimicrobial use programs in fattening pig farms, providing valuable insights into the emergence and dissemination of ESBL-producing strains. The findings emphasize the urgent need for targeted strategies to mitigate the public health risks associated with antimicrobial resistance in the food production chain.

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# IMPLEMENTING A MODIFIED AIR WASHING SYSTEM TO PREVENT BIOAEROSOL EMISSION FROM THE EXHAUST AIR OF PIG BARNS

#### N. Volkmann<sup>1</sup>, N. Lisa<sup>1</sup>, N. Kemper<sup>1</sup>, J. Schulz<sup>1</sup>

<sup>1</sup>University of Veterinary Medicine Hannover, Foundation, Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour

# **Background and Objectives**

The air of pig fattening farms contains high amounts of bioaerosols, ammonia and dust. Usually, the primary purpose of exhaust air treatment is the reduction of ammonia, dust and odour. Since dust is reduced by washing or by passing biofilters, exhaust air treatment additionally has the potential to reduce bioaerosol concentrations. This study aimed to investigate the reduction of potentially harmful bioaerosol contents by an exhaust air washer with multiple cleaning stages in a field study.

# Material and Methods

Air samples were taken with impingers from a three-stage-system (stage 1 washer, stage 2 chemical washer, stage 3 biofilter) installed at a finisher barn. The third stage was a modified biofilter, half filled with wood chips (WC) and half with paper pads (PP). Air samples were taken by impingement. Concentrations of bacteria, Streptococci and endotoxins were measured in the untreated air, behind stage 2 and behind both biofilter materials, each. To compare the differences of filtration performance between the stages, a Wilcoxon rank sum test was conducted.

#### Results

The average concentration of mesophilic bacteria in the barn air was 5.47 log<sub>10</sub>cfu/m<sup>3</sup>. This concentration was reduced to -1.4 log<sub>10</sub>cfu/m<sup>3</sup> behind PP and to -1.6 log<sub>10</sub>cfu/m<sup>3</sup> behind WC. Similar reduction efficiencies were achieved for Streptococci (mean: 4.5 log<sub>10</sub>cfu/m<sup>3</sup>, behind PP: -1.5 log<sub>10</sub>cfu/m<sup>3</sup>, behind WC: -1.6 log<sub>10</sub>cfu/m<sup>3</sup>) and endotoxins (mean: 2.7 log<sub>10</sub>EU/m<sup>3</sup>, behind PP: -0.9 log<sub>10</sub>EU/m<sup>3</sup>, behind WC: -1.2 log<sub>10</sub>EU/m<sup>3</sup>). Reductions behind PP differed significantly from WC. Significant differences between bacterial and endotoxin contents were also found in comparison of the second and third filter stages.

### **Discussion and Conclusion**

The results showed that the treatment with a three-stage-system can reduce the examined bioaerosol contents efficiently (>80%). Humans, animals and nature in the vicinity of barns are less exposed to harmful bioaerosols when such systems are installed. Thus, this treatment contributes to prevention in line with the One Health principle.

The project was supported by funds of the Federal Ministryof Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

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# USE OF PHYTOGENICS FOR PIGLETS IN THE NURSERY PHASE AS A NATURAL PERFORMANCE ENHANCER TO REPLACE GROWTH-PROMOTING ANTIBIOTICS.

E. Miotto Ternus <sup>1</sup>, J. Cristani <sup>1</sup>, S. Davi Traverso <sup>1</sup>, C. Ficagna <sup>2</sup>, D. Paiano <sup>2</sup>, A. Da Silva <sup>2</sup>, I. Praxedes Campagnoni <sup>3</sup>, F. Matté <sup>3</sup>, <u>J. Paulo Sato</u> <sup>4</sup>

<sup>1</sup>Department of Veterinary Medicine, Universidade Estadual de Santa Catarina, UDESC, Lages - SC, Brazil. <sup>2</sup>Department of Zootechnology, Universidade Estadual de Santa Catarina, UDESC, Chapecó - SC, Brazil. <sup>3</sup>Vetanco Brasil, VETANCO, Chapecó - SC, Brazil. <sup>4</sup>Dr. Bata Brazil, Dr. BATA, Chapecó - SC, Brazil.

#### **Background and Objectives**

Many countries have banned the use of growth-promoting antibiotics in animal feed. Therefore, the search for alternatives to replace antibiotics in pig feed has grown, with emphasis on the use of phytogenics. Therefore, the objective of this research was to evaluate whether the addition of phytogenics based on chicory (Cichorium intybus), carob seed (Ceratonia siliqua), and fenugreek seed (Trigonella foenum-graecum) to the diet of piglets in the nursery phase has a positive effect on the immunological and oxidative response and growth of piglets.

# **Material and Methods**

One hundred five intact male piglets were used, weaned at an average of 26 days. Five treatments were used with 7 replications and 3 animals per replication. The treatments were: T1, Negative control; T2, 0.5 kg phytogenic/ton; T3, 1.0 kg phytogenic/ton; T4, 1.5 kg phytogenic/ton and T5, Colistin (200 ppm). Blood collections were carried out (days 14 and 40), as well as weighing of the animals (days 1, 7, 14, 40). The blood cell count, metabolic variables, antioxidant activity, and immunological biomarkers were evaluated in the blood.

#### Results

Within 40 days, considering the parameters body weight, daily weight gain, and average daily intake, it was observed that piglets from T4 and T5 were statistically equal and superior to T1, respectively. Effect of treatment on total leukocyte and lymphocyte counts, being lower in T4 animals compared to T1 and T5. Higher levels of glucose (T3 and T4) and urea (T4) were observed in piglets compared to T1. Higher concentration of globulins was found in the blood of piglets from T3 and T4 when compared to T1. This increase is a consequence of the higher concentration of IgA. The levels of tumor necrosis factor on day 40 were lower in piglets at T3 and T4, as well as the concentration of interleukin 10 had a dose-dependent effect, being higher in T4. A dose-dependent effect was also observed for superoxide dismutase activity and total antioxidants, being greater in T4, followed by T3 when compared to T1.

# **Discussion and Conclusion**

We conclude that the phytogenic works as a performance enhancer and also has antioxidant action and stimulates a humoral immune response.

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

# DEVELOPMENT OF AN INTELLIGENT DECISION SUPPORT SYSTEM FOR THE COMPLEX OPTIMIZATION OF THE ANTIBIOTIC USE IN PORK PRODUCTION

L. Gombos<sup>1</sup>, V. Laszlo<sup>1</sup>, L. Búza<sup>2</sup>, G. Szur Veronika<sup>3</sup>

<sup>1</sup>University of Györ, Wittmann Antal Plant-, Animal- and Food Sciences Multidisciplinary Doctoral School, Mosonmagyaróvár, Hungary

<sup>2</sup>Topigs Norsvin

<sup>3</sup>Vadocdoki Ltd. Research Development, Hungary

# **Background and Objectives**

Correct and effective decision making processes in antibiotic use of farm animals cannot be made without authentic data collecting, adequate comprehensibility and control during the entire process. The goal of the research was to develop an intelligent, web-based decision support system that can effectively monitor antibiotic use and animal health status of farms in real time. In addition, it also helps fulfill legal obligations and support a successful decision-making process.

# **Material and Methods**

The data and its related parameters have been collected from 12 large scale farrow to finish farms since 2020. Data collection was divided into three groups: antibiotic use in detail, decision influencing and supporting factors. The followings were taken into consideration during the development of the project: animal health approach, support for preparing an antibiotic reduction plan and effective support of the authorities's antibiotic reduction efforts. Our priorities included online accessibility, easy usage and traceability. Futhermore an integrated document storage and an option to create easy-to-understand statistics and reports highlighting the problems of the farm, were implemented.

#### Results

The ammount of antibiotic use of the farms varied between 3,3 - 952 mg/PCU. It was possible to precisely determine the production parameters related to antibiotic use, which can be applied and monitored in all farms. The scoring and weighting of the parameters within the categories allows to express the reduction process as well as benchmarking between farms with numbers. The software is web-based and user friendly, the tutorial is simple, and the reports are easy-to-understand both for the farm management and for the authorities.

# **Discussion and Conclusion**

By 2030, the amount of antimicrobials used in farm animals must be cut by half. Based on feedback from authorities and farm experts, the current version of the program already provides useful information for analyses. The web-based digital support system has proved to be able to increase the effectiveness of decision making and improve production efficiency.

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

# USE OF LIQUID ORGANIC ACIDS FOR SUCKLING PIGLETS IMPROVES THEIR PERFORMANCE IN NURSERY

A.P. P Pavaneli <sup>1</sup>, R. Souza <sup>1</sup>, L. Sartori <sup>1</sup>, A. P Lozano <sup>1</sup>, <u>J. Paulo Sato <sup>2</sup></u>, I. Praxedes Campagnoni <sup>3</sup>, F. Matté <sup>4</sup>, J. Cristani <sup>5</sup>, S. Davi Traverso <sup>5</sup>, E. Miotto Ternus <sup>5</sup>

<sup>1</sup>InsideSui Research Services, Insidesui, Patrocínio - MG, Brazil.

<sup>2</sup>Dr. Bata Brazil, Dr. BATA, Chapecó - SC, Brazil.

<sup>3</sup>Vetanco Brasil, VETANCO, Chapecó, SC, Brazil.

<sup>4</sup>Vetanco Brasil, VETANCO, Chapecó - SC, Brazil.

<sup>5</sup>Department of Veterinary Medicine, Universidade Estadual de Santa Catarina, IDESC, Lages - SC, Brazil.

# **Background and Objectives**

Acidifiers are associated with better performance in pig farms (1). Organic acids (OA) regulate the gastric pH, have an antimicrobial effect on the gastrointestinal tract, increase pancreatic secretion, and provide better nutrient digestibility (2,3). Water acidifiers have been used for suckling piglets in some farms but without a well-defined concept. This study evaluated the effects of offering a blend of OA in drinking water for suckling piglets on their performance in maternity and nursery.

# **Material and Methods**

Thirty-five litters were divided into 2 treatments, based on sow's parity order, average litter weight, and number of piglets/pen: Control, litters did not receive acid (n=17); and BlendOA, litters received acid (n=18). A blend of OA was offered via drinking water (350mL/1000L) in circular troughs from cross-fostering (1 day) to 10 days old. Individual and litter weight and number of piglets per sow were evaluated at 1, 10, and 20 days old (weaning). Average daily gain (ADG) and mortality were obtained for the first 10 days old, and for total period. In nursery, 288 piglets studied during lactation (144/treatment) were housed in collective pens (12 piglets/pen) and had their daily feed intake (DFI), ADG, and feed conversion ratio evaluated. Data were analyzed by ANOVA and means compared by Tukey test, Minitab 19.

#### Results

No differences were observed between treatments during maternity phase (p>0.05). In nursery, animals previously supplemented with OA showed higher DFI at the end of the first week (140 vs. 112g; p<0.01), second week (371 vs. 338g; p<0.05), 14 days (263 vs. 232g; p<0.01), 21 days (347 vs. 320g; p<0.05), and total period (720 vs. 686g; p=0.085). They also showed higher ADG at the end of the first week (83 vs. 42g; p<0.01), 14 days (218 vs. 186g; p<0.01), 21 days (277 vs. 253g; p<0.05), and total period (483 vs. 458g; p<0.05).

# **Discussion and Conclusion**

These results suggest a better preparation and gastrointestinal health of weaned piglets when treated with OA in farrowing house, probably due to microbiota modulation. The use of OA for piglets up to 10 days of age improve the feed intake and weight gain in nursery.

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

# ALVEOLAR ECHINOCOCCOSIS IN FATTENING PIGS - IMPORTANCE OF OFFICIAL MEAT INSPECTION FOR DIAGNOSIS

R. Igelbrink <sup>1</sup>, <u>T. Frey</u> <sup>1</sup>, I. Schwabe <sup>2</sup>, M. Prot <sup>3</sup>, F. Reimus <sup>4</sup>, R. Oehme <sup>5</sup>, F. Löwenstein <sup>6</sup> <sup>1</sup>Pig Health Service Baden-Württemberg, Fellbach, Germany <sup>2</sup>Chemical and Veterinary Analysis Agency Stuttgart, Germany <sup>3</sup>Veterinary and Food Inspection Office Rastatt, Germany <sup>4</sup>Tierärzte Team Tiefenbach GmbH, Crailsheim, Germany <sup>5</sup>State Health Department of Baden-Württemberg, Stuttgart, Germany <sup>6</sup>State Institution for Swine Breeding of Baden-Württemberg, Boxberg, Germany

# **Background and Objectives**

Echinococcosis is a worldwide parasitic zoonosis. Causative agents are Cestoda (Tapeworms) of genus Echinococcus. In Germany, Echinococcus (E.) multilocularis is mainly found as the pathogen causing alveolar Echinococcosis in humans. Final hosts like foxes, domestic dogs and cats are the main risk for infections of humans. Pigs can also become infected as accidental host. In 2022 in Southern Germany, up to 100 % of the livers of different slaughter groups from one herd were condemned due to parasitic lesions (milk spots) over a six months period. Intensified antiparasitic metaphylaxis with fenbendazole of the fatteners had no effects on the incidence of parasitic lesions during slaughter. Further investigations were carried out at slaughter and on the farm to determine the cause of the liver lesions.

#### **Material and Methods**

From two slaughter groups 6 livers were pathological examined. For epidemiological and parasitical examination, fecalsamples were collected from semi-feral domestic cats near the feed mixer and in the corridor of the barn.

#### Results

Pathologically oligofocal fibrotic inflammation were observed in the livers. Histopathologically, chronic granulomatous hepatitis with massive involvement of eosinophilic granulocytes and central parasitic structures of a helminth could be detected. Examination of the liver lesions by PCR revealed evidence of E. multilocularis. In fecal-samples of cats, parasitologically cestode eggs were detected. Genome fragments of E. multilocularis could not be amplified by PCR. Mycobacterium spp., Ascaris suum and other possible pathogens could not be detected by PCR and bacteriological examination.

# **Discussion and Conclusion**

The present case showed the importance of official meat inspection in diagnosing E. multilocularis infections in pigs. A diagnosis in the herd is not possible due to the absence of clinical symptoms in pigs. Serologically tests for pigs are not available. Subsequent epidemiological investigations can identify entry routes into the herd. In the present case, cats were the most likely vector for an entry. In view of the long incubation period (up to 15 years) of alveolar echinococcosis in humans, early detection is essential. Based on the One Health concept, the detection of liver lesions typical of E. multilocularis during official meat inspection can contribute to human health in risk areas.

VPH - One Health: Veterinary Public Health and Sustainable Pig Production

# IMPACT OF LAWSONIA INTRACELLULARIS VACCINATION ON ANTIBIOTIC USAGE IN FATTENING FARMS

<u>C. Renken</u><sup>1</sup>, R. Tabeling<sup>1</sup>, F. Von Und Zur Mühlen<sup>1</sup> <sup>1</sup>Intervet Deutschland GmbH; MSD Animal Health, Unterschleißheim, Germany

# **Background and Objectives**

The use of antibiotics as antimicrobial growth promoters in the EU was banned from 2006 onwards. Since then, only metaphylactic and therapeutic use of antibiotics is allowed. Due to the aim of further improvements in the prudent use of antibiotics in livestock production, national regulations on documentation and reduction plans have been put in place. The aim of this study was to evaluate the impact of Lawsonia intracellularis vaccination on reduction of antibiotic costs for enteric use.

#### Material and Methods

Clinical data (ileitis-related signs), performance (feed conversion ratio; FCR), and antibiotic usage for enteric purposes (measured indirectly as treatment cost) were recorded in 8 farms with a history of subclinical or clinical ileitis. Pigs from non-vaccinated (NV) and vaccinated (PL) (intramuscularly/intradermally Porcilis®Lawsonia/ID; at 3-11 weeks of age) batches were included. NV batches, used as historical control, were compared to PL vaccinated batches after implementing Lawsonia intracellularis vaccination to control ileitis.

# Results

In all farms, ileitis-related signs were improved clinically after the start of PL vaccination. Mean FCR was 2.81 and 2.71 in non-vaccinated and vaccinated batches, respectively. Average antibiotic treatment costs for enteric reasons (n=7 farms) were reduced by 68.3% (50.0– 95.2 %) in vaccinated batches compared to non-vaccinated batches. No group in-feed medication for enteric purposes was necessary anymore on these farms after the introduction of Lawsonia intracellularis vaccination. In one exceptional farm, enteric treatment costs were higher (factor 9.7) in vaccinated batches, as vaccination was administered at the beginning of fattening period, leaving no time for the onset of immunity before the pigs showed clinical ileitis-associated symptoms (3 days after vaccination).

# **Discussion and Conclusion**

Under the conditions of this report, Lawsonia intracellularis vaccination reduced ileitis-related clinical signs, improved FCR and led to a reduction of antibiotic costs for enteric purposes, as an indicator of antibiotic usage. This suggests that Lawsonia intracellularis vaccination may be a reliable tool for a more efficient and sustainable pig production.

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# ACTIONANTIBIO: A MULTI-SECTOR ONLINE RESOURCE CENTRE PUBLISHING THE PROJECTS OF THE FRENCH ECOANTIBIO PLANS

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<sup>1</sup>IFIP-Institut du porc, Domaine de la Motte au Vicomte, BP 35104, 35651 Le Rheu

#### **Background and Objectives**

The French Ecoantibio plans have funded over 100 research and development projects to provide knowledge and operational tools to reduce the use of antibiotics in the pig production, other livestock productions and pet animal sectors. The results were disseminated by each project manager as projects were finalized without any centralized communication channel. Therefore, many people such as veterinarians, technicians, and farmers who could have benefited from the information provided by the Ecoantibio plans were unable to access it. The aim of this work is to ensure wide, structured and effective dissemination of the results of the Ecoantibio plans.

# **Material and Methods**

A multi-sector and multi-partner web portal (ActionAntibio) dedicated to communicating the projects of the Ecoantibio French plans was created by Ifip in partnership with all French technical and scientific organizations involved in reducing the use of antibiotics: AFVAC, AVEF, ANSES, INRAE, ITAVI, IDELE, GDS France, SIMV and SNGTV. The information of each project is displayed via an informative summary sheet that follows a standardized model of presentation. The web portal features various filters by species, keywords and six themes, facilitating the user experience and ensuring that the relevant information can be found quickly. The six themes are: monitoring of antibiotic use and resistance, training tools and promotion of good practices, health prevention practices, medical prevention practices, disease detection and monitoring, and alternatives to antibiotics. The user can access more detailed results about each project via web links displayed on the summary sheet. It's a freely accessible web portal at www.actionantibio.fr.

#### Results

This website includes 82 sheets, 22 of which are about pig. In one year, there were more than 1500 visits to the site, 1000 different users connected and 1800 technical sheets were downloaded. It is now the website recommended by the Ministry of Agriculture to disseminate the actions carried out within the framework of the French Ecoantibio plans.

# **Discussion and Conclusion**

New sheets will be added to the website as Ecoantibio projects are finalized and other projects aimed at reducing antibiotic use. This web portal contributes to the reasoned prescription of antibiotics, the knowledge of controlling infectious diseases measures and to fight against antibiotic resistance.

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# EFFECTS OF DIETARY SUPPLEMENTATION WITH A BLEND OF ORGANIC ACIDS AND MANNAN OLIGOSACCHARIDES ON THE HEALTH STATUS OF NURSERY PIGLETS CHALLENGED BY SALMONELLA TYPHIMURIUM

J.C. Vieira Furtado <sup>1</sup>, A. Miranda Da Silva <sup>1</sup>, N. De Oliveira Telesca Camargo <sup>1</sup>, A. Zem Fraga <sup>1</sup>, K. Ludwig Takeuti <sup>2</sup>, A. Beatriz Da Rosa <sup>2</sup>, R. Da Rocha Bonera Dos Santos <sup>2</sup>, D. Magnabosco <sup>1</sup>, I. Andretta <sup>1</sup>, S. Davi Traverso <sup>3</sup>, E. Miotto Ternus <sup>3</sup>, I. Praxedes Campagnoni <sup>4</sup>, F. Matté <sup>4</sup>, <u>J. Paulo Sato <sup>5</sup></u>

<sup>1</sup>Faculty of Agronomy, Federal University of Rio Grande do Sul, Porto Alegre - RS, Brazil.

<sup>2</sup>University Feevale, Porto Alegre - RS, Brazil.

<sup>3</sup>Department of Veterinary Medicine, Universidade Estadual de Santa Catarina, UDESC, Lages - SC, Brazil.

<sup>4</sup>Vetanco Brasil, VETANCO, Chapecó - SC, Brazil.

<sup>5</sup>Dr. Bata Brazil, Dr. BATA, Chapecó - SC, Brazil.

#### **Background and Objectives**

Feed additives may be a strategy to minimize the impact of sanitary challenges on pig health. This study was performed to evaluate the effect of organic acid combined with mannan oligosaccharide (OAM) supplementation on nursery pigs challenged by Salmonella Typhimurium (ST).

# **Material and Methods**

Twenty-eight male piglets (28-day-old) were allocated to a 19-day trial. Pigs were housed individually, and feed and water were provided ad libitum. On day 5, all pigs were orally inoculated with 5 mL of a broth with  $2 \times 10^{\circ}$  CFU/mL ST. Pigs received one of the 4 treatments: control (CON; without supplementation), total supplementation (TS; inclusion of 2 kg/ton OAM during the entire experiment period), low-dose supplementation (LDS; inclusion of 1 kg/ton OAM in the post-challenge period), and high-dose supplementation (HDS; inclusion of 2 kg/ton OAM in the post-challenge period). Rectal temperature was monitored twice a day. Fecal samples were collected on days 1, 3, 5, 7, and 14 after challenge to evaluate fecal ST shedding (Most Probable Number quantification). Means were compared using variance analysis followed by Tukey's test at P≤0.05.

# Results

Treatments did not affect rectal temperature (P=0.44). The maximum frequency of piglets with fever (i.e., temperature greater than 39.8 °C) was observed at 3 days post-challenge with a gradual reduction until the 14-day post-challenge. On day 3 post-challenge, greater frequency (P<0.05) of pigs with fever was observed for CON (75%), followed by LDS (25%), HDS (15%), and TS treatment (0%). At the end of the experiment, the frequency of positive pigs for ST was 63% for CON, 25% for HDS, 13% for TS, and 0% for LSD. During the entire trial period, greater average ST quantification was observed for CON (237) than supplemented treatments (56, 61, and 70 for TS, LDS, and HDS; respectively, P=0.04).

### **Discussion and Conclusion**

Organic acids are known for their antimicrobial effects and several action modes were reported, impacting both the survival and virulence of the bacteria. When combined with prebiotics, the impacts on microbiota modulation can be even more pronounced. Thus, the supplementation of AOM may alleviate the negative effects of ST challenge for nursery pigs.

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# CONCENTRATION OF TIAMULIN IN SOW MILK EVALUATED BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (UHPLC-MS/MS)

P. Cybulski<sup>1</sup>, A. Gajda<sup>2</sup>, M. Bilecka<sup>2</sup>, A. Jablonski<sup>3</sup>

<sup>1</sup>Goodvalley Agro S.A., Przechlewo, Poland

<sup>2</sup>Department of Pharmacology and Toxicology, National Veterinary Research Institute, Pulawy, Poland <sup>3</sup>Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences—SGGW, Warsaw, Poland

### **Background and Objectives**

Tiamulin (TIA) is a semisynthetic derivative of a naturally occurring antibiotic pleuromutilin. TIA is commercially available under a variety of invented names, either in injectable solutions, or as water soluble and premix formulations, and extensively used in the treatment of diseases in pigs. According to the available publications, the passage of TIA into sow milk has not been studied yet. Therefore, the aim of our investigation was to analyse the concentrations of TIA in milk samples collected from lactating sows after intramuscular applications.

# Material and Methods

The investigation was carried out in a herd with 8000 sows. A group of 5 multiparous sows was given deep intramuscular injections of Tiamowet inj. (Vetoquinol Biowet, Poland) at 12 mg of TIA per kg for 3 consecutive days. Milk samples were collected manually 9 times: 3 h, and at systematic intervals, i.e. 1, 2, 3, 4, 5, 7, 14, 22 days after the first administration. For the quantification of TIA an ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method was developed and validated. The analysis was performed using Shimadzu Nexera X2 (Shimadzu, Japan) system connected to the QTRAP® 4500 triple quadrupole mass spectrometer (AB Sciex, USA).

#### Results

The mean concentration of TIA in samples collected 3 h after the first administration was 1043  $\mu$ gL<sup>-1</sup>. The mean TIA level at day 1, 2, 3, and 4 was 876  $\mu$ gL<sup>-1</sup>, 902  $\mu$ gL<sup>-1</sup>, 1061  $\mu$ gL<sup>-1</sup>, and 660  $\mu$ gL<sup>-1</sup>, respectively. TIA concentrations at the following days were significantly lower. The samples collected at day 22 were negative.

#### **Discussion and Conclusion**

To the best of the authors' knowledge, this is the very first work analysing concentration of TIA in milk samples collected from sows after an intramuscular application. Effective passage of TIA into sow milk was demonstrated. Potential influence of contaminated milk on suckling piglets' health needs to be further investigated.

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# EFFECT OF SUPPLEMENTATION WITH A BLEND OF ORGANIC ACIDS AND MANNAN OLIGOSACCHARIDES IN NURSERY PIGLETS CHALLENGED BY SALMONELLA TYPHIMURIUM ON NITROGEN BALANCE, ORGAN WEIGHT AND CONTAMINATION AND ZOOTECHNICAL PERFORMANCE

A. Miranda <sup>1</sup>, J. C. V. Furtado <sup>1</sup>, D. V. Nogueira <sup>1</sup>, G. B. Martins <sup>1</sup>, N. O. T. Camargo <sup>1</sup>, A. Z. Fraga <sup>1</sup>, D. Magnabosco <sup>1</sup>, I. Andretta <sup>1</sup>, K. Ludwig Takeuti <sup>2</sup>, S. Davi Traverso <sup>3</sup>, E. Miotto Ternus <sup>3</sup>, I. Praxedes Campagnoni <sup>4</sup>, <u>J. Paulo Sato <sup>5</sup></u>, F. Matté

<sup>1</sup>Faculty of Agronomy, Federal University of Rio Grande do Sul, Porto Alegre - RS, Brazil. <sup>2</sup>University Feevale, Porto Alegre - RS, Brazil. <sup>3</sup>Department of Veterinary Medicine, Universidade Estadual de Santa Catarina, UDESC, Lages - SC, Brazil. <sup>4</sup>Vetanco Brasil, VETANCO, Chapecó - SC, Brazil.

<sup>5</sup>Dr. Bata Brazil, Dr. BATA, Chapecó - SC, Brazil.

# **Background and Objectives**

The presence of Salmonella spp. in pig production systems has been a global concern for economic and public health reasons. Therefore, the objective of this study was to evaluate the effect of supplementation of organic acids and mannan oligosaccharides in piglets challenged by S. Typhimurium.

# **Material and Methods**

Twenty-eight male piglets (28-day-old) were randomly attributed to one of four different treatments: control treatment (CON), fed with non-supplemented diets; CON + 2 kg/ton of an additive containing organic acids and mannan oligosaccharides during the total period (pre-challenge and post-challenge); CON + 1 kg/ton of additive only in the post-challenge period; and CON + 2 kg/ton of additive only in the post-challenge period. The pre-challenge and post-challenge periods lasted 5 and 14 days, respectively. Animals were challenged orally with 5 mL of a broth with 2 × 10° CFU/mL of Salmonella Typhimurium. Performance, nitrogen balance, organ weight, and bacterial recovery in the liver and spleen were evaluated. Means were compared using variance analysis followed by Tukey's test at P≤0.05 and P≤0.10.

# Results

There was no effect of supplementation with additives on performance variables and nitrogen balance. The absolute and relative weights of the liver, heart, spleen, and kidney were lower (P<0.05) in the groups supplemented with organic acids and mannan oligosaccharides. However, the absolute and relative weight of the lung did not differ between treatments. Despite the supplementation strategy, organic acids and mannan oligosaccharides reduced the number of positive animals from 38 to 17% (mean value of the three supplemented treatments, which were similar) in the liver (P<0.05). The S. Typhimurium recovery was also reduced by 3.49 to 2.49 UFC/g in the liver (P<0.05).

# **Discussion and Conclusion**

Although supplementation with organic acids and mannan oligosaccharides is not completely preventive to the infection in pigs exposed to Salmonella spp., the current results indicated that this additive can reduce the number of animals carrying Salmonella spp. in the production system and, thus, benefit the biosecurity actions.

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# ANTIMICROBIAL RESISTANCE OF SALMONELLA SPP. ISOLATED FROM SWINE IN BRAZIL

J. Viktor De Paula Barros Baêta <sup>1</sup>, G. Pinheiro Paes <sup>1</sup>, <u>L. Fernando Dos Santos <sup>1</sup></u>, D. Lúcio Dos Santos <sup>1</sup>, W. Vieira Guimarães <sup>1</sup>, J. Lúcio Dos Santos <sup>1</sup> <sup>1</sup>*MICROVET - Microbiologia Veterinária Especial* 

#### **Background and Objectives**

Salmonellosis is a major foodborne illness threat to public health worldwide, and has been caused by different serovars of Salmonella. Pigs are reservoirs for many Salmonella serotypes that affect humans, and reports of antimicrobial resistance in Salmonella isolates from swine production have increased over the years. Currently, the national and international market is demanding an increasingly updated herd health status. Given this scenario, salmonellosis is a disease that deserves attention. Therefore, this summary seeks to address the antimicrobial resistance profile of Salmonella strains isolated from pigs in Brazil.

# **Material and Methods**

A total of 6242 isolates of Salmonella spp. (serovars S. Choleraesuis (n=4162) and S. Typhimurium (n=1832), recovered from sick animals during the period of 2019 to September 2023, in the Veterinary Diagnostic Laboratory of Microvet were used in this study. After obtaining the pure culture, confirmation of the isolates was performed using a MALDI-TOF mass spectrophotometer. To evaluate the resistance profile, the samples were subjected to the diffusion method on Mueller Hinton agar plates, in which antibiotics from the classes Aminoglycosides, Cephalosporins, Chloramphenicol, Colistin, Lincosamides, Macrolides, Penicillins, Quinolones, Sulfonamides, Tetracycline and Tiamulin, were tested. The isolates were categorized as sensitive, resistant or multiresistant according to the number of classes for which resistance was observed.

#### Results

A total of 5,631 (90.21%) isolates revealed resistance to two or more classes of antibiotics (multiresistance), 43 isolates (0.69%) revealed resistance to at least one class of antibiotics and 568 isolates (9.1%) were sensitive to all antibiotic classes tested. Chloramphenicol, Macrolides, Tetracycline and Penicillins showed resistance cases above 80% and an increase in resistance over the years.

#### **Discussion and Conclusion**

It was possible to observe an increasing trend in cases of multi-resistant bacteria and a tendency towards the reduction of sensitive Salmonella spp, in this period. The swine industry faces significant challenges related to the spread of multidrug-resistant Salmonella strains, which not only compromises animal welfare but also poses a threat to food safety and human health. The use of alternative methodologies to conventional antibiotic therapy, such as the use of autogenous vaccines, have been a viable option for controlling salmonellosis in Brazil.

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# EUROPEAN WILD BOARS (SUS SCROFA) AS POTENTIAL RESERVOIR OF (ZOONOTIC) VIRAL AND BACTERIAL DISEASES IN AUSTRIA

<u>A. Lang</u><sup>1</sup>, S. Revilla-Fernández<sup>1</sup>, F. Schmoll<sup>1</sup>, T. Sattler<sup>2</sup> <sup>1</sup>Institute for Veterinary Disease Control, AGES – Austrian Agency for Health and Food Safety, Mödling, Austria <sup>2</sup>Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany

# **Background and Objectives**

Wild boars can be carriers of various infectious diseases with zoonotic potential or a danger to domestic pigs. With the increase in wild boar populations in Europe over the last few decades and the associated increase in direct and indirect human-wild boar contacts, the risk of disease transmission has also increased. Yet, there is little data on the spread of the pathogens of concern in wild boars, as they are rarely systematically sampled. However, this has changed due to the spread of African swine fever (ASF) in Europe in recent years and the subsequent development of preventive surveillance programs. In Austria, all wild boars found dead and samples of hunted wild boars sent in voluntarily are now tested for ASF as a preventive measure. The aim of this study was to use the wild boar samples from the ASF surveillance to investigate the prevalence of other relevant pathogens in the Austrian wild boar population, namely causing Aujeszky's disease, hepatitis E, influenza, leptospirosis and brucellosis.

# Material and Methods

For this study, we used the samples of 143 wild boars found dead and sent in for ASF monitoring in Austria in 2022. The organ pools consisting of tonsil, lymph nodes, spleen, liver, lung and kidney were analysed using real-time PCR for the presence of Suid herpesvirus 1 (SuHV-1), hepatitis E virus (HEV), influenza A virus (IAV), intermediate and pathogenic Leptospira and Brucella spp..

#### Results

We found SuHV-1 in 0 samples (0%), HEV in 5 samples (3,5%), IAV in 4 samples (2,8%), intermediate Leptospira in 2 samples (1,4%), pathogenic Leptospira in 0 samples (0%) and Brucella spp. in 2 samples (1,4%).

# **Discussion and Conclusion**

As the size of the wild boar population of Austria is not known and we tested exclusively animals found dead, the results can only give us an indication of the true prevalence of the investigated diseases. Although the prevalence of most pathogens tested seems low, we cannot exclude the possibility of former infections since we could not test for the presence of antibodies. Both, active and passive surveillance seems reasonable to give a true picture of pathogens in the wild boar population.

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# RELATION BETWEEN ANTIBIOTIC CONSUMPTION, PRRSV STATUS AND RESPIRATORY HEALTH STATUS OF NURSERY PIGS. A CASE STUDY.

<u>P.H. Rathkjen</u><sup>1</sup>, L.M. Jensen<sup>2</sup>, C. Alonso<sup>3</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Denmark <sup>2</sup>PORCUS Oerbaekvej 276, DK-5220 Odense <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH, Germany

## **Background and Objectives**

The objective of this study was to understand the impact of PRRS viral status and overall respiratory health in antibiotic consumption of a nursery flow during a PRRS control and elimination program.

# **Material and Methods**

A Danish sound-monitored nursery site, initially sourced with pigs from an unstable PRRSV positive sow site undergoing elimination, was selected for sampling. PRRS viral status was determined by PCR from monthly oral fluid samples from all nursery age groups (1 – 8 week post placement) from 5 months before sow herd stability, during nursery elimination phase, until 5 months of negative status. Antibiotic consumption was calculated as rolling 9-month average of Daily Doses (ADD) per 100 animals from official prescription database. Respiratory health status and %\_days\_with respiratory\_alarms (SoundTalks®, ReHS) was aggregated monthly for the analysis.

# Results

A total of 23 months of monitoring data were analyzed for the study. Time and PRRS status (≤ 1PCR positive) were highly significant variables on antibiotic consumption. On the other hand, %\_days\_respiratory\_alarms were also included in the final multifactorial regression model to understand its impact. Antibiotic consumption decreased in 30% (LSM: 9.05 vs 12.63 ADD\_9month rolling average) when the farm achieved PRRS negative status and adjusted for the other variables in the model. % Alarms were no significant and confounded by the higher number of Influenza outbreaks starting when the farm achieved negativity.

### **Discussion and Conclusion**

PRRS viral status was a strong predictor of antibiotic consumption. As long as PRRSV could be detected in some rooms, changes in monthly ADD and monthly ReHS did not change significantly. Monthly sampling revealed that the infection was maintained in the oldest 3-age-groups. As soon as the nursery site reached PRRS virus negative status, all the parameters, (i.e Monthly\_%\_PRRS\_pos, Monthly\_ADD and Monthly\_alarms) were significantly lower than any period with positive pigs on the site. Based on the results of this study, all age groups present on a nursery site should be continuously monitored, preferably at least monthly, including realtime ReHS, OF PCR and antibiotic consumption in order to understand and correct inappropriate management and flow procedures.

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# SURVEY OF 52 PIG PRODUCERS USING ORAL VACCINATION AGAINST E. COLI F4/F18 IN FRANCE : REASONS AND INSIGHTS

<u>S. Vigneron</u>, B. Fily <sup>1</sup>, J. Fernand-Genty <sup>1</sup>, V. Burlot <sup>1</sup> <sup>1</sup>Elanco France SAS – Crisco Uno, Bâtiment C, 3-5 avenue de la Cristallerie, CS 80022 - 92317 Sèvres CEDEX

#### **Background and Objectives**

Very few surveys exist on the reasons and insights of pig producers to vaccinate. Even with more recent significant drop in antibiotic use, in 2019, 63% of French pig farms were still administered antibiotics to treat post-weaning digestive infections. Coliprotec<sup>™</sup> F4/F18 (Elanco) is a live vaccine administered orally to piglets to reduce the incidence of postweaning diarrhea (PWD) due to E. coli F4/F18. The objective of this survey was to describe the reasons of piglet's vaccination and the insights of pig producers using Coliprotec<sup>™</sup> F4/F18 in France.

# **Material and Methods**

Between November 2022 and February 2023, 52 pig producers were interviewed by phone by an independent market research company. Interviews were conducted with the person responsible for health monitoring of pigs. It consisted of questions with some spontaneous answers to give or some statements to choose. The 52 pig producers had started vaccination between 2017 and 2022 and were users of the vaccine at the time of the interview.

#### Results

The reasons of piglet's vaccination were mainly to manage post-weaning diarrhea in farm (71%), to reduce mortality due to PWD (23%), to reduce the use of antibiotics (19%) and to follow specifications (16%). Concerning the insights of pig producers, the first strong point spontaneously mentioned was the ease of administration. Major advantage of vaccination after the efficiency against PWD was the reduction in the use of digestive antibiotics. 61% of producers have stopped and 29% have reduced the use of digestive antibiotics after the implementation of the vaccination. Finally, 87% pig producers interviewed would recommend vaccination for greater serenity and sustainability of pig farming.

#### **Discussion and Conclusion**

The results showed that the reasons to vaccinate were to solve issues with PWD, to reduce mortality, to limit the use of antibiotics and to follow specifications for some pig producers. The reduction of the use of antibiotics is a major advantage of oral vaccination against E. coli F4/F18 according to users. According to them, it represents a sustainable solution that pig producers are proud to implement, and which improves welfare of animals on the farm and serenity of pig producers.

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# COMPARATIVE ANALYSIS OF ANTIMICROBIAL USAGE IN FARMS HOUSING PIGS EXCLUSIVELY OR CATTLE ALONGSIDE

L. Fleischer <sup>1</sup>, J. Becker <sup>2</sup>, D. Kümmerlen <sup>3</sup>

<sup>1</sup>Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland

<sup>2</sup>University of Bern, Departement of clinical veterinary medicine, Bremgartenstrasse 109a, 3012 Bern, Switzerland <sup>3</sup>Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland.

# **Background and Objectives**

Antimicrobial resistance (AMR) is an increasing threat for human and animal health and imprudent antimicrobial usage (AMU) is a major cause for the development of AMR in livestock production. The Swiss Federal Food Safety and Veterinary Office established the national reporting system IS ABV for monitoring and quantification of AMU in Switzerland. The objectives of this study were to analyze AMU in fattening pigs using IS ABV data. Differences in AMU were investigated between farms housing pigs exclusively and mixed farms housing pigs alongside cattle. Associations of farm type and AMU were investigated for total AMU and on level of the antimicrobial substance.

# **Material and Methods**

AMU was calculated in total for all farms (n= 99) and specifically for the antimicrobial classes of penicillins and tetracyclines. Calculation was carried out using a treatment incidence (TI) based on Defined Daily Doses (DDD) by the European Medicines Agency, 2016. Statistical comparisons were performed using the Mann-Whitney-U-Test.

#### Results

From January to October 2022 there were a total of 232 prescriptions in 99 farms, including 146 prescriptions in 49 pig farms and 86 prescriptions in 50 mixed farms. A total of 4'475 out of 48'848 fattening pigs (=9.16%) were treated. A total AMU of 0.28 DDD/pig/year (min: 0.00028; max: 30.7) for pig farms and of 0.09 DDD/pig/year (min: 0.00275; max: 4.96) for mixed farms was calculated. For penicillins the mean TI for pig farms and mixed farms was 1.11 DDD/pig/year and 0.40 DDD/pig/year, respectively. The mean TI for tetracyclines for pig farms was 0.07 DDD/pig/year and for mixed farms 0.25 DDD/pig/year. No significant differences between the types of farms were found concerning total AMU or any specific active substance.

# **Discussion and Conclusion**

No association of specialization of the farmers on pigs on AMU was found in this study. Overall low AMU in combination with moderate sample size could have limited the detection of such effects.

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# NON-ANTIBIOTIC TREATMENT OF DYSGALACTIA POST PARTUM

L. Hägg Grønborg <sup>1</sup> <sup>1</sup>Distriktsveterinärerna

# **Background and Objectives**

Colostrum and milk production of the sow is essential for healthy piglets. Dysgalactia, an impaired colostrum and/or milk production days after farrowing will affect the piglets, eventually leading to illness and extended use of antibiotics. Sow management for prevention of mastitis, metritis, obstipation, bacterial intoxication, mycotoxicosis and other diseases is first choice for prevention of dysgalactia. Treatment can though be necessary. Treatment of dysgalactia without antibiotics has been described. To prevent antibiotic resistance prudent use is necessary, and non-antibiotic treatment is to be used when possible.

# **Material and Methods**

The aim of this study was to systematically review the literature on non-antibiotic treatments with positive effect on milkproduction, or -quality, available for the piglets the days after farrowing. Studies of dysgalactia postpartum in individual animals and on farm level are included. Dysgalactia is defined as all cases of reduced colostrum-, milk volume or quality, irrespective of measure or method of measurement. It should also be considered if other symptoms in the sow should be taken into account when treating dysgalactia the days after farrowing.

# Results

Studies of non-antibiotic treatment of dysgalactia are few. Oxytocin is known to promote milk let-down when there is a secretion of milk. NSAIDs have shown to reduce the effect of endotoxins and inflammation. Glucocorticoids could be an alternative anti-inflammatory treatment, but it should be considered when in lactation and to which symptoms. Metamizole is in Sweden registered for use, but peer review publications are very few. Other drugs affecting milk production or the milk available for piglets are also described but not approved for use in food animals or for farmers to administer (in Sweden).

### **Discussion and Conclusion**

Very few studies include more than one farm and a variation in definition of dysgalactia and when to treat sows is observed. A more accurate description of the sows' symptoms when treated for dysgalactia is needed in research to evaluate the possible effect of a differentiated treatment in accordance with other symptoms. Substances as NSAIDs, oxytocin, glucocorticoids and metamizole can have effect on symptoms associated with dysgalactia.
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# COMPARISON OF IN-VITRO PERFORMANCE AND IN VIVO FERTILITY OF BOAR SEMEN PRESERVED AT 17 ° IN A SEMEN EXTENDER WITH LOW ENVIRONMENTAL IMPACT SUBSTANCES ALTERNATIVE TO ANTIBIOTICS

D.C. Angel <sup>1</sup>, L. Maglie <sup>2</sup>, R. Bettini <sup>2</sup>

<sup>1</sup>Research & Development department Medinova Italy , University of Parma

<sup>2</sup> Department of Food and Drug Science, University of Parma, Parma, Italy

# **Background and Objectives**

In swine reproduction, the impending need to find alternative substances to antibiotics is undoubtedly one of the most important challenges of the present era and is a priority issue in the fight against antibiotic resistance. Alternative substances with low environmental impact such as AMPs( antimicrobial peptides) have been considered as potential alternatives in the formulation of semen extender for boar sperm storage. The issue is to maintain the sperm cells without bacterial growth at a temperature between 15 - 17 °C without affecting the reproductive parameters of the farm. The aim was to evaluate the performance in vitro and the fertility in vivo of a new semen extender formulation with substances with low ambiental impact that replace the antibiotics.

# Material and Methods

Tests were carried out on the ejaculation of a 24-month-old male Golland to analyze the effect of the two formulations (with antibiotic and with antibiotic and substances) on cell vitality and antimicrobial activity. The experimental design was based on two groups (antibiotic and antibicterial substances) where the differences between motility parameters were studied, in terms of bacterial load decrease and total number of births. Sperm motility was followed daily for four days by computer-assisted sperm analysis (CASA, Microptic, Spain). Aliquots of extended semen were used and analyzed for motility, progressive motility, acrosome, and microbiological CFU count.

#### Results

The results showed that during the 4 days of storage, sperm motility, viability, and acrosome integrity in the antibiotic group were not significantly different from the antibiacterial substances group (p < 0.05)The bacterial count (log10) of pure semen on day one was higher (p > 0.05) against the antibiotic group (p > 0.0134) and against the antibiacterial group (p > 0.0439). Between the antibiotic and antibacterial groups there was no significant difference (p < 0.05). The bacterial growth was reduced (p=0.0581) across the storage days. Fertility trait fertility parameters of 85 females did not differ significantly between the two semen groups.

## **Discussion and Conclusion**

These results provide new options for the reduction of antibiotics in the swine AI industry, indicating that it is possible to store and inseminate without reproductive effect doses treated with the enriched formulation with antimicrobial substances replacing antibiotics.

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# COMPOSTING HOUSEHOLD ORGANIC WASTES AND PIG MANURE AS A SUSTAINABLE TECHNIQUE TO RESOLVE SANITARY ISSUES IN DEVELOPING COUNTRIES

M.J. Amaya Hernández <sup>1</sup>, <u>S.E. Mendoza Elvira</u><sup>2</sup>, A. Vargas Sánchez <sup>1</sup> <sup>1</sup>Facultad de Medicina Veterinaria y Zootecnia. UNAM. <sup>2</sup>Facultad de Estudios Superiores Cuautitlán. UNAM.

## **Background and Objectives**

In developing countries, as México, the treatment of household organic waste (HOW) is difficult due to technical deficiencies<sup>2</sup>. The same, ocurrs in animal farms were pig manure (PM) acummulates and favours the development of species like flies and rodents that can harbour pathogen agents and induce diseases in susceptible animals (young pigs) and human beings<sup>1</sup>. So, it's necessary and urgent to resolve, in a sustainable manner, the treatment and disposal of both residues. Therefore, the objective of this work is to treat the HOW mixed with PM by and easy-to-follow composting process and analyze the compost obtained.

#### **Material and Methods**

Cylinder-shaped containers were built using a metal sieve where HOW mixed with PM was placed. Dry grass clippings were added as bulking agent. The relative moisture (55 %) was setted by adding tap water. For 42 days, three times weekly, the following activities were carried out: temperature measurement, extraction of all the organic matter and homogenization, moisture adjustment and re-charge of the container. Of these procedures, three replications were carried out. Subsequently, representative samples were taken (n=3) and analyzed physicochemically to determine the following characteristics: pH, electrical conductivity, nitrogen content (Dumas method) and organic carbon content (incineration, 520 °C), as well as phosphorus and potassium levels (microwave digestion/ICP).

## Results

According to the Mexican Regulations<sup>3</sup> consulted, the mean levels (%) of N, P and K ( $3.56 \pm 0.225$ ;  $1.70 \pm 0.045$ ;  $2.45 \pm 0.297$ , respectively), qualificate the compost as a high quality fertilizer. While, the carbon to nitrogen (C/N) ratio obtained as well as the temperature curve obtained indicate that the compost is stable and therefore mature.

# **Discussion and Conclusion**

These results show that treated HOW and PM by the composting technique descripted, generate a high quality fertilizer instead of the various health, social and environmental problems that arise when these organic waste are not treated.

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# PHYTOBIOTIC-PREBIOTIC FEED ADDITIVE IMPROVE GROWTH PERFORMANCE, CARCASS TRAITS, AND FECAL MICROBIOTA OF FATTENING PIGS

#### A. Juhász <sup>1</sup>, K. Posta <sup>1</sup>, K. Tso <sup>2</sup>, V. Molnár-Nagy <sup>2</sup>, Z. Bata <sup>2</sup>

<sup>1</sup>Hungarian University of Agriculture and Life Sciences, Institute of Genetics and Biotechnology, Gödöllö, Hungary <sup>2</sup>Dr. Bata Ltd, Ócsa, Hungary

## **Background and Objectives**

The purpose of this study was to determine the effectiveness of a phytobiotic-prebiotic feed additive (PPFA), which contains a combination of chicory and extracts of carob pulp and fenugreek, in the diets of fattening pigs on growth indicators, carcass characteristics, and fecal microbiota.

# **Material and Methods**

A total of 329 crossbred pigs were randomly divided into two dietary treatments, including a basal diet without additives as the control group and a basal diet supplemented with 1 kg/t PPFA as the trial group. At the end of the trial period, all pigs were conventionally slaughtered and the S-EUROP system was used to classify carcasses according to their shape and fat level. Fecal samples were collected and analyzed for lactic acid -, coliform-, total aerobic and anaerobic bacteria. Additionally, the abundance of the bacterial communities of the fecal samples were measured by high-throughput sequencing on an Illumina MiSeq platform.

## Results

The PPFA supplementation led to a significant increase in the body weight gain and average daily gain of the trial group compared to those of the control group after 70 days of feeding. Through the S-EUROP evaluation system, we also found that the fattening pigs fed PPFA significantly improved their carcass indicators. Furthermore, it was shown that PPFA regulated porcine intestinal microbiota, including promoting the growth of the beneficial commensal bacteria (i.e., Bifidobacterium and Lactobacillus) while limiting the growth of some potential pathogen bacteria (i.e., Bacteroidaceae and Campylobacteraceae).

## **Discussion and Conclusion**

Our work revealed that the consumption of the phytobiotic-prebiotic feed additive was beneficial for the production parameters as well as improved the porcine intestinal microbiota. For practical feeding and management of fattening pigs, using this PPFA as their dietary supplement can be an optimal solution for both antibiotic-free intestinal regulation and a growth-promoting feed additive. Since the study<sup>1</sup>, the PPFA has been registered under the trade name of NeoSantrix. <sup>1</sup>https://doi.org/10.3390/ani13233621

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# PARTICIPATORY APPROACH IN ANIMAL HEALTH: WHEN VETERINARIANS ADOPT A FACILITATOR POSTURE

<u>M. Leblanc-Maridor</u><sup>1</sup>, S. Di Bianco<sup>2</sup>, J. Defois<sup>3</sup>, A. Dufay-Lefort<sup>4</sup>, A. Poissonnet<sup>5</sup>, A. Wache<sup>6</sup>, C. Manoli<sup>7</sup>, A. Sigwalt<sup>8</sup> <sup>1</sup>ONIRIS, INRAE, BIOEPAR, 44307 Nantes, France <sup>2</sup>Ecole Supérieure d'Agricultures d'Angers, Unité LARESS, 55 rue Rabelais 49007 Angers

<sup>3</sup>Ecole Supérieure d'Agricultures d'Angers, Unité URSE, 55 rue Rabelais, 49007 Angers <sup>4</sup>(6) ITAVI – Institut Technique de l'Aviculture, 42 rue Georges Morel, CS 60057, 49070 Beaucouzé, France <sup>5</sup>IFIP-Institut du porc, Domaine de la Motte au Vicomte, BP 35104, 35651 Le Rheu <sup>6</sup>Institut de l'élevage, 42 rue Georges Morel - CS 60057- 49071 Beaucouzé Cedex, France <sup>7</sup>Ecole Supérieure d'Agricultures d'Angers, Unité URSE, 49007 Angers, France <sup>8</sup>Ecole Supérieure d'Agricultures d'Angers, Unité LARESS, 49007 Angers, France

## Background and Objectives

Veterinarians are identified by livestock farmers as major advisors regarding health management, even though animal health depends on many factors on which other agricultural counselors can interfere. Their scientific knowledge often prompts them to adopt an expert posture whereas livestock farmers also are in demand of discussions about practices. Within a European research program on animal health in ruminants and monogastric sectors conducted in western France, an experiment was launched to test the utility of livestock farmers meetings facilitated by vets.

## Material and Methods

Vets were previously trained on facilitation methods before organizing by themselves meetings on animal health with their clientele, whose topics were either chosen by them or the farmers. Each meeting was assessed by three methods: i) meeting's observation, ii) a questionnaire form proposed right after the session, and iii) semi-directed interviews performed later on by the research team. Our analysis material encompasses 7 meetings, attended by 23 livestock farmers and 8 vets.

## Results

Four types of meetings appear, from mostly participatory ones to meetings with a significant amount of top-down information times. The vet's initial motivations impacted both the farmers' recruitment and the meeting program. The posture of expert-facilitator contributed to a group dynamic based on a horizontality of exchanges, enabling cooperative learning. Besides, since they knew the participants' farms, vets were perceived as legitimate in the animation of the meetings, and these ones complementary to their on-farm interventions. This device allowed vets to better understand their livestock farmers' practices and to reinforce their relationship with them.

# **Discussion and Conclusion**

Changing posture is complexe and requires adequate training for vets. there is a real legitimacy for vets to facilitate exchange meetings on health topics due to their mastery of content and previous relationships with farmers. These meetings are to be seen in a long-term relationship between vet and farmers, and not only as a one-shot meeting. these meetings should be repeated to valorize the vet's training facilitation methods.

The changing posture of veterinarians therefore brings new perspectives for health management, since this original intervention mode both comfort their scientific legitimacy and their ability to understand the farmers' points of view on health practices.

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# PARTICIPATORY APPROACH: A WAY TO IMPROVE FARMER'S ANIMAL HEALTH MANAGEMENT

M. Leblanc-Maridor <sup>1</sup>, J. Defois <sup>2</sup>, S. Di Bianco <sup>3</sup>, A. Poissonnet <sup>4</sup>, A. Dufay-Lefort <sup>5</sup>, A. Sigwalt <sup>6</sup>, A. Wache <sup>7</sup>, C. Manoli <sup>8</sup>, C. Manoli <sup>8</sup>

<sup>1</sup>ONIRIS, INRAE, BIOEPAR, 44307 Nantes, France

<sup>2</sup>Ecole Supérieure d'Agricultures d'Angers, Unité URSE, 55 rue Rabelais, 49007 Angers
<sup>3</sup>Ecole Supérieure d'Agricultures d'Angers, Unité LARESS, 55 rue Rabelais 49007 Angers
<sup>4</sup>IFIP-Institut du porc, Domaine de la Motte au Vicomte, BP 35104, 35651 Le Rheu
<sup>5</sup>(6) ITAVI – Institut Technique de l'Aviculture, 42 rue Georges Morel, CS 60057, 49070 Beaucouzé, France
<sup>8</sup>Ecole Supérieure d'Agricultures d'Angers, Unité LARESS, 49007 Angers, France
<sup>7</sup>Institut de l'élevage, 42 rue Georges Morel - CS 60057- 49071 Beaucouzé Cedex, France
<sup>8</sup>Ecole Supérieure d'Agricultures d'Angers, Unité URSE, 49007 Angers, France

# **Background and Objectives**

Through the evaluation of farmer's participatory meetings led by their veterinarians on animal health topics, the objectives of this study are: (i) to see how the organization modalities influence the satisfaction, the learning and the behaviors of the farmers, (ii) to understand which are the main important factors that play a role between meetings' organization modalities and individual or situational factors, and (iii) to define if and how this type of device should be continued.

# **Material and Methods**

Each of the seven participatory meeting organized within a European research program was assessed by three methods: (i) a meeting's observation performed by an external observator, (ii) a questionnaire form proposed right after the meeting and (ii) a qualitative semi-directive interview performed later with 23 farmers, and 8 vets.

# Results

The overall perception of this approach was very positive, even though we noticed that various factors can impact the satisfaction and the learning effects, such as how is the meeting organized, how the group cooperates, how the facilitator interacts with the group. In fact, three satisfaction profiles emerged from the farmer's interviews. Ten farmers appreciated the participatory meeting without identifying any elements of dissatisfaction, eight appreciated the exchange with some little elements and only five farmers did not find it sufficiently practical or with enough precise and useful top-down information.

All farmers enjoyed the dynamic exchange between the participants and with the veterinarian, especially through the participatory activities. Vets were appreciated since they could give them preventive keys through top-down information, knowledge to make reminders about health management, and create learning on certain concepts by fostering exchanges between peers. The results also showed that the modalities of the meetings mainly influence the satisfaction, the learning process and the intention of farmers, whereas the onward changes in farmer's behavior and practices rather depend on individual and situational factors, specific to the farmer and his farm.

# **Discussion and Conclusion**

This participatory approach has been demonstrated to be a new way to improve farmer's herd health management. In the future, adjustments are needed especially for a better transfer of learning by providing a personalized support post-meeting by the veterinarian.

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# WHERE THERE IS A WILL THERE IS A WAY: REDUCTION OF ANTIMICROBIAL USE IN CYPRIOT SWINE FARMS.

M. Christoforou <sup>1</sup>, <u>G. Nikolaou</u><sup>1</sup>, K. Spanoudes <sup>1</sup> <sup>1</sup>Department of Veterinary Medicine, University of Nicosia School of Veterinary Medicine, 2414, Nicosia, Cyprus

## **Background and Objectives**

Over the previous decades, Cyprus has consistently been a place of swine antimicrobial overuse. This has been attributed to a lack of good practices, poor infrastructure, and over-prescription. Under government legislation and European framework, it was decided by the local Veterinary Services of Cyprus to implement a program for the reduction of the use of antimicrobials in Swine herds.

# Material and Methods

A program was put in place and every farmer must sign a contract with an independent veterinarian and set a protocol for the reduction of use of antimicrobial medicine in the farm.

The designated veterinarian had to make a farm profile and present a biosecurity and vaccination program while collecting the following information:

Vaccination program Size of the herd Breeding program Close or open herd ( the origin of the breeding animals ) Level of biosecurity Antimicrobial usage during the previous calendar year

In this work, the data included concern 14 farms with 9260 sows, while antimicrobial usage was calculated based on ESVAC recommendations.

## Results

Upon enrollment to the program, and within a 6-month period, a 50% decrease in average antimicrobial has been confirmed for all participating farms

(from 6956 mg/PCU to 3280 mg/PCU).

# **Discussion and Conclusion**

The hypothesis that antibiotic abuse is tightly connected with overprescription, has been confirmed.

The findings of this work, suggest that the administration of veterinary antimicrobials in the swine industry

should be closely monitored by the competent authority, to prevent unnecessary usage.

EU targets in reduction of antimicrobial use and antimicrobial resistance, can be hindered by overprescription.

The participating farms are currently upgrading their biosecurity measures and their vaccination regimes, to further reduce antimicrobial use.

The participating farms are currently upgrading their biosecurity measures and their vaccination regimes, under more vigorous surveillance schemes to further reduce antimicrobial use.

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# BIOSECURITY ASSESSMENT IN 27 PIG FARMS DURING THE PERIOD 2022-2023

J.D.L.L. Luevano-Adame<sup>1</sup>, M. Carvajal<sup>1</sup>, M. Palma<sup>1</sup>, A. Iturbide<sup>1</sup>, I. Lopez<sup>1</sup>, A. Diaz<sup>1</sup>, A. Henao<sup>1</sup> <sup>1</sup>Pig Improvement Company

### **Background and Objectives**

The measures applied in a production unit to prevent the entry and spread of diseases are known as biosecurity. The implementation of a biosecurity program in a farm contributes decreasing the risk of exposure to pathogens. The aim of this study was to rate the biosecurity level from 27 production units in order to assess the necessity to improve their protocols.

## Material and Methods

During the period from 2022 to 2023, 27 commercial pig farms from Mexico were evaluated, through a technical visit and reviewed of their biosecurity protocols and procedures, where a survey of 1000 biosecurity points was carried out. A database was created and the results were obtained as percentage from these thousand points. The evaluation contemplated the main features considered as non-negotiable in biosecurity of pig farms, such as, the mortality disposal protocol, the washing and disinfection of transportation units methods, the employment of a disinfection room, the presence of any other animals within the farm, among others.

## Results

Site 1 and Gene Transfer Center (GTC) farms were the ones that obtained the lowest rate, one of them with 30.6% of achievement. The wean to finish system presented the highest score with 90.1%. The most representative evaluated aspects that the farms did not fulfilled were: the mortality disposal protocols, the washing and disinfection of transportation units methods, the correct use of the disinfection room and the presence of other animal species such as birds and cattle.

# **Discussion and Conclusion**

The biosecurity evaluation within a production unit allows to control or mitigate the risk of disease transmission. Viral and bacterial infectious agents can remain viable and be infectious when a correct washing and disinfection process is not carried out. Obtaining a score in biosecurity allows to consider an improvement plan to reduce the exposure risk level. Considering that site 1 and GTC farms were the lowest ones, therefore, this could have a considerable economic impact in an infectious outbreak.

# REPRODUCTION

## REP-PP-01

## **REP** - Reproduction

# NECROPSY FINDINGS CORRELATED TO THE MAIN CAUSES OF SOW MORTALITY

<u>J.V. Rodrigues</u><sup>1</sup>, A.L. Ansolin<sup>1</sup>, L. Brandalise<sup>1</sup>, M.A. Zandonai<sup>1</sup>, A.L. Waltrich<sup>1</sup>, R.M. Cavalcante Filho<sup>1</sup>, A. P Siqueira<sup>1</sup> <sup>1</sup>Technical Services, Agroceres PIC, Brazil

# **Background and Objectives**

Identifying the main causes of death is still an everyday opportunity, and given the importance of the topic, studies are being carried out all over the world. The main objective of this study was to correlate the main necropsy findings with the main causes of death in sows to target actions to reduce the number of deaths.

### **Material and Methods**

The present study analyzed the results of 76 sow necropsies carried out in 2023 on three farms in Brazil. Information regarding the skin, oral cavity, snout and turbinates, abdominal cavity, heart, lungs, joints and hooves, liver, spleen, kidney, bladder, uterus, stomach, was evaluated and collected. For statistical analysis, PROC GENMOD was used to describe incidence, PROC FREQ for relative of two groups, PROC LOGISTIC for odds ratio estimation using SAS On Demands for Academics® (SAS Institute, Inc., Cary, NC).

#### Results

The findings were identified as normal (organ without alteration) and altered (containing some type of alteration). The two main causes of death identified were: 31.6% uterine prolapse and 26.6% organ torsion. For the two causes mentioned, there was a significant difference (p<0.01) for findings with changes in relation to normal observed in: abdominal cavity (ascites, peritonitis and hemoperitoneum), kidney (presence of pus in the pelvis; hemorrhagic changes), spleen (enlarged; hemorrhagic; torsion), liver (enlarged, congested, torsion). For the uterine prolapse there were significant differences (p<0.01) in: skin lesions (abscesses and scapular ulcers) and cardiac lesions (pericarditis). Changes in bladder, joints and hooves, snout and turbinates and lungs did not show significant differences (p<0.01) between altered and normal in terms of causes of death.

#### **Discussion and Conclusion**

The findings suggest that factors that increase the volume and pressure in the abdominal cavity may favor the occurrence of prolapses and are obviously involved in torsion. However, it is necessary to understand more about the primary causes of the findings and the possible involvement in the causes of death. The skin lesions indicate that the sow's body condition, especially thinness, is a risk factor for the occurrence of prolapse.

**REP** - Reproduction

# LACK OF ASSICIATION BETWEEN THE NUMBER OF STILLBORN PIGLETS AND HAEMOGLOBIN CONCENTRATION IN GILTS IN LATE PREGNANCY

<u>P. Cybulski</u>, J. Rynkowski <sup>1</sup>, R. Kondratiuk <sup>1</sup>, E. Michalik <sup>1</sup> <sup>1</sup>Goodvalley Agro S.A., Przechlewo, Poland

## **Background and Objectives**

Over the past decades, the prolificacy of swine has increased substantially. Rising performance of reproductive animals triggers a greater incidence of piglets born dead. The issue has become a matter of concern to farm managers and veterinarians. The number of stillbirths was found to be greatly influenced by a number of factors, including level of haemoglobin (Hb) during pregnancy. The Hb concentrations in sows have already been described in relation to the increased number of stillbirths; however, data presenting the same association in gilts are scarce. The objective of this study was to determine the relationship between number of piglets born alive and stillbirths in gilts, and their Hb level in late pregnancy.

### Material and Methods

A group of 134 pregnant DanBred gilts from three farms (3300, 5000, 8000 sows/herd) was subjected in this investigation. The number of sampled animals was 33, 39, and 62, respectively. The farms were PRRSV-negative and all the gilts were vaccinated against leptospirosis, porcine parvovirus, and PCV2. The samples were collected in late gestation (>111 days) from the ear and evaluated using HemoCue Hb201+ system (HemoCue AB, Ängelholm, Sweden). Anaemia was defined as Hb value below 10.3 g/dl. Data regarding the number of stillborn piglets and piglets born alive were tracked. The statistical analysis was performed using STATISTICA 13.1 (StatSoft, Cracow, Poland). The Mann–Whitney U test was utilised to assess the relationship between the parameters.

### Results

The mean Hb concentration was 10.1 g/dl (SD: 1.2), and 52.2 % (70/134) of the examined gilts were anaemic. The total number of piglets born alive per litter in anaemic and non-anaemic gilts was 15.13 (SD: 4.54) and 16.06 (SD: 2.80), respectively. The total number of stillborn piglets per litter in anaemic and non-anaemic gilts was 0.66 (SD: 1.15) and 0.50 (SD: 0.69), respectively. The differences were not found to be statistically significant (p>0.05).

# **Discussion and Conclusion**

In this study no significant correlation were found between the Hb level in pregnant gilts and the number of piglets born alive/stillbirths. Further studies are needed to determine a reliable predictor of gilts with lower reproductive levels.

**REP** - Reproduction

# INCREASE PRODUCTIVITY IN SOWS INSEMINATED WITH AGED SEMEN USING PROSTAGLANDIN F2 $\alpha$

P.P. Pwint Thu <sup>1</sup>, N. Am-In <sup>2</sup>

<sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>Center of Excellence in Swine Reproduction, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Thailand

## **Background and Objectives**

Boar semen stored for more than 48 hours reduces sow productivity. The number of viable sperm decreases, reducing the likelihood of live sperm reaching the fertilization site. Increasing the likelihood of viable sperm reaching the fertilization site can increase farrowing rate and litter size. Increasing uterine contractions during mating is one option. Research suggests that adding prostaglandin F2 $\alpha$  (PGF2 $\alpha$ ) to extended boar semen improves uterine contraction and reproductive indices in sows, without affecting semen quality. In this study, we investigated if adding PGF2 $\alpha$  to diluted old semen boosted sow output.

# Material and Methods

Data were collected from a sample of 80 sows, crossbreeds between Landrace and Yorkshire, with parities ranging from 3 to 5. The sows were evenly distributed into four groups based on the following criteria: 1) insemination with semen aged no more than 48 hours, 2) insemination with semen aged no more than 48 hours along with PGF2 $\alpha$  at a concentration of 1.76×10<sup>-7</sup> M, 3) insemination with semen aged more than 48 hours, and 4) insemination with semen aged more than 48 hours along with PGF2 $\alpha$  at a concentration of 1.76×10<sup>-7</sup> M. Litter size was compared using ANOVA, and farrowing rate was assessed via chi-square analysis.

#### Results

The results indicated that the farrowing rate, total born piglet and born alive piglets were significantly lower (P< 0.05) in Group 3 (75%,12.3 $\pm$ 1.2 and 11.8 $\pm$ 1.9) compared to Groups 1 (85%, 14.3 $\pm$ 1.2 and 13.1 $\pm$ 1.2 heads) and 2 (90%, 14.7 $\pm$ 1.5 and 13.3 $\pm$ 1.6 heads), but there was no significant difference when compared to Group 4 (80%, 13.9 $\pm$ 1.8 and 13.3 $\pm$ 1.2 heads, P $\geq$  0.05). No significant differences were observed in farrowing rate and litter size among Groups 1, 2, and 4 (P $\geq$  0.05).

# **Discussion and Conclusion**

The enhancement of uterine contractions by the inclusion of  $PGF2\alpha$  has the potential to facilitate the migration of viable sperm from aging semen to the site of fertility, a phenomenon underscored by the no different outcomes between Group 1 and Group 4. This phenomenon is conducive to sustaining production levels. As a result,  $PGF2\alpha$  formulations like Lutalyse could be integrated into the processing of aging extended boar semen to augment myometrial contractility, thereby enhancing fertility outcomes subsequent to artificial insemination procedures.

**REP** - Reproduction

# EFFICACY OF ALTRENOGEST ON ESTRUS SYNCHRONIZATION IN GILTS UNDER FIELD CONDITIONS.

I. Nimmansamai <sup>1</sup>, N. Boonraungrod <sup>1</sup>, P. Lawananon <sup>1</sup>, T. Kawongklang <sup>2</sup>, <u>D. Sperling</u> <sup>3</sup> <sup>1</sup>Ceva Animal Health (THAILAND) Co, Ltd. <sup>2</sup>Bioscience Animal Health Public Company Limited <sup>3</sup>Ceva Animal Health, France

# Background and Objectives

Gilt management is one of the important points to drives the availability of sufficient cyclic gilts and lends predictability to the breeding target and weaned pigs output. Altrenogest (AT) supplementation has been widely used for estrus synchronization in gilts and sows. Altrenogest is synthetic progesterone with the progestagenic activity and acts the similar way as natural progesterone produced by corpus luteum. The objective of this study was to prove the synchronization efficacy of AT and compare the effect on reducing non-productive day in gilt development unit between gilts with AT treatment and gilts with conventional management under the field conditions.

### **Material and Methods**

This study was conducted in commercial farm from Northern of Thailand. A total of 320 gilts (8 batches) after confirmed first estrus were included and divided into 2 groups; treatment group (4 batches; n=160) were treated 18 days with Altresyn® (20 mg AT/day) and control group (4 batches; n=160) without any treatment. After the withdrawal of AT, standard estrus detection was performed every day in the same way in both groups. The number of gilts with heat detected and days from first to last gilt mating in both groups were recorded.

# Results

Gilts in the treatment group were in heat after withdrawal of AT for 5 days on average (3-8 days) and the estrus rate of gilts in treatment group was numerically higher than control group 2.5% (98.1% vs 95.6%, p>0.05). The average range of the mating days from first to last gilt in treatment group were significantly shorter than control group; 5 (4-6 days) vs 19.8 (16-23 days) days, p<0.01.

## **Discussion and Conclusion**

This study proven Altresyn® as an effective tool for estrus synchronization of gilts under the field condition. According to the results, the higher estrus rate and predictable estrus date enable more effective introduction of replacement gilts in optimal number. The short range of first to last mated gilt within a batch leads to reducing of non-productive day and production cost. However, the use of effective gilt synchronization must be done in parallel with the good management in gilt development unit to achieve the highest efficacy and performance.

**REP** - Reproduction

# IMPACT OF FARM OF ORIGIN AND HOUSING SYSTEM FOR BOARS IN THE BACTERIAL CONTAMINATION AND QUALITY OF SEMEN DOSES.

A.L. Ansolin<sup>1</sup>, J.V. Rodrigues<sup>1</sup>, A. P Siqueira<sup>1</sup>, R.M. Cavalcante Filho<sup>1</sup>, L.C. Rodrigues<sup>2</sup>, M.G. Marques<sup>3</sup>, I. Bianchi<sup>4</sup>, J. D Kich<sup>5</sup>

<sup>1</sup>Technical Services, Agroceres PIC, Brazil <sup>2</sup>Liquid Genetics, Agroceres PIC, Brazil <sup>3</sup>Embrapa Suínos e Aves, BR <sup>4</sup>Catarinsense Federal Institute - Araquari, BR <sup>5</sup>EMBRAPA Suínos e Aves, Concórdia, Brasil

## **Background and Objectives**

Understanding the impact of boar microbiota and the conditions to which boars are exposed in relation to ejaculate contamination and sperm quality is critical to the development of control measures. The objective of the present study was to evaluate the effect of origin, housing system and age of boars on bacterial contamination and doses production.

# **Material and Methods**

Boars (n=24; 186 d) of same genetic line were divided in two groups according to the farm of origin at birth (origin A, n=12; origin B, n=12) and housed in two barns (B1 and B2), forming four groups OAB1; OAB2; OBB1; OBB2. Barn 1 had negative air pressure and 70% slatted floor, whereas barn 2 had positive air pressure and 100% slatted floor. From every, boar raw sperm samples were obtained through a semi-automatic system. Motility and morphology were performed as well as quantification of colony forming units (CFU). The data was analyzed through a multivariate linear regression (individual and interactive effects).

#### Results

There was interaction between the origin and the barn (P<0.05). OBB2 boars had a lower count of CFU of ejaculate compared to OA boars ( $1.4 \pm 0.2 \text{ vs} 1.9 \pm 0.1 \log^{10}$ , P<0.05). A negative correlation was observed between age of the boar and contamination of raw sperm sample (r= -0.27, P<0.003). The highest percentage of ejaculate discards by sperm morphology was in the OAB1 group (54% ± 3.7, P<0.05). OAB1 also presented lower total motility of raw sperm (83.8% ± 1.78, P<0.05) compared to groups OBB1/OBB2 (89.8% ± 1.77; 89.1% ± 1.77) and lower concentration/ejaculate (52.9 billion spermatozoa/mL ± 1.65, P<0.05), and a lower number of doses produced (21.3 ± 0.92, P<0.05). OAB1 presented a higher odds ratio (4.4 times) of requiring medicated intervention.

# **Discussion and Conclusion**

The origin of birth and the barn have an effect on the contamination of the ejaculate, on the boars productivity parameters and the amount of medicines used. These factors may be linked to the management carried out on the farms of origin, as well as the amount of antibiotics used and the conditions in which the boars are raised before entering the insemination centers.

**REP** - Reproduction

# A PRACTICAL USE OF A SERUM PROGESTERONE STRIP TEST IN ASSESSING THE REPRODUCTIVE STATUS OF REPLACEMENT GILTS

P. Taechamaeteekul<sup>1</sup>, K. Komolpis<sup>2</sup>, R.V. Knox<sup>3</sup>, P. Tummaruk<sup>1</sup>

<sup>1</sup>Centre of Excellence in Swine Reproduction, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

<sup>2</sup>Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok, 10330, Thailand

<sup>3</sup>Department of Animal Sciences, University of Illinois Champaign-Urbana, Champaign-Urbana, Illinois, 61801, USA

# **Background and Objectives**

Minimizing non-productive days (NPD) in swine herds is crucial for increasing the annual number of piglets weaned per sow. The interval from the entry date of a gilt until the first mating significantly influences NPD. Therefore, identification of reproductive status in these replacement gilts can enhance mating schedule efficiency. This study aimed to evaluate the effectiveness of a serum progesterone strip test in identifying the reproductive status of replacement gilts.

### **Material and Methods**

The study examined 112 crossbred Landrace x Yorkshire gilts, aged between 28 and 32 weeks. From each gilt, 5–10 ml of blood was collected from the jugular vein, and serum was separated by centrifugation at 3,000 ×g for 10 min. The serum progesterone levels were measured using both ELISA and a progesterone strip test developed by Chulalongkorn University. Gilts with serum progesterone levels of  $\ge$  5.0 ng/ml were considered puberty status. The strip test, designed for porcine serum, utilized a monoclonal antibody against progesterone in a competitive lateral flow immunological assay. A result was reported as 'positive' if the test line did not show but the control line did, and 'negative' if both lines were visible. For the test, 100 µl of serum was applied to the sample area, and the results were interpreted after 15 minutes.

#### Results

On average, gilts had serum progesterone levels of  $16.3 \pm 17.1$  ng/ml, with a range of 0.2 to 57.9 ng/ml. Among them, 48.2% (54 gilts) were pre-pubertal, and 51.8% (58 gilts) were pubertal. Prepubertal gilts had significantly lower serum progesterone levels than pubertal gilts ( $0.8 \pm 0.8$  ng/ml, ranging from 0.2 to 2.7 ng/ml, versus  $30.8 \pm 11.4$  ng/ml, ranging from 5.0 to 57.9 ng/ml, P < 0.001). The progesterone strip test's effectiveness was evaluated against the ELISA method, revealing a sensitivity of 98.2% and a specificity of 94.4%. The study also found that the percentage of pubertal gilts was 66.7%, 58.1%, 53.1%, 44.0%, and 0% in gilts aged 28, 29, 30, 31, and 32 weeks, respectively (P > 0.05).

#### **Discussion and Conclusion**

In conclusion, the progesterone strip test is practical for field use to determine progesterone levels and assess reproductive status in replacement gilts.

**REP** - Reproduction

# EFFECTS OF TWO INMUNOCASTRATION PROTOCOLS ON MORPHOLOGY IN THE REPRODUCTIVE TRACT OF AMERICAN MINIPIG GILTS

Q. María Lucía 1, M. Francisco Ernesto 2, M. Susana Elisa 3, T. María Elena 4

<sup>1</sup>Universidad Nacional Autonoma de Mexico. Facultad de Medicina Veterinaria y Zootecnia

<sup>2</sup>Universidad Autónoma del Estado de México. Instituto de Ciencias Agropecuarias y Rurales

<sup>3</sup>Universidad Nacional Autónoma de México. Facultad de Estudios Superiores Cuautitlán

<sup>4</sup>Universidad Nacional Autónoma de México. Programa Universitario de Alimentación Sostenible.

# **Background and Objectives**

Inmunocastration consists in applying a vaccine to male piglets, its function is to prevent the animal from presenting sexual maturity to reduce aggression towards other pigs and caretakers; additionally avoid boar taint in the meat of finisher pigs. However, not many studies emphasise exclusively in the morphological effect in the reproductive tract of female minipigs. Therefore, the present study was designed to investigate the effect of an FDA approved product Improvac®, which induces antibody production against gonadotropin releasing hormone (GnRH) in the reproductive tract morphology.

# Material and Methods

A Kruskall-Wallis test was employed to compare the effect inmunocastration in 15 minipig female gilts. Three treatments were evaluated. T1: control; T2: standard inmunocastration (application at 12<sup>th</sup> and 16<sup>th</sup> week of age; and T3: early inmunocastration (application at 3<sup>rd</sup>, 7<sup>th</sup>, and 12<sup>th</sup> week of age).

## Results

Significant differences were observed in length and width dimensions of the uterine horns and length dimensions of the ovaries (P<0.05) were found between T1 vs T2 and T3. Also, differences were observed in all treatments in length dimensions of the oviducts. No differences were observed in width dimensions of the oviduct and ovaries.

# **Discussion and Conclusion**

The morphological comparison revealed that inmunocastrated gilts in both protocols showed an infantilized reproductive tract vs the reproductive tract of non-treated gilts by showing a significant difference in the length of the uterine horns, oviduct, and ovaries. Incorporating animal welfare by sustaining that inmunocastration in female minipigs can be used as an alternative to surgical castration.

**REP** - Reproduction

# EVALUATION OF THE SAFETY IN PREGNANT SOWS AND THEIR PIGLETS AFTER TREATMENT WITH AMOXICILLIN-BASED PRODUCT

<u>G. Marco Stingelin</u><sup>1</sup>, A. Silva Castro<sup>1</sup>, K. Eduardo Zanoni Nubiato<sup>1</sup>, M. Garcia Borgi Lino De Sousa<sup>1</sup>, L. Cristine Penha<sup>1</sup>, D. Oliveira Carneiro<sup>1</sup>, E. Marucci Baccin<sup>1</sup> *Tearmabase Saúde Animal* 

## **Background and Objectives**

In pigs, the use of antibiotics in the final trimester of pregnancy can negatively influence the immunological competence of piglets, causing greater susceptibility to diseases and reduced performance. The aim of this study was to evaluate the reproductive safety of amoxicillin at a dosage of 20 mg/kg for 14 days included in the diet of sows on the final third of pregnancy and their respective piglets, based on clinical, hematological and biochemical evaluations.

# **Material and Methods**

Twenty pregnant sows (Sus domesticus), Camborough x AGPIC 426 genetic lineage, Agroceres PIC, from 70 days of pregnancy, between one and five pregnancy cicles, weight between 175 kg and 269 kg and in perfect health conditions were used. The animals were divided into groups G1 (control) and G2 (treated with 20 mg/kg of amoxicillin included in the SID diet, for 14 days since 76th pregnancy day). Sows and piglets were evaluated for clinical and reproductive safety. In addition, hematological (HCT, RBC, HGB, MCV, MCHC, MCH, PLT and WBC) and biochemical (ALKP, ALT, AST, BUN, ALB, PT, GLB, CREAT and GGT) evaluations were carried out in piglets on the 7 and 21 days of life. To evaluate the parametric data, the Student's t test was used and to evaluate the non-parametric data, the Fisher's test was used.

### Results

There was no statistical significance in any of the parameters evaluated on the clinical and hematological examinations between G1 and G2. To the biochemical evaluations, there were statistical differences (p<0.05) in the ALB and PT concentrations in which the piglets in the treated group had higher values than the control group, however they remained within the reference range recommended by the species. There were also no differences between the groups in any of the reproductive phases evaluated, such as pregnancy duration, premature birth rate and abortion rate with and without malformation.

## **Discussion and Conclusion**

Based on the results, it is possible to state that there were no deleterious clinical, laboratory or reproductive effects. Therefore, the administration of amoxicillin at the dosage tested in sows at the final trimester of pregnancy is safe for them and their respective piglets.

**REP** - Reproduction

# EFFECT OF PENIS FIXATION SYSTEMS ON BOAR PRODUCTIVITY AND EJACULATE QUALITY

<u>A.L. Ansolin</u><sup>1</sup>, A. P Siqueira<sup>1</sup>, J.V. Rodrigues<sup>1</sup>, R.M. Cavalcante Filho<sup>1</sup>, L.C. Rodrigues<sup>2</sup>, R.M. Cardoso<sup>2</sup> <sup>†</sup>*Technical Services, Agroceres PIC, Brazil* <sup>2</sup>*Liquid Genetics, Agroceres PIC, Brazil* 

### **Background and Objectives**

Semi-automatic collection systems are widely used nowadays, allowing optimization in the number of collections per hour per technician and reducing labor costs. The objective of this study was to verify the effect of two types of penis fixation systems on boar productivity and ejaculate quality.

# Material and Methods

Two different types of penis fixation systems from two different suppliers were used. System 1 consisted of the manual fixation of the penis in an artificial vagina controlled by air pressure. System 2 consisted of manual fixation of the penis in an artificial cervix attached to a clamp with manual adjustment of penis compression. Twenty males of the same age and genetic lineage were used, divided into 2 groups (G1: system 1, n=10 and G2: system 2, n=10). Collections were made by just one person in both groups, totaling 260 collections.

# Results

There was no difference (P>0.05) in sperm volume, concentration per mL, and number of colony-forming units (CFU/mL) between groups. There was a difference (P≤0.05) in the efficiency on penis fixation during collection, where G1 showed a lower percentage of penis escape ( $19.8 \pm 3.8 \text{ vs } 31.0 \pm 4.1$ ). Considering the boars that did not have continuous fixation throughout the collection time, G1 showed a shorter collection time (06 min. 31 sec vs 08 min. 49 sec, P<0.0001) and a tendency (P=0.09) towards lower CFU/mL (G1:  $2.4 \pm 0.13 \text{ vs } G2: 2.8 \pm 0.21$ ).

# **Discussion and Conclusion**

The percentage of penis escape in G1 was 10% lower than in G2, resulting in an average time of 02 min. 18 sec less in collections where the penis escaped at some point. The fact of a higher percentage of escape may lead to a greater risk of contamination during collection, due to the greater difficulty in re-fixing in which the artificial cervix and/or vagina touches the ventral region of the animal, which is moist and with greater presence of dirt, due to contact with the collection dummy. There is also an extra cost of around S1 for additional material per collection.

**REP** - Reproduction

# EFFECT OF IONIZED CALCIUM BLOOD LEVEL ON HEALTH AND UTERINE INVOLUTION IN THE PUERPERIUM IN FREE FARROWING SOWS

T. Holzmann <sup>1</sup>, G. Schüpbach <sup>2</sup>, <u>A. Grahofer</u><sup>1</sup> <sup>1</sup>*Clinic for Swine, Vetsuisse Faculty, University of Bern, Bern, Switzerland* <sup>2</sup>*Veterinary Public Health Institute, University of Bern, Liebefeld* 

# **Background and Objectives**

Calcium levels are critical for coordinated uterine contraction during and after farrowing. Therefore, the aim of the study was to evaluate the influence of ionized calcium blood levels post-partum on the health and the uterine involution of sows.

# Material and Methods

In total, 60 blood samples were obtained from the ear vein post-partum and ionized calcium level was tested directly on sow side using LAQUAtwin CA-11C. The body temperature, the vaginal discharge and lack of appetite were recorded for five days post-partum. Furthermore, the uterine diameter of three uterine horns was measured on day 3, 5 and 7 post-partum and again before weaning (approximately 28 days) using ultrasonography. In addition, the following sow and piglets traits were assed: parity, body condition score (BCS), backfat thickness, gestation length, number of live born piglets, number of stillborn piglets, farrowing duration, duration of placenta expulsion, litter weight, placenta weight, number of placenta parts, faecal score, and obstetrical intervention. Descriptive statistics was performed after data cleaning and Spearman rank correlation was used. The level of statistical significance was set to p < 0.05.

#### Results

The mean parity of the sow was  $4.2 \pm 1.7$  with an average total born piglets of  $15.4 \pm 3.8$ . The ionized calcium blood level post-partum significantly negatively correlated with the uterine diameter 3 days post-partum (Spearman correlation coefficient r=-0.3543; p-value=0.0116) as well with the parity of the sows (Spearman correlation coefficient r=-0.4000; p-value=0.0030). No further correlations with continuous variables and ionized blood calcium level postpartum were detected. However, a positive significant correlation between the uterus diameter 3 days post-partum and the gestation length (Spearman correlation coefficient r=0.5915; p-value=0.0001) and obstetrical intervention (Spearman correlation coefficient r=0.2984; p-value=0.0353) were identified.

# **Discussion and Conclusion**

The ionized calcium blood level, the gestation length and obstetrical intervention had a significant effect on uterine involution in free farrowing sows. Therefore, these parameters should be routinely monitored in the farrowing management to assess reproductive health post-partum and warrant early intervention.

**REP** - Reproduction

# IMPACT OF LIGHT INTENSITY IN GESTATING ROOM ON GESTATION LENGTH AND PREMATURE FARROWINGS: A DESCRIPTIVE STUDY IN ONE FRENCH FARROW-TO-FINISH HERD

A. Lebret <sup>1</sup>, <u>C. Teixeira-Costa</u><sup>1</sup>, J. Jeusselin <sup>1</sup>, C. Chevance <sup>1</sup>, V. Normand <sup>1</sup>, T. Nicolazo <sup>1</sup>, G. Boulbria <sup>1</sup> <sup>1</sup>Rezoolution Pig Consulting Services, Noyal-Pontivy, France

## **Background and Objectives**

The aim of this study was to investigate the impact of light intensity on gestation length (GL) and percentage of premature farrowings (PF) in one 300-sows French farrow-to-finish herd.

## **Material and Methods**

Gestation lengths, sow's parity and backfat thickness (BFT) before farrowing were recorded between April and September 2023. Light intensities in gestation rooms were measured using a lux meter at six different points classifying a room as dark or as bright (durations of light were the same between all rooms). The impacts of BFT and parities on GL were tested using a Kruskal-Wallis test regardless of genetic. GL averages were compared between the two groups of brightness using a Wilcoxon test. Percentages of PF (<114 days of gestation) were compared between groups using a Chi2 test. For these analyses, statistics were performed according to different genetic types: LW x Tai Zumu x Landrace (genetic 1) and LW x Landrace (genetic 2).

## Results

Our dataset included 241 farrowing data: 55 gilts, 44 parity 2 sows, 53 of parities 3 and 4 together and 89 sows of parities 5 or more. Two rooms out of three were classified as dark (means of 16 and 20 lux) and the third one as bright (43 lux). There was no effect of parity rank and BFT on GL and PF percentages. Regarding light intensity impact, farrowing data of 62 sows of genetic 1 (23 sows in the bright room and 39 in dark rooms) and 179 sows of genetic 2 (81 and 98 sows respectively) were included. GL averages in bright or dark rooms for genetic 1 were 113.78  $\pm$  1.55 days and 112.49  $\pm$  1.70 days respectively and the difference was statistically significant (p=0.001). We did not show any difference for sows of genetic 2 (114.42  $\pm$  1.14 versus 114.44  $\pm$  1.31). Regarding PF percentages, our results also showed a significant impact of light intensity for sows of genetic 1 only in dark rooms compared to bright ones (p=0.002).

## **Discussion and Conclusion**

This case report highlights the impact of light intensity on gestation length and premature farrowings. We also show a difference of susceptibility between two different sow genetic lines.

**REP** - Reproduction

# SOWS EXHIBITING ELEVATED LEVELS OF PROGESTERONE IN THEIR COLOSTRUM EXPERIENCED LONGER DURATIONS OF FARROWING

C. Srisang<sup>1</sup>, R. Boonprakob<sup>1</sup>, J. Suwimonteerabutr<sup>1</sup>, P. Tummaruk<sup>1</sup>

<sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

# **Background and Objectives**

Swine industry has utilized hyperprolific sows with anticipation to produce more piglets and thus enhance profitability. Nevertheless, a larger litter size often leads to extended duration of labor. Since colostrum progesterone levels have been used as a non-invasive measure to assess reproductive status and detect irregularities in cows and goats, the relationship between progesterone levels in sow colostrum and farrowing duration remains unclear. The aim of this research was to explore the potential link between the concentration of progesterone in colostrum and the duration of farrowing process in gilts and sows.

### **Material and Methods**

100 Landrace × Yorkshire crossbred sows were included in this study. The average parity of the sows was  $1.9 \pm 0.8$  (range:1-3). Measurements of backfat thickness were taken at 109 days of gestation with A-mode ultrasonography. The collected data included the duration of farrowing, total number of piglets born, BA, SB, MF. Colostrum samples were collected within 3 hours after the beginning of farrowing to measure progesterone levels using ELISA technique. Farrowing duration was group into two categories for analysis: normal farrowing (<300 min) and prolonged farrowing (>300 min).

### Results

colostrum progesterone levels varied among the sows: 27% had low levels (10 - 40 ng/ml), 48% of sows displayed moderate levels (41 - 99 ng/ml), and the remaining 25% had high levels (>100 ng/ml). Sows that experienced prolonged farrowing duration had average colostrum progesterone level at 113.6 ng/ml, while average colostrum progesterone level of those with normal farrowing was 72.7 ng/ml (P < 0.001). In this study, Primiparous sows with prolonged farrowing duration had significantly elevated levels of progesterone in their colostrum compared to those with normal duration (142.2 vs. 71.2 ng/ml respectively, P < 0.001). In contrast, among multiparous sows, there was no evidence for difference of colostrum progesterone levels (P = 0.387)

## **Discussion and Conclusion**

In conclusion, there is a relationship between higher colostrum progesterone levels and extended farrowing times in primiparous sows. The colostrum progesterone might be used to indicate the prolonged farrowing duration in primiparous sows for on farm management.

**REP** - Reproduction

# BENEFIT OF ADDITIONAL VACCINATION AGAINST PORCINE PARVOVIRUS 1 WITH A VACCINE BASED ON A 27A-LIKE STRAIN

P. Talaga <sup>1</sup>, E. Michalik <sup>2</sup>, R. Kondratiuk <sup>2</sup>, R. Morgenstern <sup>3</sup>, M. Tarasiuk <sup>4</sup>, <u>P. Cybulski</u> <sup>2</sup> <sup>1</sup>Private Veterinary Practice, Poland <sup>2</sup>Goodvalley Agro S.A., Przechlewo, Poland <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany <sup>4</sup>Boehringer Ingelheim Sp. z o.o., Warsaw, Poland

# **Background and Objectives**

Porcine parvovirus 1 (PPV1), precisely ungulate protoparvovirus 1, is described to cause SMEDI (stillbirths,mummification, embryonic death, and infertility) in swine. Vaccination of sows for PPV1 control is a widely used method to prevent clinical disease. However, infection is commonly not prevented by vaccination and viruses genetically change over time. "Newer" variants, named 27a-like viruses, became predominant in Europe. 27a-likeviruses show changes in the capsid protein, which negatively influences cross-neutralization with serum of sowsvaccinated with "older" vaccine strains.

# Material and Methods

The study was conducted in a farrow-to-wean farm with 3300 sows in Poland. This study was initiated as a pilotproject to explore the effect of an additional vaccination against PPV1 with a vaccine, which is based on a 27a-likestrain. Forty-eight (48) replacement gilts were included in this study. All of them were vaccinated with the standard vaccine against PPV1 used on this farm. Twenty-three (23) of those gilts were additionally vaccinated with ReproCyc© ParvoFLEX (treatment group) before first insemination. The other 25 gilts (control group) received only the standard vaccination protocol against PPV1. Comparison of selected reproductive parameters was performed between groups (liveborn, stillborn, mummified and weak-born piglets per litter).

#### Results

The number of liveborn piglets showed a numerical difference of one piglet per litter (median treatment group: 17 vs.median control group: 16). Other variables showed only minor numerical differences.

# **Discussion and Conclusion**

The number of liveborn piglets showed a relevant numerical, yet not statistically significant, difference in favor of gilts additionally vaccinated with ReproCyc© ParvoFLEX. Lack of statistical significance was conditioned by the low overall number of gilts in the investigation and the rather large variation of the parameters. Thus, this investigation will be repeated with a larger sample size.

**REP** - Reproduction

# CHARACTERIZATION OF FOLLICULOGENESIS IN CONTEMPORARY GILTS OF DIFFERENT AGES

L. P Moreira <sup>1</sup>, J. V L Ferreira <sup>1</sup>, L. C Cardoso <sup>1</sup>, A. L Shinkawa <sup>1</sup>, P. A Ferreira <sup>1</sup>, R. Nascimento <sup>2</sup>, G. A Silva <sup>2</sup>, A. P Siqueira <sup>2</sup>, <u>F. R C L Almeida</u> <sup>1</sup>

<sup>1</sup>Department of Morphology, Federal University of Minas Gerais, Brazil <sup>2</sup>Technical Services, Agroceres PIC, Brazil

## **Background and Objectives**

Modern swine industry is a highly competitive market where the most productive animals are selected to meet the demand for pork production. Producing offspring at the youngest ages is desirable for acceleration of genetic progress and reduction of production costs. Contemporary gilts show high growth rates, sexual precocity, and age at first breeding has positively been associated with sow longevity. Folliculogenesis directly affects gilts' reproductive efficiency. Thus, understanding recruitment, atresia, and selection of follicles in pigs may provide insights into approaches to limit fertility failures. Our aim was to evaluate follicular dynamics in high growth rate gilts of different ages.

#### Material and Methods

Twenty gilts (Camborough®,Agroceres PIC, Brazil) were weighed individually at selection (150 days (d) of age). According to the average daily weight gain (ADG) at selection, females with ADG greater than 600g were divided into four groups, according to the predicted age at first insemination: G1 (195-200d), G2 (200-210d), G3 (210-220 d) and G4 (>220d). Females were euthanized, on avarage, 4.3d after estrus (luteal phase) and ovaries were collected, weighed, measured, and processed for histology. Follicles, corpora lutea and albicantia were measured and follicles were classified as: F1:<3mm, F2:3-5mm, F3:>5mm. Subsequently, ovarian histological sections were evaluated, and follicles classified as: primordial, primary, preantral, secondary, tertiary and atretic. In addition, areas of secondary and tertiary follicles components were measured by the ImageJ® software. Statistical analysis was performed by ANOVA and comparison of means by Tukey Kramer's test (SAS®).

### Results

Ovarian biometrical data, corpora lutea and albicantia diameters and numbers were not affected by age. Follicles on the ovarian surface of G4 females were smaller than in G1 (P<0.05). Follicle, oocyte, antrum, and granulosa areas were similar among groups. Although average follicular population were similar among experimental groups, G1 females presented fewer secondary follicles than G4 (P<0.05).

# **Discussion and Conclusion**

By evaluating ovarian parameters, sexual maturity of those gilts was equivalent, regardless of age. Therefore, these findings strongly suggest that gilts at an early age may present ovarian development compatible with older gilts, without compromising folliculogenesis.

**REP** - Reproduction

# REDUCING WEANING FERTILE SERVICE INTERVAL AND ABORTION RATES THROUGH CLOPROSTENOL ADMINISTRATION BEFORE WEANING

<u>S. Crespo</u><sup>1</sup>, J. Gadea<sup>2</sup> <sup>1</sup>Veterinary technician cefusa & dept physiology of University of Murcia (Spain) <sup>2</sup>Dept. Physiology. University of Murcia. Spain

# **Background and Objectives**

The administration of prostaglandins has a luteolytic effect, reducing progesterone to basal levels in order to restart ovarian cyclicity and control the possible presence of persistent corpora lutea, as well as an uterotonic effect, which helps the involution of the uterus. This beneficial effect applies to sows with a high number of productive cycles. The aim of this study was to improve reproductive performance during the first 40 days of gestation using a synthetic analogue of prostaglandins (cloprostenol sodic) 12 hours before weaning.

# **Material and Methods**

In total, 709 productive sows of different parities (range from 1 to 7) were selected, and randomly assigned to a control group (n = 360) or a PGF2a group (n = 349). The PGF2a group was injected with 2 ml of cloprostenol sodium (Planate®) 12 hours before piglet removal. The sows were then inseminated by post-cervical artificial insemination at 0 and 24 hours after the onset of oestrus. Individual data were recorded for each sow and a comparative study during the first 40 days of gestation was performed, including pregnancy rate and the occurrence of purulent endometritis.Comparison between groups was performed by non-parametric statistical analysis using the Kruskal-Wallis H test (SPSS programme).

### Results

The weaning fertile service interval (WFSI) was shorter in the cloprostenol group compared to the control group (6.32 vs. 8.13; p=0.017), while the pregnancy rate (91-93%) and the occurrence of purulent endometritis (3-4%) were similar in both groups (p=0.415). However, the abortion rate was lower in the cloprostenol group (1 vs 3%; p=0.008).

## **Discussion and Conclusion**

Cloprostenol administration stimulates uterine involution and reduces the incidence of endometritis in the other hand, it can reduce the incidence of persistent corpora lutea by improving reproductive performance in sows with longer cycles (Lopez et al 2009, Crespo et Gadea 2022). Under the conditions of this study, an injection of cloprostenol 12 hours prior to weaning reduced WFSI, the number of non-productive days, and the occurrence of abortions during the first 40 days of gestation. It is important to increase reproductive efficiency, especially in free gestation sow housing systems, to improve animal welfare.

**REP** - Reproduction

# VALIDATION OF THE PROGESTERONE KIT FOR THE DETERMINATION OF PROGESTERONE IN SERUM AT FIELD LEVEL USING SAMPLES FROM A STUDY EVALUATING THE EFFICACY OF ALTRENOGEST TREATMENT FOR OESTRUS SYNCHRONIZATION IN GILTS

P. Sánchez-Giménez <sup>1</sup>, C. Mata <sup>2</sup>, M. Marcos <sup>3</sup>, M. Jiménez <sup>3</sup>, R. Menjon <sup>3</sup> <sup>†</sup>AGROPOR, Murcia, Spain <sup>2</sup>Provesa SL <sup>3</sup>MSD Animal Health

# **Background and Objectives**

Presence or absence of progesterone (P4) in gilts is useful to check cycle status specially with a specific hormonal treatment, such as induction or synchronization. Until now, it took a few days between blood collection, sending it to the corresponding laboratory and receiving the results. The objective of this study was to determine the validity of a rapid serum P4 detection kit to evaluate the efficacy of a treatment with altrenogest for synchronization of oestrus in gilts, directly on the farm

# Material and Methods

A blood sample was obtained (n=30 gilts) before starting an 18-day treatment with altrenogest (Regumate®). Sera from each sample was separated and divided into two aliquots. One aliquot was sent to a laboratory for P4 determination with an analytical method PNT-HOR-30409 (ELFA reference technique; ng/mL) and the other aliquot was tested by the MSD progesterone kit. After treatment, a blood sample was collected again, and P4 testing repeated as described above (n=40 gilts).For the rapid serum P4 detection kit, 5 drops of serum were added to a well and the result read 15 minutes later. Interpretation of this field kit is based on P4 concentration:>10ng/mL and <10ng/mL will test as positive and negative, respectively.The statistical correlation between the two methods was tested by a two-by-two comparison, using Phi (values -1 to +1)

#### Results

There was a highly significant association between the results of progesterone of both tests:

- Pre-treatment (n=30): lab results (17- & 13+) vs kit (16- & 14+): Phi=0.818 (p<0.001)
- Post-treatment (n=40): lab results (38+ & 2-) vs kit (38 + & 2-): Phi=1.000 (p=0.001)

# **Discussion and Conclusion**

This quick test has proved to have a high correlation with the results of the quantitative gold standard assay, allowing producers to obtain, at farm level and in just a few minutes, a qualitative assessment of serum progesterone levels. In this case, it was used to assess the presence or absence of progesterone, before and after treatment, to determine if the treatment had been correct and effective or not. The gilts must be positive for P4 before starting treatment (puberty), and must be negative after treatment, which indicate that finished luteal phase

**REP** - Reproduction

# INFLUENCE OF SPERM CONCENTRATION ON REPRODUCTIVE PERFORMANCE UNDER COMMERCIAL FARM CONDITIONS

H. González-Ramiro<sup>3</sup>, A. Occón<sup>2</sup>, A. álvaro Mañas<sup>1</sup>, M.J. Martínez Alborcia<sup>1</sup>, S. Blanco López<sup>1</sup>, F. Valcarce Borrás<sup>2</sup>, P. Sánchez-Giménez<sup>3</sup>, <u>J. Sanchez-Osorio<sup>2</sup></u> <sup>1</sup>*AIM IBERICA, Madrid, Spain* <sup>2</sup>*Topigs Norsvin España, Madrid, Spain* <sup>3</sup>*AGROPOR, Murcia, Spain* 

## **Background and Objectives**

Artificial insemination (AI) has contributed to a more efficient use of boars with greater genetic value. However, the optimal sperm concentration in AI doses is controversial and large differences can be observed between different countries and different suppliers of seminal doses. Objective of this study was to evaluate the effect of different sperm concentration on reproductive performance (farrowing rate and litter size) in artificially inseminated sows in a commercial farm.

## **Material and Methods**

A total of 44 ejaculates from 25 Landrace boars were collected using a semi-automatic method in a commercial boar station (AI Leon, AIM Iberica, Spain). Only ejaculates approved after semen quality assessment were used for this trial. Each ejaculate was divided into two aliquots and each aliquot was diluted to achieve the final sperm concentration (Standard-27 x10<sup>6</sup> sperm /mL or High- 20% extra sperm/mL) which was assessed using Nucleocounter® (SP100, Chemometec, Denmark). AI doses were identified including information about boar and type of concentration and delivered at the farm (La Ventica, Agropor, Spain) on the following day. At the farm, Large White gilts and sows were weighted before insemination and randomly allocated to each experimental group (350 vs 241 for Standard and High concentration, respectively). Results were statistically analysed using RStudio (version 2023.06.1). A Pearson's Chi-squared test was used to analyse homogeneity between experimental groups and Two sample test for equality of proportions for fertility analysis. To compare litter size, a MIXED procedure was used using SAS System 9.0.0 for Windows.

#### Results

No differences were observed (P>0.05) in distribution of sows by cycle, weight at insemination or number of previous inseminations between both groups of animals inseminated with Standard ( $26.2 \pm 0.52$  sperm/mL) or High sperm ( $31.8 \pm 0.57$  sperm/mL) concentration. Similar farrowing rate (89.4 vs 89.2% for Standard and High concentration, respectively. P=0.933) and litter size ( $16.10 \pm 0.70 \text{ vs } 16.40 \pm 0.71$  Standard and High concentration, respectively. P= 0.280) were observed.

# **Discussion and Conclusion**

Under commercial field conditions with good quality semen and good reproductive management at the farm, there is no benefit on increasing 20 % sperm concentration compared to standard concentrations (27x10<sup>6</sup> sperm /mL).

**REP** - Reproduction

# REPRODUCTIVE PERFORMANCE OF SOWS HOUSED IN DIFFERENT GESTATION SYSTEMS

<u>J.V. Rodrigues</u><sup>1</sup>, A.L. Ansolin <sup>1</sup>, M.A. Zandonai <sup>1</sup>, R.N. Gonsalves <sup>1</sup>, R. Cavalcante Filho <sup>2</sup>, A. P Siqueira <sup>1</sup> <sup>1</sup>Technical Services, Agroceres PIC, Brazil <sup>2</sup>Agroceres PIC, Rio Claro, SP, Brazil

## **Background and Objectives**

In recent years, there has been a significant migration from individual housing systems during sow gestation to collective housing. This study aims to compare and evaluate the main productive results in to housing models and management during gestation in sows.

# Material and Methods

The study analyzed farrowing rate and sows mortality data for the months of January to august 2023 for 160,472 sow from 42 farms in Brazil, according to the type of housing and feeding system - individual housing (IH), Electronic Sow Feeding (ESF), Stanchions (ST), floor feeding (FF), and housing management in post-implantation (PI) and pre-implantation (PRI). For statistical analysis, PROC GLM. The means were estimated with PROC MEANS for each collective housing system model. One-Way ANOVA analysis of variance using SAS On Demands for Academics® software (SAS Institute, Inc., Cary, NC).

### Results

11.9% of they were housed in IH and 88% in group-housing systems: 4.8% in ESF, 64.3% in ST and 19% in FF. In the group-housing systems, 77.8% were in PI housing management and 22.2% in PRI. The farrowing rate is higher in PI systems compared to PRI systems (89.1% and 86.9%, respectively). ESF showed a 86.8% farrowing rate, followed by ST (88.3%), FF (88.7%) and IH (89.2%). In the ESF, farms with PI management had a 92.7% farrowing rate, while farms with PRI management had 83.82%. Furthermore, in the ST systems, 89.5% farrowing rate was observed in PI management and 83.8% in PRI management. The observed sow mortality rate was 14.3% in ESF, 13.5% in FF, 12.6% in ST and 12.0% in IH systems. None of the data analyzed had a statistical difference (p>0.05).

# **Discussion and Conclusion**

The results reinforce that PRI housing systems present worse reproductive results, since sows are housed during a critical period of migration and embryonic fixation. However, this does not mean that all farms with PI systems perform poorly. It is important to emphasize attention to group formation and individual care of sows. Furthermore, in relation to sow mortality, the results showed the great importance of different management in the collective system, health inspection, feeding and floor quality.

**REP** - Reproduction

# ZEARALENONE AND PIG REPRODUCTION: EFFECTS AND A MITIGATING STRATEGY

<u>B. Doupovec 1</u>, B. Streit 1, M. Killinger 1, D. Schatzmayr 1 1dsm-firmenich, Animal Nutrition and Health, Research Center Tulln, Technopark 1, 3430 Tulln, Austria

## **Background and Objectives**

Zearalenone (ZEN) is one of the most frequently detected mycotoxins detected in more than 50% of the samples analyzed (Source: DSM World Mycotoxin Survey, 2022). Due to its structural similarity to estrogen, ZEN binds to estrogen receptors, thereby acting as an endocrine disruptor and interfering with reproductive functions. The aim of these studies was to evaluate the zearalenone hydrolase ZenA (ZENzyme®), a purified recombinant enzyme, that can catalyze the hydrolysis of ZEN into the non-estrogenic metabolite hydrolyzed ZEN (HZEN).

# Material and Methods

In the first trial 18 female piglets (age 5 weeks) were randomly allocated to three experimental groups with six replicates each. The treatments were as follows: negative control (basal diet), positive control (2000 µg ZEN/kg feed), and ZenA (2000 µg ZEN/kg feed + 50 Units ZenA/kg feed). At the end of the trial, vulvar volume and the relative weight of the reproductive tract was evaluated. For the second trial 72 weaned piglets (mixed sex) were assigned to two experimental groups with 6 replicates each: positive control (200 ppb of ZEN), and ZenA (200 ppb of ZEN + 10 Units ZenA/kg feed). Feces, urine and plasma were sampled at four timepoints and analyzed for ZEN and its metabolites.

## Results

In trial 1 the vulva volume and the weight of the reproductive tract was significantly increased in the ZEN group compared to the control animals and significantly reduced again in the ZenA group (p<0.05). The results of trial 2 showed a significant shift from the estrogenic ZEN to HZEN due to the addition of ZenA. ZEN content feces D49: ZEN 174 ng/g vs. ZEN + ZenA 100 ng/g, p<0.001, HZEN content feces D49: ZEN 7.25ng/g vs. ZEN + ZenA 182 ng/g, p<0.001). This shift was visible at all sampling points as well as in the other investigated matrices, i.e. plasma and urine.

### **Discussion and Conclusion**

The results demonstrate that ZENzyme<sup>®</sup> applied as a feed additive was effective in transforming ZEN to HZEN in the digestive tract of pigs and therefore counteracted the negative impact of ZEN in pig production.

**REP** - Reproduction

# EFFECTS OF ALTRENOGEST SUPPLEMENTATION IN LATE LACTATION ON GENITAL TRACT MORPHOLOLOGY IN PRIMIPAROUS SOWS

<u>D. K O Pires</u><sup>1</sup>, S. V Ferreira<sup>2</sup>, J. V L Ferreira<sup>1</sup>, A. L Shinkawa<sup>1</sup>, I. G A Vidigal<sup>1</sup>, F. R C L Almeida<sup>1</sup> <sup>1</sup>Department of Morphology, Federal University of Minas Gerais, Brazil <sup>2</sup>Research and development, DanBred, Brazil

# **Background and Objectives**

Low birthweight (LW) is related to high neonatal mortality due to lower energy reserves. Besides, suboptimal luteal function, which is associated with low embryonic survival, may also negatively influence litter size, leading to economic losses to the production system. Our previous study showed a reduction in LW piglets after altrenogest (ALT) supplementation in primiparous sows during late lactation. However, the reasons behind the improvement in birthweight following ALT treatment are limited. In this context, a thorough understanding of the female genital tract morphology becomes essential to elucidate those effects. The aim of the present study was to evaluate the effects of ALT supplementation in late lactation in primiparous sows on the genital tract morphology.

# Material and Methods

Ten primiparous hybrid sows (DB30, DanBred, Brazil) were randomly allocated to two treatments: females supplemented orally with 20 mg of ALT during the last 6 days of lactation, ending 24 hours before weaning (ALT;n=5), and non-supplemented sows (CON;n=5). All sows were euthanized at the time of estrus detection (4 days after weaning) and ovaries, uterine tubes and uterus were collected for morphological evaluation (-macroscopic parameters: ovarian weight and follicle diameter, vaginal and uterine tubes lengths, uterine horn weight and lengths; -microscopic parameters: proportion of the endometrial components, height and width of the uterine tubes' folds, epithelial height, and width of the connective tissue of those folds). Data were analyzed as a randomized complete design, and treatment effects were evaluated by the Tukey-Kramer's test (SAS®).

### Results

No treatment effects were observed in the macroscopic parameters of the genital tract (P>0.05). However, the uterine tubes of ALT sows were longer than those of the control group (ALT:  $35.2\pm1.3^{a}$ , CON:  $30.2\pm1.3^{b}$ ; P<0.05). ALT supplementation did not affect the uterine tubes histomorphometrical parameters. However, higher proportion of endometrial glands (ALT:  $39.4\pm2.0^{a}$ , CON:  $30.8\pm2.0^{b}$ ; P<0.05) and less connective tissue (ALT:  $54.2\pm1.9^{b}$ , CON:  $63.8\pm1.9^{a}$ ; P<0.05) were observed.

## **Discussion and Conclusion**

ALT supplementation for six days during the last week of lactation stimulated an increase in the proportion of endometrial glands, which will provide adequate resources for further fetal development, thus decreasing the proportion of LW piglets.

**REP** - Reproduction

# ROUGHNESS ANALYSIS BY ATOMIC FORCE MICROSCOPY IN SPERMATOZOA STORED WITH BOAR SEMEN EXTENDER

L. Maglie 1, D.C. Angel 2, R. Bettini 1 1 Department of Food and Drug Science, University of Parma, Parma, Italy 2 Research & Development department Medinova Italy, University of Parma

# **Background and Objectives**

Atomic force microscopy (AFM) has become the only technique capable of obtaining real-time images of the surface of a living cell with nanometer-scale resolution. With this instrument, it is possible to examine defects in the sperm head and the acrosome, responsible for fertilization, and related to the lack of functional integrity of the cell. The objective of this work was to evaluate the morphological difference in surface roughness (nm), as a direct indicator of sperm surface damage caused by bacterial growth during storage, between spermatozoa stored in three different formulations of boar semen extender.

# Material and Methods

In this work, three formulations of boar semen extender for conservation were tested: a formulation with antibacterial substances alternative to antibiotics (compounded by antimicrobial protein and peptide) currently covered by patent project. A commercial formulation with antibiotics and a formulation free from antibiotics/antimicrobial substances. After semen collection of three adult boar, the semen sample was diluted to a final concentration of 40M cells/ml in the formulations and stored at 16-18°C. Analyses were performed on frist and fifth day of semen storage to evaluate the incidence of bacterial growth on the sperm surface. The analysis was carried out by intermittent contact atomic force microscopy (icafm mode). The roughness values (nm) were obtained with the gwiddion version 2.62 software. The statistical analysis was performed with rstudio v2023.09.01 to compare the differences between the groups.

# Results

The results showed no significant difference in roughness parameters (p>0.05) between the formulations on day one of storage. At day five of storage, the roughness of the ecological formulation was  $(8.0349 \pm 1.5031nm)$  showing a statistically significant difference (p<0.01) compared to the formulation containing antibiotics (12.0455 ± 2.8597nm), 0455 ± 2.8597nm) in the same way a significant difference (p<0.001) was observed between the ecological formulation and the formulation without antibiotics (14.1444 ± 1.0979nm).

# **Discussion and Conclusion**

In conclusion, the difference in surface roughness observed between the formulations shows that conservation within the ecological formulation allows cells with superior protection and less damage evident at the spermatic membrane level and is a valid alternative for conservation of spermatic cells in the use of antibiotics.

**REP** - Reproduction

# THE EFFECT OF THE USE OF DIFFERENT TYPES OF INSEMINATION PIPETTES ON REPRODUCTIVE PERFORMANCE DURING INTRAUTERINE INSEMINATION.

<u>M. Slanikova</u><sup>1</sup>, B. Rackova<sup>1</sup>, L. Czanderlova<sup>1</sup>, M. Zizlavsky<sup>1</sup> <sup>1</sup>Sevaron, Czech Republic

## **Background and Objectives**

In an effort to use the full potential of sows, we decided to test whether a different type of insemination pipette has an effect on reproductive performance. The farm capacity is approx. 2000 sows. Farrow to weaning system, with SPF status, using the intrauterine insemination method from 2022. During the experiment, no clinical signs of the disease affecting the reproductive system were recorded. The operation was performed by the same people, and the farm regularly monitors the levels of mycotoxins in the feed.

# Material and Methods

Following parameters were monitored: pregnancy rate and number of piglets born and number of piglets weaned. For comparison, we chose two types of pipettes. The pipettes were suitable for use in intrauterine insemination. For Pipette 1 sows were inseminated from 1.6.2023 until now. Farrowing interval monitored for this group is from 23.9.2023 to 30.10.2023. Pipette 1 is a foam catheter, features a rear adapter with a cap to prevent backflow. For the Pipette 2 sows were inseminated from 1.11.2022 – 20.5.2023. Farrowing interval monitored for this group is from 26.2.2023 to 20.5.2023.Pipette 2 is catheter with a foam head and an inserted diaphragm with a pleat in the tube. Pipett's were also tested simultaneously using Pipette 1 on a small group during the monitored period for Pipette 2.

### Results

Pipette 1 group had 429 farrowed sows. 15,5 liveborn piglets and 16,8 piglets in total. Number of weaned piglets was 14, and pregnancy rate was 95,65%. Pipette 2 group had 957 farrowed sows. 14,9 liveborn piglets and 16,2 piglets in total. Number of weaned piglets was 13,6, and pregnancy rate was 94,30%.

Results for Pipette 1 during simultaneously use: 39 sows farrowed, 15.9 liveborn, 17.4 piglets in total. This group is too small for a valid comparison.

# **Discussion and Conclusion**

After evaluating the results, we came to the conclusion that, when doing intrauterine insemination, the choice of pipettes can have an effect on reproductive parameters. 1.35% difference in pregnancy rate, 0.6 piglets liveborn, 0.6 piglets in total, 0.4 piglets weaned. Although we have tried to minimize external influences, certain influences during the process that could affect the reproduction system cannot be avoided.

**REP** - Reproduction

# IMPLEMENTATION OF 18-DAY BATCH MANAGEMENT SYSTEM PRACTICED IN A TIBETAN PIG BREEDING FARM

J. Dai <sup>1</sup>, J. Zhang <sup>1</sup>, <u>D. Sperling</u> <sup>2</sup> <sup>1</sup>Ceva Animal Health China <sup>2</sup>Ceva Animal Health France

## **Background and Objectives**

Tibetan pigs are characteristic by small body size and early sexual maturity. This study was conducted in Tibetan pig breeding farm, which implemented 18-day batch management system, and the study aimed to evaluate its impact on Tibetan breeding's reproductive performance.

# Material and Methods

The trial was carried out in a nucleus farm of Tibetan genetics in Sichuan province, China. The farm used continual production system, before implementing an18-day batch management system. Eight groups of Tibetan breeding animals were established (72 sows) with average piglet weaning age 24 DOL (Days of Life). Implementation of a batch farrowing system was based on manipulation of weaning time using altrenogest (Altresyn<sup>®</sup>, Ceva Sante Animale) together with early weaning to accommodate sows in specific groups. Incoming gilts were treated with altrenogest for 18 consecutive days. Standard estrus management was applied including light stimulation, flush feeding, and boar exposure. Implementation of batch farrowing and change of the continual system lasted from April to August 2022 (5 months).

## Results

A total of 627 animals were successfully introduced into the batch management system with overall estrus rate of 93.1 %defined as the percentage of sows presenting a first standing estrus within 10 days after end of the treatment or after weaning. During the transition period, the estrus rate of weaned sows under the altrenogest treatment was numerically higher (96.4%) compared to untreated sows (diff. 3.1%, p> 0.05). The average total litter size after implementation of batch management was 9.75, where 0.71 extra piglets were produced (P< 0.05). The average born alive piglet parameter increased from 8.19 to 9.1 (p< 0.01).

# **Discussion and Conclusion**

After the implementation of 18 days management system, an improvement of reproduction performance was recorded. This study demonstrated that altrenogest could be successfully applied to Tibetan breeding animals to establish batch management system which could adapt to a variable farm layout and maximize the utilization of production capacity. Altrenogest should be applied at similar time daily, if possible, individually by drenching (18 days at 20 mg/day). The shorter and lower dose regime may increase risk of follicular cyst development (Kraeling RR et al, 1981).

**REP** - Reproduction

# SERUM PROGESTERONE CONCENTRATION IN GILTS AT DIFFERENT PHASES OF REPRODUCTIVE CYCLE

M.G. Marques <sup>3</sup>, <u>M. Quirino</u><sup>1</sup>, P. Baldessar <sup>2</sup>, A.M.G. Ibelli <sup>3</sup>, M.C. Ledur <sup>3</sup>, M.A. Mores <sup>3</sup>, T. Lucia Jr. <sup>4</sup>, R.R. Ulguim <sup>5</sup>, B.G. Gasperin <sup>4</sup>, I. Bianchi <sup>1</sup> <sup>1</sup>Federal Institute of Santa Catarina - Araguari

<sup>2</sup>Pamplona Alimentos, BR <sup>3</sup>Embrapa Suínos e Aves, BR <sup>4</sup>Federal University of Pelotas, BR <sup>5</sup>Federal University of Rio Grande do Sul, BR

## **Background and Objectives**

The decision to cull swine females due to anestrus condition is not precise since errors during estrus detection management can routinely occur, especially for gilts. A relevant proportion of gilts may have been unnecessarily culled, which could be avoided by associating regular estrus detection management with progesterone (P4) levels assessment. However, reference values are still needed for better interpretation of P4 level profile in swine females, thus this study characterized the P4 levels profile in gilts at different phases of reproductive cycle.

## Material and Methods

A total of 101 gilts (Landrace × Large White) were submitted to the conventional estrus detection management and classified into 'prepubertal' (<140-d old and no estrus signs; prepubertal phase: n = 37); 'in estrus' (140–240 d-old with estrus signs; follicular phase: n = 9); 'not in estrus' (>140-d old and no record of estrus signs; n = 55). Blood samples were collected for anayzing serum P4 levels through chemiluminescence method and gilts classified as 'not in estrus' were culled. After post-mortem ovarian evaluation they were classified into gilts in the luteal phase (n = 45) or in anestrus (n = 10).

# Results

Serum P4 concentrations (mean  $\pm$  standard error of mean; min–max) in gilts at the luteal phase (27.5  $\pm$  1.7 ng/mL; 4.1– 51.2 ng/mL) were greater than levels at all the other stages of reproductive cycle (P < 0.01). The P4 levels were similar among prepubertal phase (0.62  $\pm$  0.08 ng/mL; 0.21–1.84 ng/mL), follicular phase (0.76  $\pm$  0.12 ng/mL; 0.30–1.40 ng/mL) and anestrus condition (0.54  $\pm$  0.13 ng/mL; 0.22–1.54 ng/mL; P > 0.05). Minimum P4 concentration observed in the luteal phase was six-, five-, and seven-fold greater the average levels at prepubertal phase; follicular phase, and anestrous condition, respectively.

# **Discussion and Conclusion**

These preliminary results are useful as reference values and confirm that P4 evaluation could be used to avoid unnecessary culling of gilts, allowing identifying cyclic animals not detected in estrus. Serial blood collection during the beginning and end of luteal phase should be performed to establish a threshold that distinguishes cyclic and anestrus gilts.

**REP** - Reproduction

# THE EFFICACY OF OPTIMIZE(TM) ON REPRODUCTIVE PERFORMANCE IN A SOW HERD

<u>P. Poolperm</u><sup>1</sup>, A. Ratanamongkolmas <sup>2</sup>, S. Talummuk <sup>2</sup>, W. Deeampai <sup>2</sup>, W. Lakkanasombat <sup>2</sup>, M. Purser <sup>3</sup>, R. Musser <sup>4</sup> <sup>1</sup>Professional Training and Consultant, Co.LTD., Thailand <sup>2</sup>Elanco (Thailand) Ltd., Bangkok, Thailand. <sup>3</sup>Elanco Animal Health Ireland Limited <sup>4</sup>Elanco Animal Health, Greenfield, IN, USA.

## Background and Objectives

Highly prolific sows are common in the pig industry. Embryonic loss caused by several factors; oxidative stress is one important factor. The quality of embryos and fetuses is crucial for the health and development of newborn piglets. It has been well documented that piglets with higher birth weights grow faster and exhibit better performance during the nursery and finisher periods. This study aimed to investigate the efficacy of Optimize<sup>™</sup>, contains of bentonite (SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> and MgO), on enhancing reproductive performance of a sow herd.

## **Material and Methods**

A 2,200-sow, wean-to-finish system farm was selected for this study. Sows were randomly assigned to either the control group (C; n=105) or the treatment group (T; n=110). Sows in the treatment group received Optimize<sup>™</sup> supplementation in their feed at the dosage of 2 Kg/ton of feed, starting at breeding and continuing through the end of lactation. Sow breeding performance data, including litter size, born alive litter size, piglets weaned per litter, and average daily litter weight gain (ADLWG), were collected and analyzed.

### Results

There were no significant differences in litter size ( $11.84\pm3.12$  versus  $12.15\pm2.78$ ; p=0.43) or the number of piglets weaned per litter ( $11.07\pm0.58$  versus  $10.53\pm0.67$ ; p=0.52) between the treatment and control groups. However, weaning weight and ADLWG were significantly higher in the treatment group compared to the control group ( $7.44\pm1.51$  kg versus  $6.98\pm1.55$  kg; p<0.001; and 2,886.13±706.26 gram/day versus 2,490.48±661.49 gram/day; p<0.001, respectively).

# **Discussion and Conclusion**

The results of this study demonstrate that Optimize<sup>™</sup> supplementation could significantly improve lactation performance in sows, as evidenced by increased weaning weight of piglets and ADLWG. Eventhough, the number of pig weaned per litter is not statistical difference. This improvement in lactation performance may be attributed to the positive effects of the supplement on milk production, might be due to reducing in oxidative stress during gestation and lactation periods.

# PARASITOLOGY AND PARASITIC DISEASES

## PAR-PP-01

PAR - Parasitology and Parasitic Diseases

# VALIDATION OF A NEW SAMPLING METHOD FOR A REAL-TIME PCR DETECTION OF CYSTOISOSPORA SUIS FROM ENVIRONMENTAL AND FECAL SAMPLES

<u>S. Bartelt</u>, S. Wesselmann <sup>1</sup>, L. Bechmann <sup>2</sup>, K. Kipschull <sup>2</sup>, N. Guerra <sup>3</sup>, H. Karembe <sup>3</sup>, D. Sperling <sup>3</sup>, V. Cvjetkovic <sup>4</sup> <sup>1</sup>Vetxperts GmbH, Carl-Benz-Str. 21, 48734 Reken, Germany <sup>2</sup>Bio-Diagnostix, Carl-Benz-Straße 21, 48734 Reken, Germany <sup>3</sup>Ceva Santé Animale. 10 Avenue de la Ballastière. 33500 Libourne - France

<sup>4</sup>Ceva Tiergesundheit GmbH, Kanzlerstraße 4, 40472 Düsseldorf - Germany

# Background and Objectives

There are several methods for the detection of Cystoisospora suis (C.suis). Recently, a new qPCR method has been developed for the detection of C. suis-genetic material in feces from suckling piglets. However, environmental sampling using sponges has proven to be more convenient and animal-friendly but still needs to be validated in comparison to classical fecal sampling.

# **Material and Methods**

As part of a larger study, fecal samples from 2–3-week-old suckling piglets originating from 10 C. suis positive piglet producing farms (10 farrowing crates per farm) were sampled. Per farrowing crate, the investigator collected five individual fecal samples (floor) of different consistencies which were later pooled. Parallely, a scouring sponge was then used to wipe all accessible walls of the farrowing crate, followed by the floor and feces. Later, the sponges were processed as follows: buffered peptone water (4 ml) was added to sponges, then they were squeezed to collect the dissolved fecal materials. After centrifugation the pellet was collected for DNA extraction. For the fecal samples previous steps were not necessary. The statistical analysis was performed using Graph Prism. The results of both sampling methods were compared using Mann-Whitney test and Blant-Altman analysis of agreement.

### Results

All farms were positive. On average, 67% (20%-100%) of farrowing crates were positive per farm with both sampling methods. Average Ct values of positive crates were slightly higher for sponge sampling (29.57  $\pm$  3.08) than for fecal sampling (28.67  $\pm$  3.15) and were not different (p= 0.207). The mean difference in Ct value obtained was equivalent to 100-1000 copies of investigated genetic material. A good agreement between two methods was observed with a mean bias of 0.5968 in the Blant-Altman agreement test.

## **Discussion and Conclusion**

Preliminary results obtained in this pilot study have shown that there are no significant differences between the two sampling methods. However, sponge sampling presents significant advantages for the investigator and/or most importantly field vet: sponges are easier to use, less stressful for the animals, which is particularly important when working with more aggressive sows, and they are more efficient in collecting semi-liquid and liquid fecal materials. This new alternative sampling strategy may improve the diagnosis of cystoisosporosis under the field conditions.

PAR-PP-02

PAR - Parasitology and Parasitic Diseases

# EFFICACY OF FLUDOSOL® 200 MG/ML IN DRINKING WATER AGAINST MIGRATORY AND INTESTINAL LARVAL STAGES OF ASCARIS SUUM

<u>S. De Jong</u><sup>1</sup>, B. Engelen <sup>1</sup>, K. Ceyssens <sup>1</sup>, T. Vugts <sup>1</sup> <sup>1</sup>Dopharma Research, Raamsdonksveer, The Netherlands

## **Background and Objectives**

Ascaris suum is economically the most important helminth in growing-fattening pigs and a zoonotic agent. Migration of A. suum causes rejected livers at slaughter and results in higher susceptibility to respiratory pathogens. Additionally, a higher feed conversion ratio and reduced growth can be seen in farms with a high prevalence. The aim of this study was to evaluate the efficacy of flubendazole in drinking water against larval stages of A. suum in an induced infection model.

## **Material and Methods**

The study was designed as a randomized, controlled trial with four groups of 10 weaned, helminth naïve piglets (13.5-14.0 kg), never treated with anthelmintics before. Each piglet was inoculated with 1,500 embryonated eggs of A. suum (D0). To study the efficacy against migratory L3 larvae, two treatment groups were evaluated: 2.5 mg flubendazole/kg BW/day during 2 consecutive days (D6-7) and 1 mg flubendazole/kg BW/day during 5 consecutive days (D4-8). For evaluation of efficacy against intestinal larvae, a third group received 2.5 mg flubendazole/kg BW/day for 2 days (D11-12). The fourth (negative control) group did not receive any treatment. Animals were necropsied, the small intestine was opened and content was passed through a sieve (212  $\mu$ m mesh width) to collect and enumerate the intestinal larvae (D14). Efficacy was determined by reduction of intestinal stages in comparison to the control group. The geometric mean (GM) was used to prove efficacy.

### Results

Larval stages of A. suum were found in all 10 animals of the control group (GM: 325 larvae/animal). Flubendazole given at a dose of 2.5 mg/kg of bodyweight per day for 2 days was 99.95% (GM) efficacious in reducing both the number of migrating L3 and intestinal larvae (p<0.0001). The dosage of 1 mg/kg bodyweight per day for 5 days was 93.97% (GM) efficacious in reducing migrating L3 larvae (p<0.0001).

# **Discussion and Conclusion**

Fludosol® 200 mg/ml, given at a dose of 2.5 mg flubendazole/kg BW during 2 consecutive days, is very efficacious in reducing the number of migratory and intestinal A. suum larvae. The total dose of 5 mg flubendazole divided over 5 days was slightly less efficacious and is therefore not preferred as treatment schedule.

PAR-PP-03

PAR - Parasitology and Parasitic Diseases

# DETECTION OF CYSTOISOSPORA SUIS ON BELGIAN FARMS SUSPECTED OF COCCIDIOSIS FROM 2021 TO 2023, USING A REAL-TIME PCR.

<u>P. De Backer</u><sup>1</sup>, E. De Jonghe<sup>1</sup> <sup>1</sup>Ceva Santé Animale, Brussels, Belgium

### **Background and Objectives**

Porcine coccidiosis caused by Cystoisospora suis (C.suis) is one of the most important causes of diarrhea and poor growth in young piglets worldwide. A real-time PCR is a sensitive tool to detect C.suis in fecal samples or environmental fecal samples using a sponge. The environmental sampling protocol with sponges has the advantage that it is easy to perform and animal friendly. The aim of this study is to investigate the occurrence of C.suis on Belgian farms using a real-time PCR.

# **Material and Methods**

As part of Ceva Santé Animale's ongoing diagnostic service, veterinarians could send fecal samples or environmental fecal samples collected with a sponge, from piglets suspected of coccidiosis, to the lab of Diergezondheidszorg Vlaanderen (Belgium). From September 2021 until September 2023 248 samples of 66 farms were investigated, 156 fecal samples and 92 environmental fecal samples. The samples were analysed by a real-time PCR assay for rapid detection and quantification of C.suis. The positive PCR results are divided in 4 categories according to their Ct-value. The categories for Ct-values between  $\geq$ 38 and  $\leq$ 40,  $\geq$ 35 and <38,  $\geq$ 30 and <35 and <30 are respectively categorized as very weak positive, weak positive, moderate positive and strong positive.

### Results

From the 248 examined samples 108 were positive (43,6%). The percentages of the four categories of the positive PCR results very weak positive, weak positive, moderate positive and strong positive are respectively 4%, 17%, 44% and 35%. In total 43/66 (65%) farms were positive for C.suis in at least one sample. The percentage of the positive farms classified as very weak positive, weak positive, moderate positive and strong positive according to the most positive sample found on that farm are respectively 2%, 9%, 37% and 51%.

# **Discussion and Conclusion**

C.suis was detected on 65% of the farms. These results are in line with previous Belgian data that showed a 72,7% herd prevalence. 51% of the farms had at least one sample within the category strong positive. This demonstrates that C.suis is still an important pathogen in Belgium. The PCR is a valuable, sensitive tool to detect this important pathogen of piglets.

## PAR-PP-04

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# AN OUTBREAK OF MUCOHEMORRHAGIC DIARRHEA IN AN ORGANIC FATTENING FARM IN NORTHERN ITALY: THE CONTRIBUTION OF HYSTOPATHOLOGY TO THE DIAGNOSIS

<u>G. De Lorenzi</u><sup>1</sup>, R. Zuliani<sup>2</sup>, S. Bariselli<sup>1</sup>, G. Pupillo<sup>1</sup>, C. Torreggiani<sup>1</sup>, L. Franchi<sup>3</sup>, A. Luppi<sup>1</sup> <sup>1</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy <sup>2</sup>Università di Parma, Italy <sup>3</sup>Progeo - Italy

## **Background and Objectives**

An outbreak of mucohemorrhagic diarrhea occurred in an organic fattening farm (2000 pigs) in August 2023 in Northern Italy. Pigs involved were around 40 kg BW, morbidity was 4% and lethality was not significant. Two dead pigs with a history of diarrhea and hematochezia were submitted to Reggio Emilia IZSLER laboratory for diagnostic investigations.

# **Material and Methods**

Necropsies were performed following standardized procedures. Bacteriology was performed on kidney, spleen, small intestine and colon content using different bacterial media under aerobic and anaerobic conditions. PCR for L. intracellularis was performed on ileum content and fecal flotation on colon content. Tissue samples collected for histopathology were fixed in 10% formalin and 5 µm sections obtained were stained with haematoxylin and eosin.

## Results

At necropsy, enterotyphlocolitis involving the ileum, cecum, and colon was observed. PCR for L. intracellularis, bacteriology for Brachyspira spp. and fecal flotation were negative. Salmonella Derby was isolated from the intestinal content of one pig. Histopathological examination of both pigs showed fibrino-necrotic enteritis, suppurative colitis and trichyuriasis with numerous larvae of Trichuris spp. in the colon mucosa and infiltration of lymphoplasmacytic elements and eosinophils into the lamina propria.

## **Discussion and Conclusion**

This case report describes an outbreak of mucohemorrhagic diarrhea in an organic fattening farm. Histopathology demonstrated Trichuris spp. larvae infestation and typhlocolitis in both pigs, despite fecal flotation resulted negative. The presence of adult worms can be confirmed detecting the eggs in fecal flotations, but false negatives may occur due to sporadic egg shedding by females and when the infection is in the prepatent phase (oviposition begins 6–7 weeks post infection). Before their arrival in the fattening unit, pigs were treated twice with fenbendazole, which is effective against adults but not effective against larvae. Histopathology revealed fibrino-necrotic enteritis due to S. Derby in one out of two pigs. Trichuris spp. may provide an entry point for pathogens and can act synergistically with other pathogens. The most common approach in the diagnosis of trichuriasis is fecal flotation, while complementary analyzes are usually not performed. However fecal flotation hasn't always diagnostic value. When applicable, histopathology represents an effective tool for the diagnosis of trichuriasis in pigs.
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## EFFICACY OF FLUDOSOL® 200 MG/ML IN DRINKING WATER AGAINST ADULT STAGES OF ASCARIS SUUM

<u>S. De Jong</u><sup>1</sup>, B. Engelen <sup>1</sup>, K. Ceyssens <sup>1</sup>, T. Vugts <sup>1</sup> <sup>1</sup>Dopharma Research, Raamsdonksveer, The Netherlands

#### **Background and Objectives**

Ascaris suum is economically the most important helminth in growing-fattening pigs and a zoonotic agent. The aim of anthelmintic treatment of A. suum is reducing lesions, improving growth and reducing the feed conversion ratio. Because adult Ascaris females are highly productive egg-shedders and eggs are very resilient in the environment, an additional important goal of treatment is the reduction of infection pressure at the farm. The aim of this study was to evaluate the efficacy of a flubendazole in drinking water in an induced infection model. Adult worm count and eggs per gram faeces (EPG) were used to assess the efficacy of flubendazole. The study was done according to GCP, VICH GL9 and VICH GL16.

# Material and Methods

The randomized, controlled trial included three groups of 7 weaned, helminth and anthelmintic naïve piglets (8-9 weeks, 9-20 kg). Each piglet was inoculated with 1,500 embryonated eggs of A. suum divided over 3 days (D0-2). On days 49, 51 and 53 before treatment, EPG (mean EPG in log<sub>10</sub> of 3.09 (95% CI: 2.85 - 3.33)) was determined to randomly allocate the animals into three study groups (D53). Pigs were treated with 2,5 mg flubendazole/kg bodyweight/day during 2 days (D56-57) or 1 mg flubendazole/kg bodyweight/day during 5 days (D56-60), administered through the drinking water. The control group was experimentally infected and remained untreated. Prior to treatment (D49, 51 & 53) and prior to necropsy (D67), faecal samples were collected rectally and investigated for the presence of A. suum eggs, using the McMaster method. Animals were necropsied and helminths in the intestines were enumerated (D67). Infection was adequately established; mean adult worm count/animal in the controlgroup (D67) was 46 (95% CI: 30-62).

#### Results

Flubendazole given in a 5 mg total dose in the 2-day and 5-day treatment scheme both showed an efficacy of 100% (GM) in reducing adult worm counts (p=0.0006). Additionally, both treatments were respectively 100% and 99,99% (GM) efficacious in reducing the EPG (p=0.0006).

#### **Discussion and Conclusion**

Fludosol® 200 mg/ml was very efficient in eliminating adult worms and reducing the EPG. Flubendazole is highly suitable for the treatment of A. suum infections in pigs and lowering the infection pressure in the environment.

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# COCCIDIOSIS: IS PERIPARTUM EXCRETION FROM SOWS OR ENVIRONMENTAL OOCYSTS THE MAIN SOURCE OF INFECTION OF PIGLETS?

<u>P. Sánchez-Giménez</u><sup>1</sup>, F. Murciano<sup>2</sup>, A. Cuellar-Flores<sup>2</sup>, A. Martínez-Moreno<sup>1</sup>, G. Ramis<sup>2</sup> <sup>1</sup>AGROPOR, Murcia, Spain <sup>2</sup>Dpto, Producción Animal., Facultad de Veterinaria., Universidad de Murcia, Spain

#### **Background and Objectives**

Coccidiosis caused by Cystoisospora suis is still the main parasitic disease occurring in intensively reared piglets. Although control programmes have been carried out in most farms, based on biosecurity, hygiene and prophylaxis by oral or parenteral toltrazuril, the parasite persists in the populations. It is speculated that sows may have a peak of excretion at the time of peripartum and this may be the main source of contamination. The aim of this work was to determine whether excretion of C. suis occurs in periparturient sows and its correlation with the appearance of the parasite in faeces in piglets.

### **Material and Methods**

The study was carried out on a farm of 2,000 sows in south-eastern Spain specialising in the sale of piglets at 6 kg (at weaning). Thirty-six sows were sampled from five days before farrowing until four days after farrowing. After farrowing, piglets were individually tagged and sampled on days 0, 5, 10, 15 and 20 post farrowing. Samples were placed in sterile containers and kept refrigerated until further analysis.From the faecal samples, DNA was isolated and a real-time PCR that discriminates the coccidian species based on melting temperature was performed.

### Results

There was a very low frequency of coccidial excretion in the sows, which was quantified as 6.1%, 6.9%, 4%, 2.9%, 6.3%, 0%, 0%, 0%, 0%, 3% and 0% for each one of the sampling days. None of the sows had excretion on two consecutive days. The mean Ct was between 32.9 and 37 cycles, with a minimum of 28.51 cycles. However, in piglets a frequency of 0%, 20%, 60% and 70% of piglets sampled was found, so that from day 5 onwards infestation occurs in many piglets. Seventy percent of the piglets had one or more consecutive positive samplings. All samples had a dissociation temperature corresponding to C. suis.

#### **Discussion and Conclusion**

Although it has been speculated that farrowing stress could produce a peak of coccidial excretion by sows, from which piglets would become infected, this phenomenon was not observed in this study. However, piglets do become infested in the farrowing pen, as evidenced by the presence of coccidia in 70% of piglets at weaning.

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# EVALUATION OF THE PREVALENCE OF CYSTOISOSPORA SUIS IN GERMAN PIGLET PRODUCING FARMS USING A NOVEL REAL-TIME PCR

<u>H. Lösing</u><sup>1</sup>, S. Wesselmann <sup>1</sup>, L. Bechmann <sup>2</sup>, K. Kipschull <sup>2</sup>, N. Guerra <sup>3</sup>, D. Sperling <sup>3</sup>, V. Cvjetkovic <sup>4</sup> <sup>7</sup>Vetxperts GmbH, Carl-Benz-Str. 21, 48734 Reken, Germany <sup>2</sup>Bio-Diagnostix, Carl-Benz-Straße 21, 48734 Reken, Germany <sup>3</sup>Ceva Santé Animale, 10 Avenue de la Ballastière, 33500 Libourne - France <sup>4</sup>Ceva Tiergesundheit GmbH, Kanzlerstraße 4, 40472 Düsseldorf - Germany

#### **Background and Objectives**

Cystoisospora suis (C. suis) can lead to diarrhoea and reduced weight gain in suckling piglets, especially together with Clostridium pefringens Type A. Last studies focusing on the occurrence of C. suis in German farms are outdated. In this respect, we want to give a more actual and accurate picture on the presence of C. suis in German farms using a novel, more sensitive method.

#### Material and Methods

Present work is an interim evaluation of a larger study, involving 30 (out of totally 60), randomly chosen piglet-producing farms all over Germany: 17 from the Northwestern (NW) part, eight from the East (E) and the remaining five from the Southern region (S). Per farm, suckling piglet feces from each farrowing pen (ten per farm) was processed as following: five fecal samples were collected from the floor and pooled into one collection tube, before being scored and analysed by a novel qPCR.

# Results

Out of all the 296 litters assessed, a total of 118 (39.9%) were positive for C. suis, which is approximately every 2.5 litter. Ct values in positive litters were 28.42 on average ( $\pm$ 3.53).Overall, 18 (60%) of all farms were tested positive with an average of 65.6% positive litters per farm. The highest in-herd prevalence was found in E (100%), followed by S (60%) and NW (41.2%).

#### **Discussion and Conclusion**

Compared to last results published in 2005, there was a trend towards lower prevalence for C. suis in German piglet producing farms. This might be attributed to numerous factors, such as higher awareness of the pathogen as well as better understanding of disinfection and metaphylactic measures. Upon completion of the study, we shall give a more detailed insight, by considering most important factors which might have an effect on the presence and/or absence of C. suis.

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# SARCOPTIC MANGE DIAGNOSIS IN BREEDING PIGS: CLINICAL EVALUATION GRID AND TEST OF TWO DETECTION TOOLS

F. Launay <sup>1</sup>, P. Duval <sup>2</sup>, M. Couteau <sup>2</sup>, J. Fernand-Genty <sup>3</sup>, B. Fily <sup>3</sup>, <u>S. Vigneron <sup>3</sup></u>, V. Burlot <sup>3</sup> <sup>1</sup> Chêne Vert – Conseil Vétérinaire, 4 Rue Théodore Botrel, 22600 Loudéac, France <sup>2</sup>Chêne Vert – Conseil Vétérinaire, 2 rue Pierre Harel, 35133 Lécousse, France <sup>3</sup>Elanco France SAS – Crisco Uno, Bâtiment C, 3-5 avenue de la Cristallerie, CS 80022 - 92317 Sèvres CEDEX

#### **Background and Objectives**

Sarcoptes scabiei var. suis is responsible for pig sarcoptic mange. It leads to reduction of growth, feed efficiency and fertility leading to economic loss and could also be involved in tail biting. In recent years, some severe cases of scabies in breeding animals have been detected with a good response to treatment. However, few tools are used to regularly monitor the livestock status. The objectives of this study were to develop an updated clinical assessment grid for scabies, to test it on a heavily infested farm and to test two diagnostic tools: serology and scrapings of the inner surface of the ear.

#### **Material and Methods**

A clinical assessment grid was developed based on clinical signs of chronic scabies: hyperkeratosis and earwax presence. The rating scale was determined by mutual agreement among veterinary practitioners. The grid was tested in an infested farrow-to-finish farm of 100 sows. 26 sows were scored and blood sampled to measure hemoglobinemia and perform serological analysis (Sarcoptes-ELISA-2001-Pig kit). Backfat thickness measurements were performed too. For ear scrapings, 12 sows were collected. Mann-Whitney test was used.

#### Results

The results of the clinical assessment grid showed that gilts were less affected by hyperkeratosis than multiparous sows. Comparison of sows with hyperkeratosis score 2 and 3 showed that sows with less hyperkeratosis also had lower earwax score, as well as greater backfat thickness (p<0,05). For the ear-scraping test, only one sow was positive and had high scores on the grid (score 3 for hyperkeratosis and earwax). For serology, only one sow was tested positive but was negative with ear-scraping test.

# **Discussion and Conclusion**

The clinical assessment grid developed would be useful to monitor, raise awareness and validate prevention around sarcoptic mange. A link between hyperkeratosis and backfat thickness scores has been observed and is consistent with the literature, more data would be required to confirm it. For the detection tools, results were disappointing. Ear-scraping is 100% specific but has low individual sensitivity. Serology, due to these same characteristics, seems to be reserved for monitoring herds free of scab. More data are needed to improve sarcoptic mange diagnosis on farm.

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# STABILITY OF FLUDOSOL®, A 20% FLUBENDAZOLE SUSPENSION FOR USE IN DRINKING WATER

<u>S. De Jong</u><sup>1</sup>, B. Engelen <sup>1</sup>, H. Jaadar <sup>1</sup> <sup>1</sup>Dopharma Research, Raamsdonksveer, The Netherlands

#### **Background and Objectives**

It is well-known that benzimidazoles are insoluble in water and widely used to treat A. suum. However, drinking water application is a very suitable way to administer the correct dosage to all to be treated animals, stressing the importance of a stable and homogeneous pre-solution. In addition, there is increasing societal pressure on the use of deworming products in the feed because of cross-contamination and increasing resistance against deworming agents. The aim of this study was to investigate the stability of the 20% flubendazole (FLB) with a patented technology for good dispersion in drinking water, based on a visual and homogeneity test.

# **Material and Methods**

The visual and homogeneity tests were performed four times during 24 hours (at 0h, 4h, 8h, 24h). The FLB suspension was prepared in standardized hard water, high pH (185 mg/L calcium carbonate, pH 8.3) and soft water, low pH (21 mg/L calcium carbonate, pH 6.0). For both standardized solutions, a concentration of 75 ml and 7,9 ml product/L was prepared and not stirred during 24 hours (Guideline EMEA/CVMP/540/03). Homogeneity in pre-solution was assessed based on the concentration of FLB in the upper and lower part of graduated glass cylinders of 1 liter, using ultra high performance liquid chromatography in mg FLB/ml solution. Results are shown in percentage of FLB compared to the 100% concentration of T=0h.

# Results

Visually all suspended solutions remained unclear and fully dispersed at all times. No sedimentation was seen. In the upper and lower part of the columns, 100-103% and 91-97% respectively of the FLB was found in both low and high concentration in soft water at T=24h. In hard water, the concentration FLB in the upper and lower part were respectively 98% and 101% for both the low and high FLB concentration.

# **Discussion and Conclusion**

The Fludosol® pre-solutions remained very stable and homogeneous during 24 hours. Thus, enabling the FLB suspension to be well distributed over all animals, resulting in correct dosing of every animal and improved animal welfare for no water restriction before treatment is needed. Because of a minor decrease in homogeneity in soft water, we recommend to stir the prepared pre-solution after 12 hours.

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# PREVALENCE OF ASCARIS SUUM AND OESOPHAGOSTOMUM SPP. IN UNDERPERFORMING DANISH SOW HERDS

J.B. Sanden <sup>1</sup>, H. Bundgaard <sup>1</sup>, <u>L. Kunstmann</u><sup>2</sup>, L. Claerhout <sup>3</sup>, L. Meedom <sup>2</sup> <sup>1</sup>Porcus <sup>2</sup>Huvepharma NV, Denmark <sup>3</sup>Huvepharma NV Antwerp. Belgium

### **Background and Objectives**

A prevalence study of Ascaris suum and Oesophagostomum spp. in Danish sow herds, that underperform compared to average levels, was performed within in a large Danish pig practice. Inclusion criteria for sampling were increased sow mortality, loss of appetite and too few weaned piglets per sow. Herds with PRRS, Influenza, obvious management mistakes and / or low feed quality were not sampled. Eleven in-door sow herds with unsatisfactory performance during lactation were selected for parasitological examination of faeces.

### **Material and Methods**

Samples were taken from 5 sows and 5 gilts per herd during the first 7 days after farrowing. The samples were sent for analysis at the veterinary laboratory at the pig research centre in Kjellerup, Denmark.

EPG counts were reported as:

Ascaris suum: none (0), low (<500), moderate (500-5000), high (>5000)

Strongylidea (Oesophagostomum spp.): none (0), low (<200), moderate (200-2000), high (>2000)

Egg shedding is described as intermittent for both Ascaris suum and Oesophagostomum spp.

#### Results

Herd prevalence:

Herd level Ascaris suum 72,4%: none 3, low 3, moderate 4, high 1.Herd level Strongylidea 72,4%: none 3, low 3, moderate 1, high 4. Summary statistics by Yates corrected Chi-square: Prevalence Ascaris suum in gilts 31%, Ascaris suum in sows 29%, Relative Risk = 1.02, Not significant. Prevalence Strongylidea in gilts 55%, Strongylidea in sows 44%, Relative Risk = 1.04, Not significant.

### **Discussion and Conclusion**

Our observations found a relative high prevalence of nematodes in poor performing sow herds. We did not find any difference in EPG between sows and gilts. We found Strongylidea (Oesophagostomum spp.) being just as likely to be diagnosed as Ascaris suum. The clinical impact of Strongylidea (Oesophagostomum spp.) in in-door housed sow herds, could be underestimated, and needs further studies.

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# OBSERVATIONAL STUDY OF CYSTOISOSPORA SUIS ON FARM POSITIVITY BY QPCR METHOD AND COMPASISON WITH STANDARD FLOTATION METHOD IN CHINA

Z.Y. Zhang <sup>1</sup>, J. Zhang <sup>1</sup>, F. Zhao <sup>2</sup>, D. Ye <sup>1</sup>, <u>D. Sperling</u> <sup>3</sup> <sup>1</sup>Ceva Animal Health China <sup>2</sup>Ceva SSIU China <sup>3</sup>Ceva Animal Health France

#### **Background and Objectives**

Cystoisospora suis is an important protozoan parasite causing varying degrees of diarrhea in young piglets, resulting in economic losses. Quantitative Polymerase Chain Reaction (qPCR) is one of the most sensitive diagnostic method previously described for detection of specific genes of C. suis. The aim of this study is to assess the C. suis farm positivity in industrial pig farms in China using a new qPCR method and compare the qPCR technique with the standard flotation.

### **Material and Methods**

261 litters from 23 industrial pig farms in different regions of China were sampled from 1<sup>st</sup> September 2022 to 30<sup>th</sup> August 2023. Faeces were collected from piglets of 7-21 days of age, with a minimum of 10 randomly selected litters sampled per farm. All the samples were divided in two aliquots and sent to Ceva SSIU Hangzhou China where a qPCR method was performed, based on a modified protocol (Hommonay et al, 2021) and to Fujian Agriculture and Forestry University for evaluation of oocysts presence by flotation technique.

#### Results

Using the qPCR method, 78% of pig farms (at least one litter) and 41% of samples were positive for presence of oocysts. 52.17% of farms (12/23) had more than 50% collected samples positive/farm. The farms located in southern area showed higher frequency of positive samples (55.00%) compared to northern area located farms (20.79%). Using the floatation method, 22% of pig farms and 3% of samples were positive for the presence of oocysts. All positive samples detected by floatation method were also positive by qPCR method.

#### **Discussion and Conclusion**

The farm and litter positivity for the C. suis in selected Chinese farms detected by qPCR were high and similar to figures recently described in EU countries. The qPCR assay would provide a more sensitive diagnostics method for parasite detection, especially for diagnosis of subclinical infection, where classical flotation technique may lead to false negative result.

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# PREVALENCE OF COCCIDIA IN SUCKLING PIGLETS IN CHINA IN 2022

F. Zang <sup>1</sup>, T. Guo <sup>1</sup>, T. Lei <sup>1</sup>, <u>T. Tan <sup>1</sup></u> <sup>1</sup>*Elanco China* 

# **Background and Objectives**

Piglets coccidiosis is a significant parasitic disease, causing diarrhea in suckling piglets worldwide, with Isospora suis being its causative agent. There are limited nationwide reports regarding the prevalence of this disease in China. Technical department from Elanco Animal Health employed the saturated urea flotation method to investigate the nationwide prevalence, aiming to provide foundational data for the formulation of prevention and control strategies against coccidiosis for Chinese pig farms.

#### **Material and Methods**

In 2022, the technical team of Elanco Animal Health visited 26 provinces across China, sampling 809 pig farms and collecting a total of 14,524 fecal samples. The saturated urea solution flotation method was employed for this investigation. As reported, this method effectively removes most fats and proteins from piglet feces, eliminating interference in microscopic examination and enhancing detection rates.

#### Results

The results indicate a high prevalence of Isospora suis in Chinese pig farms. We also analyzed the relationship between the farm's age, farm size, sampling season and positivity rates. Finally, we summarized the positivity among pig farms in different province. The positivity rate in pig farms for coccidiosis in 2022 was 70.3%, and different farm ages showed positivity rates ranging from 58% to 83%, with a general trend that the positivity rate increases when the farms have been used longer.

#### **Discussion and Conclusion**

The infection rate of coccidia was slightly higher in the summer and autumn compared to winter and spring, with Q1-Q4 positivity rates being 60.9%, 73.4%, 73.9%, and 63%, respectively. The age at which piglets in different farms became infected varied. Oocysts were detected as early as 7 days of age, peaking in oocysts release at two weeks of age, and could be detected throughout the sucking period, which is consistent with previous reports.

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# DETERMINATION OF TRANSMISSION OF AFRICAN SWINE FEVER VIRUS BY VARIOUS FLY SPECIES IN SWINE FARM IN PENINSULAR MALAYSIA

J.X. Lee <sup>1</sup>, P.T. Ooi <sup>2</sup>, M.W.C. Fong <sup>2</sup>, M.I. Nur Mahiza <sup>2</sup>, A.R. Yasmin <sup>2</sup>, S.E. Low <sup>2</sup>, S.X. Lau <sup>2</sup>, B.W. Sia <sup>3</sup>, D.W. Ang <sup>3</sup>, S.L. Soh <sup>3</sup>, E.H.C. Cheah <sup>1</sup>, K.Y. Kam <sup>1</sup>, <u>R.L.H. Yong <sup>1</sup></u> <sup>1</sup>Boehringer Ingelheim (Malaysia) Sdn. Bhd.

<sup>2</sup>Faculty of Veterinary Medicine, Universiti Putra Malaysia <sup>3</sup>Agritech Enterprise Sdn. Bhd, Malaysia

# **Background and Objectives**

African swine fever (ASF) is a devastating viral disease that causes significant economic losses in swine industry worldwide and even threaten the food security. A study from Mellor et al. revealed that stable fly (Stomoxys calcitrans) is a mechanical vector of ASFV transmission. Therefore, the objective of this study is to determine the transmission of ASFV by various fly species in swine farm in Peninsular Malaysia.

#### **Material and Methods**

This study was conducted in 4 swine farms with different ASF status. Upon sample collection, 1 farm was free from ASF whereas another 3 farms were post-ASF within 6 months. Flies from sewage pool, feed house, finisher pens and sow pens were collected using 4 sticky fly traps in each location over 24 hours. Then, fly species identification was performed under a stereomicroscope based on the variation in antennae, wing venation, body colour and stripes. 5 flies of the same genus from each location were selected and pooled by genus. Then, real-time PCR was conducted using IDEXX RealPCR\* ASFV DNA test with positive validity of Ct value <38.

#### Results

2254 flies were collected in this study and 3 species of fly were classified. 98% (2218 flies) were identified as Musca sp., 1% (22 flies) Chrysomya sp. and 1% (14 flies) Stomoxys sp. A total 25 pools were tested with qPCR and the results showed that all ASF positive flies were Musca sp (4/25 pools positive). Interestingly, Farm 1 was ASF free but Musca sp. pool from sewage pool was positive (Ct value 35.39). Furthermore, Musca sp. pool from Farm 2 sewage pool, Farm 3 feed house and Farm 4 sow pens were tested positive with Ct value 36.77, 36.73 and 34.29 respectively.

# **Discussion and Conclusion**

Positive detection of ASF in Musca sp. conclude that Musca sp. is a potential mechanical vector of ASFV. Insufficient Chrysomya sp. and Stomoxys sp. samples collected in this study could contributed to negative qPCR results. It also demonstrated that Musca sp. is the most abundant fly genus among 4 swine farms in Peninsular Malaysia. Therefore, an effective fly control strategy should be implemented to reduce the risk of ASF transmission in a farm.

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# THE FIRST DETECTION OF ANTI-NEOSPORA CANINUM ANTIBODIES IN THE POLISH PIG POPULATION

<u>A. Augustyniak</u><sup>2</sup>, A. Dors<sup>2</sup>, R. Niemyjski<sup>1</sup>, M. Pomorska-Mól<sup>2</sup> <sup>1</sup>Agri Plus Sp. z o.o., Poland <sup>2</sup>Department of Preclinical Sciences and Infectious Diseases, Poznan University of Life Sciences, Poland

#### **Background and Objectives**

Neosporosis is an infectious animal disease of worldwide occurrence caused by the protozoal parasite Neospora caninum. The clinical symptoms of invasion were observed mainly among dogs and cattle. For cattle, it is considered one of the most important causes of infertility, abortion, and neonatal mortality. The reproductive disorders were also observed in several other species, including sheep and goats. The published data indicate that N. caninum can cross the sows' placenta during each stage of gestation and that invasion can lead to some clinical symptoms in sows. However, the potential consequences of such infection in pigs remain unclear. Our study aimed to determine the seroprevalence of N. caninum in the Polish pig population.

#### **Material and Methods**

A total of 902 pigs sera were collected from 13 commercial farms located in different regions of Poland. The farms varied in size, production type, health, and hygiene status. Samples were obtained from random pigs of different age groups. Blood samples from 259 piglets, 200 weaners, 150 fatteners, 70 gilts, and 223 sows were collected. Samples were tested using the commercial ELISA test to detect anti-N. caninum antibodies following the manufacturer's instructions. Seroprevalence of N. caninum infection was calculated at the individual and farm levels including age categories. Subsequently, the overall seroprevalence and seroprevalence among particular groups were estimated via Epitools (https://epitools.ausvet.com.au/ciproportion).

#### Results

At the farm level, 7.7% (1/13; 95% CI 1.37-33.31) of the selected farms were positive. At the individual level, the overall prevalence was 0.11% (95%; CI 0.02-0.63), wherein the antibodies against N. caninum have only been found in one serum sample, which had been collected from gilt. The prevalence in the group of gilts was 1.43% (95% CI 0.25-7.76).

#### **Discussion and Conclusion**

The results of this study provide the first evidence of N. caninum antibodies in the Polish pig population. The obtained seroprevalence is, however, lower compared to results from other countries. Contrary to the previous studies, we did not detect antibodies among fatteners. Since N. caninum can infect pigs, further studies are necessary to elucidate the potential effect of N. caninum on reproduction in sows.

# MISCELLANEOUS AND CLINICAL CASES

MIS-PP-01

MIS – Miscellaneous and Clinical cases

# ECONOMIC IMPACT DUE TO NURSERY E. COLI BREAKS UNDER FIELD CONDITIONS

<u>E. Magalhaes</u><sup>2</sup>, K. Kuecker<sup>1</sup>, X. Portal<sup>1</sup>, K. Dion<sup>2</sup>, M. Almeida<sup>2</sup>, D. Rosero<sup>3</sup>, D. Holtkamp<sup>2</sup>, G. De Souza E Silva<sup>2</sup>, G. Trevisan<sup>2</sup>, D. Correia Lima Linhares<sup>2</sup>

<sup>1</sup>The Hanor Company

<sup>2</sup>lowa State University, Veterinary Diagnostic and Production Animal Medicine <sup>3</sup>lowa State University, Department of Animal Science

Background and Objectives

Escherichia coli infection increases productivity and economic losses in growing pigs (John M. Fairbrother, 2019). This study aimed to measure the impact of E. coli outbreaks in commercial groups of growing pigs from a large US swine production system based on the changes in productivity while measuring the economic opportunity lost due to worse performance.

#### **Material and Methods**

A retrospective study analyzed 582 nursery groups stocked from January 2022 to March 2023. The study linked diagnostics, management, and performance data from growing pigs to sow farm health & productivity of the originating weaned batches. Groups were categorized into "E. coli outbreaks" (n=51) and "No E. coli outbreak" (n=531) based on clinical signs and diagnostics. The outcomes studied were mortality and average daily weight gain (ADWG) in the nursery phase. Causal inference analysis was employed to measure the impact of E. coli on the outcomes while controlling for confounders (PRRS and PED outbreaks, weaning age, stocking weight, and other pathogens). Economic opportunity lost due to high mortality was estimated using the groups` performance, a market carcass price of U\$70/per 45kg of carcass weight, and a feed cost of U\$0.20/kg.

#### Results

The overall nursery mortality for all 582 groups was 4.0% and 1.04 lbs. for ADWG. The results of the causal inference analysis for the impact of E. coli breaks were as follows: increased nursery mortality from 3.09% to 5.36%, decreased nursery ADWG from 479 grams per day to 433, and increased mortality economic losses from U\$3.16/head to U\$5.47/head. The worsened overall growth performance in E. coli-affected nursery groups represented a lost economic opportunity of U\$1.88/head.

# **Discussion and Conclusion**

coli-associated disease is expected to decrease the performance of pigs through the growing phase. This study provides the field impact of E. coli challenges in nursery pig populations through a causal inference analysis, demonstrating that this pathogen continues to impact the swine industry significantly. The outbreaks of E. coli in this study population represented approximately \$115,000 for the 51 groups in this population if considering 1,200 pigs placed/group.

MIS – Miscellaneous and Clinical cases

#### ANOREXIA AND VAGINAL DISCHARGE IN A SIX-YEAR-OLD BREEDING SOW – A CASE REPORT

T.J. Nicolaisen<sup>1</sup>, F. Kiene<sup>1</sup>, J. Buchallik-Schregel<sup>1</sup>, S. Neubert<sup>1</sup>, A. Von Altrock<sup>1</sup>, I. Hennig-Pauka<sup>1</sup>

<sup>1</sup>Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Foundation, Bischofsholer Damm 15, 30173 Hannover, Germany

#### **Background and Objectives**

Breeding sows with anorexia are commonly presented to swine practitioners. Long-lasting anorexia regularly is a cause of culling for individual sows and reasons often remain unidentified. This case report is about a former breeding sow that was presented due to anorexia and vaginal discharge at the clinic for swine and small ruminants of the University for Veterinary Medicine Hannover.

#### Material and Methods

In January 2023, a six-year-old sow (274 kg) that showed oestrus associated vaginal discharge since March 2022 and recurrent anorexia for 1.5 months was presented at our clinic. The clinical case was worked up using the follwoing diagnostic tools: clinical examination, haematology and clinical chemistry, ultrasound, diagnostic laparotomy and pathological examination.

#### Results

A reduced body condition score of 1.5 and purulent vaginal fluid were detected during clinical examination. Haematological examination revealed a leucocytosis. Subsequent ultrasound visualized dilated uterine horns filled with hypoechoic fluid and multiple hyperechoic spots. An abdominal mass of unknown extent, which could not be assigned to a specific organ, was found. At the owner's request, a diagnostic surgical attempt was conducted. During surgery, a uterus highly filled with fluid, a uterus-associated tumour occupying large parts of the abdomen, multiple orange-sized liver tumours and several solid masses in the Omentum majus were found. The pig was euthanized due to the poor prognosis. A pathological examination was conducted in which a high-grade suppurative metritis, a uterine leiomyoma (50 cm in diameter), adenomas of the liver and fat tissue necrosis in the Omentum majus were found.

#### **Discussion and Conclusion**

In general, uterine neoplasms are rarely seen in pigs, but among these, leiomyomas are the most common tumours. Leiomyomas are described more often in miniature pet pigs and are considered a geriatric disease, whereas reports in farm pigs are rare. The authors are not aware of any case in farm pigs in which a porcine leiomyoma was described concurrent with suppurative metritis. Nevertheless, a connection between these two diseases is likely. In older breeding sows with anorexia and infertility, tumours of the reproductive tract should be considered as a rare but possible differential diagnosis.

MIS – Miscellaneous and Clinical cases

# IT IS POSSIBLE TO ESTIMATE PIGLET-LEVEL PRRSV PREVALENCE FROM ORAL FLUID SAMPLING IN FARROWING ROOMS

O.H. Osemeke<sup>1</sup>, E. De Freitas Costa<sup>2</sup>, V. Weide<sup>3</sup>, G. De Souza E Silva<sup>4</sup>, S. Jayaraman<sup>4</sup>, D. Correia Lima Linhares<sup>4</sup> <sup>1</sup>Iowa State University Veterinary Diagnostic & Production Animal Medicine

<sup>2</sup>Department of Epidemiology, Bioinformatics and Animal Models, Wageningen Bioveterinary Research, Lelystad, the Netherlands

<sup>3</sup>Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, Farroupilha, RS, Brazil ⁴Iowa State University, Veterinary Diagnostic and Production Animal Medicine

#### **Background and Objectives**

Family oral fluids (FOF) are fluids obtained from a cotton rope chewed by sows and their suckling litters. FOF has emerged as a method for detecting PRRSV RNA at the litter level, which differs from conventional samples that typically reveals PRRSV RNA at the piglet level. Understanding the relationship between PRRSV prevalence at the individual piglet level and the litter level within farrowing rooms remains underexplored. This study sought to characterize the interrelation between the proportion of viremic piglets (PPV), True Litter Prevalence or proportion of litters with  $\ge$  1 viremic pig (TLP), and Apparent Litter Prevalence estimated by FOF positivity (ALP).

#### **Material and Methods**

A predictive model was built using data from a previous study to understand how the proportion of viremic piglets within a litter relates to the likelihood of obtaining a positive FOF from that litter. The heterogeneity of PRRSV-positive pigs within sampled rooms from the referenced study was assessed and scaled, with a median value of 0.61 set as a baseline. Simulations were conducted to mimic farrowing rooms, each containing a fixed number of litters. These simulations varied the PPV across a range from 0 to 50%. The number of piglets within each litter was drawn from an empirical distribution obtained from the litter sizes observed in the sampled rooms. The number of viremic piglets in each litter was determined using a recursive binomial model, factoring in a clustering parameter spanning from 0 (random distribution of PRRSV-positive pigs across litters) to 1 (signifying a maximum clustering of PRRSV-positive pigs within a minimal number of litters).<sup>1</sup>

#### Results

There was a consistent linear relationship between piglet-level and litter-level prevalence. When PPV values were 1%, 5%, 10%, 20%, and 50%, TLP values were 5.36%, 8.93%, 14.29%, 23.21%, and 53.57%, respectively. While ALP values were 2.06%, 6.48%, 11.25%, 21.60%, and 51.56%,

### **Discussion and Conclusion**

This study matches the different prevalence metrics and serves as a practical guide for determining sample sizes. Furthermore, it presents a structured framework to estimate the proportion of viremic pigs within a farrowing room based on the PRRSV-RT-qPCR positivity rate obtained from FOF samples.

MIS – Miscellaneous and Clinical cases

# VALIDATION OF A NEW ASSAY FOR THE MEASUREMENT OF CRP IN THE SALIVA OF PIGS

<u>A. Ortin Bustillo</u><sup>1</sup>, E. Llamas <sup>1</sup>, M.J. López Martínez <sup>1</sup>, S. Martínez Subiela <sup>1</sup>, A. Tvarijonaviciute <sup>1</sup>, L. Franco Martínez <sup>2</sup>, J.J. Cerón Madrigal <sup>1</sup>, A. Muñoz Prieto <sup>1</sup>

<sup>1</sup>Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence "Campus Mare Nostrum", University of 9 Murcia, 30100, Espinardo, Murcia, Spain

<sup>2</sup>1Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence "Campus Mare Nostrum", University of 9 Murcia, 30100, Espinardo, Murcia, Spain 2 Moorepark Animal and Grassland Research Center, Teagasc, Irish A

#### **Background and Objectives**

C-reactive protein (CRP) is a protein related to inflammatory conditions, and in many animal species, such as pigs, is classified as a major acute phase protein. Therefore, CRP is a sensitive biomarker of inflammation widely used in the laboratory to diagnose and monitor inflammatory processes. The main objective of this work was to develop a reliable high sensitive method to measure CRP in porcine saliva to increase the use of saliva as a biological sample to evaluate the health status of this species.

# **Material and Methods**

A direct sandwich assay using AlphaLISA technology was developed. The reproducibility, accuracy, and sensitivity of the assay were evaluated by the coefficient of variation of intra- and inter-assay (CV%), the linearity under dilution (LUD) and recovery, and the low limit of quantification (LLOQ), respectively. In addition, the ability of the assay to detect inflammation was evaluated in a pilot study with five pigs having acute inflammatory conditions (D) and five healthy pigs (H).

#### Results

The average imprecision of intra- and inter-assay for assay were  $3.96\pm2.17\%$  and  $11.03\pm1.39\%$ , lower than the maximum acceptable for immunoassay methods (CV <10-15\%, respectively). LUD tests presented a linear regression equation with a determination coefficient close to 1 (R<sup>2</sup>>0.99). The average recovery percentage was 100.34\pm8.15\% (inside the acceptable clinical range of 80-120%), and the LLOQ was  $0.86 \mu g/L$ . Pigs from group D showed significantly higher levels of CRP in saliva compared to H group (P-value = 0.0079; median 33.53 and 3.43  $\mu g/L$ , respectively).

#### **Discussion and Conclusion**

CRP can be measured in the saliva of pigs by alphaLISA technology with adequate precision, accuracy and sensitivity, and it could improve the use of the saliva as a non-invasive sample to assess health state of swine.

MIS – Miscellaneous and Clinical cases

# WHEY PROVISION THROUGH THE WATER SYSTEM AS CAUSE OF SALT POISONING

<u>S. Engel</u><sup>1</sup>, C. Sanderman <sup>1</sup>, T. Tobias <sup>1</sup>, S. Klarenbeek <sup>1</sup> <sup>7</sup>Royal GD, Deventer, the Netherlands

#### **Background and Objectives**

Several nine-week-old fattening pigs were sent in for necropsy, with the anamnesis describing neurological symptoms. The newly introduced animals from three sections exhibited the following symptoms while alive: trembling, reduced appetite and recumbency. The differential diagnosis for these signs points to infectious pathogens such as streptococci and E. coli. Pathological examination could not confirm the proposed infectious causes, but instead, a non-infectious cause was identified as the cause of death after histological examination. The case aims to raise awareness of non-infectious causes of clinical signs of the nervous system.

# **Material and Methods**

Macroscopic post-mortem examination was performed followed by microscopy (HE staining). PCR testing was performed for the following pathogens: Mycoplasma Hyopneumoniae, Mycoplasma hyorhinis, PCV2, Influenza virus and PRRSv.

#### Results

The pigs were diagnosed with eosinophilic meningoencephalitis, associated with salt poisoning or obstructed water intake. The meningoencephalitis was characterized by an increased presence of eosinophilic granulocytes in the brain, concurrent with neuronal necrosis in the cerebral cortex. The results were considered pathognomonic for salt intoxication. Signs of salt intoxication in pigs include cessation of eating (but with persistent thirst) and restless behavior. Animals may appear deaf and blind, and various other nervous signs may occur, similar to those in bacterial meningitis. Typical signs include tremors of the snout initially, spreading to clonic spasms of the neck, opisthotonus, walking backward, sitting, lying on the side, and generalized clonic spasms.

#### **Discussion and Conclusion**

In this specific case, a high supplementation of whey in combination with a limited amount of water was found to be the cause of the excessive intake of sodium. Following a decrease in whey supplementation and giving ad libitum access to water, the problems were not observed anymore. Rebalancing the pigs' fluid levels should be done slowly. Water intake should initially be limited and distributed over several hours until unrestricted intake is possible again. The case emphasizes the importance of careful dietary management and monitoring of water supply systems to prevent salt poisoning or water shortage-related issues in pig farming.

MIS – Miscellaneous and Clinical cases

# OUTBREAK OF ARTHRITIS IN PIGS WITH INVOLVEMENT OF MYCOPLASMA HYORHINIS AND MYCOPLASMA HYOSYNOVIAE

<u>M. Merenda</u><sup>1</sup>, M. Gastaldelli<sup>1</sup>, A. Tondo<sup>1</sup>, G. Foiani<sup>2</sup>, C. Tonelli<sup>3</sup>, P. Mondin<sup>4</sup>, D. Prataviera<sup>1</sup>, V. Righetti<sup>1</sup>, R. Bardini<sup>5</sup>, G. Nai<sup>1</sup>, D. Vio<sup>6</sup>

<sup>1</sup>Mycoplasma Unit, SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Buttapietra (VR), Italy <sup>2</sup>Laboratorio di Istopatologia, SCS3, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy <sup>3</sup>Private Practice Veterinarian <sup>4</sup>Huvepharma NV, Antwerp, Belgium <sup>5</sup>Trouwrittion Mozzoopo (VR), Italy

<sup>5</sup>Trownutrition, Mozzecane (VR), Italy

<sup>e</sup>Laboratorio di Patologia e Benessere della Specie Suina, SCT4, Istituto Zooprofilattico Sperimentale delle Venezie, Cordenons (PN), Italy

# **Background and Objectives**

Porcine infectious arthritis is caused by different bacteria, among which Mycoplasma hyorhinis (MHR) and M. hyosynoviae (MHS) play an important role. Both these species are commensals of the higher respiratory tract, but they can also be associated to arthritis in post-weaning and growing pigs. Here is reported a clinical case of lameness and arthritis in a farrow-to finish pig farm.

#### **Material and Methods**

Five carcasses and 9 joints were conferred from an antibiotic-free farm located in Northern Italy, for diagnostic investigations. Bacteriological and histopathological examinations were performed, as well as mycoplasma isolation. In addition molecular tests targeting viruses and bacteria commonly found in commercial pig farms were carried out. Minimal Inhibitory Concentration (MIC) of Florfenicol, Lyncomicin, Spectynomicin, Erythromycin, Tylosin, Tilmicosin, Spiramycin, Tiamulin, Enrofloxacin and Oxytetracyclin was determined for MHR and MHS isolates.

#### Results

MHR and MHS were isolated from 4 and 3 synovial fluids, respectively. MHR isolates were also obtained from 2 pericardial swabs in pigs with pericarditis and a MHR-MHS coinfection was found in a joint. No other bacteria were isolated from joints. Glaesserella parasuis was excluded via PCR. Histopathological examination of MHR/MHS-positive joints revealed a severe chronic fibrinous arthritis, with abundant lymphoplasmacytic perivascular infiltrates, erosive and suppurative foci. Both MHR and MHS displayed low MIC values with the exception of Enrofloxacin and Erythromycin. In addition, MHS exhibited a high Oxytetracyclin MIC value. Therefore, sows were treated with tiamulin before and after farrowing with good clinical results. Finally, an autogenous vaccine was deviced and its efficacy is at the moment under evaluation.

#### **Discussion and Conclusion**

According to these findings, MHR and MHS should always be considered in the differential diagnosis of pig arthritis. Even though much effort is required for obtaining viable mycoplasma strains, these are fundamental to study their antimicrobial susceptibility, epidemiology and pathogenicity. In addition, viable strains can be used as autogenous vaccines, an alternative to antimicrobial treatments for the control of infectious diseases, as recommanded by the "One Health" vision.

MIS – Miscellaneous and Clinical cases

# THE EFFECT OF IMPROVAC® VACCINATION AGAINST GONADOTROPHIN-RELEASING FACTOR ON BODY WEIGHT GAIN AND CARCASS QUALITY OF FEMALE FATTENING PIGS

R.O. De Castro<sup>1</sup>, S. Udompoka<sup>2</sup>, S. Kaewbubpa<sup>3</sup> <sup>1</sup>Charoen Pokphand Foods Phils. Corp. <sup>2</sup>Feed Technology Office, CPF Philippines <sup>3</sup>Animal Health and Technical Services Office, CPF Philippines

#### **Background and Objectives**

Improvac® was initially developed as an immunological alternative to physical castration of male pigs. More recently, studies on female fattening pigs showed that it also allows more efficient productivity. The induced suppression of estrus during late finishing phase can avoid the associated reduction in feed intake by maturing gilts. This study was conducted to observe if the immunization of synthetic GnRH would have effect on weight gain and carcass quality of female fattening pigs via the suppression of ovarian activity.

#### **Material and Methods**

A total of female pigs (n = 178) were housed in a fattening building divided into two groups by body size: large (88 heads) and small (90 heads). Pens were assigned in randomly alternate fashion between vaccinated pigs (Tx, n = 89) and unvaccinated control pigs (Cx, n = 89). Each pig was tagged and weighed the same day at 11 weeks of age (Average: Tx = 24.50 kg; Cx = 24.52 kg). Pigs in Tx pens were vaccinated subcutaneously with 2 ml per dose, on the same day at 11 and 17 weeks of age respectively. All pigs in the study were fed ad libitum. Final individual weighing was done during market day at 24 weeks of age for both groups. (Average: Tx = 100.00 kg; Cx = 97.03 kg). Carcass quality assessment was randomly sampled for each group (n = 10), based on the Lenden-Speck Quotient sccre calculation.

#### Results

Body weight gain of vaccinated pigs averaged higher by 2.99 kgs. (P = 0.02) versus the unvaccinated control group. Average total feed intake of Tx group was also higher by 1.51 kgs. (P = 0.6). LSQ score of Tx was at 0.176 while Cx was at 0.200 (P = 0.2). It was also noted that the vaccinated group had 0% ovarian follicular growth (9-25mm length), while 100% of the control group had ovarian follicles (25-32mm length).

#### **Discussion and Conclusion**

The suppression of ovarian activity led to a significant advantage in body weight gain and carcass quality. This advantage led to a USD 6.46 more profit / head after considering the feed and vaccination cost, and USD 3.56 / kg liveweight price during the study.

MIS – Miscellaneous and Clinical cases

# THE EFFECTS OF DOSE TIMING / INTERVALS ON GROW-FINISH PERFORMANCE, CARCASS CHARACTERISTICS, CARCASS CUTTING YIELDS, AND MEAT QUALITY OF MARKET GILTS IMMUNIZED AGAINST GNRF

L. Van De Weyer <sup>1</sup>, B. Bohrer <sup>2</sup>, B. Hansen <sup>3</sup>, S. Pollmann <sup>4</sup>, A. Aldaz <sup>5</sup> <sup>1</sup>Zoetis Canada Inc, Kirkland, QC <sup>2</sup>Dept of Animal Sci, The Ohio State University, Columbus, Ohio <sup>3</sup>BCH Consulting LLC, Atlantic, Iowa <sup>4</sup>DSP Consulting LLC, Alpine, Utah <sup>5</sup>Zoetis Inc, Parsippany, NJ, USA

# **Background and Objectives**

Improvest-immunization (IMP) provides temporary suppression of ovarian function and estrus in market gilts. The product requires two injections administered at least 4 weeks apart, with full suppression of ovarian function demonstrated 4-10 weeks after second injection. Recent research has shown the importance of monitoring the time-interval between the injections and between the second injection and harvest. The objective was to determine the effects of dose-timing on grow-finish performance, carcass-cutting-yields, and meat quality of IMP-market gilts when days on feed remained constant.

#### Material and Methods

The study consisted of 1,056 market gilts (average starting weight of 31.7 kg) in 48 pens (22 pigs/pen) with experimental treatments arranged as a 2 × 2 factorial design with main effects of interval between the first and second IMP (**D1-D2**; 4-week interval or 6-week interval) and time between the second IMP and slaughter (**D2-slaughter**; weighted average of 34-35 days post-second injection [**SHORT**] or weighted average of 48-49 days post-second injection [**LONG**]). Following slaughter, 288 carcasses (24/treatment for each marketing event; pigs near the population average) were selected for evaluation of carcass cutting yields and meat quality. Data were analyzed with PROC MIXED of SAS, with pen serving as the experimental unit.

#### Results

There were limited interactions between  $D_1$ - $D_2$  and  $D_2$ -to-slaughter, with nonsignificant interactions (P  $\ge 0.07$ ) for average daily feed intake (ADFI), average daily gain (ADG), and feed:gain (F:G) ratio during the grow-finish period, hot carcass weight, backfat thickness, or carcass primal weights. Limited differences existed for the main effect of  $D_1$ - $D_2$ . There were significant differences (P < 0.01) for  $D_2$ -slaughter for ADFI, F:G ratio, fat quantity, and fat quality. SHORT had lower ADFI (2.47 kg versus 2.53 kg) and more efficient F:G (2.69 versus 2.74) compared with LONG, while LONG had 0.78 mm greater backfat thickness, 0.15 kg heavier trimmed bellies, and 1.30 units lower iodine value.

#### **Discussion and Conclusion**

Overall, this study illustrated the trade-offs associated with altering the duration of time between  $D_2$ -slaughter. Advantages in feed efficiency exist when the  $D_2$ -slaughter is shortened while advantages in fat quantity and quality exist when the  $D_2$ -slaughter period is lengthened.

MIS – Miscellaneous and Clinical cases

# UTILIZING AN INTENSIVE SURVEILLANCE PROGRAM TO COMPLETE A PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS ERADICATION IN A SINGLE SITE FARROW TO FINISH FLOW

<u>T. Snider</u><sup>1</sup> PIC Europe

#### **Background and Objectives**

Routine surveillance testing detected the presence of the Porcine Reproduction and Respiratory Syndrome virus (PRRSv) in a 1250 sow farrow-to-finish genetic nucleus. PRRSv was characterized as 99.8% homologous (ORF5) with the strain in Ingelvac PRRS MLV® (Boehringer Ingelheim). The eradication program incorporated an intense surveillance program to guide and confirm the process. The objective of this report is to demonstrate the value of intense surveillance to improve the likelihood of a successful eradication.

# **Material and Methods**

Following partial depopulation and the implementation of offsite weaning, replacement gilts were retained, and the herd closed. The highly homologous commercial vaccine was used to expose all animals. A McRebel program was implemented and intensive surveillance commenced using PRRS RT-PCR (Tetracore). Weekly samples were collected and submitted to an accredited laboratory (TiHo Hannover Foundation, Bakum, Germany). Samples included: 120 processing fluids (PF), 3670 pre-weaning sera (pools=6), 892 post-weaning sera (pool=5), 746 post-weaning oral fluids (OF), and 38 sentinel group OF. In total, 1694 PCRs were completed. After attaining stable production, PRRS-PCR positives were investigated intensely. Any positive crate or pen groups triggered the testing of 100% of the group individuals. All positive groups were immediately culled. Crate and room status were tracked graphically on a cycle-by-cycle basis using EXCEL® (Microsoft Corp).

#### Results

After stable production was achieved, the herd experienced ten PCR positive events in three PF groups, five pre-weaning sera groups, one post-weaning sera group, and one post-weaning OF group. The tracking of individual crate status illustrated that the same crate was infected on subsequent cycles on two occasions. Overall, individual pig testing in PCR positive groups showed that between only 1 - 3% of the group were responsible for the positive event in PF and OF groups.

# **Discussion and Conclusion**

PRRSv transmission can continue at a low prevalence, even after the minimum requirements for stable production are met. High intensity surveillance increases the likelihood of detection and provides the opportunity to prevent transmission to the susceptible population. The diagnostic evidence indicates that transmission from unstable previously-exposed females and the environment are both likely transmission pathways.

MIS - Miscellaneous and Clinical cases

# AFRICAN SWINE FEVER: SUCCESSFUL KEYS TOWARDS ASF PREVENTION AND CONTROL

A. Boonsoongnern <sup>1</sup>, P. Boonsoongnern <sup>1</sup>, Y. Woonwong <sup>1</sup>, N. Thanantong <sup>1</sup>, T. Kaminsonsakul <sup>1</sup>, S. Phuttapatimok <sup>1</sup>, N. Rattanavanichrojn <sup>1</sup>, <u>P. Poolperm <sup>1</sup></u>, P. Jirawattanapong <sup>1</sup> <sup>1</sup>Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, 10900

#### Background and Objectives

The impact of African Swine Fever (ASF) on pig production leads to significant economic losses during viral outbreaks.Due to the absence of a vaccine or effective treatment, early and accurate diagnosis, along with the eradication of infected animals, remains crucial for preventing and controlling ASF. Biosecurity measures and management practices play a pivotal role in mitigating ASF risks. This study aimed to assess the effectiveness of biosecurity measures employed on a pig farm, focusing on key practices that contribute to successful ASF prevention and control.

#### **Material and Methods**

The study was observed on 1,500 sow farrow-to-finish commercial farm utilizing an open housing system for the breeding herd and an evaporative cooling system for nursery-finishing pigs. Following a complete depopulation inFebruary 2022 due to substantial ASF-related losses, the farm underwent extensive renovations to prevent bird and insect entry. Thorough cleaning with sodium hydroxide and glutaraldehyde disinfectants was implemented. After the cleaning process, all houses were swabbed and tested for ASF virus (ASFV) contamination using the qPCR technique before introduction of new pigs. Eighty-three days after the depopulation, the farm resumed production without the breeding herd. The initial group of weaned pigs from an external source was brought into the farm facility as part of the reproduction strategy. Wean-to-finish performance parameters including ADG, FCR, and % Losses, have been monitored. Additionally, pen swab practices have been implemented after the cleansing process in each house.

#### Results

The farm began its reproduction activities using wean-to-finish pigs in February 2022. Average performance parameters measured in 2022 (n=11 batches) and 2023 (n=30 batches) were ADG (793 and 747 g/d), FCR (2.33 and 2.39), and percentage losses (1.05 and 0.96). All swab samples for the past two years were negative for ASFV.

#### **Discussion and Conclusion**

The implemented disinfection protocol effectively eliminated ASFV contamination from the farm environment. It conveys those crucial renovations, including bird and insect protection, individual pen separation, and enhanced biosecurity measures, were essential for the farm's successful reproduction. This study highlights the importance of adhering to a comprehensive biosecurity protocol to ensure It effectively communicates sustainable pig production in the face of ASF threats.

MIS – Miscellaneous and Clinical cases

# CLINICAL EVALUATION OF SKIN LESIONS OF PORCINE EAR NECROSIS SYNDROME AND RESPECTIVE RISK FACTORS IN GREEK FARROW-TO-FINISH FARMS.

<u>D. Floros</u><sup>1</sup>, G. Papadopoulos<sup>2</sup>, R. Farmaki<sup>3</sup>, A. Lallas<sup>4</sup>, E. Tzika<sup>1</sup>, P. Tassis<sup>1</sup> <sup>1</sup>Farm Animals Clinic, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece <sup>2</sup>Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece <sup>3</sup>Companion Animal Clinic, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece <sup>4</sup>First Department of Dermatology, School of Medicine, Aristotle University of Thessaloniki, Greece

#### **Background and Objectives**

Porcine ear necrosis syndrome (PENS) affects pigs globally, usually after weaning. Lesions of the syndrome may vary from mild and localized to severe with ulcers and necrotic tissue development. Several bacteria, viruses, and management practices have been linked to PENS, but the specific etiology and pathogenesis are still unknown. The objective of the study was to analyze dermatologically, based on image dataset, the clinical findings of PENS in weaned piglets in Greek farrow-to-finish farms.

# **Material and Methods**

The study was conducted in 15 farrow-to-finish Greek farms, where the manifestation of PENS was established. Five weaned pigs with skin lesions of different degrees, from each farm were selected and examined. In total 150 ear pinnae were clinically examined. Images from the dorsal and the ventral side of each ear pinnae were collected from every study animal with the use of a photo camera. Skin lesions compatible with PENS were later analyzed and categorized based on two scoring systems as regards to their extent and severity. Chi-square analysis and Kruskall-Wallis test were used to analyze the recorded data, while correlations between parameters were investigated with Spearman's rank in IBM SPSS Statistics, 26.0.

#### Results

Findings demonstrated that 8/150 (5.33%) pinnae had compatible to PENS lesions at the greatest extent level, and 35/150 (23.33%) ear pinnae had lesions at the greatest severity level. From the examined animals, 58/150 (38.67%) ear pinnae were erected, and the rest 92/150 (61.33%) were semi-erected. A positive association between the severity and the extent of the lesions (P<0.001) was detected, whereas a positive correlation between erected or semi-erected ears and the extent of lesions (r=0.214, P=0.009) was also reported. On the contrary, the correlation between ear position and the severity of the lesions was insignificant. An absent of interaction was observed between linear excoriations and the severity of the lesions.

# **Discussion and Conclusion**

Results of the study confirm the extensive prevalence of PENS in Greek farms, as well as the variability of clinical findings. Erect or semi-erect ears could be significantly related with the extent of PENS lesions and further assessment is necessary to evaluate their possible role as a risk factor in PENS.

MIS – Miscellaneous and Clinical cases

# HISTOLOGIC CONFIRMATION OF MYOCARDITIS IN PIGLETS WITH PFTS.

P. Lopes <sup>1</sup> <sup>1</sup>FMV-ULHT

# **Background and Objectives**

PFTS (Periweaning Failure to Thrive Syndrome) in nursery piglets has been described since 2011 and so far the cause for this condition has not been identified.

In the studied Portuguese farms the nursery mortality rates due to PFTS range from 3,6% to 12,1%.

Symptoms of anorexia, severe weight loss and mortality begin post-weaning at 5-6 weeks of age.

Dilated cardiomyopathy is a common finding in necropsy.

Our investigation aimed to find histologic confirmation of myocarditis and its causes, and to do a differential diagnosis with PMWS (PCV2).

# Material and Methods

Two swine farms with diagnosed PFTS in nursery piglets were selected.

Both farms vaccinate piglets for PCV2 (21 days) and PRRS (42 days).

Necropsies of 32 piglets with symptoms of PFTS were performed.

In the piglets without other macroscopic lesions, the hearts with dilated cardiomyopathy were collected. One half was fixed in saline formaldehyde 10%, for histopathology, and the other half sent for PCR of Encephalomyocarditis virus (EMCV), PRRS and PCV2.

Inguinal lymph nodes were sent for Q-PCR for PVC2.

30 blood samples from live pen mates were sent for PCR of EMCV, PRRS, and PCV2.

#### Results

Necropsies showed that 68% of the dead piglets with cachexia (suspected PFTS) had bilateral dilatation of the heart, but no macroscopic lesions in other organs.

Results from histopathology identified mononuclear myocarditis in the myocardium and subepicardial area of 66% of the hearts. Inclusion bodies and suspicious infectious particles were not identified in the samples studied.

PCR in the heart tissues were all negative for EMCV, PRRS and PCV2.

Q-PCR for PCV2 in lymph nodes was negative.

PCR in blood samples were negative for EMCV and PCV (Q-PCR), but positive for PRRS, European vaccine strain in 2/6 pools.

### **Discussion and Conclusion**

Mononuclear myocarditis was confirmed in the hearts of piglets with dilated cardiomyopathy, that showed PFTS symptoms. Clinical symptoms are compatible with heart faillure.

The presence of EMCV, PRRS and PCV2 virus was not confirmed by PCR in the heart tissue.

The cause for this myocarditis and dilated cardiomyopathy is unknown.

The clinical condition looks like circovírus PMWS, but was not diagnosed in this case (PCV2 negative results).

MIS – Miscellaneous and Clinical cases

# IRON DEFICIENCY ANEMIA (IDA) AND HEMOGLOBIN (HB) STATUS AT WEANING PIGLETS IN INDUSTRIAL FARMS IN COLOMBIA: AN OBSERVATIONAL STUDY

J. Calveyra <sup>1</sup>, A. Vallejo <sup>1</sup>, N. Castro <sup>1</sup>, <u>D. Sperling</u> <sup>1</sup> <sup>7</sup>CEVA ANIMAL HEALTH

#### **Background and Objectives**

A piglet is born with limited iron stores, and sow milk is a poor source of iron. Therefore, piglets require exogenous iron supplementation during the first week after birth. The aim of the observational study was to assess the frequency of iron deficiency anemia (IDA) and hemoglobin (Hb) levels at weaning, in industrial farms in Colombia.

#### Material and Methods

Twenty randomly selected farms were included in the study (ranging from 70 to 1230 sows) covering the most important production areas in Colombia (Centro, Eje Cafetero, Valle, Antióquia). A minimum of 10 litters or 10% of litters per batch (size of the farm exceeding 1000 sows) from sows of different parity (average: 3.2) have been evaluated. Within each litter, blood was collected from a large piglet (weaning weight >6 kg), a medium piglet (5-6 kg), and a small piglet ( $\leq$ 5 kg). In total 1071 piglets at weaning were sampled (18 to 24 DOA, average). The Hb concentration was measured with the Hemocue<sup>®</sup>. Piglets were classified as follows: Hb levels < 9g/dl were considered as anemic, Hb levels ≥ 9 g/dl and ≤ 11 g/dl suboptimal and Hb levels > 11 g/l were optimal. To evaluate the variance between groups, Bartlett's test of homogeneity of variances was used. Mean blood Hb values were assessed using one-way analysis of variance and were expressed as means± standard variation. A P value < 0.05 was considered as statistically significant.

#### Results

Of the farms sampled, 85% had anemic piglets. The mean Hb level was  $10.5 \pm 1.7$  g/dl with a CV of 16.4%. 18.9% of the piglets sampled were anemic at weaning, and 41.1% were suboptimal. The group of large piglets had significantly more anemic piglets compared to the group of medium and small piglets (32% vs 18.3% vs 5.9%) (p<0.05). There was no difference in IDA/HB levels in relation to parity or gender.

### **Discussion and Conclusion**

IDA is a frequent problem on industrial farms in Colombia despite the regular use of iron-based products during the first week of age of piglets. Future studies, evaluating the risk factors and more detailed management practices would provide more detailed information and possible corrective measures.

MIS – Miscellaneous and Clinical cases

# MEASURING THE ECONOMIC OPPORTUNITY OF HIGHER WEAN-TO-FINISH MORTALITY BY TIMING PATTERNS

<u>E. Magalhaes</u><sup>1</sup>, X. Portal<sup>2</sup>, G. Trevisan<sup>1</sup>, G. De Souza E Silva<sup>1</sup>, D. Holtkamp<sup>1</sup>, D. Rosero<sup>3</sup>, D. Correia Lima Linhares<sup>1</sup> <sup>1</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine <sup>2</sup>The Hanor Company <sup>3</sup>Iowa State University, Department of Animal Science

Background and Objectives

Swine wean-to-finish (W2F) mortality is a major driver of profitability in swine operations. This study investigated the impact of different W2F mortality patterns and their respective economic losses in a U.S. production system.

#### Material and Methods

This study assessed the economic impact of wean-to-finish (W2F) mortality rates exceeding a 5% baseline for groups of growing pigs within a swine production system. Algorithms based on a full-budget model from Iowa State University<sup>1</sup> were developed for 1,965 groups (~3 million pigs) marketed between January 2022 and February 2023, divided into "high" (n=491) and "Iow" W2F mortality (n=1,474) groups. Additionally, groups were categorized as "late" or "early" based on the timing of mortality, with "early" having nursery mortality above 30% of the complete W2F mortality, and "late" below 30%. Economic losses were calculated per group using fixed feed costs U\$0.31/Kg and carcass price (\$70/45Kg).

#### Results

The average mortalities per category and the respective economic losses compared to a 5% mortality baseline were: groups classified as "high & early" (n=349) had 13.7% W2F mortality and economic loss of U\$ 5.84/head; groups classified as "high & late" (n=142) had 13.4% W2F mortality and economic loss of U\$ 6.79/head; groups classified as "low & early" (n=1079) had 7.9% W2F mortality and economic loss of U\$ 2.20/head; groups classified as "low & late" (n=395) had 8.0% W2F mortality and economic loss of U\$ 2.58/head.

#### **Discussion and Conclusion**

Although "high & late" closeouts had lower W2F mortality compared to "high & early", it incurred higher economic losses (\$6.79 vs. \$5.85 per head, respectively). This nearly \$1/head difference highlights that early mortality is relatively less costly due to lower feed consumption. If the "high and late" groups had been classified as "early," the costs would have been \$161,880 less for the 142 groups, assuming 1,200 pigs per group. Furthermore, the economic difference between "low & late" and "high & late" groups was \$4.21, potentially resulting in a \$717,384 reduction for the high-mortality groups if they had lowered their mortality rate. The study underscores the importance of developing an automated economic model for such systems.

#### MIS – Miscellaneous and Clinical cases

# IMMUNIZATION AGAINST GNRF IMPROVES UNIFORMITY OF CARCASS WEIGHT AND OPTIMIZES BACKFAT THICKNESS IN MARKET GILTS

L. Van De Weyer <sup>1</sup>, B. Bohrer <sup>2</sup>, B. Hansen <sup>3</sup>, S. Pollmann <sup>4</sup>, A. Aldaz <sup>5</sup> <sup>1</sup>Zoetis Canada Inc, Kirkland, QC <sup>2</sup>Dept of Animal Sci, The Ohio State University, Columbus, Ohio <sup>3</sup>BCH Consulting LLC, Atlantic, Iowa <sup>4</sup>DSP Consulting LLC, Alpine, Utah <sup>5</sup>Zoetis Inc, Parsippany, NJ, USA

#### **Background and Objectives**

Immunological suppression of estrus in market gilts is being increasingly adopted by the swine industry. The objective was to assess the mean differences and distribution of carcass weight and optical probe readings (backfat thickness, muscle depth, and predicted lean yield) of gilts immunized with Improvest (IMP gilts, also known as Improvac) compared with untreated gilts (UNT gilts) and physically castrated barrows (PC barrows).

# **Material and Methods**

A total of 924 pigs were raised in a commercial finishing barn in mixed-sex pens with 22 pigs per pen. Half of the gilts in each pen were randomly selected to be immunized against GnRF (IMP gilts) and the other half remained untreated (UNT gilts). At the completion of the finishing period, all pigs in each room were marketed over a 28-day period with equal proportions of each treatment marketed on each day (a ratio of 1:1:2 for IMP gilts, UNT gilts, and PC barrows, respectively). Carcass served as the experimental unit for all analyses and data were analyzed as a general linear mixed model with PROC MIXED of SAS. Distributions for each parameter were created in Microsoft Excel.

#### Results

Hot carcass weight was greater (P  $\le$  0.02) and standard deviation was improved in IMP gilts (98.2 ± 5.1 kg) and PC barrows (99.2 ± 4.4 kg) when compared with UNT gilts (95.8 ± 6.6 kg). Backfat thickness was greater (P < 0.01) in IMP gilts (17.1 ± 3.4 mm) and PC barrows (18.1 ± 4.4 mm) when compared with UNT gilts (14.8 ± 3.4 mm); however standard deviation was not different between IMP and UNT gilts.

# **Discussion and Conclusion**

Hot carcass weight and backfat thickness for IMP gilts were intermediate in their mean values when compared with PC barrows and UNT gilts, suggesting that the gap in carcass uniformity between barrows and gilts can be narrowed when gilts are managed with Improvest. Furthermore, the standard deviation for hot carcass weight was improved by 22.7% in IMP gilts (standard deviation = 5.1 kg) when compared with UNT gilts (standard deviation = 6.6 kg). This reduction in standard deviation should increase premiums for producers selling pigs on a grid-based marketing system and improve system efficiencies for pork processors.

MIS – Miscellaneous and Clinical cases

# ISOLATION OF CLOSTRIDIUM NOVYI SENSU LATO PRODUCING BOTULINUM NEUROTOXINS TYPE C FROM A CLAW LESION OF A GESTATING GILT

B. Cordioli <sup>1</sup>, L. Palazzolo <sup>1</sup>, M. Garbuio <sup>1</sup>, A. Rizzardi <sup>1</sup>, A. Guolo <sup>1</sup>, I. Drigo <sup>1</sup>, E. Girotto <sup>1</sup>, M. Vedana <sup>1</sup>, E. Schiavon <sup>1</sup>, L. Bano <sup>1</sup>

<sup>1</sup>Istituto Zooprofilattico Sperimentale delle Venezie

#### **Background and Objectives**

Ataxia and lateral recumbency were noticed in a gestating gilt of a multiplier farm. No antibiotics were administered and the gilt died three days later the appearance of the clinical signs. The necropsy performed on farm revealed a mild claw lesion on the right back foot. After skinning the claw lesion appeared to be extended to subcutaneous tissues and muscles, reaching the medial muscular plane of the thigh with irregular edges, brown-reddish exudate and fetid odour. Internal organs showed early autolysis and no gross lesions were observed. The presumptive diagnosis was a severe bacterial subacute necrotic myositis.

#### Material and Methods

Bacterial examinations were performed from muscles affected and the bone marrow by plating conventional media incubated in aerobiosis and anaerobiosis at 37°C. In addition, samples were also inoculated in Fortified Cooked Meat Medium (FCMM), heat-shocked and incubated at 37°C in anaerobic conditions. Forty-eight hours later the broth was plated on differential agar media, incubated in aerobiosis and anaerobiosis for 48h. Bacterial cultures were identified by means of MALDI TOF MS. Based on the bacteriological results, a qPCR targeted on the botulinum neurotoxins (BoNTs) encoding genes was performed on the FCMM.

#### Results

The bacteriological examination performed on bone marrow resulted negative. Pasteurella multocida, Streptococcus dysgalactiae, Escherichia fergusoni, Clostridium (C.) perfringens, C. tertium and C. novyi sensu lato were isolated from the muscles. PCR conducted on the FCMM revealed the presence of C. novyi sensu lato pruducung non-mosaic BoNTs C. The toxigenic strain was also isolated.

#### **Discussion and Conclusion**

The presence of BoNTs type C producing Clostridia has been already described in healthy pigs but only one outbreak of food-borne botulism has been reported. In the present case report, BoNTs were not investigated in biological samples, but C. novyi sensu lato type C have been detected in a claw lesion involving the inner muscles. This finding, coupled with the symptomatic picture, leads to suspect a wound botulism form, similar to episodes in humans. Thus, botulism could be underestimated in swine pathology and it could be included in the differential diagnosis of paralytic syndromes observed in pigs with wounds.

MIS – Miscellaneous and Clinical cases

# VARIATIONS IN MICROBIOTA OF PIGLETS TAKING FEED SUPPLEMENTED WITH A SYMBIOTIC

<u>S. Isusi</u>, J.A. Murillo<sup>2</sup>, M. Escuder<sup>1</sup>, J.L. Platero<sup>1</sup>, B. Gonzalez-Guijarro<sup>3</sup>, A. Muñoz-Luna<sup>3</sup>, G. Ramis<sup>4</sup> <sup>†</sup>*TECNOVIT-FARMAFAES, Spain* <sup>2</sup>*Ingaso Farm* 

<sup>3</sup>Departamento de Producción Animal. Universidad de Murcia <sup>4</sup>Dpto, Producción Animal., Facultad de Veterinaria., Universidad de Murcia, Spain

#### Background and Objectives

The microbiome in piglets is changing and is subject to numerous factors such as nutrition, pathogens present, management, biosecurity, etc. A balanced microbiota or eubiosis will maintain piglet health while dysbiosis often leads to enteric disease. In this work we have studied the differences in microbiota in piglets fed a feed containing a symbiotic.

### **Material and Methods**

Piglet faeces were sampled at weaning (n=20) from four farms of the same company at weaning and re-sampled two weeks after feeding a prestarter supplemented with Reforce containing fructo-oligosaccharide (FOS), mannanoligosaccharide (MOS) and Clostridium butyricum. The four farms were in different locations and were multi-phase farms with off-farm sites. They did not share site 2 with each other.DNA was isolated and metagenomics was performed by sequencing of the 16s ribosomal genes V1-V9. Results were analysed by Kronas and expressed as relative abundance.

#### Results

At the genus level, significant increases were observed in 31 different genera, including an increase in Prevotella (5.81 vs 13.72%, p=0.002), Lactobacillus (22.32 vs 35.8%, p=0.031), Clostridium (4.23 vs 8.54%, p=0.044) and a decrease in Bilophila (12.22 vs 0.19%, p<0.001), Faecalibacterium (13.23 vs 6.91%, p=0.001), among others. Specifically, a significant decrease was observed in Escherichia coli (0.16% vs 0.01%, p=0.027), Campylobacter coli (0.06 vs 3.17%, p=0.029) and C. butyricum (0.0 vs 1.95%, p=0.006) or Gemminger formicilis (0.95 vs 10.95%, p<0.001). Of the four farms studied, three showed the same changes in microbiota and almost in the same dimension while a fourth showed differences with the others, e.g. a decrease in Clostridium (8.6 vs 6.89%, p=0.023).

#### **Discussion and Conclusion**

The addition of a symbiotic to the prestarter has produced changes in the microbiota that include the significant presence of the probiotic included in the feed, and an increase in bacteria of genera such as Lactobacillus or Prevotella, which are key to the maintenance of intestinal eubiosis and enteric health of the piglets.

MIS - Miscellaneous and Clinical cases

# A CASE OF GENERALIZED EXUDATIVE EPIDERMITIS IN SUCKLING PIGLETS REMEMBERING SOME BASICS

N. Clemens <sup>1</sup>, D. Cassart <sup>2</sup>, <u>M. Laitat <sup>1</sup></u> <sup>1</sup>Swine clinic - University of Liège - Belgium <sup>2</sup>Department of Pathology - University of Liège - Belgium

#### **Background and Objectives**

In a 9 sows farrow-to-finish pig farm, a case of exudative epidermitis was detected in two 10-day-old fostered piglets.

#### Material and Methods

One piglet (P1), affected by severe and generalized skin lesions was weak and had a low rectal temperature (37.2°C). It was euthanized after blood sampling. Samples were taken for histopathology. The second piglet (P2) was in the acute phase of the disease, with mild skin lesions but a high rectal temperature (40.8°C). He received meloxicam and amoxycillin intramuscularly as well as a skin ointment based on honey and essential oils and recovered within a week.

#### Results

Before treatment, blood sampling was collected from the affected piglet and from the two other not affected fostered piglets of the same litter, in a way to compare their IgG serum concentration: it was respectively 0,8-0,9 and 10,1-10,4 g/L in affected and normal piglets. Swabbing for bacteriological confirmation was performed on the skin under the most severe crusts, on the stimulated precrural lymph node of P1 and in the blood of both affected piglets. An aerobic microbial culture confirmed the presence of Staphylococcus hyicus and Staphylococcus aureus under crusts (+++) and in the lymph node (+++) of P1. Staphylococcus hyicus (+) was also detected in the blood of P1. The strain isolated was sensible to all 15 tested antibacterials but tetracyclines. Among the 8 resident piglets of the litter, lesions developed on the skin of the ear wearing the ear tag and/or around the castration wound of three piglets. The sow developed mild lesions on the skin of the udder. The 3 piglets were given skin ointment and recovered after a few days; the sow recovered without treatment.

#### **Discussion and Conclusion**

This case remembers us that generalized exudative epidermitis in suckling piglets is a septicaemic condition characterized by severe skin lesions associated to Staphylococcus hyicus. Two main contributing factors - the lack of colostrum protection and skin abrasion or trauma (fighting for milk, castration wounds, lesions close to the ear tag) – have probably played an important role in this case. To treat severely affected piglets, parenteral associated to local treatment should be given during the acute phase.

MIS – Miscellaneous and Clinical cases

# ALTERNATIVE METHOD TO MEASURE BODY TEMPERATURE OF PIGS UNDER EXPERIMENTAL CONDITIONS

K. Sonalio <sup>1</sup>, F. Boyen <sup>2</sup>, B. Devriendt <sup>3</sup>, F.A. Moreira Petri <sup>1</sup>, L.G. De Oliveira <sup>1</sup>, M. Dominiek <sup>4</sup>

<sup>1</sup>São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil

<sup>2</sup>Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Belaium

<sup>3</sup>Department of Translational Physiology, Infectiology and Public Health, Faculty of Veterinary Medicine, Ghent University, Belgium

<sup>4</sup>Unit of Porcine Health Management, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

#### **Background and Objectives**

The assessment of rectal temperature (RT) in pigs, considered the gold standard for body temperature, is labor-intensive and may not reflect resting temperature accurately. Alternative methods, such as thermal transponders, are gaining interest. We aimed to correlate RT with intramuscular temperature (IMT) in pigs under experimental conditions.

# **Material and Methods**

Three independent experiments were conducted. In Exp1, 26 piglets received intramuscular implants of a classic thermal chip on the right side of the neck. Exp2 involved 53 piglets with the same chip, and Exp3 included 52 piglets with the mini thermal implant. RT and IMT were measured by the thermochip for five, three, and 10 consecutive days in Exp1, Exp2, and Exp3, respectively. RT was measured with a digital thermometer, while IMT was assessed using a portable reader. Data were analyzed with GraphPad Prism 10, including Spearman correlation.

#### Results

In Exp1, RT ranged from 38.5 to  $40.9^{\circ}$ C, and IMT ranged from 38.6 to  $41.2^{\circ}$ C. Exp2 recorded RT between 38.6 and  $40.6^{\circ}$ C and IMT between 38.6 and  $41.1^{\circ}$ C. In Exp3, RT ranged from 37.2 to  $41.2^{\circ}$ C, and IMT ranged from 38.6 to  $41.1^{\circ}$ C. Median IMT values were 39.8°C for Exp1 and 2 and slightly lower at 39.5°C for Exp3. Median RT values were 39.4°C, 39.6°C, and 39.2°C for Exp1-3, respectively. Coefficient of variation (CV) in Exp1 was slightly higher for IMT (CV=1.16) than RT (1.06), while in Exp2, the CV for IMT and RT was 0.91. In Exp3, the CV was 1.04 and 1.25 for IMT and RT, respectively. IMT was higher than RT in Exp1 to 3, with differences of  $0.25^{\circ}$ C,  $0.17^{\circ}$ C, and  $0.46^{\circ}$ C, respectively. Strong correlations (r=0.53) between IMT and RT were observed in Exp1, while weaker correlations (r=0.23) were reported in Exp2 and Exp3 (p<0.05).

#### **Discussion and Conclusion**

Repeated RT assessments in pigs, particularly in experiments, are inconvenient and may not reflect resting temperature accurately due to movement and handling stress. In this study, IMT was consistently higher than RT, showing strong correlations and suggesting thermal chips as a precise alternative in pig body temperature assessment.

MIS – Miscellaneous and Clinical cases

# PROGRESSION OF THE SLAUGHTER DATA IN ELEVEN FRENCH PIG HERDS HAVING IMPLEMENTED PARENTERAL VACCINATION IN REPLACEMENT OF OTHER STRATEGIES AGAINST LAWSONIA INTRACELLULARIS

C. Renoult <sup>1</sup>, J. Rouillier <sup>1</sup>, I. Delaunay <sup>1</sup>, E. Chabeauti <sup>1</sup>, K. Thirouard <sup>1</sup>, D. Dagorn <sup>1</sup>, P. Veugeois <sup>1</sup>, M. Rigaut <sup>2</sup>, F. Preault <sup>2</sup>, D. Duivon <sup>2</sup>, J. Trebault <sup>2</sup>, L. Daluzeau <sup>2</sup>, <u>F. Voisin</u> <sup>2</sup> <sup>1</sup>HYOVET, 5 P.A. Carrefour du Penthièvre, 22640 Plestan, France <sup>2</sup>MSD Animal Health, 49 Beaucouzé, France

#### **Background and Objectives**

The aim of this study was to illustrate the performance of parenteral vaccination against Lawsonia intracellularis (L.i.) in several pig farms under French conditions.

# **Material and Methods**

A group of 11 French conventional farms having implemented vaccination against L.i. at weaning (Porcilis® Lawsonia) were included. Selection criteria for these herds included the presence of a history of proliferative enteropathy, identification of the pigs with the week of their birth, to be the first ones to use the above vaccination strategy, and the availability of slaughterhouse data. Live weight (LW) at slaughter, age at slaughter, and average daily gain (ADG) was recorded for two periods: previous 6 months before the implementation of the new prevention strategy, and 6 months after such implementation. Comparison to contemporary slaughters was included for both periods. Wilcoxon rank sum test was used as statistical analysis.

# Results

Among the farms included, 6 did previously use oral vaccination, and 5 relied on nutrition or antibiotics as a former strategy against L.i. A total of 20.890 and 21.333 pigs were respectively recorded for the periods before and after. Following the implementation of the parenteral vaccination, LW at slaughter and ADG significantly increased by 1.45kg, and 14 g/d respectively, and the age at slaughter was significantly reduced by 1.8d (p<0,05). Comparing both periods (before vs after), there was a significant reduction of the average difference between the included animals and the contemporary slaughters for LW at slaughter (-1.67vs.-0.71kg, p<0.05) and lean meat (+0.18vs.-0.004%, p<0.05).

#### **Discussion and Conclusion**

Under the conditions of this study, the performances at slaughter were improved after parenteral vaccination against L.i. at weaning in comparison to previous strategies in 11 of the first farms that had chosen this strategy in France.

MIS – Miscellaneous and Clinical cases

# EVALUATION OF AN INJECTABLE COMBINATION OF GLEPTOFERRON AND TOLTRAZURIL IN THE CONTROL OF ANEMIA AND PERFORMANCE OF PIGLETS AT WEANING

A. Vallejo <sup>1</sup>, J.C. Mesa <sup>2</sup>, C.E. Rendón <sup>3</sup>, <u>D. Sperling</u> <sup>1</sup>, J. Calveyra <sup>1</sup> <sup>1</sup>CEVA ANIMAL HEALTH <sup>2</sup>Independent statistical consultant <sup>3</sup>Cercafé Cooperativa de Porcinocutores de Eie Cafetero

#### **Background and Objectives**

Anemia and coccidiosis are two disorders that affect weight gain of piglets in pre and post weaning period. The objective of this study was to evaluate the effect of an injectable combination of Gleptoferron and Toltrazuril on anemia and weaning weight of piglets on a Colombian commercial farm.

# **Material and Methods**

The study was carried out on a farm located in the Eje Cafetero region of Colombia with 600 sows. 89 sows at the gestation were selected and randomly distributed into the 2 groups: 43 to Group 1: Control (according to farm practice) (piglets received 2 mL (200 mg of iron) of Injectable Iron Dextran on the 1st day and 1 mL of Oral Toltrazuril on the 7th day of age) and 46 to Group 2: piglets received 1.5 mL Forceris® Injectable (Ceva Animal Health, France) between 24 and 96 hours of age. 30 piglets from different litters were randomly selected from each group at weaning for Hemoglobin measurement, being classified according to the accepted categories. Initial weight, weaning age, mean parity order, and weaning weight were analyzed by one-way analysis of variance. Statistical analysis Anemia was performed using the Chi-Square test.

#### Results

No statistically significant difference was observed in the sow parity (G1 = 2.72 / G2 = 3.4); in the piglets born alive per litter, in the initial average weight (G1 = 1.43 / G2 = 1.39), showing that the initial conditions were similar for both treatments. There was no difference in age at weaning (G1 = 28.23 / G2 = 28.15; P=0.8909). However, there was a significant difference in weaning weight (G1 = 7.38; G2 = 7.96, P<0.001). There was a significant difference in frequency of anemic piglets at weaning: G1 had 30% of anemic animals and G2 had 3% of anemic animals (P <0.05).

#### **Discussion and Conclusion**

In the group of piglets treated by injectable product, a lower risk of being weaned with anemic or sub-anemic status under conditions presented study. Better Hb status of piglets at weaning influenced the zootechnical performance with a positive impact of 580 g at weaning.

MIS – Miscellaneous and Clinical cases

# **MYCOTOXINS IN COMMERCIAL BREEDING HERDS, A SILENT PROFIT KILLER?**

<u>V. Van Hamme</u><sup>1</sup>, R. Neto<sup>1</sup>, S. Ravichandran<sup>1</sup>, R. Van Gelderen<sup>2</sup>, N. Luyten<sup>1</sup> <sup>7</sup>*KEMIN EUROPA NV* <sup>2</sup>*Porc business bv* 

#### **Background and Objectives**

Mycotoxins are toxic compounds produced by moulds present on feed and raw materials. Pigs are susceptible to a large range of mycotoxins such as deoxynivalenol (DON), zearalenone (ZEA), T-2 and fumonisins (FUM), less for ochratoxin (OTA) or aflatoxin. A mycotoxin prevalence study was conducted to assess the importance of (sub)clinical mycotoxicosis in breeding herds.

#### **Material and Methods**

The study was performed for 1 year (September 2022 to August 2023) on 6 Dutch breeding herds: 3 herds without any sign that could indicate mycotoxin presence and 3 others suspected of mycotoxin presence, as management was not optimal or clinical symptoms were appearing periodically such as necrotic tails, an indication for T-2 presence. Gestation, lactation, and piglet starter feeds of each herd were sampled every quarter and analyzed for FUM, OTA, T-2/HT-2, DON, and ZEA by LC-MS/MS.

#### Results

A total of 98 samples were evaluated. There was no difference in DON, ZEA, OTA and FUM prevalence among farms. Across all samples, 64% of samples were positive for DON, 66% for T-2/HT-2, 58% for ZEA, 37% for OTA, and 30% for FUM. 66% of samples were contaminated with 2 or more mycotoxins. 10% of samples exceeded the risk level for DON. 10% exceeded the risk level for ZEA. 0 samples exceeded risk levels for FUM or OTA. The 2 farms reporting necrotic tails showed T-2 / HT-2 contamination levels up to 140 ppb. Mycotoxin prevalence showed a seasonal pattern, with the highest contaminations in summer 2023, especially for FUM.

#### **Discussion and Conclusion**

In this study, subclinical mycotoxicosis due to ZEA and DON is present quite often, as all farms reported one or more feeds exceeding the risk level without clinical symptoms. The appearance of necrotic tails could be linked to higher HT/T-2 levels. Today, there are no EU guidance levels for T-2 available in pig feeds, only indicative levels for the sum of T-2 and HT-2 toxins in cereals and cereal products for compound feed. This study indicates that although no guidance levels are available, the presence of T-2/HT-2 should not be neglected in pig feed.

MIS - Miscellaneous and Clinical cases

# COMPARISON OF TREATMENT OUTCOMES BETWEEN THREE DIFFERENT MEDICATIONS OF TULATHROMYCIN IN A COMMERCIAL SWINE FARM

A. Prapattanakullawong <sup>1</sup>, M. Rattana <sup>1</sup>, <u>W. Navasakuljinda <sup>1</sup></u>, C. Muangpaisarn <sup>1</sup> <sup>1</sup>Zoetis (Thailand) Limited, Bangkok 10500, Thailand

#### **Background and Objectives**

The use of tulathromycin injectable, a prolonged duration of activity antibiotic as the preferred medication for addressing respiratory bacterial infections in weaned pigs has been well accepted in Thailand over the years. However, recently there were reported treatment failures with certain tulathromycin medication that raise some concerns. The objective of this study was to compare the treatment outcomes of three different medications of tulathromycin under the same field conditions in a commercial swine farm.

# **Material and Methods**

The study was conducted on a farrow-to-finish farm with 3,200 sows' capacity and without routine PRRS vaccination. Swine Respiratory disease (SRD) associated with Actinobacillus pleuropneumoniae, Pasteurella multocida, and Mycoplasma hyopneumoniae has previously been diagnosed. A total of 2,300 weaned pigs were then randomly ear-tagged and administered either Draxxin® (Treatment A) or generic tulathromycin (Treatment B or C) at dose of 2.5 mg per kilogram of bodyweight (20 mg/pig), as part of a metaphylaxis program at 26 days of age. Each pig was individually weighed at the beginning and the end of the nursery phase, feed intake and mortality rates were also recorded.

#### Results

The results indicated that pigs in Treatment A had significantly (p<0.05) better average weight gain per pig (0.77 and 0.97 kg) respectively and ADG increase (36 and 42 grams) respectively, lower mortality 4.84% versus 6.6% and 8.24% respectively and shorten the number of days in the nursery barn by 1 day, in comparison with pigs in Treatment B and Treatment C.

#### **Discussion and Conclusion**

The use of tulathromycin medications on this farm, within the same program and environment, revealed varying treatment outcomes across different brands. The results indicate that the Treatment A yielded the best treatment outcomes for weaned pigs in face of complex SRD situation. Further investigation is warranted to compare the pharmacokinetics and pharmacodynamics of different tulathromycin medications.

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# EFFECTS OF A NEW WATER PIPE CLEANING PROTOCOL ON WATER PIPE DEPOSITS AND HEALTH STATUS OF NURSERY PIGS

T. Handt <sup>1</sup>, K. Aumann <sup>2</sup>, O. Hansen <sup>3</sup>, J. Ceron <sup>4</sup>, <u>I. Hennig-Pauka</u> <sup>1</sup> <sup>1</sup>Field Station for Epidemiology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany <sup>2</sup>Aumann Hygienetechnik, Goldenstedt, Germany <sup>3</sup>Convet GmbH&Co KG, Monheim, Germany <sup>4</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia

#### **Background and Objectives**

Drinking water might be impacted by detaching deposits from pipes. Pipe cleaning is recommended, but no routine protocols are available. A potential positive effect might be expected for nursery pigs considered sensitive to endotoxins or pathogens washed off from biofilms. A water pipe cleaning protocol was tested in this study on two farms in the nursery.

### Material and Methods

Water pipes were rinsed with a 42°C solution containing tartaric acid (4Cidal®) for 30 minutes followed by a 10 minute flow pulse period of 2 bar. This two-step cleaning protocol was repeated for three times. Control and trial compartments in two farms (farm A: 100 pigs per compartment, 25 pigs/pen, farm B: 400 pigs/compartment, 100 pigs/pen) were examined in three subsequent batches, in which three pipes were analysed for deposits prior to and after cleaning. In each compartment 100 pigs were scored for clinical signs over the study period and 12 pigs were sampled (blood, saliva samples) at arrival and end of nursery. Both farms were evaluated separately with respect to saliva stress markers (amylase, cortisol, chromogranin, oxytocin), acute phase proteins haptoglobin and CRP as well as average daily weight gain by two-factorial analysis of variance (fixed effect: treatment, random effect: batch).

#### Results

Pipe deposits in the trial compartments were macroscopically reduced but not completely removed by cleaning (n=18). Salmonella antibodies were not differing at the end of nursery between pigs from both compartments (non-parametric ANOVA). Saliva stress markers and acute phase proteins did not differ between trial and control compartments. Average daily weight gains were higher in compartments with cleaned pipes (farm A: trial (n=281): 497+0.11 g, control (n=287): 474+0.10g, p=0.002/ farm B: trial (n=278): 360+0.11 g, control (n=275): 342+0.10g, p=0.04).

#### **Discussion and Conclusion**

Water pipe cleaning by mechanical impulses and chemical detergents was partly effective in removal of deposits. Hypothetized health effects of pipe cleaning were not obvious and might be superimposed by other impacting factors. For prevention of infection from potential water pipe deposit reservoirs (e.g. E.coli, salmonella) pipe cleaning can supplement other preventive measures.

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# IMPROVED TASTE PANEL MEASUREMENTS OF PALATABILITY OF LOINS FROM MARKET GILTS IMMUNIZED AGAINST GONADOTROPIN-RELEASING FACTOR IS NOT MIRRORED BY LABORATORY MEASUREMENTS

<u>K. Vonnahme</u><sup>1</sup>, R. Swalla<sup>1</sup>, D. Amodie<sup>1</sup>, M. Mellencamp<sup>1</sup> <sup>7</sup>Zoetis

#### **Background and Objectives**

Pork loins that have increased intramuscular fat are more desirable as it increases palatability. Gilts administered antigonadotropin-releasing factor (anti-GnRF) had loins that had enhanced markers of palatability in a trained taste panel. Our objective was to determine if anti-GnRF impacted other parameters of meat quality in loins from gilts at differing times post second dose (PSD) of anti-GnRF when tested in a laboratory setting.

#### Material and Methods

Gilts were either not administered anti-GnRF (Control) or were administered anti-GnRF (Improvest®) at 9- and 19-weeks of age. Gilts were marketed at 39-, 46-, and 53-days PSD of anti-GnRF. Loins were randomly selected each day, vacuum packaged, and stored at 4°C until analysis. Minolta chromameter readings [L\* (lightness), b\* (yellowness), and a\* (redness)], pH, drip loss (i.e., purge loss), Instron Star-probe (i.e., tenderness), and intramuscular fat analyses were performed and data analyzed with SAS.

#### Results

Treatment or day did not impact L\* or b\*, however, a day effect (P<0.01) was observed for a\* with loins harvested 53 days PSD (14.75±0.10) being larger than loins collected at 39 (14.25±0.10) and 46 days (14.31±0.10), which did not differ. While there was no effect of treatment, loin pH on day 39 was lower (P<0.01;  $5.63\pm0.01$ ) than on day 46 ( $5.75\pm0.01$ ) and 53 ( $5.73\pm0.01$ ), which did not differ. There were no effects of treatment, day, or their interaction (P>0.16) on tenderness (i.e., Instron Star-probe) or drip loss. A day effect (P=0.0204) for cook loss was observed with greater cook loss on day 39 ( $23.26\pm0.34\%$ ) compared to day 46 ( $21.93\pm0.34\%$ ); day 53 was intermediate ( $22.77\pm0.34\%$ ). There was a treatment by day interaction (P=0.0325) for intramuscular fat with loins from anti-GnRF gilts having more intramuscular fat compared to control at 46 days PSD. There were no differences at 39- or 53-days PSD.

#### **Discussion and Conclusion**

Parameters of meat quality are not greatly affected by anti-GnRF in gilts when harvested after 39 days PSD. The increased loin tenderness which was determined by a trained taste panel was not observed by the Instron method. Further work is needed to determine if any palatability measurements can be determined by the general consumer. ©2023 Zoetis Services LLC. All rights reserved. TI-1026

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# EFFECTS OF A ALGAE β-1,3-GLUCAN ON PIG PERFORMANCE IN A FINISHING HERD IN FRANCE

A. Nalovic <sup>1</sup>, D. Marchand <sup>1</sup>, M. Plessis <sup>1</sup>, V. Kerherve <sup>2</sup>, S. Thominiaux <sup>2</sup>, V. Van Hamme <sup>3</sup>, <u>R. Neto <sup>4</sup></u> <sup>1</sup>Reseau Cristal, France <sup>2</sup>Kemin France <sup>3</sup>KEMIN EUROPA NV <sup>4</sup>Kemin Europe NV

### **Background and Objectives**

Social, immunological, nutritional and environmental stressors can lead to poorer performance and inflammatory pathways which lead to sickness and potentially abnormal behaviour such as tail bite Nordgreen et al (2020). The objective of this study was to assess the effect of a  $\beta$ -1,3-glucan (BG) of algae origin (Euglena gracilis) on performance and lesions in a finishing pig farm.

# **Material and Methods**

Growers with an average age of 75 days and 30.5 kg body weight (n= 628) were allocated to one of two diets with two rooms per treatment for 130 days, with a density of 0,78  $pig/m^2$ , 15 pigs/pen. Control group (C) (n=316) received a commercial diet and a BG group (BG) (n=312) received a diet supplemented with BG (AletaÔ) (200 g/ton). Initial body weight, average pen body weights (BW) at day 70 of the of the study and before slaughter (approximately 130 days) and pig mortalities were measured. Tail lesions were scored approximately every 15 days, from entry, by the same investigator all along via the scoring system adapted from Honeck et al. (2019). Data were analysed in the Fit Model function of JMP 16. Differences were considered significant at P<0.05.

#### Results

There was no significant difference between both groups BG and C at the start, approximately 30.5 kg liveweight (P>0.5). At 70 days of the study, pigs in the C group weighed 96.3 and 97.0 kg for gilts and boars respectively, pigs in the BG weighed 97.8 and 97.3 kg for gilts and boars respectively (P>0.05). At the end of the study average pig weight for C group was 122.3 (112.8 days) and 123.3 kg (108.9 days), for the BG group 124.2 (111.8 days) and 124.0 kg (107.8 days) for gilts and boars respectively (P<0.1). The average percentage of pigs with tail biting lesions in group C was significantly higher than for pigs in group BG for the whole duration of the trial (16.1 and 12.9 % respectively, P<0.005).

# **Discussion and Conclusion**

Supplementing the diet of growing and finishing pigs with an algal  $\beta$ -1,3-glucan may be a beneficial intervention to support the health and welfare of the animals through modulation of inflammatory processes that lead to improved growth.
MIS – Miscellaneous and Clinical cases

# HOW OBSERVER EXPERIENCE CAN BIAS MEASUREMENTS OF SOW BODY CONDITION

<u>R. Carnevale</u><sup>1</sup>, B. Bracco Donatelli Muro<sup>2</sup>, M.S. Monteiro<sup>3</sup>, G.P.J. Janssens<sup>4</sup>, C.A. Pospissil Garbossa<sup>2</sup>, N. Nollet<sup>5</sup>, A. Cools<sup>6</sup>, S. Millet<sup>7</sup>

<sup>1</sup>University of São Paulo

<sup>2</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>3</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>4</sup>Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium <sup>5</sup>Inagro, Department of Animal Production

<sup>e</sup>Faculty of Veterinary Medicine, Ghent University

ILVO (Flanders Research Institute for Agriculture, Fisheries and Food), Scheldeweg 68, 9090 Melle, Belgium

# **Background and Objectives**

Evaluating the body condition (BC) of sows in modern pig herds has become essential to maximize the proportion of sows within the optimal BC, avoiding, therefore, overweight, and underweight sows. There are different methods to assess sow BC, and in the literature, there is no consensus if these methods correlates or not among them. The observer bias is arguably one of the most important variables affecting the correlation among these methods. Experienced and inexperienced observers may differ regarding the accuracy of body condition measurements. Thus, the aim of this trial is to analyze if observer experience can affect the accuracy and the correlation of different methods to assess BC of sows.

#### Material and Methods

Observers were divided into EXP (experienced observers, veterinarians or animal scientist who had already been trained to perform sows' body condition measurements) and INEX (inexperienced observers, observers with no prior training to measure sows' body condition). The measurements were performed in 12 gilts with similar age and at the same gestational age (100 days of gestation). The methods to measure gilts' body condition were visual body score (BCS) (ranging from 1 – 5), backfat thickness (BT) and loin depth (LD) by ultrasound and caliper. All gilts were weighed at day 100 of gestation. The relation between the different methods was analyzed Spearman's correlation and statistical significance was set at p<0.05.

# Results

For EXP group all the measurement were correlated (p<0.05). For the INEX group gilts weight and LD were not correlated (p<0.05) with BT, as well as LD and BCS (p<0.05). Additionally, all the correlation coefficients were numerically higher for EXP group.

# **Discussion and Conclusion**

Measurements assessed by the ultrasound seems to rely on more experience of the observer, requiring more technique and previous knowledge to achieve a correlation between measurements. The result of this trial supports the notion that the experience of the observer can in fact bias the evaluation of different methods of assess BC and that the methods to assess sow BC should be chosen based on who is performing the analysis, to ensure an accurate measurement.

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# MANDIBULAR TUSK EXTRACTION IN A VIETNAMESE POT-BELLIED PIG - A CASE REPORT

<u>T.J. Nicolaisen</u><sup>1</sup>, A. Bienert-Zeit<sup>2</sup>, J. Niepold<sup>1</sup>, L. Wittenberg-Voges<sup>2</sup>, M. Hellige<sup>2</sup>, J. Buchallik-Schregel<sup>1</sup>, F. Kiene<sup>1</sup>, M.G. Wagener<sup>1</sup>, B.U. Bauer<sup>1</sup>, A. Von Altrock<sup>1</sup>, M. Ganter<sup>1</sup>, I. Hennig-Pauka<sup>1</sup>

<sup>1</sup>Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Foundation, Bischofsholer Damm 15, 30173 Hannover, Germany

<sup>2</sup>Equine Clinic, University of Veterinary Medicine Hanover, Foundation, Bünteweg 9, 30559 Hanover, Germany

#### **Background and Objectives**

Tusks are defined as continuously growing, long pointed teeth that protrude from the closed mouth. Porcine tusks are large, rootless and curve shaped maxillary and mandibular canines. Reports about dental pathology and oral surgery in miniature pet pigs, both in general and for tusk pathology in particular, are rare. This case report describes the surgical extraction of a mandibular tusk in a male Vietnamese pot-bellied pig due to periodontal disease with apical abscess.

# **Material and Methods**

A fifteen year-old, castrated male Vietnamese pot-bellied pig was presented to the clinic due to a swelling of the left mandible in September 2023. This clinical case was processed using clinical and oral examination, X-ray, microbiological examination and dental surgery.

# Results

During clinical examination, a orange-sized fluctuating swelling was detected on the outside, medioventral part of the left mandibule. Additionally, a hard, bony swelling was found intra-orally near the suspected apical end of the left mandibular tusk. Pus oozed near the tusk when pressure was put onto the swelling. Feed intake was unaffected. X-ray revealed a severe enlargement of the tooth with signs of hypercementosis and suspicion of subsequent tusk infection with abscess formation. The abscess was opened trough the skin and malodorous pus was drained. Fusobacterium necrophorum was isolated in microbiological examination. Antimicrobial treatment (Lincomycin, Tulathromycin) was started prior to dental surgery. Tusk extraction was conducted under general and local anaesthesia. The surgical access was enlarged via trephination at the tusk's apical end. The tooth was mechanically loosened and completely extracted in six pieces after dissecting the bulbous apical end. Curettage and subsequent irrigation of the dental alveolus was conducted and it was sealed with honey gauze. The pig was discharged from hospital in good general condition nine days after surgery. Eight weeks after surgery, healing process (closure of dental alveolus) is not complete, but uncomplicated.

### **Discussion and Conclusion**

This case report shows that serious dental diseases also occur in pigs. It was shown that a total tusk extraction is possible but should be performed by a specialized veterinary dentist. In this case, the pathological intra-alveolar large size and highly curved shape of the tusk made extraction complicated and challenging.

MIS – Miscellaneous and Clinical cases

# IMMUNOLOGICAL PROFILE OF COLOSTRUM SAMPLES FROM A FORMERLY ASF-POSITIVE FARM IN THAILAND

S. Sopin <sup>1</sup>, <u>Y. Woonwong</u> <sup>2</sup>, P. Jirawattanapong <sup>2</sup>, P. Boonsoongnern <sup>2</sup>, A. Boonsoongnern <sup>2</sup> <sup>1</sup>Veterinary Clinical Study Program, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, 73140 <sup>2</sup>Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, 10900

#### Background and Objectives

African swine fever (ASF) is a highly contagious viral disease caused by the ASF virus (ASFV). Because of its severity, diagnostic techniques, especially the monitoring of disease status in farms previously affected by ASF, have been given priority. The Enzyme-linked Immunosorbent Assay (ELISA) is the method used for detecting anti-ASFV antibodies. While serum and plasma are typically considered suitable samples for this assay, colostrum was also assessed for the detection of specific antibodies. The aim of this study was to diagnose and monitor ASF infection using colostrum.

# **Material and Methods**

A farrow-to-finish farm of 430 sows in Thailand, used an open housing system with bird net protection. This farm had encountered an ASF infection confirmed by qPCR in February 2022. For following the ASF infection, colostrum samples were collected between May 2022 and October 2023 to detect antibodies using an in-house ELISA. Moreover, sow's blood samples in 2023 were ASF detected using qPCR. A total of 498 samples were collected and categorized by the number of repetitions: the first-time collection (n=413), the second-time collection (n=81), and the third-time collection (n=4). Descriptive analysis of the laboratory results was conducted for farm monitoring.

#### Results

At the end of December 2022, all ELISA results from the farm were negative, which correlated with the absence of any clinical signs related to ASF. Surprisingly, the percentage of positive samples gradually increased from 4.46% in March to 13.51% in October 2023. Additionally, a noteworthy 9.88% of repleted samples from the second and third collections turned out positive compared to the first collection. Importantly, all blood samples from sows at the farrowing date, taken from January to October 2023, tested negative for ASF using the qPCR.

### **Discussion and Conclusion**

Colostrum samples were found to be suitable for detecting ASF antibody responses. Collecting colostrum was a simpler procedure compared to blood for ELISA testing. However, the results indicated that the farm might have reinfected with ASFV in 2023. It underscores that, despite the absence of abnormalities detected by qPCR and clinical signs observation, there were notable findings in the ELISA results. Hence, farmers should consider implementing a monitoring strategy involving the collection of colostrum samples for ELISA antibody testing.

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# LONG-TERM (RE)PRODUCTIVE PERFORMANCE OF A NAÏVE HERD INFECTED WITH PORCINE EPIDEMIC DIARRHEA VIRUS

R. Nathaniel Alec<sup>1</sup>, M. Francisco Ernesto<sup>2</sup>, T. María Elena<sup>3</sup>

<sup>1</sup>Universidad Nacional Autonoma de Mexico. Facultad de Medicina Veterinaria y Zootecnia <sup>2</sup>Universidad Autónoma del Estado de México. Instituto de Ciencias Agropecuarias y Rurales <sup>3</sup>Universidad Nacional Autónoma de México. Programa Universitario de Alimentación Sostenible.

#### **Background and Objectives**

Porcine Epidemic Diarrhea Virus (PEDV) is currently considered to be one of the four emerging coronaviruses relevant in pork production. the emergence of less virulent PED strains with the capacity to persist in shoats could turn PEDV into an endemic disease. The objective of this study was to assess the long-term productive and reproductive performance of a naïve group of sows infected with Porcine Epidemic Diarrhea Virus (PEDV), along with the associated cost per weaned piglet.

# Material and Methods

A repeated-measurement design was employed to compare the (re)productive performance over multiple farrowing cycles in a farm with 170 sows, located in State of Mexico. The analysis encompassed five (re)productive cycles: a) Farrowing pre-PEDV outbreak; b) Farrowing during the outbreak; c) First post-PEDV farrowing; d) Second post-PEDV farrowing; and e) Third post-PEDV farrowing. The cost per weaned piglet was estimated using a general cost formula.

#### Results

Significant differences in the number of weaned piglets and unproductive days were observed amid the outbreak (P < 0.05). Before PEDV, sows weaned an average of 10.55 piglets, compared to 3.49 during the outbreak. In the three subsequent post-PED farrowings, the average number did not exceed 9.60. The number of stillborn piglets increased (0.62) in the first post-outbreak farrowing. Piglet cost in following post-outbreak were USD\$23.82, 23.64 and 23.66, compared to 22.03 before PED.

#### **Discussion and Conclusion**

In the productive and economic analysis, incorporating a review of sow productivity, revealed that the cost per weaned piglet remained elevated for at least three subsequent farrowings post-outbreak control. Since the emergence of highly virulent strains in Asia and North America, PEDV continues to be a relevant pathogen in pork production.

MIS - Miscellaneous and Clinical cases

# PREDICTION AND EVALUATION OF STILLBORN PIGLETS AT THE BIRTH OF MULTIPAROUS SOWS

<u>D. Daniel A</u><sup>1</sup>, M. Francisco Ernesto <sup>2</sup>, T. María Elena <sup>3</sup>, H. José Guadalupe <sup>4</sup>, B. Ricardo <sup>4</sup>, O. María E <sup>4</sup>, R. Antonio <sup>5</sup> <sup>1</sup>Universidad Nacional Autonoma de Mexico. Facultad de Medicina Veterinaria y Zootecnia <sup>2</sup>Universidad Autónoma del Estado de México. Instituto de Ciencias Agropecuarias y Rurales <sup>3</sup>Universidad Nacional Autónoma de México. Programa Universitario de Alimentación Sostenible. <sup>4</sup>Colegio de Postgraduados. Recursos Genéticos y Productividad <sup>5</sup>Universidad de Murcia. Facultad de Veterinaria

#### **Background and Objectives**

In sows, assistance at farrowing helps reduce stillborn piglets due to anoxia; In industrial conditions, where the number of animals is large, this assistance capacity is limited. Generating predictive models from the available information makes it possible to predict the response of the sows regarding the presence of stillborn piglets. The objective of this study was to generate a prediction model with a Bayesian approach to determine the factors that influence the presence of stillborn piglets (PSP) and estimate the probability that this phenomenon manifests itself

# Material and Methods

The data of 2,415 births of 822 sows from different farms, with Landrace, Yorkshire breeds and their crosses, five variables related to the current birth, five with the performance of the previous birth, were analyzed, and with cross validation (groups = 5) obtained the model the response variable (PSP, 1: presence and 0: otherwise).

#### Results

The results showed that only litter weight had a negative effect (p<0.01) on the presence of PSP; while litter size at birth and parity number had a positive effect (p<0.01) on PSP. The observed frequency of PSP was 0.29 and the probability estimated by the model was 0.20, an apparent underestimation in the probability, but in the categorization with obtaining and using the optimal cut-off point of 0.39 (this represents the decision frontier probability, where the phenomenon is classified as 0 or 1 based on the estimated probability), it improves predictive efficiency with an area under the receiver operating curve of 0.747 for training and 0.748 for testing. P(Y=1/X), where: is the probability that there is presence of stillbirths at birth (PSP), and takes a value of one, X1=partum number, X2=size of the litter at birth and X3=weight of the litter at birth.

# **Discussion and Conclusion**

It is concluded that the probabilistic model with Bayesian approximation adjusted together with the categorization criterion, presented good predictive efficiency to predict stillborn piglets. The implementation of the model obtained in the information management software will allow technical assistance to be promptly directed to the sows that require it with good efficiency

MIS – Miscellaneous and Clinical cases

# TWO (2) COMBINATION PORCINE CIRCOVIRUS 2 AND MYCOPLASMA HYOPNEUMONIAE VACCINE IN 4 KOREAN FARMS

J. Jeong <sup>1</sup>, G. Kang <sup>1</sup>, C. Bae <sup>1</sup>, H. Chae <sup>1</sup>, Y. Oh <sup>1</sup>, <u>C. Maala <sup>2</sup></u>, L. Lecznieski <sup>3</sup>, M. Genzow <sup>4</sup>, E. Schacht <sup>3</sup> <sup>1</sup>Boehringer Ingelheim Vetmedica Korea <sup>2</sup>Boehringer Ingelheim Animal Health ROPU ASKAN <sup>3</sup>Boehringer Ingelheim Animal Health GmbH <sup>4</sup>Boehringer Ingelheim Vetmedica GmbH

### **Background and Objectives**

**Background and objectives:**Porcine Circovirus 2 and Enzootic Pneumonia are economically damaging diseases, affecting mortality or growth rates in pigs. Vaccination is one major way to prevent or lessen the impact of these 2 diseases. In Asia, where a lot of pathogens need to be prevented, the effective prevention of these diseases is critical. These field efficacy studies compare the efficacy four trials evaluating two different vaccine treatments in farms in South Korea

### **Material and Methods**

**Material and methods:** Four different commercial farrow-to-finish single site farms, ranging from 180 to 650 sows in various provinces of South Korea. The efficacy parameters measured as mortality % and body weight gain as well as group homogeneity (reduced weight variation within the group) were evaluated, based on two vaccine treatments: a Mixable PCV2 and M. hyopneumoniae vaccine (Mix) and a Ready to Use (RTU) counterpart. An economic analysis was performed considering the cost/benefit of the different treatments. The return on investment (ROI) was calculated using an economic calculator. Weights were analyzed using ANOVA and Histogram distribution curves for uniformity

#### Results

**Results:**The Mix was statistically superior in Average body weight (Farm A: +3.2 kg; Farm B: +2.7 kg; Farm C: +4.6 kg; Farm D: +5.9 kg) and the 3 out of 4 farms have more uniform weights in terms of variation. Final weights were all statistically significant in favor of the Mix.The Return on Investment for FLEXcombo treatment were 2.47:1 Farm A, 8.7:1 Farm B 12.89:1 Farm C. 13.9:1 Farm D.

# **Discussion and Conclusion**

**Discussion and conclusion:**There was a 4.2 kg difference in favor of the Mix group with a lower Standard Deviation, confirming a better group homogeneity. The difference of both Final weight and Average Daily Gain between groups was also statistically significant (P <0.0001)These results were consistent with previous studies in Asia

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# PREVALENCE OF IRON DEFICIENCY ANEMIA IN SUCKLING PIGLETS ON BRAZILIAN FARMS - AN UPDATE

J. Calveyra 1, F. Betiolo 2, M. Walter 2, C. Postal 2, J. Candeias 2, S. Moro 2, C. Sartori 2, A. Horst 2, P. Filsner 2, D. Sperling

<sup>1</sup>CEVA ANIMAL HEALTH <sup>2</sup>Ceva Animal Health, Brazil

#### **Background and Objectives**

Iron supplementation is a necessary management practice established in the first days of a piglet's life. Even so, iron deficiency anemia (IDA) still pose the challenge for modern pig farms. This study aims to evaluate the current prevalence of IDA on industrial pig farms in Brazil, comparing with results from previous studies. A secondary study aim was to compare Hb levels in piglets treated with iron dextran and gleptoferron.

# **Material and Methods**

During the period of year 2022 and 2023, 39 farms were randomly selected and included in the study. The farms have varied from 400 to 5850 sow population and all use injectable iron. Ten litters of different sow parity (3.2 in average) were randomly selected. In each litter, 3 piglets were selected according to the size: large, medium and small. In total, 1200 piglets were sampled from 400 batches at weaning (from 17 to 30 days of age). Piglets were classified according to hemoglobin levels (Hb): anemic, suboptimal and optimal. The influence of size of the piglet, and parity order was evaluated by ANOVA.

#### Results

The average Hb level at weaning was 11.6 g/dl and the distribution of the percentage of anemic, suboptimal and optimal categories was 5%, 29% and 66%, respectively. On average, large piglets were more likely subanemic compared to small and medium-sized piglets. (p < 0.05). There was no statistically significant effect of parity.

#### **Discussion and Conclusion**

The presence of anemic and subanemic animals at weaning was confirmed. This study provided an update on IDA status at weaning on Brazilian commercial farms. Comparing with the results from 2021, in which all farms used Iron Dextran, the farms sampled in this study were subjected to treatment with Iron Dextran (N=19) or Gleptoferron (N=20). The differences in the absorption activity and bioavailability of these two molecules is a determining factor in the reduction of iron deficiency anemia in suckling piglets. In this study, animals treated with Iron Dextran showed 7% anemia and 31% subanemia, while animals treated with Gleptoferron showed 3% anemia and 25% subanemia. IDA should be considered an emerging challenge, and assessment of the hematinic status and Hb levels of piglets should be part of the standard monitoring procedure on farms.

MIS – Miscellaneous and Clinical cases

# PREVALENCE STUDY OF IRON DEFICIENCY ANEMIA IN SUCKLIG PIGLETS IN COSTA RICA.

F. Chacon <sup>1</sup>, J. Calveyra <sup>1</sup>, <u>D. Sperling</u> <sup>1</sup> <sup>1</sup>CEVA ANIMAL HEALTH

#### **Background and Objectives**

A common challenge in newborn piglets is Iron deficiency anemia (IDA). One of the most relevant and frequent parameter to identify IDA is hemoglobin level at weaning (Hb). The aim of this study was to assess the prevalence of IDA in Costa Rica.

#### **Material and Methods**

During the period of year 2023, 16 commercial farms in Costa Rica, representing 60% of national production were included in the study. The farms (350-6500 sows) were geographically distributed from the south to the north/Atlantic zone of the country.A total of 429 piglets at weaning were included in the survey. Piglets were selected according to previous recommendations: large, medium and small per litter. The litters (minimum of 10 litters/30 piglets per farm) represented sows of different parities (average 3,6 parity). Hb levels were measured with the analyzer Hemocue® Hb 201+ at weaning. Piglets were classified as follows: levels of Hb < 9 g/dl are considered indicative of anemia, Hb  $\ge$  9 g/dl and < 11 g/dl, subanemia, and Hb  $\ge$  11 g/dl, optimal. All animals sampled in the study received a dose of iron dextran from 1 to 5 days of life. The influence of size of the piglet, and parity were evaluated by ANOVA.

#### Results

The average hemoglobin level was 10.4 g/dl. The percentage of anemic, suboptimal and optimal animals was 20%, 41% and 39%, respectively.Regarding the size of the piglets sampled, there was an effect on the frequency of IDA: 23% of the larger piglets were anemic, followed by 21% of the medium piglets and 16% of the small piglets at weaning (p < 0.05). There was no statistically significant effect of parity on IDA at weaning.

#### **Discussion and Conclusion**

This study demonstrated a high prevalence of IDA in animals at weaning, observed in previous studies in other production countries in Latin America. The management of IDA prevention should be reviewed on farms, including the protocol (day of administration) and type of the iron product. This is the first study assessing iron mnagement and IDA prevalence in commercila farms in Costa Rica.

MIS – Miscellaneous and Clinical cases

# EFFICACY COMPARISON STUDY OF ENTERISOL® ILEITIS VACCINATION IN KAGOSHIMA BERKSHIRE IN JAPAN

<u>S. Nikaido</u><sup>1</sup>, E. Sakamoto<sup>1</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Japan Co., Ltd.

#### **Background and Objectives**

Kagoshima Berkshire, which are raised mainly in Kagoshima Prefecture, are a breed unique to Japan that is an improvement of the Berkshire breed. These pigs are fed a diet containing sweet potatoes. The breed is known for strong flavor and high quality It develops slower than other breeds (white pigs). To improve the average daily weight gain (ADWG), the efficacy of the vaccine Enterisol Ileitis® on production performance was evaluated.

# Material and Methods

The evaluation study was carried out at a two-site farm in Kagoshima Prefecture housing 2,300 sows that tested positive by Lawsonia ELISA after 120 days of age. For each test group, 3,060 piglets (test group) and 3,029 piglets (control group) were vaccinated orally at weaning on an average of 35 days of age. For evaluation, mortality rate, feed conversion rate, ADWG, average fattening days, and average market weight compared to the control period were statistically analyzed using the MAFF meat distribution statistics using a t-test.

#### Results

FCR improved significant (4.32 control vs 4.039 vaccinates; P<0.05) resulting in 26,7 kg less feed used per vaccinated pig. Carcass weight (kg meat) increased from 77.76 kg in the control group to 80.15 for the vaccinated pigs (P<0.05) resulting in 2,39 kg more meat per pig produced. Variability in ADWG and average market weight was also improved. ADG increased from 525 gram/day to 541 gram/day with a reduced standard deviation for the vaccinated animals of 23,2 gram/day when compared to the unvaccinated controls with a standard deviation of 28,8 gram/day. Mortality was not changed significant (6,0% control vs 5,9% vaccinates; p=0.86).

# **Discussion and Conclusion**

This study describes the impact of subclinical ileitis in the slow growing breed of Kagoshima Berkshire pigs. Calculations of the economic effects based on productivity indicators, domestic feed/pork prices, and vaccine resulted in an economic benefit of US\$17.01 per vaccinated pig when compared to the control group. This was because of a higher carcass weight and a lower FCR.

#### MIS - Miscellaneous and Clinical cases

# MELATONIN SECRETION IN SOWS UNDER NATURAL AND ARTIFICIAL LIGHTING IN DIFFERENT SEASONS IN A SUBTROPICAL CLIME (SOUTH-EAST OF SPAIN)

P. Sánchez-Giménez <sup>2</sup>, L. Martínez-Alarcón <sup>1</sup>, F. Murciano <sup>3</sup>, R. Fernández-Rodríguez <sup>2</sup>, E. Rodríguez-Hernández <sup>3</sup>, G. Ramis <sup>3</sup>

<sup>1</sup>Instituto Murciano de Investigación en Biomedicina (IMIB), Murcia, Spain <sup>2</sup>AGROPOR, Murcia, Spain <sup>3</sup>Dpto. Producción Animal.. Facultad de Veterinaria., Universidad de Murcia. Spain

#### **Background and Objectives**

Lighting is undoubtedly linked to the secretion of melatonin, a photoperiod-dependent hormone. The aim of this study was to determine the rate of melatonin secretion in sows after weaning under different temperature and lighting conditions to see its possible influence.

# **Material and Methods**

The experiment was carried out in a commercial farm with 9,600 Landrace x Large White sows, housed in cages immediately after weaning. The sampling was carried out before and after a modification of the building and two different times of the year: all-natural lighting and no temperature control in summer (SNL), and environment-controlled with artificial lighting (16L:8D), both in summer (SAL) and winter (WAL). Blood samples were obtained at 24 hours post-weaning every 3 hours (1.00 to 22.00). The amount of melatonin was quantified by ELISA using a Melatonin ELISA Kit (Enzo Life Sciences, NY, USA).

# Results

There were significant differences between the three groups taking mean daily melatonin (p<0.0001). The SNL had much higher quantification than the other two. Differences among groups were observed in all samplings except at 4.00 and 7.00. When comparing groups, differences were verified between SNL and WAL at 1.00 (p=0.013), 10.00 (p=0.015), 13.00 (p=0.01), 16.00 (p<0.0001), 19.00 (p<0.0001) and 22.00 (p<0.0001). There were also differences between SNL and SAL at 16.00 (p=0.003), 19.00 (p<0.0001) and 22.00 (p<0.0001) and between SAL and WAL at 19.00 (p=0.003) and 22.00 (p=0.005).In SNL group, the maximum quantification was observed at 10.00 and the lowest at 7.00 and an elevation again in the afternoon. However, in the WAL sows there is a peak at 4.00 and there is practically no melatonin production throughout the day. Sows in the SAL group have a maximum between 22.00 and 1.00 and a minimum at 13.00.

#### **Discussion and Conclusion**

The rhythmic secretion of melatonin is constant in virtually all mammals and is documented in the pig as well. Our results suggest that it is strongly influenced by environmental conditions, and that control of temperature and light can successfully regulate it, eliminating the effects of summer. And there may be a link between abnormal secretion patterns and hormones involved in ovulation and overt expression of oestrus.

MIS – Miscellaneous and Clinical cases

# STREPTOCOCCUS SUIS - CAN AUTOGENOUS VACCINES PROVIDE AN OPPORTUNITY TO SOLVE THIS PROBLEM?

<u>I. Spiekermeier</u><sup>1</sup>, S. Nowaczyk<sup>2</sup>, W. Luzynski<sup>3</sup> <sup>1</sup>SAN Group Biotech Germany GmbH, Hoeltinghausen, Germany <sup>2</sup>Uslugi Weterynaryjne lek.wet. Szymon Nowaczyk, Mieszków, Poland <sup>3</sup>Cedrob, S.A., Ciechanów, Poland

#### **Background and Objectives**

Infection by Streptococccus suis (S. suis) globally constitutes a pervasive health challenge in swine herds. Economic impacts primarily emanate from clinical manifestations such as meningitis, arthritis, and endocarditis. Beyond the approach of mitigation acute infections through antibiotic intervention, alternative strategies warrant consideration. In this regard, the ensuing discourse wants to present a case report delineating the outcomes derived from the deployment of a monovalent autogenous S. suis vaccine in a 6000-sow farm. This autogenous vaccine improved the overall health of treated animals and thus the production metrics.

# Material and Methods

Animals exhibiting clinical signs of disease were primarily observed among rearing piglet with a mortality rate of 2.99 % and 1.3 % meningitis cases on average. Pathogenic S. suis out of these animals (n=17) were extracted from the central nervous system, joints, and pericardium by bacterial culture techniques. Isolate typing was carried out using molecular biology methodologies, while pathogen selection was facilitated by mass spectrometry analysis. The predominant serotypes identified were serotype 7 and 9. After initial vaccine deployment, a routine sampling of clinically significant animals has been consistently performed, and the vaccine formulation has undergone continuous refinement. Initial immunization of piglets was accomplished through active immunization, with subsequent batches employing passive immunization via sow vaccination before farrowing.

#### Results

Diminishment in clinical manifestations, as evidenced by a significant reduction in post-weaning mortality by 1 % (p<0.009) and meningitis cases by 0.7 % (p<0.009) comparing groups before and after vaccination could be observed. Amoxicillin group treatment could be reduced from four to one time per rearing group after vaccine implementation. Alterations in production metrics like daily weight gain could not be observed. These findings are validated through analyses of farm-specific data.

#### **Discussion and Conclusion**

The presented case report demonstrates that the implementation of a herd-specific, autogenous vaccination against S. suis can lead to a substantial decrease in disease prevalence, even in scenarios where multiple serotypes contribute to clinical outbreaks. Key factors contributing to the success include conscientious pathogen selection and the ongoing updating of the vaccine. Consequently, this strategy can contribute to the amelioration of animal welfare and a reduction in the reliance on antibiotic therapies.

MIS – Miscellaneous and Clinical cases

# MYCOPLASMA HYOPHARYNGIS IDENTIFIED IN ARTHRITIS OF WEANERS AND THE DEVELOPMENT OF A QPCR ASSAY

<u>D. Földi</u><sup>1</sup>, E.Z. Nagy<sup>1</sup>, G. Tóth<sup>2</sup>, L. Makrai<sup>3</sup>, L. Gombos<sup>4</sup>, M. Gyuranecz<sup>1</sup> <sup>1</sup>HUN-REN, Veterinary Medical Research Institute, Budapest, Hungary <sup>2</sup>University of Veterinary Medicine, Budapest, Hungary <sup>3</sup>Autovakcina Kft., Budapest, Hungary <sup>4</sup>University of Györ, Wittmann Antal Plant-, Animal- and Food Sciences Multidisciplinary Doctoral School, Mosonmagyaróvár, Hungary

# **Background and Objectives**

After complete depopulation and disinfection, a Hungarian farm was repopulated with 200 gilts and 1700 finishers from the same population but different farms. Two days later, diarrhea and septicaemia caused by Salmonella Typhimurium was detected among the finishers. In total the disease caused about 10% loss. After the first farrowings, among the 21-25-day old piglets purulent arthritis was detected, and by 45-50 days caused 30% loss (culling or death). There were no response to several antibiotic treatments. Joint and intestine samples of the affected animals were sent for diagnostic culture of mycoplasmas and other bacteria.

#### **Material and Methods**

From the joint samples bacteria other than mycoplasmas were cultured on blood and chocolate agar plates at  $37^{\circ}$ C in the presence of 5% CO<sub>2</sub>. The intestine sample was cultured on Rappaport-Vassiliadis and Rambach agar plates. Identification of the cultured bacteria was accomplished by biochemical test and mass spectrometry. Culture of Mycoplasma sp. was carried out in MolliScience GM media at  $37^{\circ}$ C with 5% CO<sub>2</sub>. Mycoplasmas were identified by sequencing the intergenic spacer region of the 16S-23S rRNA genes. A qPCR targeting the 16S rRNA gene of M. hyopharyngis was developed and validated.

#### Results

From the joint samples from which mycoplasmas were not cultured Staphylococcus haemolyticus, S. hyicus and Aerococcus viridans were recovered. In one joint sample besides Trueperella pyogenes and Streptococcus agalactiae, M. hyopharyngis was isolated. The developed qPCR assay proved to be a specific and sensitive novel diagnostic tool.

#### **Discussion and Conclusion**

This case emphasizes the importance of considering mycoplasmas in the differential diagnosis of arthritis. This is the second isolation of M. hyopharyngis from arthritic joints, which highlights the necessity of a better understanding of this often-overlooked species. The developed qPCR method can simplify future prevalence studies and diagnosis of this minor pathogen.

MIS – Miscellaneous and Clinical cases

# MITIGATING STREPTOCOCCUS SUIS-RELATED MORTALITY WITH THE HELP OF PROBIOTIC MIYA-GOLD® (CLOSTRIDIUM BUTYRICUM)

G. Hoornenburg <sup>1</sup>, <u>L. Kunstmann</u><sup>2</sup>, L. Meedom <sup>2</sup>, W. Van Der Veken <sup>3</sup> <sup>1</sup>Porcus, Denmark <sup>2</sup>Huvepharma NV, Denmark <sup>3</sup>Huvepharma NV, Belgium

#### **Background and Objectives**

In 2022, a herd of 600 sows (Actinobaccilus Pleuropneumoniae serotype 6, Mycoplasmae hyopneumoniae and PRRS – type 1 positive) experienced high mortalities compared to the previous year's baseline levels (+3 to 5%). Meningitis and arthritis were observed and diagnosed as Streptococcus suis serotype 7 and serotype 9 and beta-haemolytic Streptococci. Cases were treated with sulfa-tmp or tiamulin by injection. Post weaning diarrhoea was present and treated by aminoglycosides according to antibiogram. In the 1<sup>st</sup> quarter of 2023, mortality increased further to an average of 8%. Peaks of 10-12% mortality were seen in the worst weeks. The farm had installed a continuous electrochemically activated (ECA) water treatment system. A buffer unit for weak weaned piglets was present in the middle of the post-weaning facilities as well. Multiple interventions were applied for 17 weeks to combat the increased mortality.

# **Material and Methods**

Interventions included:

-A Clostridium butyricum probiotic was added to all weaning feeds (2 kg Miya-Gold®/ton).

-Four weeks later, the same probiotic was included in all pre-weaning feeds.

-The amount of piglet feed formulations was reduced from four to three. All piglet transport through the buffer unit was

stopped.

-The ECA treatment was discontinued postweaning.

-Needle-free treatments were prioritised.

Weekly postweaning mortalities and causative clinical diagnosis were recorded as response parameters. Results from the 1<sup>st</sup> quarter of 2023 (before interventions, 13 weeks) were compared to those of the 2<sup>nd</sup> quarter of 2023 (after interventions, 17 weeks). Statistical analysis was performed with Students t-test.

### Results

Average weekly mortality significantly declined from 8,1% to 4,5% (P=0,0009). Streptococcus suis related mortalities also decreased significantly: meningitis went from 3,9% to 1,7% (P=0,02) whilst arthritis recorded a decrease from 1% to 0,5% (P=0,0002).

# **Discussion and Conclusion**

Our observations and interventions support "leaky gut" could be a part of Streptococcus suis pathogenesis in pigs around weaning. In this case a statistically significant reduction in overall mortality and Streptococcus suis related mortalities were obtained by managing PRRS, ensuring antibiotic efficacy by discontinuing ECA water treatment and optimizing intestinal gut health. The mortalities were restored to the original low-level baseline.

MIS – Miscellaneous and Clinical cases

# EVALUATION OF TYLVALOSIN AND TILMICOSIN TO MANAGE A NATURAL PORCINE RESPIRATORY DISEASE COMPLEX CHALLENGE IN A COMMERCIAL FINISHING SETTING

<u>J. Madigan</u><sup>1</sup>, N. Lauterbach<sup>2</sup> <sup>1</sup>Pillen Family Farms, Columbus, USA <sup>2</sup>Pharmgate Animal Health, Wilmington, USA

#### **Background and Objectives**

Porcine respiratory disease complex (PRDC) is a multi-pathogen disease process that commonly includes viral and bacterial pathogens. Treatment with antimicrobials is often needed. The objective of this project was to evaluate two water soluble antibiotics, Aivlosin® (62.5% w/w Tylvalosin as Tylvalosin Tartrate; ECO Animal Health) and Pulmotil® (tilmicosin, Elanco), to control a natural PRDC challenge in a commercial finishing setting.

# **Material and Methods**

A finishing site with eight barns (Barns 1-8) in the Midwestern United States was selected. Pigs from multiple sow farms negative to porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV) and Mycoplasma hyopneumoniae (MHP) were placed into a single nursery site. At nine weeks of age, a lateral, wild-type PRRSV was diagnosed at the nursery site. Pigs were then moved into the selected finisher barns. Four barns received tilmicosin at the labeled rate of 200 parts per million (ppm) whilst other four barns received tylvalosin at the labeled rate of 50 ppm. Both treatments were administered ad libitum in water for five consecutive days at placement into the finisher barns. Prior to administering medication, necropsies were performed on three random pigs. Sections of lung were submitted to Iowa State University Veterinary Diagnostic Laboratory to confirm the presence of PRDC pathogens. Production information was collected for 22 weeks and includes percent mortality, number of treatments, percentage of cull animals at marketing, and truck weights at marketing from each barn.

#### Results

Lung samples confirmed the presence of PRDC. The samples were positive by PCR to PRRSV (Ct 15.8) and Mycoplasma hyorhinis (Ct 16.7). Additionally, Glaesserella parasuis and Bordetella bronchiseptica were cultured from lung samples. No significant statistical difference was seen between treatment groups for percent mortality, number of treatments, percentage of culls, or truck weights at marketing.

# **Discussion and Conclusion**

Understanding the tools that are available to veterinarians when faced with PRDC is critical to limiting the impact this multifactorial disease complex has on growing pigs. In this evaluation, Aivlosin® and tilmicosin performed similarly in managing the health of pigs in a commercial setting in the face of a natural PRDC challenge.

MIS - Miscellaneous and Clinical cases

# SOW MORTALITY ECONOMICS IS GETTING WORSE.

P. Lopes <sup>1</sup> <sup>1</sup>FMV-ULHT

# **Background and Objectives**

Looking at data from an European database (Spain, Italy, Portugal) covering 1.5 million sows in 200 pig production companies, between 2018-2023 sow mortality rate (on-farm death plus culling, not including slaughtered sows) increased from an average of 9,9% (SD 3,6%) to 16,4% (SD 5,6%).

In a group of 100 Portuguese swine farms it increased from 7,8% (SD 1,94%) to 10,53% (SD 2,87%).

The aim of this study is to determine the importance of the increased cost of sow mortality in the cost of the weaned piglet in this group of Portuguese farms.

# **Material and Methods**

Data from production costs (2018-2023) of 59870 sows from over 100 Portuguese farms were analyzed.

The average parity of dead/culled sows was estimated to be 2,8 parities.

Main causes for sow mortality in these farms were: sudden death (unknown cause), infectious arthritis, uterine and rectal prolapses, and post-partum anorexia non responsive to treatment.

The cost of one dead sow was calculated adding the value of purchase of a new gilt, the feed consumed, fixed costs (energy, labor, housing, other), medication, breeding.

#### Results

The cost of replacing an old sow for a new gilt (Portuguese database) increased from  $59 \in$  to  $74 \in$  (+25,4%), for a replacement rate of 51% per year.

The cost of one dead/culled sow with 2,8 parities in 2018 was 891€, in 2023 is 1235€ (+ 38,6%) .

Concerning sow mortality rate in 2023, the best/worse 15% farms (+/- 1 SD) ranged from 7,6% to 13,3%.

For a 500 sow farm, producing 28,8 weaned piglets/ year, this difference in sow mortality alone adds a cost of 2,4€ per weaned piglet.

# **Discussion and Conclusion**

The following conclusions apply only to the study group of Portuguese farms (60K sows).

On-farm sow mortality rate has increased significantly in the last 5 years, from an average of 7,8% to 10,53%.

The cost of on-farm dead/culled sows increased 38,6% in 5 years (1235€/sow in 2023).

A difference of +5,7% in annual sow mortality rate had an extra cost of 2,4€/piglet weaned (comparing low (7,6%) to high (13,3%) mortality in 2023).

MIS – Miscellaneous and Clinical cases

# WHAT IS THE BEST DISINFECTION PROTOCOL FOR INJECTION DEVICES: AN OBSERVATIONAL STUDY

E. Abily<sup>1</sup>, C. Teixeira-Costa<sup>2</sup>, J. Jeusselin<sup>3</sup>, C. Chevance<sup>3</sup>, V. Normand<sup>3</sup>, T. Nicolazo<sup>2</sup>, G. Boulbria<sup>3</sup>, M. Brissonnier <sup>3</sup>, J. Favrel<sup>3</sup>, J. Da-Costa<sup>3</sup>, A. Lebret<sup>3</sup> <sup>1</sup>Oniris VetAgroBio, Nantes, 101 route de Gachet, 44300 Nantes, France

<sup>2</sup> Rezoolution Pig Consulting Services, Noval-Pontivy, France

<sup>3</sup> PORC, SPECTIVE, Swine Vet practice, Noval-Pontivy, France

# **Background and Objectives**

The aim of this trial was to investigate the impact of two modalities of cleaning and disinfection of injection devices on bacterial contamination. Indeed, sow neck injuries are commonly linked with bacterial contamination at injection.

# Material and Methods

Thirty nine syringes from 14 commercial herds have been collected. A randomization table allowed to assign one modality of cleaning and disinfection to one syringe. Two modalities were tested: the first one (Group 1), internal and external cleaning with warm water and soap and, for the second one (Group 2), the same as Group 1 followed by a disinfection with wet steam. Before and after cleaning, 2 mL of sterile water were used to rince each syringe to investigate internal contamination and external contamination was evaluated after swabbing the surface of each syringe. Swabs were suspended in 2 mL sterile distilled water (pH 7). Samples were 10-fold serially diluted three times in sterile distilled water and plated using the pour plate method on plate count agar medium and incubated at 37°C. Heterotrophic plate counts were recorded 24 hours later.

# Results

Before cleaning and disinfection, respectively 12, 14 and 13 syringes presented a low (0-10 UFC/mL), moderate (11-5000) and high (>5000) internal contamination. Regarding external contamination, 21 presented a low (0-5000) and 17 a high (>5000) level of contamination (one missing data). In group 1, 8/18 syringes had a moderate to high level of internal contamination after cleaning procedure. In group 2, 16 syringes had a low level of internal contamination and 5 a moderate one after disinfection even if 14 /21 of them presented a moderate to high level of contamination before. Regarding external contamination, in group 2, all syringes (n=7) highly contaminated before disinfection had a low level of contamination after. In group 1, it represented only 8/10 syringes.

# **Discussion and Conclusion**

Our results highlight the high level of contamination of syringes in the field. Our study needs further investigations but we show that cleaning with soap followed by a steam disinfection seems to be an efficient solution to maintain a reasonable level of injection device contamination after usage without using any chemical product.

# BACTERIOLOGY AND BACTERIAL DISEASES

# BBD-PP-01

BBD – Bacteriology and Bacterial Diseases

# MOLECULAR EPIDEMIOLOGY OF GLAESSERELLA PARASUIS STRAINS COLLECTED FROM DISEASE ASSOCIATED CASES IN NORTH AMERICAN SWINE PRODUCTION SYSTEMS REVEAL HIGH LEVELS OF SPECIES HETEROGENEITY

R. Mugabi <sup>1</sup>, A.P. Silva <sup>1</sup>, X. Hu <sup>1</sup>, M. Gottschalk <sup>2</sup>, V. Aragon <sup>3</sup>, N. Macedo <sup>1</sup>, O. Sahin <sup>1</sup>, R. Main <sup>1</sup>, P. Harms <sup>5</sup>, A. Tucker <sup>4</sup>, G. Li <sup>6</sup>, M.J. Clavijo <sup>7</sup> <sup>1</sup>*lowa State University, Veterinary Diagnostic and Production Animal Medicine* <sup>2</sup>*University of Montreal, Canada* <sup>3</sup>*Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, UniversitatAutònoma de Barcelona (UAB), Campus, Bellaterra, Barcelona, Spain* <sup>4</sup>*University of Cambridge, Cambridge, UK* <sup>5</sup>*PIC North America, Hendersonville, TN 37075* <sup>6</sup>*lowa State University, Ames, USA* <sup>7</sup>*lowa State University* 

#### **Background and Objectives**

Glaesserella parasuis is the causative agent responsible for Glässer's disease in pigs. The high prevalence of non-typeable strains and limited discriminatory power of serotyping pose challenges in identification of pathogenic trains. The goal of this study was to conduct genetic characterization of G. parasuis strains isolated from disease-associated cases using whole-genome sequencing.

#### **Material and Methods**

In this published study, we conducted WGS on 218 field clinical isolates and 15 G. parasuis reference strains. We determined multilocus sequence types, serotypes, core-genome phylogeny, antimicrobial resistance (AMR) genes, and putative virulence genes.

#### Results

In silico WGS serotyping identified 11 out of 15 serotypes, with the most commonly detected ones being 7, 13, 4, and 2. MLST identified a total of 72 sequence types (STs), with 66 of them being novel. The most prevalent among these was the newly discovered ST454, a strain associated with high mortality events in the US. All isolates carried at least one group 1 vtaA gene, except for serotype 8 (ST299 and ST406), serotype 15 (ST408 and ST552), and non-typeable strain (ST448). Some group 1 vtaA genes showed significant associations with specific serotypes or STs. The core-genome phylogeny revealed three lineages (LI, LII, and LIII), and isolates within the LIIIA sublineage were found to lack all vtaA genes. The virulence gene lsgB was found in 8.3% of isolates, primarily serotype 5/12. While most isolates carried the bcr, ksgA, and bacA genes, the following antimicrobial resistance genes were detected at lower frequencies: blaZ (6.9%), tetM (3.7%), spc (3.7%), tetB (2.8%), bla-ROB-1 (1.8%), ermA (1.8%), strA (1.4%), qnrB (0.5%), and aph3''Ia (0.5%).

# **Discussion and Conclusion**

Core-genome phylogeny provided the best strain discrimination, compared to serotyping. These findings will contribute to an improved understanding of molecular epidemiology and virulence in G. parasuis, paving the way for future developments in diagnostic tools, autogenous vaccines, antibiotic use evaluation, disease prevention and control.

BBD - Bacteriology and Bacterial Diseases

# MOLECULAR SEROTYPING AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF ACTINOBACILLUS PLEUROPNEUMONIAE ISOLATED IN ITALIAN PIG FARMS FROM 2015 TO 2022

<u>F. Guarneri</u><sup>1</sup>, C. Romeo<sup>1</sup>, F. Scali<sup>1</sup>, S. Zoppi<sup>2</sup>, N. Formenti<sup>1</sup>, A.M. Maisano<sup>1</sup>, S. Catania<sup>3</sup>, M. Gottschalk<sup>4</sup>, G.L. Alborali<sup>1</sup> <sup>1</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy <sup>2</sup>Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy <sup>3</sup>Istituto Zooprofilattico Sperimentale della Venezie, Legnaro, Italy <sup>4</sup>Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Québec, Canada

#### **Background and Objectives**

Actinobacillus pleuropneumoniae (APP) is a gram-negative bacterium and a major cause of respiratory diseases in pigs. The acute form of the disease is highly contagious, often fatal, and causes significant economic losses. Based on capsular antigens, 19 APP serotypes are currently recognised. Serotype diversity and antimicrobial resistance (AMR) of APP strains circulating in north Italian farms from 2015 to 2022 were retrospectively evaluated to investigate the epidemiology of APP in the area.

# Material and Methods

A total of 572 strains isolated from outbreaks occurring in 337 different swine farms were analysed. The strains were analysed by PCR to define the serotypes and by broth microdilution to determine the minimum inhibitory concentration (MIC) of 11 antimicrobials.

#### Results

The majority of isolates belonged to serotypes 9/11 (39%) and 2 (28%). Serotype diversity increased over the study period, from four different serotypes isolated in 2015 to nine in 2022. The most common resistances were against tetracycline (53% of isolates) and ampicillin (33%), followed by enrofloxacin, florfenicol and trimethoprim/sulphamethoxazole (23% each). Multidrug resistance (MDR) was common, with 31% of isolates showing resistance to more than three antimicrobial classes. Resistance to the different classes and MDR varied significantly depending on the serotype, with serotype 9 showing the highest MDR rates (46%).

### **Discussion and Conclusion**

Our evaluation of APP strains revealed serotype-dependent AMR patterns and an increasing serotype diversity over the years. Although serotypes 9/11 and 2 were the most common, other serotypes seem to be emerging, especially in areas with higher pig density. Resistance to aminopenicillins, sulphonamides and tetracyclines was high. These antimicrobials should be used with caution to avoid a further increase of AMR. Resistance to amphenicols and fluoroquinolones was of concern and should be monitored also considering their relevance for human therapy. Although pig vaccination can mitigate disease severity, it is hindered by the increasing strain diversity, and antimicrobial treatment remains thus pivotal for effective APP control. This study highlights therefore the importance of serotyping APP to implement tailored antimicrobial therapies, which can be effective against the disease without promoting the spread of AMR.

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# ACTINOBACILLUS PLEUROPNEUMONIAE SEROTYPE 15 LONGITUDINAL PERSISTENCE IN NATURALLY INFECTED PIGS AFTER AN OUTBREAK

M. Almeida<sup>1</sup>, A. Michael<sup>2</sup>

<sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

<sup>2</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

### **Background and Objectives**

Actinobacillus pleuropneumoniae serotype 15 (App15) was responsible for a severe outbreak of respiratory disease with high mortality in finishing sites in central IA, USA at the end of 2021 involving multiple companies in a small geographic area. The atypical regional spread raised questions about the dynamic of this strain within pig populations. This study aimed to determine the duration of App15 persistence in convalescent pigs using different sample types.

#### **Material and Methods**

In a three-barn site, each containing 1200 pigs, 67 pigs (one per pen) were tagged and sampled weekly for 6 weeks using nasal swabs (NS), tonsil scrapings (TS), and serum samples (SS); pen-based oral fluids (OF) were also collected. Sampling started 3 weeks after the reported outbreak. Swabs and OF were tested for App detection by rtPCR, while serum was screened with the Swinecheck mix-APP ELISA.

#### Results

All 67 pigs tested App positive in TS  $\geq$  1 during the sampling period. Twenty-five pigs (37.3%) tested positive for TS in all time points. Only 23 pigs (34.3%) were positive in NS  $\geq$  1 during the sampling period. Forty-six of 48 (95.8%) were App positive in TS at the last time point, 8 weeks after the reported outbreak. All 48 pigs tested negative for App in NS on the last time point. Fifty-three of 73 pens (72.6%) tested App positive in OF  $\geq$  1 during the sampling period. Fifty-three of 23 pens (20.5%) only tested positive once. Detection in NS was highest at the first sampling point, with 51.2% of samples testing positive, while TS tested positive at 53.3% of samples in the first week, increasing positivity rate until the last time point with 95.8% positive rate. OF samples had the highest detection rate at the second sampling point with 54.2%, but notably 9 OF samples (14.1%) were found positive at the last sampling point. Seropositivity decreased from 93% on week 4 to 33% on week 8.

#### **Discussion and Conclusion**

Tonsil scrapings had the highest detection rate for detection of all time points evaluated and represent the best sample type for ante-mortem detection of APP.

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# DETERMINATION OF MINIMAL INHIBITORY CONCENTRATION OF TILMICOSIN AND TIAMULIN AGAINST THAI ISOLATES OF ACTINOBACILLUS PLEUROPNEUMONIAE, PASTEURELLA MULTOCIDA, GLÄESSERELLA PARASUIS AND STREPTOCOCCUS SUIS.

<u>P. Assavacheep</u><sup>1</sup>, S. Samngamnim<sup>1</sup>, S. Simjee<sup>2</sup>, A. Ratanamongkolmas<sup>3</sup>, W. Deeampai<sup>3</sup> <sup>1</sup>Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand <sup>2</sup>Elanco Animal Health, Greenfield, IN, USA <sup>3</sup>Elanco (Thailand) Ltd., Bangkok, Thailand.

### **Background and Objectives**

Major respiratory bacterial diseases found in pig production in Thailand include Actinobacillus pleuropneumoniae (A. pleuropneumoniae), Pasteurella multocida (P. multocida), Gläesserella parasuis (G. parasuis), and Streptococcus suis (S. suis). Tilmicosin and tiamulin have been widely used for the treatment and control of porcine pneumonia. Therefore, minimal inhibitory concentration of tilmicosin and tiamulin against Thai isolates of these bacteria was evaluated.

# **Material and Methods**

MIC testing of tilmicosin and tiamulin against field Thai isolates of A. pleuropneumoniae, P. multocida, G. parasuis, and S. suis was determined. A total of 200 Thai isolates, including fifty isolates each of A. pleuropneumoniae, P. multocida, G. parasuis, and S. suis, collected from commercial pig farms and necropsy cases during 2020-2022, were used. Tilmicosin was tested for MIC against A. pleuropneumoniae, P. multocida, and G. parasuis, whereas tiamulin was examined for S. suis.

#### Results

All A. pleuropneumoniae isolates, which were serotypes 5 and 2, had an average MIC of  $45.76 \pm 16.49 \,\mu$ g/ml, and MIC<sub>50</sub> and MIC<sub>90</sub> of 32 and  $\geq 64 \,\mu$ g/ml, respectively. The average MIC for P. multocida was  $26.56 \pm 26.66 \,\mu$ g/ml, with MIC<sub>50</sub> and MIC<sub>90</sub> of 8 and  $\geq 64 \,\mu$ g/ml, respectively. Most of the G. parasuis isolates, which were serotypes 5 and 4, had an average MIC of  $4.88 \pm 15.76 \,\mu$ g/ml and MIC<sub>50</sub> and MIC<sub>90</sub> of 0.125 and 4  $\mu$ g/ml, respectively. The average MIC of tiamulin against S. suis was  $64.60 \pm 37.16 \,\mu$ g/ml, with MIC<sub>50</sub> and MIC<sub>90</sub> of 64 and 128  $\mu$ g/ml, respectively.

#### **Discussion and Conclusion**

A notable increase in tilmicosin resistance among the bacteria studied in the Thai pig population in recent years should be noted. The observed increase in tilmicosin resistance among these bacteria in the Thai pig population in recent years may be attributed to the restrictions imposed on certain commonly used antimicrobials by Thai regulations since 2018. Although the MIC breakpoint of tilmicosin and tiamulin for G. parasuis and S. suis has not yet been established by CLSI, interpretative criteria for A. pleuropneumoniae and Haemophilus somnus, together with the epidemiologic cut-off value (ECV) and pharmacodynamic cut-off (COPD), might be suggested. Tilmicosin and tiamulin resistance should be further monitored.

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# EVALUATION OF THE ANTIMICROBIAL SUSCEPTIBILITY OF MYCOPLASMA HYOPNEUMONIAE ISOLATED FROM PIGS IN BRAZIL

S. Kuchiishi <sup>1</sup>, R. Frandoloso <sup>2</sup>, <u>J.A. Caon</u> <sup>3</sup>, J. Uriarte <sup>3</sup> <sup>1</sup>Centro de Diagnóstico de Sanidade Animal (CEDISA), Concórdia SC Brazil <sup>2</sup>Laboratory of Microbiology and Advanced Immunology, University of Passo Fundo, Passo Fundo RS Brazil <sup>3</sup>ECO Animal Health, The Grange, 100 High Street, London, UK

#### **Background and Objectives**

Mycoplasma hyopneumoniae (M. hyo) is one of the most prevalent respiratory pathogens in pig production causing enzootic pneumonia with significant production and economic losses. The objective of this study was to determine the Minimum Inhibitory Concentration (MIC) of several antibiotic active ingredients against eleven M. hyo strains isolated from pig farms in Brazil

# **Material and Methods**

Eleven M. hyo isolates from farms with respiratory clinical signs compatible with enzootic pneumonia between 2017 and 2020 from the Brazilian states of Minas Gerais, Paraná, Rio Grande do Sul and Santa Catarina were tested in this study. The strains were isolated from diseased lungs submitted to CEDISA and AFK Imunotech. The microdilution method described by Hannan et al. was followed using Sensititre <sup>™</sup> Microplates (Thermo Scientific<sup>™</sup>). After growing the M. hyo strains in FRIIS Liquid Medium, samples were incubated at 37°C until bacterial growth was confirmed by pH change. The inoculum was then diluted and incubated for 14 days at 37°C before inoculating the microplate wells. The mycoplasma inoculum contained between 10<sup>3</sup> and 10<sup>5</sup> CCU (Colour Changing Units)/ml. Each microplate contained nine active ingredients at different concentrations: tilmicosin, tiamulin, enrofloxacin, doxycycline, erythromycin, lincomycin, chlortetracycline, tylvalosin and tylosin tartrate. The MIC value was defined as the lowest drug concentration capable of inhibiting M. hyo growth.

#### Results

The MIC<sup>50</sup>/MIC<sup>90</sup> and range (in brackets) ( $\mu$ g/mL) were 2/4 (0.25-4) for tilmicosin, 0.06/0.25 (< 0.015-0.25) for tiamulin, 0.25/2 (< 0.03-2) for enrofloxacin, 0.5/1 (< 0.12-2) for doxycycline, 32/>64 (16-> 64) for erythromycin, 0.25/1 (< 0.12-16) for lincomycin, 8/16 (0.5-16) for chlortetracycline, <0.015/0.06 (< 0.015-0.12) for tylvalosin and 0.12/0.5 (< 0.06-1) for tylosin tartrate.

### **Discussion and Conclusion**

The M. hyo isolates had different sensitivity profiles to the tested antibiotics. Based on the results of MIC<sup>50</sup>/MIC<sup>50</sup>, tylvalosin had the lowest MIC values followed by tiamulin, tylosin tartrate and tilmicosin in agreement with other researchers along with a narrow MIC range. These findings suggest that tylvalosin would be a highly effective antibiotic for the treatment of M. hyo and enzootic pneumonia in Brazil.

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# GENETIC DIVERSITY AND DISTRIBUTION OF VIRULOMES AND RESISTOMES OF STREPTOCOCCUS SUIS IN PIGS IN NORTH AMERICA THROUGH WHOLE GENOME SEQUENCING

R. Rupasinghe<sup>1</sup>, R. Mugabi<sup>2</sup>, G. Li<sup>2</sup>, M.J. Clavijo<sup>2</sup>, B. Martínez-López<sup>1</sup>

<sup>1</sup>Center for Animal Disease Modeling and Surveillance (CADMS), Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, CA, United States

<sup>2</sup>Department of Veterinary Diagnostic & Production Animal Medicine (VDPAM), Iowa State University, Ames, IA, United States

### **Background and Objectives**

The evolutionary arms race between host and pathogens like Streptococcus suis drives pathogens to continually circulate, evolve, and produce highly virulent and resistant to existing therapeutics/vaccines. We aimed to evaluate the population structure and virulome and resistome profiles of 725 S. suis strains isolated from 405 swine farms in North America from 2014 to 2022 using whole genome sequences.

### **Material and Methods**

Genetic diversity was assessed using four typing methods: in-silico serotyping, Multilocus sequence typing, clonal complex classification, and maximum likelihood-based core-genome phylogenomics. Virulence-associated genes (VAGs) from the virulence factor database and antimicrobial-resistant genes (ARGs) from CARD and ResFinder databases were obtained. Multiple correspondence analysis and hierarchical clustering of principal components (HCPC) were conducted to explore the gene profiles of heterologous groups.

#### Results

Common molecular types were ST1(31.2%) and ST28(18.1%); serotypes 1(19.6%) and 9(8%); CC1(31.2%), CC28(21%), and singletons(8.4%); and CL1(31.2%), CL2(25.5%), and CL3(25%), each of which was significantly different from other according to their gene profiles ( $p \le 0.05$ ). Serotypes 2(10.5%) and 1/2(7.3%) were significantly different from other serotypes ( $p \le 0.05$ ) but not from each other (p > 0.05), inferring similar gene profiles between them. HCPC classified strains into three clusters; G1(52.3%), G2(12%), and G3(35.7%), which largely corresponded to VAGs rather than ARGs. G1 was mainly characterized by the presence of pavA, tet(32), tet(44), hhly3, ssa, srtA, etc., G2 by the presence of sat4A and srtB and the absence of SSU\_RS09525, ofs, and VM2, and G3 by the absence of plr/gapA, srtA, eno, dppIV, cpsB, capsule\_related, etc., (>85% strains across-cluster). VAGs: sly, epf, mrp, VM1, and VM2, were significantly more present in G1(74.9%, 39.6%, 96.6%, 97.9%, and 98.7%, respectively) and G3(not-significant, not-significant, 97.7%, 96.1%, and 96.1%, respectively), but less present in G2(9.2%, 2.3%, 13.8%, 0%, and 0%, respectively) ( $p \le 0.05$ ).

# **Discussion and Conclusion**

Detection of emerging variants, pathogenic strains (e.g., G1 and G3 strains), and distinct virulome profiles in heterologous groups provide a better picture of disease dynamics and virulence mechanisms, which will help to build effective preventive and control strategies (e.g., identify bacterial strains to develop novel autogenous vaccines).

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# WHOLE GENOME SEQUENCING SUPPORTS AN EPIDEMIOLOGICAL APPROACH TO CARRY OUT PRUDENT USE OF ANTIMICROBIALS FOR ACTINOBACILLUS PLEUROPNEUMONIAE

A. Vilaró <sup>1</sup>, E. Novell <sup>1</sup>, J. Baliellas <sup>1</sup>, V. Tarancón <sup>1</sup>, J. Guitart-Matas <sup>2</sup>, L. Migura-García <sup>2</sup>, <u>L. Fraile <sup>3</sup></u> <sup>1</sup>Grup de Sanejament Porci, Lleida, Spain <sup>2</sup>IRTA, Centre de Recerca e Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain <sup>3</sup>University of Lleida, Lleida, Spain

# **Background and Objectives**

Actinobacillus pleuropneumoniae (APP) causes significant economic losses to the swine industry. Antibiotic treatment can be challenging due to its clinical urgency and the turnover of antimicrobial susceptibility results from the diagnostic laboratory. The aim of this study was to evaluate the vertical transmission of APP within integrated systems as a proxy for optimising antimicrobial treatment in the field, using whole genome sequencing (WGS).

# **Material and Methods**

169 APP isolates from Spanish origin with known epidemiological relationships were selected to perform antimicrobial susceptibility testing, minimal inhibitory concentration (MIC) for twelve antibiotics of seven different families. Comparative genomic analysis was carried out using WGS to assess genetic similarity between different isolates. Moreover, phenotype (susceptible or resistant)-genotype (presence/absence of resistance genes or mutations in the case of quinolones) concordance for epidemiological cut off values (ECOFFs) and clinical breakpoints was evaluated with Cohen's Kappa statistics. A kappa coefficient (k) value above 0.6 was classified as high agreement. A Chi-Square Test was carried out to decipher the association between epidemiological relationship and phylogenetic group established by WGS. All the data analysis was carried out with JMP®, v13 (SAS Institute Inc., Cary, NC, USA, 1989–2019).

### Results

Concordance between presence of resistance genes (genotype) vs antimicrobial susceptibility pattern (phenotype) was high (>0.7) for amoxicillin and florfenicol using ECOFFs and clinical breakpoints for its interpretation. This agreement was lower than 0.6 for the rest of tested antibiotics (tetracyclines, sulfamides and fluoroquinolones) and not possible to analyse for macrolides, cephalosporins and pleuromutilins due to the low number of phenotypic resistant cases available in the database. On the other hand, isolates that shared a common origin (selection sow farm) were genetically similar, than those sharing a different origin, suggesting a vertical transmission of the pathogen within integrated systems.

## **Discussion and Conclusion**

Genomic comparative analysis enhances the understanding of transmission and epidemiological relation of this species and suggests vertical transmission of the pathogen, including the resistance genes, within integrated systems. These results validate an epidemiological approach for prudent use of antimicrobials using MIC as the most suitable pharmacodynamic parameter under field conditions.

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# ANTIMICROBIAL EFFICACY OF APRAMYCIN ADMINISTERED BY DRINKING WATER IN PIGS EXPERIMENTALLY INFECTED WITH BETA-HEMOLYTIC ESCHERICHIA COLI

A.S. Castro <sup>1</sup>, M.G.L. De Souza <sup>1</sup>, K.E.Z. Nubiato <sup>1</sup>, L.C. Penha <sup>1</sup>, D.O. Carneiro <sup>1</sup>, <u>G.M.S. Stingelin <sup>1</sup></u> <sup>1</sup>Farmabase Saúde Animal

#### **Background and Objectives**

Colibacillosis, caused by the Gram Negative bacteria - Escherichia coli, is an important disease in post-weaning piglets, it causes diarrhea, weight loss and even death in infected animals, generating significant economic losses in pig farming worldwide. Apramycin is an aminoglycoside antimicrobial with effect against Gram Negative bacteria. Therefore, the aim of the present study was to evaluate the efficacy of apramycin administered by drinking water at a dose of 20 mg/kg, once a day for 5 days, in pigs experimentally infected with Escherichia coli.

# **Material and Methods**

Twenty-four piglets (Sus domesticus) between 4.2 and 9.7 kg and 25 days of age were used, divided into two groups of 12 animals each, control group (T01) and treated group (T02). All animals were experimentally infected with a strain of beta-hemolytic Escherichia coli isolated from a clinical case. Two days after, a rectal swab was performed to confirm infection in both groups. After the infection confirmation, the treatment has been started in group T02 at the dosage mentioned. The animals were clinically daily evaluated, a rectal swab was performed on the last day of treatment and all animals were euthanized one day after the end of treatment, for evaluation and bacterial count in the gastrointestinal tract. Fisher's test was performed to qualitative data and t-test for quantitative data using the Sigma plot program.

#### Results

There was statistical difference (p<0.05) between groups in the diarrhea on the last day of treatment; positive animals in the rectal swab on 3 and 5 days of treatment (T01: 12, 12; T02: 6, 5 respectively), presence of macroscopic lesions postnecropsy (T01: 7; T02: 2) and bacterial counts on the intestinal contents (T01: 3792 CFU/g; T02: 28 CFU/g), mesenteric lymph nodes (T01: 13324 CFU/g; T02: 65 CFU/g g) and stomach mucosa (T01: 848 CFU/g; T02 418 CFU/g). In all the parameters, the results were significantly lower in T02 group.

#### **Discussion and Conclusion**

The treated group presented a reduction in the clinical signs and a decrease of the etiological agent during and after treatment. It is possible to conclude that apramycin was effective against colibacillosis at the dosage tested.

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# EVALUATION OF STREPTOCOCCUS ZOOEPIDEMICUS PATHOGENICITY IN NURSERY PIGS

<u>N. Macedo</u>, E. Burrough <sup>1</sup>, K. Krueger <sup>1</sup>, G. Li <sup>1</sup>, O. Sahin <sup>1</sup>, R. Main <sup>1</sup>, L. Bradner <sup>1</sup>, J. Goncalves <sup>1</sup>, A.P. Silva <sup>1</sup>, S. Brockmeier <sup>3</sup>, K. Lantz <sup>2</sup>, S. Hau <sup>3</sup>, P. Sitthicharoenchai <sup>4</sup>, R. Derscheid <sup>1</sup> *'lowa State University, Ames, USA* 

<sup>2</sup>Animal and Plant Health Inspection Service United States Department of Agriculture

<sup>3</sup>Agricultural Research Service, USDA

<sup>4</sup>Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

### **Background and Objectives**

Streptococcus equi subsp. zooepidemicus (SEZ) caused high mortality in pigs in the U.S. in 2019 for the first time, followed by other severe outbreaks in North America, which warranted further investigation. This study evaluated the pathogenicity of three SEZ strains in experimentally inoculated nursery pigs.

# **Material and Methods**

The strains used in this study included a strain involved in cases of high mortality outbreak (TN), the reference ATCC 35246 strain, which is genetically closely related to TN and caused high mortality events in Asia, and a strain isolated from a feral pig (AZ), genetically different from the others. Challenged pigs (n=8) received either TN, ATCC, or AZ strain, intranasally. The control pigs (n=6) were mock inoculated. Pigs were monitored daily, and a post-mortem evaluation was performed up to 10 DPI (days post-inoculation) as needed.

#### Results

Respiratory and systemic clinical signs were observed beginning 1 DPI in all pigs challenged with the TN and ATCC strains. Pigs challenged with the AZ strain had mild fever. SEZ was detected in the nasal cavity and tissues by culture and PCR up to 9 DPI in TN and ATCC groups but only in the nasal cavity at 1 DPI in the AZ group. Bacteremia was detected in pigs from TN and ATCC groups up to 4 DPI. The mortality rate (8/8) of the TN group was statistically higher (P<0.001) than the control (0/6) and AZ groups (0/8), but not different from the ATCC (4/8) group. Lung consolidation and polyserositis were evident in all pigs in the TN group and 7/8 pigs in the ATCC group. Pigs challenged with the AZ isolate had enlarged lymph nodes. Histopathologically, serositis, vasculitis, tonsillitis, meningitis, encephalitis, and pneumonia were observed in TN and ATCC groups. Interestingly, the most common finding in pigs challenged with the AZ strain was mild encephalitis.

#### **Discussion and Conclusion**

This is the first study to reproduce the disease in nursery pigs with SEZ. Additionally, pathogenicity differences between genetically different SEZ swine strains were described. This experimental model will allow for further investigation of the pathogenesis of SEZ in swine and the development of methods for preventing and controlling this emerging pathogen.

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# EVALUATION OF THE SENSITIVITY OF FECAL PCR AND DIRECT PLATE CULTURE FOR THE DETECTION OF MONOPHASIC SALMONELLA ENTERICA SEROVAR I 4,[5],12:I:-

<u>F. Leite</u><sup>1</sup>, N. Macedo<sup>2</sup>, L. Gimenez-Lirola<sup>3</sup>, G. De Souza E Silva<sup>4</sup>, K. Crawford<sup>5</sup>, K. Schwartz<sup>6</sup>, S. Bearson<sup>7</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Inc, USA

<sup>2</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

<sup>3</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

<sup>4</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

<sup>5</sup>VRI-AMVC, Ames, IA

Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>7</sup>USDA, ARS, National Animal Disease Center, Food Safety and Enteric Pathogens Research Unit, Ames, IA

# **Background and Objectives**

Salmonella infection can lead to clinical disease in pigs and pose human risk as a foodborne pathogen. The purpose of this study was to compare the diagnostic sensitivity of qPCR and direct plate culture in pigs experimentally inoculated with monophasic Salmonella I 4,[5],12:i:-.

#### **Material and Methods**

Forty pigs were randomly assigned to one of two treatment groups, negative control and Salmonella inoculated. Fecal samples were collected individually from all pigs and tested at -1, 2, 3, and 7 days post inoculation (dpi) for the presence of Salmonella by 1) enrichment culture (EC) with selective antimicrobials specific for the challenge strain, 2) direct plate culture (DPC), and 3) qPCR. EC served as the positive control for fecal shedding status. Clinical signs were monitored and recorded daily for each pig.

### Results

All fecal samples collected at -1 dpi tested negative for Salmonella; all negative control group fecal samples remained negative throughout the study. All (60/60) fecal samples from inoculated pigs were EC-positive for Salmonella. DPC had a sensitivity of 56% (34/60) which was significantly greater than that of qPCR, which detected 6.7% (4/60) of positive fecal samples (p<0.05). Seventeen of the inoculated pigs had diarrhea at the time of sampling. In those pigs, DPC detected Salmonella in 82% (14/17), whereas qPCR detected only 23.5% (4/17) of samples as positive (p<0.05). When considering the identification of clinical disease, DPC and qPCR had positive predictive values (PPV) of 41.18% and 100% and negative predictive values (NPV) of 88.46% and 76.79%, respectively.

# **Discussion and Conclusion**

Although fecal qPCR had a high PPV, it only identified Salmonella in very few cases of diarrhea and demonstrated very low sensitivity in detecting fecal shedding. DPC demonstrated a high NPV suggesting negative results would be found mostly in cases when clinical disease due to Salmonella is absent. Neither qPCR nor DPC accurately reflected a pig's true Salmonella shedding status. These findings should be considered when selecting the diagnostic test to perform and when interpreting results.

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# INOCULATION OF NASAL COLONIZERS IN NEWBORN PIGLETS CAN RESTORE THE NASAL MICROBIOTA FROM THE LONG-TERM EFFECT OF CEFTIOFUR TREATMENT OF SOWS

M. Blanco-Fuertes <sup>1</sup>, <u>M. Sibila</u> <sup>1</sup>, G. Franzo <sup>2</sup>, P. Obregon-Gutierrez <sup>1</sup>, F. Illas <sup>3</sup>, F. Correa-Fiz <sup>1</sup>, V. Aragón <sup>1</sup> <sup>1</sup>*IRTA Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Spain* <sup>2</sup>*Dept. of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy.* <sup>3</sup>*Selecció Batallé, Av. Segadors s/n, Riudarenes, 17421 Girona, Spain* 

#### **Background and Objectives**

The nasal microbiota plays an important role in the respiratory health of piglets, but it is also a reservoir of pathogens that can cause polyserositis, such as Glaesserella parasuis, Mycoplasma hyorhinis or Streptococcus suis. Metaphylactic antibiotic treatment has been used to control these diseases. This way of administration is no longer acceptable since may increase antibiotic resistances and also compromise the microbiota of young animals. Here, we assessed the effect of ceftiofur and inoculation of nasal probiotics on the respiratory health of piglets and the nasal microbiota.

# Material and Methods

In a swine farm with respiratory problems, where ceftiofur was applied to pregnant sows or piglets, we followed the piglets of 60 sows from birth to 8 weeks of age. Nasal swabs were collected at birth, and at 7, 15, 21 and 49 days of age, and were used for pathogen detection, 16S rRNA sequencing and shotgun metagenomics. Clinical signs and production parameters were also recorded during the study.

#### Results

The administration of ceftiofur induced dysbiosis and colonization by environmental taxa at day 7. Ceftiofur treatment had a longer-term effect on the piglet's nasal microbiota when it was administered to the sow than directly to the piglet. This effect was partially reverted by inoculation of selected nasal colonizers to newborn piglets and was accompanied by a reduction in the number of animals showing clinical signs (mainly lameness). The prevalence of resistance genes increased over time in all groups but was significantly higher at weaning when the antibiotic was administered to the sows. Also, ceftiofur treatment induced the selection of more beta-lactams resistance genes when it was administered directly to the piglets.

# **Discussion and Conclusion**

Ceftiofur treatment by itself did not improve the health status or the productivity of the piglets, supporting the elimination of metaphylactic treatments without damaging production. The clinical benefit observed in inoculated piglets born to treated sows may be due to a reduction of the transmission of pathogens from the sows, which were replaced by the inoculated natural members of the microbiota. The inoculation of beneficial colonizers might represent a strategy to improve pig's health by using a non-invasive alternative to antibiotics.

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# LONGITUDINAL STUDY ON THE ROLE OF COLOSTRUM UPTAKE FOR BACTERICIDAL IMMUNITY AGAINST STREPTOCOCCUS SUIS AFTER PREPARTURIENT SOW VACCINATION WITH AN AUTOGENOUS VACCINE

T. Middendorf 1, J. Hallbauer 2, A. Maas 1, C.G. Baums 2

vivet Schweinegesundheit GmbH, 59590 Geseke, Germany

<sup>2</sup>Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, Leipzig University, 04103 Leipzig, Germany

#### **Background and Objectives**

Streptococcus suis causes frequently severe herd problems in piglet rearing with meningitis and arthritis as main pathologies. These herd problems are often caused by distinct invasive S. suis pathotypes. There is no approved S. suis vaccine in Europe. The use of autogenous vaccines is very common. These autogenous vaccines are often applied prior farrowing. Preparturient sow vaccination with an autogenous S. suis serotype 2 bacterin has been shown to protect piglets until the 6th week-of-life against the homologous strain. In this study we investigated the working hypothesis that there are significant differences in bactericidal immunity against S. suis between piglets within a herd vaccinated with an autogenous S. suis bacterin pre farrowing. These differences are presumably related to specific antibody levels in colostrum as well as quantity of colostrum uptake.

# **Material and Methods**

In this ongoing project we determine colostrum uptake quantitatively in piglets of a herd with 450 Hypor sows that uses preparturient vaccination with a S. suis serotype 1 and 2 bacterin to control S. suis disease in hybride piglets (PIC 408 boars). Parity defines three equally represented groups of sows in this project: gilts, younger old sows (2nd to 4th litter) and older old sows (5th or later litter). Three piglets are examined from each selected litter. Overall, 72 piglets are specifically investigated at predefined time points between birth and the 10th week-of-life. In addition to clinical screenings, serum and saliva samples are investigated in ELISA to read out specific IgG, IgM and IgA antibody levels against different S. suis antigens. Bactericidal assays are conducted to determine opsonizing antibodies leading to killing of specific S. suis pathotypes.

# Results

Preliminary data suggests that litter affiliation is a major determinant of bactericidal immunity in suckling piglets. Data of bactericidal immunity in porcine blood will be correlated with data on specific antibody levels in colostrum and the quantity of colostrum uptake. Detection of the invasive pathotype in saliva samples will be compared with specific IgA levels.

# **Discussion and Conclusion**

Data will be discussed in relation to management of herds including cross-fostering and selection of sows with high levels of opsonizing antibodies after preparturient sow vaccination.

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# NEW EPIDEMIOLOGICAL SCENARIO OF INFECTIONS CAUSED BY BACTERIA OF THE GENUS ACTINOBACILLUS IN BRAZILIAN SWINE PRODUCTION

G. Pinheiro Paes <sup>1</sup>, J. Viktor De Paula Barros Baêta <sup>1</sup>, <u>L. Fernando Dos Santos <sup>1</sup></u>, D. Lúcio Dos Santos <sup>1</sup>, W. Vieira Guimarães <sup>1</sup>, J. Lúcio Dos Santos <sup>1</sup>

<sup>1</sup>MICROVET - Microbiologia Veterinária Especial

# **Background and Objectives**

Bacteria of the genus Actinobacillus are agents of global importance for the swine industry, causing respiratory and systemic diseases. Currently, records of increased mortality due to Actinobacillus suis (A. suis) infection in pigs in Brazil have been reported. A.suis has the potential for causing lesions that resemble those caused by APP due to the similar production of ApxI and ApxII toxins. This similarity can pose a challenge in accurately diagnosing A.suis infections, potentially leading to diagnostic errors and underestimation of A. suis diagnostic. The objective of this work was to analyze the prevalence of APP and A.suis isolation in Brazilian swine production.

#### Material and Methods

From January 2019 to September 2023, a total of A.suis (n=1345) and A. pleuropneumoniae (n=2067) isolates were identified in the Veterinary Diagnostic Laboratory of Microvet database, coming from sick animals. The identification of isolates was carried out using a MALDI-TOF mass spectrophotometer. Assessment of the pathological aspects (macro and microscopic) of the lung samples was carried out to characterize the lung lesions.

#### Results

The number of diagnosed cases of A. pleuropneumoniae showed a slight decreasing trend over the analyzed years, while the number of cases of A. suis increased considerably, rising from 12.37% (2019) to 27.8% (2022). Samples isolated from abscess, lung and pleuritis together represented 84.8% and 59.6% of the total isolation of APP and A. suis, respectively. The microscopic lesions analyzed were similar for both agents and consisted of necropurulent bronchopneumonia with oat cells and fibrinopurulent and hemorrhagic pleuritis. There was a prevalence of A. suis isolation in finishing animals (71-180 days of age), increasing from 7.8% (2019) to 21.7% (2022).

### **Discussion and Conclusion**

The analysis of the number of cases with infections caused by A. suis showed an interesting, and perhaps new, trend, where the majority of isolations occurred at finishing. Episodes of increased mortality due to infections caused by A. suis may elucidate new disease patterns in swine production, in which the perception of A. suis as a pathogen in the swine industry has been changing over the years. And strategies to prevent and control the disease should consider using autogenous vaccines.

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# ORAL FLUID AS AN ALTERNATIVE TOOL TO DETECT LAWSONIA INTRACELLULARIS UNDER FIELD CONDITIONS

<u>S. Lopez</u><sup>1</sup>, L. Daluzeau <sup>2</sup>, D. Duivon <sup>2</sup>, L. Eon <sup>2</sup>, J. Sevin <sup>3</sup>, F. Voisin <sup>2</sup> <sup>1</sup>UNIVET SANTE ELEVAGE, rue Monge, 22600 Loudéac, France <sup>2</sup>MSD Animal Health, 49 Beaucouzé, France <sup>3</sup>Socavet, 75 boulevard de penthièvre, 22600 Loudéac, France

#### Background and Objectives

Lawsonia intracellularis (LI) is still causing significant damage in pigs. Faecal samples are usually tested by qPCR as an ordinary measure of the bacterial load, and hence, of the infection pressure. Saliva sampling method is recently getting more attentions due to its advantage as being more user friendly by veterinarians. The same applies for FTA cards where genetic material present in saliva is fixed. The aim of this study was to compare the sensitivity of qPCR when performed on saliva fixed in FTA cards, raw saliva, and faecal samples.

#### Material and Methods

Cross-sectional profiles were carried out on fatteners from 35 farms at 3 different ages. At each age, two chewing ropes were placed, and saliva collected, while 10 individual rectal swabs were taken from the same pigs. A drop of each saliva was then placed on an FTA-card spot. All in all, 210 FTA-cards spots, 210 oral fluids of raw saliva and 350 rectal swabs were all tested by qPCR for LI in the BactoReal Lawsonia kit of Ingenetix. PCR Cut-off was Cq=50. A Pearson Chi-squared test was applied to compare the qualitative PCR results (positive vs. negative) from the three methods. A two-factor analysis of variance model considering the effects of the profile (one age in one farm) and the method was applied to compare the PCR quantitative results.

#### Results

The mean, median, minimum, and maximum ages at sampling were 121, 119, 72 and 182 days respectively. Percentage of positive results were 72, 55 and 42 for FTA-cards, raw saliva, and swabs respectively, and were significantly different (p=0.0002). Average Cq was 40, 43 and 46 for FTA-cards, saliva's, and swabs, respectively, and were significantly different (p<0.0001). Cq median was 37, 44 and 50 for FTA-cards, saliva's, and swabs respectively.

#### **Discussion and Conclusion**

Under the conditions of our study, the type of sample has a significant influence on the result of the PCR test for Lawsonia intracellularis. Individual fecal swabs appear to be much less sensitive than salivary samples. Saliva is known to be more sensitive as it may represent environmental infection pressure.

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# ORAL VACCINATION AGAINST LAWSONIA INTRACELLULARIS BY GEL

J. Nerem <sup>1</sup>, A. Sponheim <sup>3</sup>, D. Hanson <sup>2</sup>, E. Little <sup>2</sup>, J. Rustvold <sup>3</sup>, R. Edler <sup>2</sup>, F. Leite <sup>3</sup> <sup>1</sup>Pipestone Veterinary Services, Pipestone, MN <sup>2</sup>Pipestone Research, Pipestone, MN <sup>3</sup>Boehringer Ingelheim Animal Health USA Inc.

# **Background and Objectives**

Oral vaccines have traditionally been administered through the drinking water and by oral drench. Oral drenching allows for vaccination in the sow farm; however, it requires the handling of each pig individually. Here we evaluated a novel method of oral administration by use of a gel. Advantageously, similar to drinking water, utilizing the gel allows pigs to be administered vaccine without being individually handled, saving time and labor. This study had the objective of evaluating the efficacy of oral administration of Lawsonia vaccine (Enterisol® Ileitis) by gel.

# **Material and Methods**

A randomized, controlled trial was conducted with four different treatment groups: 1) Non-vaccinated; 2) Lawsonia vaccinated by gel; 3) Lawsonia vaccinated by drinking water; 4) Lawsonia and Salmonella vaccinated by gel. Each treatment group was comprised of 45 litters and 540 pigs. The gel vaccine mixture was applied to the farrowing crate mat to allow for its consumption. Treatment group 3 received the same Lawsonia vaccine in the drinking water at 6 weeks of age. Experimental Lawsonia challenge was applied at 12 weeks of age. At this time point, each individual pig was weighed, and all four treatment groups were mixed per pen. Three pigs directly challenged with Lawsonia were added per pen to allow for the transmission and challenge of Lawsonia equally to all four treatment groups. The study ended when pigs were 20 weeks of age at which point each individual pig was weighed.

#### Results

Treatment group 1 had the lowest average daily weight gain (ADG) from challenge to final weight at 0.826 kgs. This was significantly (p<0.001) lower than the ADG observed in vaccinated treatment groups 2, 3 and 4 which were of 0.929, 0.916, and 0.920kgs, respectively. The percentage of removal and mortality in treatment group 1 was 7.45%, being significantly (p<0.01) higher than that observed in treatment groups 2 (4.31%), 3 (4.11%) and 4 (3.17%). No significant differences were observed between the vaccinated treatment groups in ADG, removal and mortality.

# **Discussion and Conclusion**

These results indicate that the oral administration of Lawsonia vaccine by gel is efficacious, with a similar efficacy to that of traditional vaccination through the drinking water.

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# PK/PD AND CLINICAL RELATIONSHIPS OF VETMULIN INJECTION (TIAMULIN BASE) ADMINISTERED TO PIGS FOR THE TREATMENT OF MYCOPLASMAL ARTHRITIS

<u>U. Klein</u><sup>1</sup>, M. Gyuranecz<sup>2</sup>, S. Catania<sup>3</sup>, L. Claerhout<sup>1</sup>, W. Depondt<sup>1</sup>, M. Gyuranecz<sup>4</sup> <sup>1</sup>Huvepharma NV Antwerp, Belgium <sup>2</sup>Veterinary Medical Research Institute, Budapest, Hungary <sup>3</sup>Istituto Zooprofilattico Sperimentale della Venezie, Legnaro, Italy <sup>4</sup>MolliScience Kft., Biatorbagy, Hungary

# **Background and Objectives**

Mycoplasmal arthritis infections in pigs can lead to significant economic losses due to lameness, swollen joints and poor growth. Tiamulin (Vetmulin<sup>®</sup> - Huvepharma NV) Injectable is registered for therapy of mycoplasmal arthritis infections. The pharmacokinetics (PK) of tiamulin after parenteral administration was compared, the synovial fluid concentrations (SFC) related to the minimum inhibitory concentration determined by testing of one hundred and six Mycoplasma hyosynoviae (Mhs) isolates and tiamulin's clinical efficacy were evaluated based on infection study data.

# **Material and Methods**

Tiamulin plasma concentration and SFC beside other specimen were determined in a pharmacokinetic study with forty healthy pigs (mixed breed, equally male/female, age 3-7 days) medicated once with tiamulin base at 15mg/kg bw. Plasma and synovial fluid concentrations were determined at pre-defined time points over 24hours. Tiamulin MICs (MIC<sub>90</sub>, MIC ranges) were determined based on testing of Mhs strains isolated 2018-2023 from various tissues (synovial fluid, lung, tonsils) in several EU countries (20 isolates/Austria/Belgium/Italy, 25 isolates/Germany, 21 isolates/Hungary).

#### Results

The tiamulin SFC was recorded at a consistent plateau concentration between 2-12 hours p.i. (average  $0.6\mu$ g/ml). A peak (Cmax) of  $0.77\mu$ g/ml was determined 4 hours p.i. Plasma tiamulin concentration were higher with a mean ratio plasma:SFC of 1.6:1. Tiamulin MIC<sub>90</sub> (<0.039) and MIC range (<0.039) for all tested Mhs strains were the same. No country-specific differences in the tiamulin susceptibility patterns of the Mhs strains were identified. Mhs strains from lung and synovial fluid show the same susceptibility as those isolated from tonsils. The tiamulin synovial fluid concentration exceeds the MIC<sub>90</sub> for approximately 24h recommended dose interval. The PK/PD results are in line with efficacy study data generated based on tiamulin parenteral administration in pigs at different age groups.

#### **Discussion and Conclusion**

Tiamulin Injection PK correlates well with its indication for mycoplasmal arthritis treatment. The use of PK/PD relationship data is an effective tool in predicting the efficacy of Tiamulin Injection in the case of mycoplasmal arthritis/joint infections in pigs. Clinical trial data verify the PK/PD data.

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# STUDY OF THE IMPACT OF GILT ACCLIMATION ON EPIDEMIOLOGY OF MYCOPLASMA HYOPNEUMONIAE IN FARMS IN BRITTANY, FRANCE

B. Boivent <sup>1</sup>, P. Gambade <sup>1</sup>, S. Lopez <sup>2</sup>, S. Martins <sup>3</sup>, <u>J. Mora Franques</u> <sup>4</sup> <sup>1</sup>Sanders Bretagne Av. de Kergroise, 56100 Lorient, France <sup>2</sup>Univet Santé Élevage 2 bis Imp. Monge, 22600 Loudéac, France <sup>3</sup>ECO Animal Health The Grange, 100 The High Street, Southgate, London, United Kingdom <sup>4</sup>ECO Animal Health Ltd

# **Background and Objectives**

Uncontrolled Mycoplasma hyopneumoniae (Mhp) infection status on gilt entry to the farrowing unit is a known risk for increased Mhp prevalence in piglets at weaning, making gilt acclimatation a critical step in pig production. This study evaluated the prevalence of Mhp in Mhp-free gilts during acclimation on three French farms from the Brittanny Area.

# Material and Methods

Selected farrow-to-finish farms were in Brittanny, France. Farms A, B and C had 120,200 and 230 sows with 8, 8 and 14 gilts entering per batch, respectively. The source farms maintained negative Mhp status throughout the trial. Gilts were not treated with antibiotics during quarantine or upon entry to the destination farm. On Farm A, the quarantine building was separated from the breeding barn. On Farms B and C, there were quarantine rooms within the breeding barn. The quarantine facilities were managed all-in-all-out with cleaning and disinfection between batches. Three gilt batches for Farms A and B and two gilt batches for Farm C were tested. Tracheal mucus was collected from all gilts at the end of quarantine period and the week before entering farrowing rooms. Mhp quantitative PCR was performed on each sample (LABOFARM, Loudéac).

#### Results

On Farm A, Batch#1, 87.5% and 71.4% gilts were Mhp-positive at the end of quarantine and prior to farrowing, respectively. All gilts from Batch#2 were Mhp-negative at both collection points. All gilts from Batch#3 were negative at the end of quarantine but 11.1% were positive prior to farrowing. On Farm B, all gilts were Mhp-negative at both collection points. On Farm C, Batch#1, 93% of gilts were Mhp-positive at the end of quarantine and all were Mhp-negative prior to farrowing. In Batch#2, all gilts were Mhp-negative at both collection points.

# **Discussion and Conclusion**

In this study, the quarantine process did not result in Mhp-negative gilts becoming positive in a consistent manner during the study period. Shedding gilts were also detected prior to farrowing on several occasions making it likely that their litters would be infected by weaning. These batches will be followed into their second parity to confirm their Mhp status over time.

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# UPDATE ON STREPTOCOCCUS SUIS SEROTYPES ASSOCIATED WITH SYSTEMIC DISEASE IN BRAZIL

V. Agnoletto Ribeiro <sup>1</sup>, E. Bresolin <sup>1</sup>, S. Ramos Prigol <sup>2</sup>, B. Fernandes Lenhard <sup>1</sup>, G. Paraboni Frandoloso <sup>2</sup>, J.A. Guizzo <sup>2</sup>, S. Satomi Kuchiishi <sup>3</sup>, A.P. Mori <sup>4</sup>, M. Tamanini <sup>4</sup>, M. Weiss <sup>2</sup>, L.C. Kreutz <sup>5</sup>, <u>R. Frandoloso <sup>5</sup></u> <sup>1</sup>Laboratory of Microbiology and Advanced Immunology, University of Passo Fundo, Passo Fundo, Brazil <sup>2</sup>AFK Imunotech, Passo Fundo, Brazil <sup>3</sup>CEDISA, Concórdia Brazil <sup>4</sup>Vaxxinova, Vargem Grande Paulista, Brazil <sup>5</sup>Laboratory of Microbiology and Advanced Immunology, University of Passo Fundo & AFK Imunotech, Passo Fundo, Brazil

### **Background and Objectives**

Streptococcus suis is an early colonizer of the upper respiratory tract of pigs, which in specific situations (virulence of the clinical strain and lack of protective immunity) can produce an acute-severe systemic disease in piglets during the first weeks of nursery. S. suis has assumed leading position among the sanitary challenges of nursery phase in Brazil. Here, our goal was to perform capsular molecular typing of S. suis associated with outbreaks of systemic disease.

# Material and Methods

A total of 417 outbreaks of systemic disease (arthritis and meningitis) caused by S. suis in 32 – 63 days-old piglets were analyzed. The outbreaks occurred in the years 2020 (n=23), 2021 (n=82), 2022 (n=199), and 2023 (n=113) in the Brazilian states of Mato Grosso-MT (n=16), Minas Gerais-MG (n=36), Paraná-PR (n=174), Rio Grande do Sul-RS (n=61), Santa Catarina-SC (n=123) and São Paulo-SP (n=7). During necropsy, samples from systemic sites were collected and plated on blood agar plates. Subsequently, colonies were molecularly analyzed (PCR) to confirm genus, species, and capsule type (Okura et al., 2014). The results are expressed as the prevalence (%) of S. suis serotypes (SV) associated with systemic disease in piglets in Brazil.

# Results

Seventeen serotypes of S. suis were identified in addition to a significant number of non-typeable (NT) strains. Serotypes 9, 2 and 1/2, 1 and 14, and 7 were the most prevalent, found in 61.2%, 9.1%, 4.8% and 3.4% of the outbreaks studied, respectively. The prevalence of other SV was: SV3 (2.2%); SV12 (1.2%); SV4, SV16 and SV18 (1.0%); SV8, SV23, and SV31 (0.5%); SV11, SV21, SV24, SV27 and SV29 (0.2%); and NT 12.7%.

# **Discussion and Conclusion**

Outbreaks of systemic disease caused by S. suis in nursery piglets have become the main health challenge in Brazilian pig farms. Here, S. suis serotype 9 was associated with 61.2% of the outbreaks, and it was distributed in the states of PR, SC and RS, in which 85.85% of the outbreaks occurred. In this scenario, management strategies combined with the use of effective vaccines should pave the way to reduce losses caused by S. suis in Brazil.

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# USE OF TECHNOLOGICAL TOOLS TO ASSESS MOVEMENT AND BODY TEMPERATURE OF PIGLETS EXPERIMENTALLY INFECTED WITH M. HYOPNEUMONIAE

K. Sonalio<sup>1</sup>, F. Boyen<sup>2</sup>, B. Devriendt<sup>3</sup>, I. Chantziaras<sup>4</sup>, I. Santamarta<sup>5</sup>, L.G. De Oliveira<sup>6</sup>, M. Dominiek<sup>7</sup>

<sup>1</sup>Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Belgium

<sup>2</sup>Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University <sup>3</sup>Department of Translational Physiology, Infectiology and Public Health, Faculty of Veterinary Medicine, Ghent University, Belgium

<sup>4</sup>Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

<sup>5</sup>Laboratorios Syva SAU, León, Spain.

<sup>6</sup>São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil <sup>7</sup>Faculty of Veterinary Medicine, Ghent University

#### **Background and Objectives**

Measurements of M. hyopneumoniae-related outcome parameters can be difficult, expensive, and time consuming, in both research and in field conditions. In addition to well-established methods, technological tools are becoming available to monitor various aspects of relevant animal- and environment-related features, often in real-time. Therefore, this study aimed at assessing whether certain parameters, such as animal movement and body temperature using microchips correlate with classical parameters.

#### **Material and Methods**

For this, 32 weaned piglets were equally allocated to three infection groups and a control group (NC). A thermal microchip was implanted intramuscularly in the neck. Pigs in the infected groups received a different high virulent M. hyopneumoniae strain on D0 and the same low virulent strain on D1. The respiratory disease score (RDS) was assessed daily, and the degree of movement by the animals was continuously measured by the healthy climate device. Mycoplasma hyopneumoniae DNA (M. hyo-DNA load), specific antibodies (anti-M. hyo antibodies), macroscopic (MLCL) and microscopic (MLL) lung lesions, and percentage of air were evaluated at D28. Finally, intramuscular temperature (IMT) was assessed daily.

#### Results

The movement was affected after the infection, in which the NC had 28.68% more chances of movement than the infected animals. The average values for IMT post-inoculation in the NC and infected animals were 39.59 °C and 39.70 °C, respectively. A median difference of 0.25 °C in intramuscular temperature was observed, where IMT was slightly higher than the RT. Statistical differences between the infected group and NC were observed for RDS, MLCL, MLL, dPCR, and ELISA. Moderate (0.42 to 0.59), strong (0.63 to 0.76), and very strong (0.84) correlations, were observed between most parameters, except for IMT post-inoculation.

#### **Discussion and Conclusion**

The results showcase that M. hyopneumoniae infection significantly reduced the movement of piglets; and that moderate to strong correlations were observed between all parameters, indicating a direct relationship between them. Therefore, we suggest that changes in movement can be a reliable indicator of M. hyopneumoniae infection in pigs under experimental conditions, and that a selected group of parameters, such as RDS, MLCL, MLL, M. hyo-DNA load, anti-M. hyo antibodies, and movement, is sufficient for the assessment of M. hyopneumoniae infection.

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# A PERFORMANT INDIRECT ELISA FOR THE DETECTION OF ANTI-M. BOVIS PORCINE ANTIBODIES IN SERUM OR FILTER PAPER SAMPLES

L. Olagnon <sup>1</sup>, A. Pages <sup>1</sup>, A. Limozin <sup>1</sup>, <u>L. Comtet</u> <sup>1</sup>, P. Pourquier <sup>1</sup> <sup>1</sup>IDvet – Innovative Diagnostics, Grabels, France

#### **Background and Objectives**

The Mycobacterium tuberculosis complex bacteria cause Tuberculosis (TB) in various hosts, including wild boar (Sus scrofa), a natural carrier of the disease. The ID Screen® Porcine Tuberculosis Indirect ELISA is specifically designed to detect antibodies against Mycobacterium bovis in porcine (wild boar and pig) serum and plasma. This flexible new iELISA also offers a performant filter paper sample application, allowing an easy and cost-effective sample collection. The kit includes microplates coated with Mycobacterium bovis recombinant protein and an anti-porcine IgG horseradish peroxidase (HRP) conjugate.

# **Material and Methods**

Diagnostic specificity was assessed using 651 pig samples—425 from wild boars and 226 from domestic pigs—originating from Bovine TB-free areas. Diagnostic sensitivity was evaluated on 16 wild boar serum samples, with 2 from France and 14 from Spain. The positive status of the French samples was determined through culture, while those from Spain were confirmed positive using another commercial ELISA (Kit A). Correlation with a commercially available ELISA kit (Kit A) was determined by testing 419 samples from both negative and infected herds. Additionally, 16 samples, comprising highly positive and negative samples, were tested in parallel on serum and filter paper samples (FPS). Threshold samples were included to assess the limit of detection.

#### Results

ID Screen® Porcine Tuberculosis Indirect measured specificity was 99.7 % Cl<sub>95%</sub> [98.9 -99.8], n=651. The percentage of correlation between ID Screen® Porcine Tuberculosis Indirect and kit A was greater than 98%, indicated very high correlation with Kit A. Comparable results were obtained for all samples, regardless of the sample type, meaning that serum and FPS can be used equivalently.

### **Discussion and Conclusion**

The ID Screen® Porcine Tuberculosis Indirect ELISA kit offers high specificity and efficient detection of positive animals, correlating well with other ELISA tests. Results are obtained in just 90 minutes, highlighting its speed and reliability for detecting porcine antibodies against Mycobacterium bovis. The kit is compatible with FPS, simplifying and reducing the cost of sample collection. The elution protocol allows fast sample treatment, and results indicate that FPS is a reliable alternative to serum samples for porcine TB detection.
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# CHANGES IN THE GUT MICROBIOME AT DIFFERENT STAGES OF SWINE DYSENTERY

L. Pérez-Pérez <sup>1</sup>, <u>H. Argüello</u><sup>1</sup>, S. Gómez-Martínez <sup>1</sup>, P. Rubio <sup>1</sup>, A. Carvajal <sup>1</sup> <sup>1</sup>Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, León, Spain

#### **Background and Objectives**

Swine dysentery (SD) is one of the most relevant enteric porcine diseases, characterized by a mucohaemorrhagic diarrhoea associated mostly with Brachyspira hyodysenteriae. Little is known about the role of other enteric bacteria in the large intestine in the disease pathogenesis. The objective of this study was to characterise the changes in the composition and diversity of the gut microbiome in early and acute SD.

## Material and Methods

Thirty-two 7-week-old pigs were randomly allocated into control (n=16) and infected (n=16) groups, the last orally challenged in three consecutive days with 1E+08 B. hyodysenteriae bacteria. Faecal and apex mucosal samples of animals at early infection (n=8), 24h after the first qPCR positive faeces to B. hyodysenteriae, or by the onset of mucohaemorrhagic diarrhoea, acute infection (n=8), were sequenced by Shotgun (faeces) or whole 16S rRNA (apex mucosal).

# Results

Both in faeces and mucosa, structural analysis of vector spatial distribution regression (P=0.001) and PERMANOVA (P<0.01) tests showed the influence of group in the samples ordination. Indeed, while at the mucosa differences were only observed for acute infection and control groups, ordination differences in faeces were observed between control and early infection compared to acute infection group. In line with this, species richness and Shannon alpha diversity index showed significant differences (P<0.001, P<0.05) in acute infection faeces and although in mucosa were not significant, a trend was observed. Regarding changes at taxonomic level, acute infection group showed an increased abundance of Campylobacter hyointestinalis, Acetivibrio ethanolgignens, Roseburia inulinivorans, Prevotella copri clade D and Prevotella pectinovora in faeces, in contrast to Megasphaera elsdenii and Limosilactobacillus reuteri that were lowered. On the other hand, in the mucosa, in addition to Acetivibrio ethanolgignens increased Campylobacter fetus and Tuzzerella, but abundance of Streptococcus alactolyticus, Lactobacillus amylovorus, Lactobacillus johnsonii, Megasphaera elsdenii and some species of Prevotella were lower.

# **Discussion and Conclusion**

The lower diversity and species richness observed in acute infection group, together with spatial differences with the other groups, show the impairment of faecal and mucosal microbiomes composition in pigs suffering SD, providing relevant information to the knowledge in pathogenesis and the involvement of the microbiome.

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# DETECTION OF ANTIBODIES INDUCED BY ORAL LIVE VACCINE AGAINST PORCINE LAWSONIA INTRACELLULARIS USING THE SLIDE STREPTAVIDIN BIOTIN (SSAB) INTERACTION METHOD

R. Uemura 1, M. Sueyoshi 1, K. Ishii 2, T. Ueda 2

<sup>1</sup>Laboratory of Animal Health, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Japan <sup>2</sup>Boehringer Ingelheim Animal Health Japan Co., Ltd.

## **Background and Objectives**

Enterisol® lleitis is a live attenuated bacterial vaccine intended to reduce the loss of body weight gain in pigs due to Lawsonia intracellularis infection. It is supposed to induce effective immunity by 3 weeks after the vaccine is properly administered orally. In most cases, however, the ELISA method now in use cannot measure vaccine antibody titer in serum. Therefore, we attempted to detect vaccine induced antibody in serum using the following method.

# **Material and Methods**

At two swine farms in Japan, the vaccine was administered to 10 pigs each at 3 weeks old, and blood samples were collected 4, 6, and 8 weeks later to measure serum antibody titers by ELISA. Serum antibodies were also attempted to be detected by the slide Streptavidin Biotin (sSAB) method. In this method, the live vaccine solution is solid-phased on a slide as antigen and reacted with the test serum, and the antibody in the test serum is evaluated by microscopic visualization using avidin-biotin interaction.

## Results

All samples tested negative for ELISA except one sample at 8 weeks after vaccine administration. In the sSAB method, on the other hand, 5 of the 20 pigs tested positive 4 weeks after vaccine administration, 7 after 6 weeks, and 2 after 8 weeks. In addition, 3 tested positive after 6 weeks but turn to negative after 8 weeks, and another 3 tested negative over the entire period of this study.

## **Discussion and Conclusion**

These results suggest the sSAB method can detect vaccine antibodies in serum with higher sensitivity than ELISA. On the other hand, since this method is a qualitative evaluation, it cannot be shown as an effective antibody titer, and it is necessary to consider how to ensure the specificity. Another challenge may be that it takes time and effort for slide preparation and microscopic observation, which cannot be automated. Although vaccine intake in drinking water administration may vary among individual pigs, we believe that this method can be used to evaluate whether vaccines are being administered appropriately on a herd basis.

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# DIAGNOSTIC APPROACHES TO PROPIDIUM MONOAZIDE-REAL-TIME QPCR FOR PORCINE PROLIFERATIVE ENTEROPATHY

H. Cho <sup>1</sup>, D. Tark <sup>2</sup>, D. Yoo <sup>3</sup>, B. Seo <sup>4</sup>, S. Lee <sup>4</sup>, C. Kim <sup>4</sup>, Y. Oh <sup>5</sup>

<sup>1</sup>College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan, Republic of Korea <sup>2</sup>Laboratory for Infectious Disease Prevention, Korea Zoonosis Research Institute, Jeonbuk National University, Iksan, Republic of Korea

<sup>3</sup>College of Veterinary Medicine, Chonnam National University, Gwangju, Republic of Korea

4WOOGENE B&G CO., LTD., Seoul, Republic of Korea

<sup>5</sup>College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon, Republic of Korea

#### **Background and Objectives**

Lawsonia intracellularis (LI) is an intracellular entero-pathogen causing two main clinical manifestations in pigs: an acute hemorrhagic form often called proliferative hemorrhagic enteropathy (PHE), and a more chronic proliferative form often referred as proliferative intestinal adenomatosis (PIA). The subclinical form of PHE could not have been recognized, which can be developed as subacute or chronic forms at any moment under stressful condition. Diagnosis of subclinical LI infection was dependent on serology and/or DNA quantification by qPCR. However, methods have limitations. Conventional qPCR could not differentiate viable LI from dead one. Thus, we evaluated whether PMA real-time qPCR is suitable for the early detection of viable LI in fecal samples of subclinical PHE and PIA cases.

#### Material and Methods

Field strains of L. intracellularis was grown in IEC-18 cells. Isolation of L. Intracellularis. LSI VetMAXTM Lawsonia intracellularis kit (Life technologies, USA) was used in this study. Dead LI were prepared by exposing live LI to 70% isopropyl alcohol for 30 min. Seven samples with dead:live ratios of 0:100 (live control), 10:90, 30:70, 50:50, 70:30, 90:10, and 100:0 (dead control) were prepared for real-time qPCR and cell culture. A total of 40 fecal samples from 20 clinical and 20 subclinical LI infection cases from weaners were subjected to qPCR with or without PMA and compare the delta Cts between culture-positive and culture-negative samples.

## Results

A total of 100 fecal samples from 10 clinical and 10 subclinical LI infection cases from weaners were subjected to qPCR with or without PMA and compare the deltaCTs between culture-positive and culture-negative samples. In 50 clinical LI case samples, the median deltaCT value of PMA-treated or non-PMA treated were 1.5 and 1.1, respectively, whereas that in 50 subclinical LI case samples was 5.8 and 1.7. It is postulated that subclinical LI infection cases contained more dead LI than those of clinical LI infection cases.

## **Discussion and Conclusion**

PMA real-time qPCR is a useful approach for differentiating dead from live LI in fecal samples and for determining higher risk in subclinical LI infection cases. Acknowledgement: This research was supported by a grant from the Next-Generation BioGreen 21 Program (PJ01322301), Rural Development Administration, Korea.

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# HIGH LEVELS OF ANTIMICROBIAL RESISTANCE IN LISTERIA MONOCYTOGENES IN PRODUCTS DERIVED FROM FREE-REARED PIGS

<u>A. Romero-Salmoral</u>, F. Jurado-Martos<sup>2</sup>, F. Cardoso-Toset<sup>2</sup>, L. Gomez-Gascón<sup>1</sup>, A. Galán-Relaño<sup>1</sup>, I. Luque<sup>1</sup>, C. Tarradas<sup>1</sup>

<sup>1</sup>Departamento de Sanidad Animal, UIC Zoonosis y Enfermedades Emergentes ENZOEM, Campus de Excelencia Internacional en Agroalimentación ceiA3, Universidad de Córdoba, España <sup>2</sup>CICAP- Centro de investigación agroalimentaria, Pozoblanco, Córdoba, España

### **Background and Objectives**

Listeria monocytogenes is an important food safety concern, also for the increasing detection of antimicrobial-resistant (AMR) isolates. This opportunistic pathogen can be isolated from the tonsils, gut and faeces of healthy pigs. The contamination of raw pork products and ready to eat (RTE) food has been detected, and large food-borne outbreaks with special importance for young, old, pregnant, and immunocompromised sensitive groups has been detected in Europe.A trend of increasing AMR has been observed in L. monocytogenes in animals. However, there is no official surveillance system for antimicrobial resistance in Listeria monocytogenes isolates exists, and published breakpoints for commonly used antimicrobials are limited. This work aims to provide information on the AMR of L. monocytogenes isolated from raw pork products.

#### Material and Methods

Forty-four L. monocytogenes strains from food products derived from free-range pig were isolated according to ISO 11290. The Minimum Inhibitory Concentration (MIC, MIC50 and MIC90) was determined against eight antimicrobials (Ampicilin, Cefotaxime, Erythromycin, Gentamicin, Penicilin, Sulfamethoxazole-Trimetoprim, Tetracycline and Vancomycin), following the methodology proposed by the CLSI (2019). Sensitivity to antibiotics was determined with cut-off points published by EUCAST (Sulfamethoxazole-Trimetoprim, Penicillin, Ampicillin and Erythromycin) was determined. GraphPad-Prism 8.0.1 software was used for statistical analysis.

### Results

MIC90 values were higher for Cefotaxime and Erythromycin ( $64 \ \mu g/mL$ ), compared to those obtained for Penicillin (8  $\mu g/mL$ ), Sulfamethoxazole-Trimetroprim (2  $\mu g/mL$ ), Ampicillin (2  $\mu g/mL$ ), Tetracycline (1  $\mu g/mL$ ), Vancomycin (0.5  $\mu g/mL$ ) and Gentamicin (0.25  $\mu g/mL$ ). According to the EUCAST, high levels of antimicrobial resistance against Sulfamethoxazole-Trimethoprim (100%), Erythromycin (47.7%), Ampicillin (45.45%) and Penicillin (25%) were detected. A total of 14 (31,81%) isolates were multidrug resistant (resistant to 3 or more antimicrobial).

# **Discussion and Conclusion**

High levels of antimicrobial resistance of L. monocytogenes isolates derived from free-reared pigs have been detected, with the risk for animal, human and environment health. The antimicrobial resistance patterns of the isolates should be included in the eurosurveillance programs, considering the emergence of this pathogen in Europe.

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# INSIGHTS INTO SEROTYPE AND SEQUENCE TYPE DIVERSITY OF STREPTOCOCCUS SUIS ISOLATES IN SWINE NEUROLOGIC DISEASE

J. Santos Streauslin<sup>1</sup>, K. Schwartz<sup>2</sup>, R. Derscheid<sup>3</sup>, D. Magstadt<sup>3</sup>, E. Burrough<sup>3</sup>, P. Gauger<sup>3</sup>, L. Schumacher<sup>4</sup>, M. Rahe <sup>5</sup>, A. Michael<sup>3</sup>, P. Sitthicharoenchai<sup>5</sup>, C. Siepker<sup>3</sup>, F. Matias Ferreyra<sup>6</sup>, M. Almeida<sup>7</sup>, R. Main<sup>8</sup>, L. Bradner<sup>2</sup>, X. Hu<sup>2</sup>, G. Li <sup>7</sup>, D. Nielsen<sup>9</sup>, A.P. Silva<sup>8</sup>, O. Sahin<sup>8</sup>, B. Arruda<sup>10</sup>

<sup>1</sup> Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>2</sup>Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>3</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>4</sup>Department of Pathology, Charles River Laboratories, Ashland, Ohio, USA

<sup>5</sup>Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

<sup>6</sup>Kansas State Veterinary Diagnostic Laboratory, Department of Diagnostic Medicine/ Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA

<sup>7</sup>Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, United States

<sup>8</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

<sup>o</sup>Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, United States Department of Agriculture, Agricultural Research Service, Ames, Iowa, USA

<sup>10</sup>Agricultural Research Service United States Department of Agriculture

#### **Background and Objectives**

Streptococcus suis poses significant challenges to swine health management due to its complex eco-epidemiology, incomplete implementation of a thorough diagnostic investigation to ascertain an isolate's clinical validity and limited information on the serotypes and sequence types associated with neurologic disease in the United States. Our study, spanning 2015 to 2019, aimed to address these gaps by analyzing isolates from the central nervous system (CNS) obtained through diagnostic submissions to lowa State University Veterinary Diagnostic Laboratory.

# **Material and Methods**

Metadata (n=29), including histologic evaluation, was aggregated and evaluated for 2,379 isolates from the CNS. We selected isolates with histologic evidence of CNS infection and employed standard and advanced techniques to identify S. suis serotypes. Furthermore, we selected 145 isolates for whole-genome sequencing to identify sequence type and five commonly used virulence-associated genes (VAGs: epf, mrp, sly, ofs, and srtF).

#### Results

Of the 2,379 S. suis strains originating from the CNS, nearly 60% originated from cases that did not have histologic evidence of CNS disease. Of the 1,032 submissions with evidence of CNS disease, all 29 S. suis serotypes were identified, in addition to four reclassified serotypes. Serotypes 1 and 7 emerged as predominant causes of CNS infection (32% of submissions). Additionally, 51 sequence types (STs), including 15 novel STs, were detected, with ST1 being the most frequently detected. Most disease-associated isolates lacked five commonly used VAGs. Additionally, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) misidentified 7% of isolates.

# **Discussion and Conclusion**

This study provides valuable insights for improved diagnostic and preventive strategies for managing swine Streptococcus suis infections. Our findings challenge the use of clinical signs and site of isolation alone for ascertaining the clinical validity of an S. suis isolate. Importantly, the unreliability of commonly used VAGs in defining the virulence capacity of S. suis isolates supports the continued use of classic tools such as microscopic evaluation. Additionally, the misidentification of isolates by MALDI-TOF MS suggests the need for complementary identification methods. The diversity of S. suis serotypes and STs associated with CNS infections identified in this study underscores the need for a more nuanced understanding of this pathogen.

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# PLANT-BASED PREMIX OF FEED ADDITIVES REDUCES BRACHYSPIRA HYODYSENTERIAE FECAL SHEDDING IN EXPERIMENTALLY INFECTED PIGS

M. Araujo <sup>1</sup>, P. Correia <sup>1</sup>, J. Barbosa <sup>1</sup>, M. Lourenço <sup>1</sup>, B. Oliveira <sup>1</sup>, L. Santos <sup>1</sup>, N. Oliveira <sup>1</sup>, D. Biasibette <sup>1</sup>, J. Bošnjak-Neumüller <sup>2</sup>, J. Raj <sup>2</sup>, M. Vasiljević <sup>2</sup>, <u>R. Guedes <sup>1</sup></u> <sup>1</sup>Departamento de Clinica e Cirurgia Veterinaria, Escola de Veterinaria, UFMG <sup>2</sup>PATENT CO DOO., Serbia

#### Background and Objectives

Essential oils (EO) are phytochemicals added to animal feed as potential substitutes for antimicrobial growth promoters<sup>1</sup>. Only recently the in vitro effects of EO over the causative agent of Swine Dysentery (SD), Brachyspira hyodysenteriae, have been evaluated<sup>2</sup>. However, no study has elucidated the in vivo effect of EO supplementation on this matter, which was addressed in this study.

# **Material and Methods**

Forty 21 days old piglets were assigned to the following groups (10 per group), housed in separated rooms: Positive (P), not supplemented and challenged with a pathogenic strain of B. hyodysenteriae; Negative (N), not supplemented and not challenged; B1, supplemented with 1kg/ton of a plant-based premix of feed additives (the formulation is propriety of PATENT CO. D.O.O Mišićevo) and challenged; and B2, supplemented with 2kg/ton and challenged. Eighteen days feed adaptation was offered prior challenge with a pathogenic B. hyodysenteriae strain. Stool samples were collected, and qPCR for B. hyodysenteriae was performed at different time points after the challenge.

#### Results

On the fifth and eight days after the initial challenge, both supplemented groups have reduced B. hyodysenteriae fecal shedding in comparison to P group (P<0.05), with a zero median for both supplemented groups and days, while P group had a median of 5.58 and 5.93, respectively, for the logarithmic conversion of mean Ct values.

## **Discussion and Conclusion**

Throughout this study, fecal shedding levels of both supplemented groups were intermediate between P and N groups, revealing a reduction in B. hyodysenteriae shedding, consequently reducing environmental contamination. This reduction can help control clinical cases of SD<sup>3</sup>, thus, reducing its impacts on productivity<sup>4</sup>. Although largely unknow, the effects of gut microbial communities over Brachyspira infections could represent an important factor on the SD development.

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## TEMPORAL TRENDS OF S. SUIS, G. PARASUIS, M. HYORHINIS, A. SUIS, AND M. HYOSYNOVIAE

<u>A.P. Serafini Poeta Silva</u>, M. Almeida <sup>1</sup>, A. Michael <sup>2</sup>, M. Rahe <sup>3</sup>, C. Siepker <sup>2</sup>, D. Magstadt <sup>2</sup>, P. Piñeyro <sup>4</sup>, B. Arruda <sup>5</sup>, N. Macedo <sup>6</sup>, O. Sahin <sup>6</sup>, P. Gauger <sup>1</sup>, K. Krueger <sup>7</sup>, R. Mugabi <sup>1</sup>, J. Santos Streauslin <sup>1</sup>, G. Trevisan <sup>1</sup>, D. Linhares <sup>1</sup>, G. De-Sousa-E-Silva <sup>8</sup>, E. Fano <sup>9</sup>, R. Main <sup>1</sup>, K. Schwartz <sup>10</sup>, E. Burrough <sup>1</sup>, R. Derscheid <sup>2</sup>, P. Sitthicharoenchai <sup>3</sup>, M.J. Clavijo <sup>11</sup> <sup>1</sup>*Iowa State University* 

<sup>2</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>3</sup>Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

<sup>4</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

<sup>5</sup>Virus and Prion Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA

<sup>6</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

<sup>7</sup>Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, United States

<sup>8</sup>Iowa State University College of Veterinary Medicine

<sup>9</sup>Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA; Current affiliation: Pipestone Veterinary Services, Pipestone, MN, USA

<sup>10</sup>Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>11</sup>Veterinary Diagnostic and Population Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, Pig Improvement Company, PIC®, Hendersonville, TN, USA

## **Background and Objectives**

The impact of swine bacterial agents is widespread throughout the swine production chain, resulting in detrimental effects on pig health and welfare, and decreased productivity. This study estimated temporal patterns of agent detection and disease diagnosis in porcine tissue specimens submitted to the Iowa State University Veterinary Diagnostic Laboratory. The study also described the diagnostic value of tissue specimens for disease diagnosis, polymicrobial diagnosis, and associations between phase production and disease diagnosis.

## **Material and Methods**

This retrospective study utilized comprehensive datasets (~27k bacteriological cases, ~20k PCR tests, ~16k histopathological reports) collected over 6 years. Regression models were used to estimate temporal trends and associate specific pathogen diseases to pig production phase.

# Results

S. suis was the most frequently detected and diagnosed agent, e.g., 22% of all cases with infectious etiology included a S. suis disease diagnosis. S. suis and G. parasuis bronchopneumonia increased by 6% and 4.3%, respectively, while S. suis endocarditis increased by 23% yearly. M. hyorhinis and A. suis serositis increased yearly by 4.2% and 12.8%. A significant upward trend in M. hyorhinis arthritis cases was observed. In contrast, M. hyosynoviae arthritis decreased by 33% on average/year. Isolation from the lungs was less predictive of S. suis disease diagnosis compared to joints, serosal fibrin, and heart valves. Approximately 70% of S. suis isolates originated from the lungs. Still, only 40.3% of those isolates were used to diagnose S. suis bronchopneumonia. In comparison, 17.2% of S. suis isolates originated from CNS, and 48.1% of those were used to diagnose meningoencephalitis, indicating that a high frequency of isolates obtained from CNS/lungs originated from cases that do not have enough evidence for diagnosis. Only 4% of S. suis isolates originated from joints, but 62.1% were diagnosed with arthritis. Similar findings were observed with the other four pathogens. S. suis arthritis was significantly associated with suckling pigs, meningitis with early nursery, and endocarditis with growing pigs.

## **Discussion and Conclusion**

The high co-detection frequency with other infectious etiologies, such as PRRSV and IAV, highlights that establishing effective viral control programs is imperative to minimize the impact of these bacterial agents.

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# THE STUDY OF METABOLOMICS ON PORCINE GASTRIC ULCER CAUSED BY INFECTION OF HELICOBACTER SUIS

P.J. Lin<sup>1</sup>, H.H. Chiang <sup>1</sup>, D.Y. Lo<sup>1</sup>, H.C. Kuo<sup>1</sup>, C.F. Wu<sup>1</sup> <sup>1</sup>Department of Veterinary Medicine, National Chiayi University, Chiayi city, Taiwan

## **Background and Objectives**

The pathogenic mechanisms underlying Helicobacter suis (H. suis)-induced gastric ulcer in pigs remain to be fully elucidated. This study used metabolomics and Next-Generation Sequencing (NGS) to unravel the alterations in cellular processes triggered by H. suis infection. Furthermore, multi-omics correlation analysis enabled the identification of potential biomarkers associated with porcine gastric ulcers.

## Material and Methods

Pigs were classified into subclinical, chronic and acute phase based on gross pathological examination, and tissues of gastroesophageal region were collected for NGS and the resulting transcriptome was analyzed by Ingenuity Pathways Analysis (IPA) software. Metabolomics was performed for the serum from H. suis PCR-positive and negative pigs, and the data was analyzed by MetaboAnalyst 5.0 and Metacore software.

## Results

Metabolic profiling revealed differential expression of tyrosine, methionine nicotinuric acid, adenosine, and 4hydroxyphenylpyruvic acid between the groups, with tyrosine and tryptophan biosynthesis identified as the common metabolic pathways. NGS profiling indicated that the acute phase of infection was primarily associated with cell adhesion and mobility. Subsequent IPA analysis demonstrated significant differences in the expression of TNF, IFNG, LCK, and GCG, which may be linked to tyrosine and methionine metabolism. Metacore analysis further revealed that tyrosine and methionine are associated with the SLC family and integrins, with c-SRC playing a crucial role.

## **Discussion and Conclusion**

The crucial role of tyrosine and methionine metabolism in H. suis-induced pathogenesis has been elucidated through this analysis. This metabolic dysregulation is also implicated in the acute infection of H. suis, leading to the disruption of cell junctions mediated by the SLC family and c-Src on the cell membrane. This disruption results in alterations in cellular functions, notably cell adhesion and transport of cell membrane components. Furthermore, H. suis exhibits gene expression patterns similar to those of H. pylori in inflammation, apoptosis, and cell motility.

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# VACCINATION WITH AN ESCHERICHIA COLI F4/F18 VACCINE REDUCES MORTALITY AND TI100 DURING THE POST-WEANING PERIOD

<u>F. Vangroenweghe</u><sup>1</sup> <sup>1</sup>Elanco - BU Food Animals - Benelux

#### **Background and Objectives**

Post-weaning Escherichia coli diarrhea (PWD) remains a major cause of economic losses for the pig industry. Therapy to combat PWD typically consists of antibiotic treatment through drinking water. The emergence of antimicrobial resistance to E. coli and new EU regulations urges the need for alternative control strategies such as immunization. The aim was to evaluate the effect of an oral live non-pathogenic Escherichia coli vaccine on mortality and TI<sub>100</sub> (treatment incidence over 100 days) during the post-weaning period.

# **Material and Methods**

We evaluated vaccination with an oral live non-pathogenic E. coli F4/F18 under field conditions in 3 consecutive batches (n = 6,651 piglets) against standard antimicrobial treatment in 4 historical control batches (n = 8,809 piglets) on a farm with severe problems related to PWD (9.1% mortality) and subsequent S. suis meningitis. Mortality and antimicrobial use related to treatment of PWD and S. suis meningitis were registered. Data were analyzed using JMP 15.0.

#### Results

Vaccine-treated groups demonstrated significant reduction in mortality (9.1% to 2.1%; -76.5%; P = 0.0003) and TI<sub>100</sub> (73.8 to 32.9; -55.0%; P = 0.0041). The proportion of antimicrobial treatment for clinical signs of post-weaning diarrhea significantly decreased (29.8 to 7.8%; P = 0.001). Most remaining antimicrobials were dedicated to treatment of S. suis meningitis (92.2%).

# **Discussion and Conclusion**

The present study demonstrated efficacy of an oral live non-pathogenic E. coli vaccine for active immunization of piglets against PWD under field conditions. Vaccine-treated groups showed a significant improvement in both mortality (-76.5%) and antimicrobial use (-55%). Moreover, there was a significant reduction in antimicrobial treatment for S. suis. Therefore, vaccination against PWD may be considered a valuable alternative to consolidate piglet performance while meeting the new EU requirements concerning prudent use of antimicrobials in intensive pig production.

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# VARIABLE NUMBER TANDEM REPEATS TYPIFICATION OF MYCOPLASMA HYOPNEUMONIAE USING NEXT-GENERATION SEQUENCING

F. Aguiar 1, M.S. Goslar 1, A. Balbinot 1, N. Fochesatto 1, L. Coldebella 1, A.C. De Lara 2, J. Zuffo 2, T.M. Ce 2, 0

<sup>1</sup>Animal Health laboratory, JBS Foods, Seara, Brazil <sup>2</sup>Ciex Agropecuário, JBS Foods, Itajaí, SC, Brazil

# **Background and Objectives**

Mycoplasma hyopneumoniae is the etiologic agent of enzootic pneumonia in swine, a prevalent respiratory disease widely disseminated in producing countries. Genetic variability between strains is mainly determined through Variable Number Tandem Repeats (VNTR) of P97 and P146 adhesin genes, mhp138 and mhp684, respectively. To classify VNTR types, evaluating the size or nucleotide sequence of adhesin loci is necessary. This study aimed to use Next-Generation Sequencing (NGS) for M. hyopneumoniae typification.

#### Material and Methods

Twenty-eight intratracheal mucus samples from four Brazilian states, previously qPCR positive for M. hyopneumoniae, were selected. The two adhesin VNTR loci, P97-RR1 and P146-RR3, were amplified using PCR conditions outlined by Vranckx et al. (2011), with overhanging adapters from the 16s rRNA Illumina<sup>®</sup> Protocol added to the loci-specific primer. Sequencing libraries of purified amplicons were prepared using the Nextera<sup>™</sup> XT kit and sequenced using the MiSeq System<sup>™</sup>, limited to samples up to 550 bp. The sequences were aligned to quantify the number of repeats (NR), forming VNTR<sub>P97-P146</sub> types for UPGMA analysis.

## Results

Amplification of RR1 and RR3 segments in all samples generated bands ranging from 350-480 bp and 320-400 bp, respectively. The repetitive tandem in the P97-RR1 locus comprise the amino acid sequence AAKP[EV], while the P146-RR3 region contain a polyserine (S). Sequencing of both genes was essential to form VNTR-types, resulting in 10 different genotypes among 23 VNTR-typed samples. Two main similarity clusters (>90%) occurred: Cluster I contained VNTR<sub>4-20</sub> samples exclusively from Southeast Brazil, while Cluster II comprised three genotypes differing in P146 loci serines.

# **Discussion and Conclusion**

Greater homogeneity and/or identical VNTR-types were demonstrated in herds with close geographic proximity or belonging to the same production flow. This aligns with our findings, especially concerning Cluster II, which comprises samples from various locations in the southern region of Brazil. Remarkably similar samples from distant locations suggest the introduction of new genotypes through infected animals or fomites. This study highlights M. hyopneumoniae's variability and its implications for coinfection control, vaccination strategies, and strain dynamics using NGS technology. Amplicons NGS enables precise and optimized typing, providing crucial insights into pathogen evolution, essential for effective management.

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# VIRULENCE PROFILE OF STREPTOCOCCUS SUIS ISOLATES FROM SWINE IN THE SOUTHERN REGION OF BRAZIL: IMPLICATIONS FOR PUBLIC HEALTH

M. Maria Cavalcante Granja<sup>1</sup>, T. Viana Fialho Martins<sup>1</sup>, G. Pereira Paschoalini<sup>1</sup>, J. Viktor De Paula Barros Baêta<sup>1</sup>, <u>L.</u> <u>Fernando Dos Santos<sup>1</sup></u>, D. Lúcio Dos Santos<sup>1</sup>, W. Vieira Guimarães<sup>1</sup>, J. Lúcio Dos Santos<sup>1</sup> *MICROVET - Microbiologia Veterinária Especial* 

## **Background and Objectives**

Streptococcus suis is a zoonotic pathogen that could affect pigs and humans, causing diseases in both species. Typically is transmitted to humans through direct contact with infected pigs or contaminated environments. The virulence genes fhb and sadP play essential roles in the virulence of S. suis and are related to transmission to humans. Fhb is involved in survival and pathogenicity, while sadP contributes to adhesion to the human intestinal mucosa. Therefore, the objective of this study is to investigate the frequency of these genes in S. suis isolates from pigs in Brazil due to their importance for public health.

#### Material and Methods

Twenty one isolates of S. suis from different organs, with prevalence of brain and joint of diseased pigs from the Southern region of Brazil were evaluated. The DNA from these samples, processed by the MicroVet Diagnostic Lab, was extracted using a commercial kit and sequenced using NGS technology. The identification of the fhb and sadP genes was performed through bioinformatics analyses.

## Results

The sequences of the S. suis isolates confirmed the presence of the pathogen in the sequencing, and virulence factors were able to be identified. In addition to the primary virulence genes, the fhb and sadP genes were identified in the evaluated S. suis isolates. Out of the 21 isolates assessed, 17/21 had the fhb gene and 06/21 had both fhb and sadP genes. High genetic variability of the fhb and sadP genes was observed among the isolates.

#### **Discussion and Conclusion**

We observed a high frequency of the fhb gene in S. suis isolates, consistent with previous findings using other molecular techniques. The fhb gene plays a fundamental role in the pathogenicity of S. suis, with strong affinity for human Factor H (hFH). The 28.5% frequency of the sadP gene is worrying due to its ability to adhere to the human intestinal mucosa. Therefore, the handling of pigs by veterinarians must be cautious and prevention strategies, such as pig vaccination, play a crucial role in minimizing the impact on pig health and, consequently, reducing the risk of transmission to humans.

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# COMBINATION OF BIOMARKERS IN SALIVA TO DIFFERENTIATE BETWEEN PIGS WITH STREPTOCOCCUS SUIS INFECTION AND HEALTHY PIGS

M.J. Lopez-Martínez <sup>1</sup>, E. Llamas-Amor <sup>2</sup>, E.G. Manzanilla <sup>3</sup>, J. Ceron <sup>4</sup>, A. González Bulnes <sup>5</sup>, E. Goyena <sup>6</sup>, A. Muñoz-Prieto <sup>7</sup>, F. Tecles <sup>2</sup>

<sup>1</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Campus de Espinardo s/n, 30100 Murcia, Spain <sup>2</sup>Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum, University of Murcia, Espinardo, Murcia, 30100, Spain.

<sup>3</sup>Pig Development Department, Teagasc - Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland & School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

<sup>4</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia

<sup>5</sup>Departamento de Producción y Sanidad Animal, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, C/Tirant lo Blanc, 7, Alfara del Patriarca, Valencia, 46115, Spain.

<sup>e</sup>Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain

<sup>7</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (INTERLAB-UMU), Department of Animal Medicine and Surgery, Veterinary School, Regional Campus of International Excellence Mare Nostrum, University of Murcia, 30100 Murcia, Spain

## **Background and Objectives**

Streptococcus suis infection has been demonstrated to produce changes in analytes related to stress, inflammation, redox status, and muscle damage in the saliva of pigs. These can be considered potential biomarkers for this disease<sup>1</sup>. This study aimed to evaluate the optimal combination of biomarkers that could better differentiate between pigs with S. suis infection and healthy pigs.

## Material and Methods

A profile of analytes including biomarkers of stress, inflammation, redox status and muscle damage, was measured in a group of healthy pigs (n=28) and in a group of pigs with clinical signs compatible with S. suisinfection and positive at bacterial isolation and characterization (n=28). A receiver operating characteristic (ROC) curve<sup>2</sup> was calculated for a variable combination of analytes that showed better performance in a previous report.<sup>1</sup>

#### Results

The combination of the biomarkers adenosine deaminase (ADA) activity, total protein (TP) concentration and alphaamylase (sAA) activity in saliva provided the best performance to differentiate between healthy and diseased groups, yielding an area under the curve of 0.983 (p < 0.001), 100% sensitivity, 90.5% specificity and an accuracy of 93.7% (Fisher exact test p < 0.001).

## **Discussion and Conclusion**

This study indicated that a combination of a biomarker of lymphocyte function such as ADA, an immune system such as TP and a biomarker of stress such as sAA showed the best performance to differentiate between pigs with disease due to S. suis and healthy pigs. Further studies with a large number of animals and including animals with other diseases, would be desirable to evaluate the potential of this combination. Also, the use of other approaches, such as algorithms or machine learning, should be explored to evaluate the possible use of salivary biomarkers to detect animals with different diseases.

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# COMPARISON OF MINIMUM INHIBITORY CONCENTRATION TESTS OF GAMITHROMYCIN AND TULATHROMYCIN FOR MAJOR BACTERIAL PATHOGENS IN PIGS ISOLATED IN KOREA

<u>Y. Oh</u><sup>1</sup>, H. Lee<sup>2</sup>, H. Lee<sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Korea Ltd. South Korea <sup>2</sup>Optipharm, Korea

# **Background and Objectives**

Zactran<sup>®</sup> is a new substance, gamithromycin which is a long-acting antibiotic that is effective for the treatment of major bacterial pathogens in swine. In order to evaluate the sensitivity of Tulathromycin and Gamithromycin to major domestic bacterial pathogens, the existence of genes encoding macrolides, lincosamides, streptogramines (MLS) resistance was confirmed for the experimental strains, and the MIC by broth microdilution method (according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) MIC test results were compared.

# **Material and Methods**

Sixty-four clinical isolates of 9 pathogens such as Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens type A, Actinobacillus suis Bordetella bronchiseptica, Glaesserella parasuis Pasteurella multocida type A and D, Streptococcus suis, Staphylococcus hyicus and aureus in swine were applied.

### Results

Haemophilus parasuis, A. pleuropneumoniae, Clostridium perfringens type A, Actinobacillus suis, Bordetella bronchiseptica, and Pasteurella multocida showed high susceptibility to both antibiotics, but partial susceptibility to streptococcus suis and strong resistance to staphylococcus spp. Gram-positive cocci S. suis and S. hyicus had resistance genes to erythromycin and macrolides. Gamithromycin showed lower MIC50 values than tulathromycin against Haemophilus parasuis, A. pleuropneumoniae, Clostridium perfringens type A, Actinobacillus suis, Bordetella bronchiseptica and Pasteurella multocida. The MIC50 values of Gamithromycin against Streptococcus suis was 0.0625, significantly lower than tulathromycin. Four out of eight isolates were highly susceptible to gamithromycin in the absence of ermA and ermB resistance genes.

## **Discussion and Conclusion**

The higher antibacterial activity of gamithromycin compared to tulathromycin, a widely used macrolide antibiotic, is likely due to being a new antibiotic molecule, which has never been used before in Korea. The emergence of bacterial resistance highlights the importance of proper management of antibiotic use and treatment methods. Adhering to recommendations for antibiotic use and appropriate control of antibiotic use helps to minimize the development of bacterial resistance and improve the effectiveness of antibiotic therapy.

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# COMPARISON OF THE GUT MICROBIOME COMPOSITION BETWEEN LAWSONIA INTRACELLULARIS VACCINATED AND NON-VACCINATED PIGS IN A SUB-CLINICALLY INFECTED PIG HERD

<u>Y. Oh</u><sup>1</sup>, S. Lee<sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Korea Ltd, South Korea <sup>2</sup>Hue Swine medical center, South Korea

## **Background and Objectives**

Lawsonia intracellularis (LI) infection in pigs can cause several clinical manifestations such as retarded growth, hemorrhagic diarrhea and mortality. In this study, the composition of the gut microbiome between LI vaccinated and non-vaccinated pigs was investigated to determine the efficacy of the vaccine on the host and the gut microbiome in a pig herd with LI sub-clinical infection.

# **Material and Methods**

A sub-clinically infected farm with LI was selected. Pigs were divided into a vaccinated and a non-treated control group. Each group had a total of 38 pigs and Enterisol ileitis® was vaccinated at 3 weeks of age. Pigs were weighed individually, and stool samples were collected from both groups at 3, 8, and 24 weeks of age. From stool samples, DNA was extracted and V3-4 region of 16S rRNA was analyzed using NGS (Miseq, Illumina) to determine the composition of pig's gut microbiome. The diversity of the gut microbiome (alpha diversity) and comparison between groups (beta diversity) were analyzed to determine species richness (Chao1 index) and species evenness (Shannon index).

#### Results

The seroconversion to LI infection occurred at 20 weeks of age (SVANOVIR® L. intracellularis/lleitis-Ab). The average daily weight gain was 0.598 kg/day for the control group and 0.639 kg/day in the vaccinated group (p value = 0.07). There was no significant difference in the composition of the whole gut microbiome between vaccinated and control group. However, in the vaccinated group, microbes related with host digestion function, especially belonging to the family Rikenellaceae showed significantly higher relative abundance compared to the control group at 8 weeks of age. Microbes related with digestion (family Prevotellaceae, Lactobacillaceae, Ruminococcaceae and Oscillospraceae) were shown higher relative abundance in the vaccinated group in comparison with the control group at 8 weeks and 24 weeks of age.

### **Discussion and Conclusion**

The results of this study suggest that vaccination with an oral live LI vaccine successfully increased the productivity of pigs by leading to a gut microbiome enriched with digestion-related taxa.

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# CORRELATION OF PULMONARY BIOAVAILABILITY OF VALNEMULIN AND MINIMUM INHIBITORY CONCENTRATION FOR RESPIRATORY PATHOGENS

<u>G.M.S. Stingelin</u><sup>1</sup>, M.R. Pirágine <sup>1</sup>, D.O. Carneiro <sup>1</sup>, E.M.B. De Souza <sup>1</sup>, L.G. De Oliveira <sup>2</sup> <sup>1</sup>Farmabase Saúde Animal <sup>2</sup>São Paulo State University (UNESP). School of Agricultural and Veterinary Sciences. Jaboticabal. Brazil

### **Background and Objectives**

A way to prove the efficiency of a given antibacterial drug against a microorganism is by conducting pharmacokinetic profile studies after administering the molecule in vivo, and comparing these results with the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), performed in vitro. For the effectiveness of antimicrobials against respiratory diseases, it is possible to correlate the concentrations found in the lung with the MIC and MBC of the microorganisms of interest. Valnemulin is a semi-synthetic derivative belonging to the pleuromutilin group, for exclusive use in veterinary medicine, indicated for the treatment of the Porcine Respiratory Disease Complex (PRDC) caused by Mycoplasma hyopneumoniae, Pasteurella multocida, Glaesserella parasuis, Streptococcus suis and Actinobacillus pleuropneumoniae. The aim of this study was to correlate the drug concentration in the lung after oral administration with MIC and MBC and to evaluate its effectiveness.

## **Material and Methods**

The determination of MIC and MBC for valnemulin was performed. The bacterial species chosen for this study were selected by their importance in pig farming, and Brazilian strains from clinical isolates were chosen. For that, 40 young pigs with an approximate body weight of 30 kg (20 males and 20 females), and four animals were used for each collection. Valnemulin tissue concentrations in lung at 0.5h, 1h, 2h, 3h, 4h, 5h, 6h, 8h, and 12h after oral administration of 10mg/kg /live weight by gavage were evaluated by liquid chromatography coupled with mass spectrometry (UPLC/MS/MS).

## Results

Observing the tissue concentration curve over 12 hours, it is noticed that valnemulin is rapidly absorbed and distributed after oral administration to pigs, reaching an average maximum pulmonary concentration above 52  $\mu$ g/g (Cmax) after 4h (Tmax). Comparing the pulmonary concentration obtained with the MIC and MBC of each microorganism, the average concentration is higher than the MIC and MBC of all bacteria evaluated for a period higher than 12h.

## **Discussion and Conclusion**

The correlation between pulmonary bioavailability and MIC/MBC studies proves that the administration of valnemulin to pigs orally at a dose of 10 mg/kg/live weight/day reaches tissue levels capable of inhibiting and eliminating respiratory pathogens of interest in pig farming.

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# DETECTION OF COLONIZATION AND VIRULENCE FACTORS OF E. COLI IN WEANED PIGS

L. Czanderlova<sup>1</sup>, M. Zizlavsky<sup>1</sup>, M. Slanikova<sup>1</sup>, B. Rackova<sup>1</sup> Sevaron, Czech Republic

#### **Background and Objectives**

Diseases caused by pathogenic strains of E. coli are a major problem, especially after the ban on ZnO. The aim of our study was to map the distribution of E. coli strains with virulence factors in pig farms in the Czech Republic in the category of weaned piglets.

## **Material and Methods**

Samples were taken either from faeces and rectal swabs from animals with visible diarrhoea or swabs of small intestines and mesenteric lymph nodes from dead pigs with enteritis. Samples collected from July 1, 2022 to September 30, 2023 were included in the study. A total of 191 strains were isolated from weaned piglets (age category weaning until 4 weeks after weaning). PCR detection for the presence of fimbrial adhesins F4, F5, F6, F41, F18, non-fimbrial adhesins AIDA1 and eaeA and determination of genes for the production of toxins STa, STb, LT, stx1 and stx2 were performed in all strains.

# Results

Only 45 strains (23.6%) were non-pathogenic in piglets in this category. Of the fimbrial adhesins, F18 was the most common, which was detected in 50 strains (26.2%), F4 was detected in 32 cases (16.8%). Nonfimbrial adhesin AIDA1 was detected in 26 strains (13.6%), eaeA in 3 cases (1.6%). Of the toxins, STb was the most frequently detected in 72 strains (37.7%), followed by STa in 50 strains (26.2%) and LT in 49 cases (27.7%). Stx2 was detected in 31 strains (16.2%). Other factors have not been identified.

# **Discussion and Conclusion**

The finding of pathogenic strains in piglets after weaning is very varied and various combinations of adhesins and other virulence factors have been recorded. ETEC rather than STEC strains prevailed. Adhesin-free Stx2 is usually part of the normal gut microbiota. Edema disease is caused by a combination of F18 and/or AIDA plus stx2.

Careful laboratory diagnosis is necessary for strains isolated from piglets after weaning, as pathogenic E. coli causes different diseases depending on adhesins and virulence factors. This information is important for the selection of the type of solution to health problems and for the appropriate selection of the vaccination strategy in this category of pigs, which is most at risk of E. coli infections.

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# EFFECT OF POOLING TRACHEAL SWAB FROM SWINE TO MYCOPLASMA HYOPNEUMONIAE DETECTION BY QPCR

C. Pissetti <sup>1</sup>, K. Prior <sup>1</sup>, <u>G.M.R. Simão</u><sup>2</sup>, L.H.G. Saraiva <sup>2</sup>, T.D.F. Freitas <sup>2</sup>, K.L.D. Carmo <sup>2</sup>, R. Pigozzo <sup>2</sup>, J. Ferreiro <sup>3</sup>, M.J. Clavijo <sup>4</sup>, E. De Freitas Costa <sup>5</sup>

<sup>1</sup>Centro de Diagnóstico de Saúde Animal (CEDISA), Concórdia, SC, Brazil

<sup>2</sup>Agroceres PIC, Rio Claro, SP, Brazil

<sup>3</sup>Agroceres PIC Argentina, Buenos Ayres, Estado de Buenos Ayres, Argentina

<sup>4</sup>Veterinary Diagnostic and Population Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, Pig Improvement Company, PIC®, Hendersonville, TN, USA

<sup>5</sup>Department of Epidemiology, Bioinformatics and Animal Models, Wageningen Bioveterinary Research, Lelystad, the Netherlands

# **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) infection and co-infections can significantly affect the productivity and profitability of swine production systems. Therefore, monitoring herd status and testing for early detection in replacement animals is essential. However, the sampling and sensitivity of in vivo diagnostic tools create a challenging diagnostic. This study investigates the effect of pooling tracheal swabs for detection by qPCR.

#### Material and Methods

Positive and negative tracheal swabs were used. After pre-treatment of individual swabs, 35 pools with ten swabs were created, using 20µL from one swab-positive plus 20µL from nine swabs-negative, totaling 200µL. The negative swabs used were selected after confirmation by qPCR and were the same in all pools. DNA was extracted by IndiMag Pathogen kit through automated extraction. The qPCR in-house was performed in QuantStudio™6-Flex.The effect of pooling the sample on the average Ct was assessed with a generalized linear mixed model employing an identity link function. The sample ID was used as random effects. Approximate maximum likelihood inference was based on numeric integration via Gauss-Hermite rule, as implemented in R in routine Imercens from library Ime4cens.

# Results

Thirty-five Mhyo-positive swabs were selected, with Cts between 21 and 34. Out of the 35 pooled samples, 5.71% were right censured (Ct undetermined). The average Ct of individual samples was 28.31 (27.45 – 29.16 95% Cl), and for the pooled samples was 31.98 (31.28 – 32.67 95% Cl). The 3.67 Ct difference between the individual and pooled samples was significant at 95% confidence level.

# **Discussion and Conclusion**

Tracheal swab samples offer the highest diagnostic sensitivity for this agent, however at a higher cost. Pooling swabs can be an effective approach to reduce the cost of testing, enabling the sampling of a greater number of animals from the herd. Considering that recent infections tend to be Ct < 30, when pooled into 10 swabs there is an increase of approximately 3.67, which would still yield a positive result. This increase is expected due to the pooling effect but likely does not affect early detection on naïve farms.

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# FECAL MICROBIOTA COMPOSITION OF KRSKOPOLJE PIG BREED RAISED IN TWO DIFFERENT PRODUCTION SYSTEMS

#### B. Papic <sup>1</sup>, J. Plut <sup>2</sup>, <u>M. Stukeli</u><sup>2</sup>

<sup>1</sup>Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia <sup>2</sup>Clinic for Reproduction and Large Animals, Clinic for Ruminants and Pigs, Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia

## **Background and Objectives**

Krskopolje breed is the only preserved Slovenian autochthonous pig breed known for its robustness and meat quality. The aim of this study was to evaluate the fecal microbiome of Krskopolje breed, which has not yet been investigated.

## **Material and Methods**

A total of 18 pigs kept at three different locations and fed organic commercial feed were included into the study: (i) pigs kept on pasture together with small ruminants (group L1), (ii) pigs kept indoors and without other farm animals and fed only commercial feed (group L2), and (iii) pigs kept on pasture with cattle (group L3). Fecal samples were collected rectally in all four seasons of 2022, from grower to finisher phase. Groups L2 and L3 were raised on the same organic farm and maintained by the same livestock workers, whereas group L3 was reared on a different organic farm and were maintained by other livestock workers. A total of 72 DNA samples underwent 16S rRNA gene (V3–V4 region) sequencing using Illumina paired-end (2x250 bp) technology. Operational taxonomic unit (OTU)-based microbiome analysis was performed using QIIME 1.9.1.

# Results

Alpha diversity was comparable between study groups; the average number of observed OTUs was 1746. The two predominant bacterial phyla in all three groups were Firmicutes and Bacteroidota. Significant seasonal changes in microbiome composition were observed between all seasons (nonparametric MANOVA, p < 0.008). The microbiome composition differed significantly between study groups L1 and L2–L3, as shown by principal coordinate analysis of weighted UniFrac distances and nonparametric MANOVA (p = 0.001), whereas groups L2 and L3 did not differ significantly (p = 0.2). Linear discriminant analysis Effect Size (LefSe) identified several bacterial taxa that were most differentially abundant between the different study groups and the different seasons.

# **Discussion and Conclusion**

Pig microbiota composition is not stable over time and is influenced by external (rearing-related) factors. In this study, the production system or co-rearing with other animal species had no significant influence on the pig microbiome composition. Rather, the main observed differences in microbiota composition could be explained by the farm and livestock workers.

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# FIELD EFFICACY TRIAL OF A RECOMBINANT VEROTOXIN 2E (VT2E) VACCINE AGAINST OEDEMA DISEASE ON A COMMERCIAL FARM IN THE PHILIPPINES.

A. Salazar <sup>1</sup>, <u>J. Rubico <sup>1</sup></u>, J. Querubin <sup>1</sup>, L. Nodar <sup>2</sup>, G. Alvarez <sup>2</sup> <sup>1</sup>HIPRA Philippines, Manila <sup>2</sup>HIPRA, Amer (Girona), Spain

# Background and Objectives

Certain Escherichia coli colonising the small intestine and producing Verotoxin 2e (Vt2e) are responsible for the characteristic vascular damage and increased vascular permeability resulting in tissue oedema. This damage manifests grossly by oedema of the eyelids, and upon necropsy oedema at the periphery of the intestine. Oedema disease usually affects rapidly growing pigs during the weaner and grower production phases. The objective of this study was to evaluate the efficacy of a recombinant vaccine against Oedema Disease (OD) in pigs under field conditions.

# **Material and Methods**

The study was conducted on a 2,400-sow farrow-to-finish pig farm in the Philippines where obvious nervous signs of oedema disease were prevalent. A total of 196 piglets was used, 1ml VEPURED® was administered intramuscularly at 3 days of age (n=97), and non-vaccinated animals served as controls (n=99). Following product administration, animals were monitored up to 70 days of age. The same husbandry practices were implemented for animals in both groups throughout the study. To determine the field efficacy of the vaccine, mortality and weights were recorded. Weight variations were also determined at the end of the nursery period. A linear regression model with weaning weight as a factor and gender as a random effect was used to analyse ADWG and weight differences at 70 days and a linear regression model with gender as a random effect was used to analyse weaning weight differences.

#### Results

At 70 days of age, the weight for the vaccinated group was significantly higher than that for the control group (29.53kg vs 28.00kg respectively, with a p-value of 0.0483). Additionally, there were significantly fewer piglets weighing less than 20kg at 70 days in the vaccinated group than in the control group (p-value <0.0265). The mortality rate from weaning to 70 days old was lower in the vaccinated group than in the Control group.

### **Discussion and Conclusion**

The results of this study showed improvement in weights at 70 days old and proportions of piglets weighing less than 20kg. Mortality was also reduced. Therefore, using VEPURED® (a purified recombinant oedema vaccine) was effective in reducing both clinical and subclinical effects of oedema disease in pig production.

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# FIELD EFFICACY TRIAL OF AN INACTIVATED INJECTABLE VACCINE AGAINST LAWSONIA INTRACELLULARIS IN THE PHILIPPINES

L.D.E. Ellerma <sup>1</sup>, C. Lima <sup>2</sup>, H. Lin <sup>3</sup>, R. Magpantay <sup>4</sup>, <u>R. Del Pozo Sacristán</u> <sup>1</sup> <sup>1</sup>MSD Animal Health <sup>2</sup>Veterinarian, Sika Farm, Magalang Pampanga, Philippines <sup>3</sup>MSD Animal Health Innovation Pte Ltd, 1 Perahu Road, Singapore <sup>4</sup>MSD Animal Health Philippines

# **Background and Objectives**

Lawsonia intracellularis is the etiologic agent of ileitis in pigs. This disease is responsible for substantial economic losses worldwide. Vaccination and use of antibiotics have been the top control options for ileitis. The efficacy of an inactivated injectable vaccine against L. intracellularis when used in conjunction with antibiotics was evaluated.

# **Material and Methods**

A total of 386 piglets were used, which were assigned randomly to one of the two groups: TREATMENT (n=198), vaccinated with Porcilis® Lawsonia vaccine mixed with Porcilis® PCV Mhyo 2ml intramuscularly (IM) at 3 weeks of age and CONTROL (n=188), vaccinated against PCV & Mhyo. Both the CONTROL and TREATMENT groups received the same routine antibiotic and vaccination program and were housed in separate pens from nursery to slaughter to allow for feed consumption measurement and ear-tagged so that no comingling between vaccinated and non-vaccinated groups occurred. Average daily gain, Feed Conversion Ratio (FCR), % survivability and final weight gain were computed. On establishing the economic benefit of having an intact control program for lleitis, current prices and cost assumptions were applied.

#### Results

We found a positive ADG difference in the treatment group of 43 grams from wean to finish, which is equal to approximately 5.89 kg advantage on market weight compared to the control group. A 10 FCR points advantage was also observed in the treatment compared to control group. This advantage in FCR points contributed to at least 49% of the total net benefit gained in the study. Comparing the % survivability of the two groups, it was observed that animals that received Lawsonia vaccination has better survivability and robustness. In this study, the treatment group garnered a total of PHP292,702.95 (USD 5,210.41) net benefit over the control with a cost benefit ratio of 22.74.

## **Discussion and Conclusion**

The study shows that Lawsonia vaccination can protect pigs from lleitis and reduce economic losses associated. Significant differences in terms of production parameters (ADG, FCR and market weights) demonstrate this. In this scenario, the economic value gained was positive even if it is an add on to what routine antibiotic and vaccination program the farm currently has.

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# IMPROVING EXTRACTION AND PCR PROTOCOLS TO ENHANCE MYCOPLASMA HYOPNEUMONIAE DNA DETECTION IN ORAL FLUID SAMPLES UNDER CONTROLLED AND FIELD CONDITIONS

A.P. Serafini Poeta Silva 1, R. Rauh 2, J. Zimmerman 3

<sup>1</sup>Iowa State University

<sup>2</sup>Tetracore Inc

<sup>3</sup>Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA

# **Background and Objectives**

Deep tracheal samples (DTs) are the most reliable and sensitive for early M. hyopneumoniae (MHP) detection by PCR but they require more labor due to pig handling. Oral fluids (OFs) are easier to collect, do not involve handling, and cost-efficient. However, current extractions and PCRs demonstrated a low performance for early detection. A side-by-side comparison (Silva et al. 2022 https://doi.org/10.1016/j.prevetmed.2022.105670) showed a statistical difference in detection of a combination of 2 extractions and 3 MHP-PCRs under controlled conditions, suggesting that improvements in extraction and PCRs are needed to maximize sensitivity. This study aimed to evaluate a novel extraction protocol using OFs for MHP detection from controlled and field settings.

# Material and Methods

**Controlled study**. 39 MHP-negative 7-week-old pigs were randomized to one of 5 treatment rooms, negative control (3 pigs) and 4 rooms of 9 pigs each that differed in the MHP-inoculated pigs' proportion with MHP 232 lung homogenate at day 0 (1 MHP-inoculated:8 naïve pigs; 3 MHP-inoculated:6 naïve; 6 MHP-inoculated:3 naïve; 9 MHP-inoculated). DTs were collected twice weekly to establish individual pig MHP infection and within-pen prevalence. OFs (320 OFs) were collected daily for 59 days post-inoculation (DPI). MagMax Core (MM) and VetAlert MagBead (VAM) were the extractions. The MHP-PCR used following both extractions was the EZ-M.Hyo Real-Time PCR. Protocols were compared in terms of probability of detecting MHP in OFs as function of within-pen prevalence.

**Field study.** The performance of these extractions and PCRs will be analyzed using OFs collected in different MHP monitoring scenarios under field conditions.

# Results

All MHP-inoculated pigs tested PCR-positive in DTs by DPI 7. The best-performing protocol included the VAM extraction, e.g., the first MHP-positive OFs was on DPI 6 and 46.3% (18.7, 76.4) probability of detection at 50% within-pen prevalence, while MM resulted in 11.5% (3.2, 33.9). VAM resulted in 10% increase in probability of detection compared to the best protocol published (Silva et al. 2022).

## **Discussion and Conclusion**

OFs are convenient to collect, and the same sample can be used to test different pathogens (PPRSV and IAV). A betterperforming extraction and PCR will optimize OFs for MHP monitoring and early detection in penned pigs.

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# INVESTIGATION OF BORDETELLA BRONCHISEPTICA AND PASTEURELLA MULTOCIDA IN WEANED PIGS FROM DUTCH FARMS

<u>A. Puigredon</u><sup>1</sup>, M. Simon-Grifé<sup>1</sup>, M. Solé<sup>1</sup>, I. Galé<sup>1</sup>, E. Van Esch<sup>2</sup>, J. Beek<sup>2</sup>, I. Ballarà-Rodriguez<sup>1</sup>, J. Montané Giralt<sup>1</sup>, K. Junker<sup>3</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA Benelux <sup>3</sup>Royal GD, Deventer, The Netherlands

# **Background and Objectives**

Toxigenic Pasteurella multocida (Pm) and Bordetella bronchiseptica (Bb) are well-known causes of atrophic rhinitis. Data from the Netherlands regarding their prevalence are scarce. The aim of this study was to determine the detection rate of Bb and Pm in the noses of weaned pigs, with or without a previous history of respiratory problems, and to examine its relationship to a nasal lesion score (NLS).

# **Material and Methods**

During 2023, fifty-five piglets between 4 and 12 weeks of age, originating from 26 farms, were received at Royal GD for post-mortem examination and randomly recruited for this study. Pigs were classified into two groups based on the presence (Respiratory Group, RG, n=31) or absence (Control group, CG, n=24) of respiratory clinical signs and pneumonia. Nasal swabs were collected for culturing Bb and Pm. All Pm were tested for Dermonecrotic Toxin (DNT)-gene by PCR. Furthermore, all animals were sampled for NLS by sectioning the snouts following the European Pharmacopoeia guidelines (0-4 points for each scroll; 0-2 points for septum deviation; maximum score: 18). NLS was evaluated using Al Diagnos, an artificial intelligence tool.

#### Results

The proportion of Bb was statistically higher in the RG compared to the CG (RG=45.2% vs CG =16.7%; p<0.05) as it was for Pm (RG 74.2% vs CG 45.8%; p<0.05). Furthermore, the proportion of pigs with concurrent Bb and Pm detection was statistically higher in the RG than in the CG (25.8% and 12.5%, respectively; p<0.05). The average NLS was numerically higher in pigs colonized by both pathogens than in the non-colonized pigs (6.6 and 4.8, respectively), even though the differences were not significant, but they did show a tendency (p=0.06). All Pm cultures were DNT-negative.

# **Discussion and Conclusion**

The results showed a significantly higher proportion of Bb and Pm in pigs exhibiting respiratory signs and pneumonia, compared to control pigs. Additionally, pigs with concurrent colonization had a numerically higher NLS. Since all Pm cultures were non-toxigenic, the results of this study suggest that BB is the cause of the nasal lesions observed. These findings emphasize the need for further research to assess the beneficial effects of preventive measures, such as vaccination.

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# INVESTIGATIONS ON THE MECHANISMS OF BACTEREMIA CAUSED BY STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS

C. Weisse 1, S. Oehlmann 1, R. Bergmann 1, I. Hennig-Pauka 2, C.G. Baums 1

<sup>1</sup>Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, Leipzig University, 04103 Leipzig, Germany

<sup>2</sup>Field Station for Epidemiology, Bakum, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

## **Background and Objectives**

Streptococcus equi subsp. zooepidemicus (SEZ) is an opportunistic pathogen often causing pneumonia, arthritis, abortion and wound infections in horses, but it is also known to cause diseases in very different species, including pigs and humans.SEZ is currently not considered a major porcine pathogen in Central Europe. However, in 1975 an SEZ epidemic occurred in the pig farms of Sichuan Province in China leading to 300,000 dead pigs (Feng, 1977). Subsequently SEZ has emerged as a major swine pathogen in China and was also reported in cases of sudden death and respiratory disease of pigs in Canada and USA (Chen et al., 2020). Recently in Northern Germany the first porcine outbreak of SEZ was reported with high morbidity and mortality in sows (Geiping et al., 2023). To better understand this current threat to porcine health we investigated the survival of SEZ in porcine blood and the role of the M-like protein SzM, a surface-associated virulence factor in SEZ.

#### **Material and Methods**

Blood was collected every two weeks from piglets (n = 6) of a commercial farm and comparatively infected with different SEZ wild type (wt) strains and their szm deletion mutants in vitro. Bacterial survival factors were calculated by plating serial dilutions at different time points. The phagocytosis and oxidative burst response of granulocytes was investigated by flow cytometry.

## Results

A specific SEZ wt strain demonstrated survival in the blood of 2 and 6-week-old piglets as well as association with granulocytes and induction of a strong oxidative burst response. The survival and the induction of ROS was dependent on the distinct SzM protein, as the isogenic mutant was efficiently killed in the same blood and induced significantly less oxidative burst. The recombinant SzM protein was found to be associated with granulocytes and lymphocytes in reconstituted blood. Another SEZ wt strain showed limited survival in porcine blood independent of SzM expression.

## **Discussion and Conclusion**

This study demonstrates distinct phenotypes of SEZ wt strains in host-pathogen-interaction in porcine blood. We postulate that different pathotypes of SEZ exists and that mechanisms of bacteremia might be very different between these pathotypes. This might have major implications for therapy and vaccination.

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# LAWSONIA INTRACELLULARIS SERUM ELISA TEST PERFORMANCE IS BETTER THAN ORAL FLUID PCR TEST

<u>J. Hulzing</u><sup>1</sup>, H. Prüst<sup>2</sup>, M. Steenaert<sup>3</sup> <sup>1</sup>Coöperatie Varkensartsen, the Netherlands <sup>2</sup>Boehringer Ingelheim AH the Netherlands BV <sup>3</sup>Boehringer Ingelheim Animal Health Netherlands bv

# **Background and Objectives**

To confirm Lawsonia intracellularis (Li) infection in a production system and to estimate the right timing for vaccination several diagnostic testing methods are available. The objective of this study: Is Li Oral Fluid testing by PCR as reliable as is Li serum sample ELISA testing.

## **Material and Methods**

In a Dutch farrow to finish herd 3 different batches of finishing pigs were included. Each batch was housed in 2 rooms of which 4 pens per room were tested. Per pen at 3 different timepoints 2 to 3 Serum samples (Sero) and 1 Oral Fluid sample (OF) were taken at 0, 4 and 12 weeks (t+0, t+4, t+12) after placement in the finishing barn respectively. Experimental unit was a pen. A pen was considered ELISA positive when per event at least one serum sample tested above cut-off OD value 30, and PCR positive when the OF sample was below cut-off ct value 40. All results were compared in a contingency table: 66 events of samples taken on the same day and in 21 respectively 22 events the Serum Samples were taken 4 respectively 12 weeks after the Oral Fluid samples.

#### Results

Sensitivity of testing OF PCR compared to Sero ELISA was 36%, 33% and 24% at t+0, t+4 and t+12 respectively. Specificity of testing OF PCR compared to Sero ELISA was 85%, 87% and 80% at t+0, t+4 and t+12 respectively.

# **Discussion and Conclusion**

The sensitivity of detecting Li in OF PCR compared to Sero ELISA was very low, resulting in false negative results. The specificity of detecting Li in OF PCR compared to Sero ELISA was rather low, resulting in false positive results. This study shows that the results from both methods are hard to compare and that for Li detection within a herd serum-ELISA is more reliable than Oral Fluid-PCR.

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# METAGENOMIC ANALYSES OF STREPTOCOCCUS SUIS STRAINS ISOLATED FROM FARMS SHOWED LOCAL DISSEMINATION.

D. Martínez Gómez <sup>1</sup>, A. Romero-Flores <sup>2</sup>, J.J. López Islas <sup>1</sup>, E.T. Méndez Olvera <sup>1</sup>, M.E. Trujillo-Ortega <sup>3</sup>, L. Hernández-Andrade <sup>4</sup>, A. Ciprian-Carrasco <sup>2</sup>, <u>S. Mendoza-Elvira</u> <sup>2</sup> <sup>1</sup>UAM-Xochimilco. México <sup>2</sup>UNAM-FESC, México <sup>3</sup>Universidad Nacional Autonoma de Mexico

4CENID Salud e Inocuidad, INIFAP

## **Background and Objectives**

Streptococcus suis is a major cause of respiratory tract and invasive infections in pigs. Several methods exist to examine strain diversity in S. suis. To date, serotyping remains the most widely used method to subtype S. suis isolates, however, serotyping by co-agglutination is dependent on the availability and quality of sera, and interpretation of results is somewhat subjective. Studying the population structure and the genetic diversity of S. suis is helpful to understand the epidemiology of this microorganism as well as reveal clones with high virulence. In this study a 23 isolates of S. suis were analyzed by PCR-based serotyping methods and metagenome techniques, to establish a molecular epidemiology system for S suis strains.

#### Material and Methods

Sixty-three samples of organs were obtained from pigs with clinical signs of S. suis infection from different regions in Mexico. The samples were processed by standard bacteriological techniques. DNA extraction was performed using a commercial DNA purification kit (Promega, USA). Serotyping was done using the two-step multiplex PCR (Okura et al. 2014). Metagenome sequencing was performed in Illumina HiSeq 2500 platform. The data was analyzed using different bioinformatic software.

## Results

The serotypes detected were 1/2, 2, 3, 5, 7, 8, 9, 17 and 23. The serotypes identified by region were Jalisco six different serotypes, Veracruz five serotypes, Puebla two serotypes and Michoacan only one serotype. The metagenome analysis by Kraken identified 4045 different genes in the 23 strains analyzed. The sequence analysis of these genes in S. suis strains showed variations between strains, however a principal component analyses showed that strains have similitudes by region.

#### **Discussion and Conclusion**

Many studies used a serotype to describe the distribution of S suis strains, however this method is limited to identify genetic variations. In our study serotypes do not show differences by region, However, using Whole-Genome Sequencing Approaches is possible identified variations between strains. This new method could be used to monitor the movement of strains or to establish possible evolutionary events that allow the generation of more virulent strains.

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# PATHOGENICITY ASSESSMENT OF KLEBSIELLA PNEUMONIAE TO PIGS

X. Huang 1, Y. Hou 1, Z. Zhu 1, L. Hua 1, H. Chen 1, Z. Peng 1, B. Wu

<sup>1</sup>National Key Laboratory of Agricultural Microbiology, The Cooperative Innovation Center for Sustainable Pig Production, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China

## **Background and Objectives**

Klebsiella pneumoniae (KP) is a zoonotic bacterium that can be found in the respiratory and digestive tracts of humans and animals. Recently, we found an increased isolation rate of KP from pig farms. However, it remains to evaluate the pathogenicity of KP to pigs.

## **Material and Methods**

KP strains were used to challenge 28-day-old weaned piglets through the auricular vein. After infection, clinical manifestations, body temperatures, bacteremia, bacterial load in oral and nasal discharge, bacterial tissue load, and pathological changes were recorded.

# Results

KP infections led to symptoms including increased body temperature, cough, runny nose, and lethargy. Bacteremia lasted for more than 3 days, while oral and nasal bacterial excretion extended beyond 14 days. The pathogen content of KP was highest in the lungs, spleen, and liver, leading to lung alveolar wall thickening, unclear structure, hepatic cell swelling, kidney tubular necrosis, and hemorrhagic glomerular interstitial inflammation.

### **Discussion and Conclusion**

KP was pathogenic to weaned piglets. In veterinary clinical practice, KP exhibits a high isolation rate in samples from the porcine respiratory tract, causing septicemia in piglets, mastitis in sows, and pneumonia in growing pigs. This pathogen has thus become one of the significant pathogens in large-scale swine farms, and its impact on swine production should not be underestimated. Furthermore, the potential transmission risk of KP between humans and animals through the food chain, water sources, and other pathways requires further investigation.

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# PRODUCTION DATA COMPARISON OF ORAL VERSUS IM VACCINATION AGAINST LAWSONIA INTRACELLULARIS IN A LARGE PRODUCTION SYSTEM IN ARGENTINA

<u>R. Jansen</u><sup>1</sup>, G. Sandoval <sup>2</sup> <sup>1</sup>Boehringer Ingelheim Vetmedica GmbH <sup>2</sup>Boehringer Ingelheim Animal Health Argentina

## **Background and Objectives**

A pig production integration in Argentina with confirmed subclinical ileitis applied oral live vaccination against ileitis from September 2021 until April 2022 at the age of 22 days. In April 2022 the integration switched to a mineral oil adjuvanted intramuscular vaccine until October 2022. Production data was monitored on a production site level (all-in all-out barns; 14 grow-finish production sites). Production data between the use of the two different vaccines were compared to check for the differences in animal performance.

# **Material and Methods**

Production data from all involved al-in all-out finisher sites were analyzed. The two treatment groups were housed under the same condition and received the same diets. ADG and FCR were normally distributed, and differences were tested with a t-test. Mortality differences were tested with a Chi-square test. Both groups contained data of over 75.000 finisher pigs.

## Results

Oral vaccination had equal performance for ADG and mortality when compared to IM vaccination with only low numerical differences (ADG 1,019 gram/day vs 1,021 gram/day, p = 0.85; Mortality 2.99% vs 3.08% p = 0.31). FCR was significantly lower in oral vaccinated herds (2.566 vs 2.610; p = <0.05).

# **Discussion and Conclusion**

Pigs had a start weight of 22.1 kg and a market weight of 127.6 kg. With a lower FCR of 0.044 for the oral live vaccinated group this resulted in a saving of 4.6 kg feed per pig marketed. This equals a financial benefit of \$1.85 lower production cost per pig under current feed prices and a lower carbon footprint due to the lower mounts of feed needed.

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# ANTIMICROBIAL EFFICACY OF A CEFTIOFUR-BASED PRODUCT, IN INTRAMUSCULAR SINGLE DOSE

A.S. Castro <sup>1</sup>, M.G.L. De Souza <sup>1</sup>, K.E.Z. Nubiato <sup>1</sup>, L.C. Penha <sup>1</sup>, <u>G.M.S. Stingelin <sup>1</sup></u>, D.O. Carneiro <sup>1</sup> <sup>1</sup>Farmabase Saúde Animal

## **Background and Objectives**

Due to the constant concern about the bacterial resistance in pig farming, it is necessary to prove the efficacy of new products against certain bacterial strains. Ceftiofur is a third-generation cephalosporin antimicrobial, with a time-dependent bactericidal effect. The aim of this study was to evaluate the efficacy of a product based on Ceftiofur (10%) at a dose of 5 mg/kg of ceftiofur, equivalent to 0.05 ml/kg of product in an intramuscular single dose, from the correlation between plasma concentration and MIC in bacteria of interest in pig farming.

# **Material and Methods**

Eight pigs (Sus domesticus) between 25 and 35 kg and 58 days of age were used. The pigs were treated with a dose of 0.05 ml/kg of the product in an intramuscular single dose. Blood samples were collected to detect plasma concentration at 0h, 1h, 2h, 3h, 4h, 6h, 8h, 12h, 18h, 24h, 32h, 40h, 48h, 54h, 60h, 66h, 72h, 84h, 96h, 108h and 120h after treatment. The MIC of 7 species of bacteria was evaluated, there were two strains per species isolated from different clinical cases, except for Salmonella choleraesuis, which was tested in only one strain. The efficacy confirmation occurred when the plasma concentration of Ceftiofur remained above the MIC, in at least 72 hours after the first quantification, corresponding to 3 days of recommended treatment.

#### Results

One hour after the treatment (1h), the presence of ceftiofur in plasma was first observed (12.413 [ $\pm$  2.744] µg/mL), to comprehend the minimum of 72 hours, the time 84h (0.294 [ $\pm$  0.059] µg/mL) was used as the cut-off point for correlation with the MIC. The MIC result obtained for the tested species was: Actinobacillus pleuropneumoniae (0.25; 0.0625 µg/mL), Escherichia coli (0.125; 0.125 µg/mL), Glaesserella parasuis (0.031; 0.0625 µg/mL), Pasteurella multocida (<0.004; 0.0622 µg/mL), Salmonella Choleraesuis (0.25 µg/mL) Salmonella Typhimurium (0.25; 0.25 µg/mL) and Streptococcus suis (0.031; 1 µg/mL).

## **Discussion and Conclusion**

Among the 7 species tested, only one strain of S. suis has showed an MIC greater than the plasma concentration of ceftiofur. Thus, the product based on ceftiofur proved to be effective against all tested species, excepting one strain of Streptococcus suis.

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# CHARACTERIZATION OF ADHESION GENES AND ANTIMICROBIAL SUSCEPTIBILITY CHANGES ON HEMOLYTIC E. COLI ISOLATES IN CASES OF PORCINE POST-WEANING DIARRHEA DIAGNOSED AT ISU-VDL FROM 2010 TO 2022

## R. C. Paiva 1, E. Burrough 1, N. Macedo 1, P. Pineyro 1, M. Almeida 1

<sup>1</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

## **Background and Objectives**

Diarrhea due to enterotoxigenic Escherichia (E.) coli (ETEC) remains one of the main enteric postweaning challenges to the US swine industry, leading to economic losses due to treatment costs, decreased weight gain, and mortality. Data from the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) has shown a high prevalence of F4 and F18 isolates and changes in antimicrobial susceptibility profiles over the last twelve years. Therefore, this study aimed to characterize fimbriae genotypes and antimicrobial susceptibility changes over time in porcine ETEC strains isolated at ISU-VDL to provide comprehensive information to swine veterinarians.

# Material and Methods

A database containing 3,240 porcine E. coli isolates recovered from ISU-VDL diagnostic cases confirmed as ETEC enteric disease (Inclusion criteria: clinical history of postweaning diarrhea, colonization of villi by coccobacilli bacteria, and laboratory testing) was analyzed. Laboratory testing included bacterial culture, genotyping based on fimbriae gene detection by PCR (F4, F18, F41, F5, AIDA, EAEA), and antimicrobial susceptibility testing using breakpoints from CLSI 2023 and NARMS. Repeated measures was used to assess statistical difference (p-value <0.05).

### Results

Isolates with F18 and F4 fimbriae genes carrying enterotoxin genes represented the most common adhesins [F18: 2,211 (68%); F4: 979 (30%)]. In contrast, 2% of the isolates contained other fimbrial genes (EAEA, F5, F41, and AIDA). An increased number of cases associated with F18 fimbria has been observed since 2019. The proportion of isolates susceptible to enrofloxacin dramatically decreased for F4 (from 2014 to 2022) and F18 (from 2017 to 2022), respectively. There was no variation in the proportion of susceptibility to ceftiofur isolates. The proportion of isolates susceptible to florfenicol, gentamicin, neomycin, sulfadimethoxine, and trimethoprim-sulphamethoxazole has decreased ( $p \le 0.05$ ) on F18 positive isolates since 2017.

### **Discussion and Conclusion**

This study revealed important changes in the frequency of detecting different fimbriae genes and antimicrobial susceptibility patterns. Identifying shifts in genetic characteristics, such as adhesins of E. coli, raises questions about driving factors behind genotype changes and aids in selecting different interventions to be applied in field conditions. Changes in antibiotic susceptibility pattern is a public health concern, and understanding these changes is important to help veterinarians in their decision-making on disease control.

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# DETECTION OF COLONIZATION AND VIRULENCE FACTORS OF E. COLI IN SUCKLING PIGLETS

L. Czanderlova <sup>1</sup>, M. Zizlavsky <sup>1</sup>, M. Slanikova <sup>1</sup>, B. Rackova <sup>1</sup> <sup>1</sup>Sevaron, Czech Republic

## **Background and Objectives**

PCR detection of virulence factors and their combinations is used to differentiate common stable strains of Escherichia coli from pathogenic ones. The aim of our study was to map the distribution of E. coli strains with virulence factors in pig farms in the Czech Republic in the category of suckling piglets.

### **Material and Methods**

Samples were taken either from faeces and rectal swabs from animals with visible diarrhoea or swabs of small intestines and mesenteric lymph nodes from dead suckling piglets with enteritis. Samples collected from July 1, 2022 to September 30, 2023 were included in the study. A total of 52 strains were isolated from suckling piglets. PCR detection for the presence of fimbrial adhesins F4, F5, F6, F41, F18, non-fimbrial adhesin AIDA1 and determination of genes for the production of virulence factors STa, STb, LT, eaeA, stx1 and stx2 were performed in all strains.

# Results

Non-pathogenic strains predominate in suckling piglets - 39 strains (75%). F4 was detected in 3 strains (5,8%), AIDA1 in 2 strains (3.8%), STb was detected in 7 cases (13.5%), STa in 6 (11.5%), LT in 2 strains (3.8%). Other factors have not been identified in suckling piglets.

## **Discussion and Conclusion**

As can be seen from the results, strains without colonization factors were found, in which only genes for the production of STa, STb and LT toxins were detected. The role of these strains is still unclear, and their contribution to the development of diarrhoea in this category also remains questionable. These strains were found on only 2 farms, confirming that the genetic makeup of E. coli strains is stable and the bacterium is not susceptible to the formation of new strains. New strains are introduced into the farms mainly from the external environment and by importing new animals. The high efficacy of commonly used vaccines against enteral coli infections, which are used in sows, is confirmed by the high detection of non-pathogenic strains in suckling piglets and also by the fact that fimbrial adhesins F5, F6, F41 were not detected at all and F4 was detected only in 3 cases. The situation with E. coli infection in suckling piglets is stable.

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# DETECTION OF MYCOPLASMA HYOPNEUMONIAE AND MYCOPLASMA HYORHINIS IN WEANED PIGLETS PRESENTING RESPIRATORY SYMPTOMS IN THE NETHERLANDS

<u>H. Prüst</u><sup>1</sup>, M. Steenaert<sup>1</sup>, M. Genzow<sup>2</sup>, C. Sanderman<sup>3</sup>, H. Kreutzmann<sup>3</sup>, K. Junker<sup>3</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Netherlands bv <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH <sup>3</sup>Roval GD. Deventer, the Netherlands

#### Background and Objectives

Mycoplasma hyopneumoniae (Mhp) is the primary causative agent of enzootic pneumonia. Different within-herd prevalences have been found at weaning in tracheobronchial swabs (TBS) or bronchoalveolar fluids ranging from 0-52%. Mycoplasma hyorhinis (Mhr) is discussed as a pathogen in porcine respiratory disease complex. The objective of this study was to investigate the detection of Mhp and Mhr in weaned piglets exhibiting respiratory disease. Furthermore, it was assessed for variations in detecting Mhp and Mhr at the trachea's start versus the end.

#### **Material and Methods**

Of all animals sent in for necropsy at Royal GD in 2023, 85 piglets were selected for inclusion in this study. Selection criteria for inclusion were an age between 4 and 10 weeks old, anamnesis of respiratory complaints, and macroscopic evidence of pneumonia, regardless of the vaccination status. TBS sampled at the tracheal bifurcation were taken from 60 piglets. The remaining 25 piglets were sampled at two locations; the tracheal bifurcation and behind the larynx. The swabs were tested by duplex PCR for presence of Mhp and Mhr. Further histopathologic examinations were done to determine pneumonia and lesions caused by Mhp.

#### Results

Out of 85 piglets, six were found positive for Mhp (7.1%; Ct 21.5 $\pm$ 3.4), confirmed by histopathology. Of the 25 piglets sampled on two locations one pig was positive on both locations. Typical histopathologic lesions could be identified. Fifty-five piglets were found positive for Mhr (78,6%; Ct 30.8 $\pm$ 4.2). Fifteen and 16 piglets were found positive for Mhr behind the larynx and at the tracheal bifurcation, respectively.

## **Discussion and Conclusion**

The results of this study indicate that prevalence of Mhp in weaned piglets with respiratory issues is low compared to other studies<sup>2</sup> and to what is perceived in the field. Farms with a later moment of infection with Mhp could, therefore, benefit from different vaccination strategies, for instance choosing a later moment of vaccination against Mhp. In the case of Mhyr, it has been confirmed that this pathogen is frequently detectable in pigs with respiratory symptoms.

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# DETECTION OF MYCOPLASMA HYOPNEUMONIAE BY PCR IN FERAL SWINE IN NORTH CAROLINA

M. Pieters <sup>1</sup>, C. Harris <sup>2</sup>, C. Cordon <sup>3</sup>, A. Loucks <sup>2</sup>

<sup>1</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

<sup>2</sup>North Carolina Department of Agriculture and Consumer Services, Raleigh, NC, USA <sup>3</sup>University of Minnesota

## **Background and Objectives**

Mycoplasma hyopneumoniae is a bacterium causing respiratory disease in pigs, leading to significant swine welfare and production issues. Thus, M. hyopneumoniae eradication has been widely applied in the US and is a growing trend in commercial farms. Feral swine, commonly referred to as wild boars, are free-ranging pigs originally introduced to the southern states for recreation purposes. The population has subsequently expended exponentially over decades. Damage to plants, crops, and wildlife habitats caused by feral swine have been well documented. However, little information is available in North America regarding the disease status of feral swine populations to pathogens such as M. hyopneumoniae. This population potentially poses a critical risk to naïve domestic pigs that may be exposed either directly to feral swine or indirectly through fomites carrying pathogens from feral swine. The objective of this study is to evaluate the disease status of M. hyopneumoniae in feral swine from North Carolina, US.

#### **Material and Methods**

Groups of feral pigs trapped and humanely euthanized in North Carolina, U.S. in 2023, have been included in this investigation. To date, eighty-eight bronchial swabs obtained from these feral swine have been tested for M. hyopneumoniae. All swabs have been processed for DNA extraction and real-time PCR using a species-specific assay. Results from this study are descriptively analyzed.

### Results

DNA extraction and PCR testing of samples is ongoing based on the collection of samples from newly trapped feral swine. Preliminary results have shown the detection of M. hyopneumoniae in 61% of the specimens. Ct values were used to estimate the relative bacterial load and varied greatly in the positive samples (low Ct 16.4; high Ct 38.4; with 69% of positive samples Ct <25).

# **Discussion and Conclusion**

Feral swine samples obtained in North Carolina, US, have tested positive for M. hyopneumoniae by PCR. Information on the detection of M. hyopneumoniae from feral swine in other US areas is not available. Similar results have been reported in other countries in Europe and South America. The potential risk for transmission of pathogens from feral swine to domestic pigs in the US is unknown and warrants further research.

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# ENTEROCOCCUS HIRAE : CHARACTERIZATION OF FRENCH STRAINS ISOLATED FROM NEONATAL PIGLET DIARRHEA

<u>E. Lewandowski</u><sup>1</sup>, H. Gantelet<sup>1</sup> <sup>7</sup>Ceva Biovac, 6 Rue Olivier de Serres, 49070 Beaucouze, France

## **Background and Objectives**

Neonatal piglet diarrhea (NPD) is a huge concern for pig producers and a health problem difficult to manage for veterinarians. As prevention is key, biosecurity rules and good management practices must be well respected. Recent publications showed that Enterococcus hirae (E. hirae) is one of the most pathogenic strains involved in NPD in French farms. No licensed vaccine is available on the market and bacterin-autogenous vaccines could be an option. As isolate selection is key, the objective of this study is to evaluate the variability of French E. hirae field strains.

# **Material and Methods**

Forty strains isolated from diarrheic piglets coming from 34 farms were selected. Among these 40 strains, 29 presented histological lesions associated with isolation, 2 none and no data was available for the 9 remaining. As no serotyping method has been developed to characterize E. hirae, we used an innovative method, Fourier transform infrared spectroscopy (FT-IR), in targeting polysaccharides which support serological classifications. This is particularly interesting for the selection of vaccine strains as it is directly linked to the phenotypic expression of molecules on the surface of bacteria, like the capsular material in Gram-positive bacteria. To increase the relevance of the comparison in this bacterial species, 11 strains isolated from clinical cases in poultry were added to this study.

#### Results

Thirty-seven strains were included in the same cluster and the 3 remaining were found in a second cluster for 2 of them and one had a unique profile. All poultry strains were discriminated into two other different clusters, so that they were distinguishable from porcine strains.

#### **Discussion and Conclusion**

Infrared spectroscopy is an interesting tool for phenotypically characterizing E. hirae because it is rapid and affordable. E. hirae appears to exhibit phenotypic variability linked to animal species. Strain characterization is key for vaccine strain selection and the low phenotypic diversity observed in the strains facilitates their choice for the use of autogenous vaccines. Field studies should allow us to confirm the relevance of this vaccination approach.

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# ESTIMATION OF MYCOPLASMA HYOPNEUMONIAE TRANSMISSION PARAMETERS USING EXPERT KNOWLEDGE ELICITATION

A. Sponheim 1, J. Alvarez 2, E. Fano 3, M. Pieters 4

<sup>1</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA

<sup>2</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Current affiliations: Centro de Vigilancia Sanitaria Veterinaria VISAVET, Universidad Complutense, Madrid, Spain; Departamento de Sanidad

<sup>3</sup>Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA; Current affiliation: Pipestone Veterinary Services, Pipestone, MN, USA

<sup>4</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

## **Background and Objectives**

Mycoplasma hyopneumoniae control and eradication efforts are growing in the U.S. Resources supporting these initiatives, including diagnostic guidelines to confirm gilt exposure to estimate shedding cessation<sup>1</sup>, or Day 0 of a herd closure program<sup>2</sup>, have been developed<sup>3</sup>. When diagnostic criteria are not achieved, or a high target prevalence is selected, complementary estimates of time to complete GDU exposure are needed. However, M. hyopneumoniae transmission dynamics are difficult to establish due to the limited performance of diagnostic tests and chronic nature of the infection. Estimates on parameters governing M. hyopneumoniae transmission have been reported, but there is variation between them. Here, we describe the results from an Expert Knowledge Elicitation (EKE) workshop aimed at eliciting distributions representing the most important parameters affecting M. hyopneumoniae transmission in the U.S. Information from this exercise will be used to adapt a published M. hyopneumoniae transmission model to reflect U.S. based production scenarios<sup>6</sup>.

#### **Material and Methods**

The elicitation was conducted following the Sheffield Elicitation Framework (SHELF<sup>4.5</sup>) and involved experts representing academia, industry, and field. Prior to the activity, experts were briefed on probabilistic judgement. A draft "evidence dossier," summarizing available evidence related to M. hyopneumoniae transmission parameters of interest, was provided ahead of the elicitation workshop, and experts had the opportunity to provide comments and additional evidence. During the workshop, a facilitator led discussion aimed at eliciting consensus probability distributions for four parameters from the group of experts by combining individual and group elicitation.

# Results

Distributions were elicited for the transmission coefficient (beta), incubation period (eta), duration of clinical period (tau), and the duration of shedding after clinical signs (gamma). The EKE led to distributions above (beta, gamma), below (tau) or in range with published data (eta), with elicited variability being wider (eta, gamma) or in range (beta, tau) compared to published estimates.

## **Discussion and Conclusion**

Scarce data is available for M. hyopneumoniae that directly applies to estimation of transmission parameters necessary to model disease spread. To our knowledge, this is the first application of an EKE using the SHELF framework to address this need. Realization of the time to complete GDU population exposure may allow for improved M. hyopneumoniae control or eradication program success.

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## EXPERIMENTAL INFECTION OF ERYSIPELOTHRIX PISCISICARIUS IN PIGS

G.M.R. Simão <sup>1</sup>, F.M. Petri <sup>2</sup>, G.S. Nogueira <sup>2</sup>, A.K. Panneitz <sup>2</sup>, E.R. Braga <sup>2</sup>, K.L.D. Carmo <sup>1</sup>, A.T. Reis <sup>3</sup>, C. Pissetti <sup>4</sup>, F. Vannucci <sup>5</sup>, <u>L.G. De Oliveira <sup>2</sup></u>

<sup>1</sup>Agroceres PIC, Rio Claro, SP, Brazil

<sup>2</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil
<sup>3</sup>Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil
<sup>4</sup>CEDISA - Centro de Diagnóstico de Sanidade Animal, Concórdia, Santa Catarina, Brazil
<sup>5</sup>Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA

# **Background and Objectives**

Erysipelothrix spp, a gram-positive bacterium, induces erysipelas in pigs, a systemic disease characterized by sudden death, fever, arthritis, and distinctive diamond-shaped skin lesions. Erysipelothrix piscisicarius (EP) causes the disease in ornamental fishes. This study aims to experimentally infect healthy pigs with EP to evaluate disease presentation in pigs.

# **Material and Methods**

Twelve 70-day-old specific pathogen-free pigs were divided into G1) 8 pigs challenged with 10<sup>s</sup> Colony Forming Units (CFU) of EP per pig; G2) 4 pigs inoculated with Feist broth (negative control). The challenge was performed via dorsal intradermal injection with strain 15TAL0474. The pigs were evaluated on body weight, rectal temperature, skin lesions, and complete blood count on D0, D7, and D14. Afterward, half of the groups were euthanized on D7 and D14 for sample collection aiming at the isolation of blood agar.

#### Results

In G1, rectal temperature significantly increased on days 1, 2, 3, 4, and 7 post-infection compared to G2 (p<0.05). The average daily weight gain (ADWG) on D7 was 0.27 kg in G1 and 0.38 kg in G2, and on day 14, it was 0.28 kg in G1 and 0.37 kg in G2. In G1, complete blood count on D14 showed erythropenia, hypohemoglobinemia, reduced red blood cell count, and lymphocytosis (p<0.05), with segmented neutrophil alterations between days 7-14 (31%). Skin lesions in G1 included erythema on days 3 (6/8), 4 (7/8), 5 (7/8), and 7 (8/8), with softly present rhomboid lesions in 4 animals and necrosis at the site of inoculation. No bacterial growth was observed.

# **Discussion and Conclusion**

The infection in G1 elicited a notable immune response, as evidenced by alterations in the complete blood count. The variations in rectal temperature and ADWG reveal an impact on animal health, magnified by the deficiency in oxygen transport caused by erythropenia during the infection process. Despite the observation of mild lesions and challenging bacteria isolation, EP inoculation induced disease in pigs. To deepen our understanding of its pathophysiology, additional laboratory tests for pathogen detection throughout the course of the disease are essential. This evaluation provides valuable insights into the clinical presentation of the disease in swine.

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## PREDOMINATING LEPTOSPIRA SEROVARS IN AUSTRIAN PIG FARMS IN THE YEARS 2020-2023

<u>R. Steinparzer</u><sup>1</sup>, K. Waldner<sup>1</sup>, T. Sattler<sup>2</sup>, F. Schmoll<sup>1</sup> <sup>1</sup>*Austrian Agency for Health and Food Safety, Mödling, Austria* <sup>2</sup>*Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Leipzig University* 

#### **Background and Objectives**

Pathogenic Leptospira species are related to reproductive disorders in pigs and can cause severe disease in human. Numerous serogroups and serovars with varying significance have been identified. Aim of the study was to give an overview of the most frequently occurring Leptospira serovars in Austrian pig farms in recent years by the detection of antibodies against the respective serovars.

## **Material and Methods**

At the Austrian Agency for Health and Food Safety, in the years 2020 to 2023 a whole of 5376 serum samples from 963 pig farms in Austria have been tested for antibodies against the Leptospira serovars Icterohaemorrhagiae, Pomona, Tarassovi, Bratislava, Grippotyphosa, Hardjo, Canicola and Wolffi by microagglutination test. Sample number was almost equally divided between the years (between 1191 and 1712 per year). The reason for testing included all samples from export examinations to clinically suspect farms.

#### Results

The most frequently detected serovar was Bratislava with 509 (9.9%) antibody positive samples. Serovars Tarassovi (123 positive samples, 2.3%), Pomona (73, 1.4%), Canicola (58. 1.1%), Grippotyphosa (56, 1.1%), Icterohaemorrhagiae (53, 1.0%), Hardjo (16, 0.3%) and Wolffi (13, 0.3%) followed. The serovars detected did not differ significantly between the years.

#### **Discussion and Conclusion**

Diagnosis can only be as exact as the analysis method selected. Since the direct detection of Leptospira is complicated, the antibody detection is mainly used for diagnostic purposes. The diagnosis, however depends on the fact that the serovars present in field are used in microagglutination test. In this study, the most frequently detected serovars were Bratislava, Tarassovi, Pomona, Canicola, Grippotyphosa and Icterohaemorrhagiae. This is in line with a study from Germany which reports results from samples between the years 2011 to 2016. The fact, however, that it is not feasible to test for antibodies against all known more than 200 Leptospira serovars in routine diagnostics, can lead to undetected serovars present in the field, especially if no recent isolates and knowledge about prevalent serovars are available.
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# PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCUS HYICUS ISOLATED FROM PIG FARMS IN SOUTH KOREA

B.Y. Jung <sup>1</sup>, K. Kim <sup>1</sup>, H.S. Shin <sup>1</sup>, A. Kim <sup>1</sup>, S. Yoon <sup>1</sup> <sup>1</sup>Bacterial Disease Division, Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea

### **Background and Objectives**

Exudative epidermitis (EE) is caused by Staphylococcus (S.) hyicus in suckling and weaned pigs. EE is characterized by exfoliation of the skin, sticky exudation, and formation of brown crusts that cover the surface of the body. Morbidity and mortality rates of EE were reported to be approximately 10–90% and 5–90%, respectively. Recent increase in outbreaks of EE poses a serious threat in pig industry in South Korea. This study aimed to investigate the toxin types and the antimicrobial susceptibilities of S. hyicus isolates from pig farms in South Korea.

# **Material and Methods**

Samples were collected from 13 EE outbreak farms (EOF) and 23 non-EE outbreak farms (NEOF) in South Korea between 2019 and 2023. A total of 771 samples consisted of external ear swabs (n=556), vaginal swabs (n=137), and airborne microbes (n=78). The samples were inoculated on sheep blood agar plates and aerobically incubated for 18–24 h at 37°C. Suspected colonies were identified by using MALDI-TOF mass spectrometry and PCR. Toxin type of isolates was determined by multiplex-PCR according to previously described method by Onuma. Antimicrobial susceptibility test was performed by disc-diffusion method with 13 antimicrobial discs.

#### Results

S. hyicus was isolated from 52.7% (406/771) of the samples tested, with a higher isolation rate from EOF (72.5%) compared to NEOF (35.0%). ExhB (65.4%) was the most prevalent toxin type in South Korea, followed by exhD (19.2%), exhA (9.6%), and exhC (5.8%). Interestingly, changes of toxin types were observed in three farms at different time periods. Antimicrobial susceptibility test was performed with 129 isolates. The highest frequency of resistance was observed with lincomycin (90.7%), followed by penicillin (72.9%), ampicillin (69.8%), florfenicol (49.6%), oxytetracycline (48.1%), gentamicin (45.7%), enrofloxacin (34.1%), neomycin (8.5%), amoxicillin/clavulanic acid (7.0%), and trimethoprim-sulfamethoxazole (0.8%). There was no isolate resistant to ceftiofur, cephalexin, and novobiocin.

#### **Discussion and Conclusion**

These findings provide important insights into the prevalence of S. hyicus from pig farms in South Korea, and highlight the need for periodical monitoring of toxin types to develop an effective toxoid vaccine against EE.

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# PREVALENCE AND EXCRETION LEVEL OF LAWSONIA INTRACELLULARIS AND BRACHYSPIRA PILOSICOLI IN FINNISH GROWER-FINISHER PIGS

<u>S. Nikunen</u><sup>1</sup>, E. Välimäki<sup>2</sup>, T. Tirkkonen<sup>3</sup>, M. Hassila<sup>4</sup>, S.L. Musse<sup>1</sup> <sup>1</sup>MSD Animal Health, Nordic, Denmark <sup>2</sup>HKScan Finland Oy, Turku, Finland <sup>3</sup>Atria Finland Plc, Seinäjoki, Finland <sup>4</sup>Snellmanin Lihanjalostus Oy, Pietarsaari, Finland

## **Background and Objectives**

Proliferative enteropathy is caused by Lawsonia intracellularis (LI), which is a common enteric pathogen found in pig production worldwide and, according to Finnish Food Authority, also in Finnish pigs. However, a LI prevalence study has not been yet conducted in Finnish conventional pig herds. In 2022, two studies were conducted with the objective to investigate (1) the prevalence and level of LI and Brachyspira pilosicoli (BP) as well as (2) the LI seroprevalence in Finnish grower and slaughter pigs, respectively.

# **Material and Methods**

In study 1, one faecal sock sample was collected from batches of pigs weighing 25-35 kg in 36 farms with a history of diarrhoeic outbreaks. No antimicrobial treatments were given at time of sampling. qPCR was performed (at L&F, Veterinary Laboratory, Denmark) to determine the bacterial shedding of LI and BP, having both qPCR tests a lower detection limit of 3 and 2 log(10) copies/gram feces, respectively.In study 2, blood samples were randomly collected at 3 major Finnish slaughterhouses by during one-two working days (100 samples from 10 farms in 1 slaughterhouse; 150-155 samples from 15 farms in each of 2 slaughterhouses). Samples were analysed for LI antibodies by ELISA (Svanova®) at CDS, The Netherlands; cut-off value for positive samples at  $\geq$ 30% inhibition.

#### Results

Prevalence and mean excretion levels in positive fecal samples (median; range) were 61.1% and 5.13 (5.29; 3.04-6.05) for LI and 44.4% and 4.04 (4.17; 2.00-6.40) for BP, both log(10) copies/gram of feces. On average 217 pigs were sampled in each batch (median: 180; range: 40-588).In total, 405 blood samples from 45 farms were collected for seroprevalence. Farm seroprevalence was 100%, whereas pig prevalence was 93.6% (variation between slaughterhouses: 91.3-96.8%).

# **Discussion and Conclusion**

Using faecal sock samples, both LI and BP were commonly found in Finnish grower pigs, though LI was more common than BP. Mean excretion of LI were found at levels known to cause proliferative lesions in the intestine and to potentially cause a loss in productivity. All finisher farms were seropositive for LI with a high prevalence, suggesting that infection becomes apparent during the grower-finisher period.

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# PREVALENCE AND SCORING OF LUNG LESIONS RELATED TO ENZOOTIC PNEUMONIA AND PLEUROPNEUMONIA IN BRAZIL: AN UPDATE

P. Filsner <sup>1</sup>, J. Calveyra <sup>2</sup>, F. Betiolo <sup>1</sup>, M. Walter <sup>1</sup>, C. Sartori <sup>1</sup>, C. Postal <sup>1</sup>, <u>R. Krejci</u> <sup>2</sup> <sup>1</sup>Ceva Animal Health, Brazil <sup>2</sup>CEVA ANIMAL HEALTH

## **Background and Objectives**

The lung scoring method is an effective way to assess respiratory health and efficiency of control programs on swine farms. Clear correlation between lung lesions, the economic impact of the disease and the efficiency of vaccination has been reported. The aim of this study was to provide the regular update on prevalence of Enzootic pneumonia (EP) and Actinobacillus pleuropneumoniae (A.p). – like lesions in Brazilian farms from 2020 to 2023 (YTD) using CEVA Lung Program.

# **Material and Methods**

Between 2020 and 2023, 1847 batches of 175535 lungs were evaluated using the CEVA Lung Program methodology with an average of 95 lungs per batch inspected. The animals in the study are from the main pig producing regions in the country. Average values were calculated for % Bronchopneumonic Lungs, % Affected Lung Surface, EP Index, AP like lesions and % Dorsal Caudal Pleurisy.

## Results

Broncho-pneumonic lungs were present in 52.8% of lungs evaluated; 3.7% was the average percentage of Affected Lung Surface. The incidence of dorsal-caudal pleurisy and cranial pleurisy were present 9% and 5.2%, respectively, in the total number of lungs evaluated. 8.9% of the evaluated lungs presented scars. Regarding the lesions related to Ap and EP they were measured using two specific indexes. The APP Index reached 0.3 and the EP index 2.24.

## **Discussion and Conclusion**

When compared with previous analysis such as the publication by Costa et al, 2019 56.1% of lugs were affected, while Calveyra et al., 2023 showed that this type of lesions ranged between 51.7 to 66.6%. The results had small variation in then Broncho-pneumonic lungs parameter. With a robust database, CLP proves to be a useful tool for monitoring and evaluating lung lesions related to Enzootic Pneumonia and Porcine Pleuropneumonia from epidemiological perspective and efficiency of control measures.

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# PREVALENCE OF ACTINOBACILLUS PLEUROPNEUMONIAE SEROTYPES ISOLATED FROM LUNG OR PLEURA SAMPLES IN FRANCE FROM 2020 TO 2023

<u>P. Berton</u><sup>1</sup>, M. Couteau <sup>2</sup>, M. Fellag <sup>3</sup>, E. Bousquet <sup>4</sup> <sup>1</sup>Virbac France, Espace Azur Mercantour, 3ème Rue, 06510 Carros, France <sup>2</sup>Bio Chêne Vert, rue Blaise Pascal, 35220 Chateaubourg, France <sup>3</sup>Labocea, Zoopôle, 7 rue du sabot, CS 30054, 22440, Ploufragan, France <sup>4</sup>Virbac SA, 13ème rue, 06510 Carros, France

# **Background and Objectives**

Actinobacillus pleuropneumoniae (APP) is a major pathogenic agent in pig production and is implicated in Porcine Respiratory Disease Complex. Biotype 1 (B1) strains are the most frequently involved in pathological outbreaks in Europe. Twelve serotypes compose B1. This study aims to update prevalence data of the different APP biotypes and serotypes from strains evidenced in lung and pleura samples sent to the laboratory within the last 3 years in France.

#### **Material and Methods**

A total of 578 isolates of APP coming from retrospective clinical cases in lung and pleura samples collected from June 2020 to October 2023 were analyzed in 2 veterinarian diagnostic labs (Lab 1 and Lab2). After bacterial culture, MALDI-TOF MS was used for bacterial identification. Serotyping was done using agglutination tests, and if necessary immunodiffusion tests were done for identifying strains that cross-reacted.

#### Results

In Lab1, 409 lung or pleura samples were analyzed from 2020 to 2023. Among them, 196 were positive for APP (48%). APP was the first bacterial agent isolated (followed by Pasteurella multocida – 26%). In Lab2, data collected only included APP positive samples (382).Serotyping was successful in 488 samples from both labs (84% of APP positive samples). APP B1S1-9-11 and APP B1S2 were the most frequently isolated (37% and 32% respectively), followed by B1S4-7 (16%) and B1S3-6-8 (13%). APP B1S5 and B1S12 were occasionally isolated. No B1S10 was identified.Concerning serogroups 1-9-11 and 4-7, serotypes could be often specified. Thus 79% of B1S1-9-11 were actually B1S9-11, while 88% and 3% among B1S4-7 were B1S7 and B1S4 respectively. A clear seasonality of APP B1S1-9-11 was observed (peaks of samples submission between October and April every year).

# **Discussion and Conclusion**

Among isolates that could be serotyped in lung and pleura samples, APP B1S2 and B1S1-9-11 were the most isolated serotypes. Some could cross-react with multiple serotyping reagents, thus identification of the exact serotype could not be performed every time (especially APP B1S1-9-11, B1S4-7, B1S3-6-8). Those results are consistent with former prevalence data of APP serotypes isolated in France, especially concerning B1S2 and B1S9-11 that are steadily the predominant ones.

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# PREVALENCE OF MYCOPLASMA HYOPNEUMONIAE AT DIFFERENT STAGES IN FIVE PIG PRODUCTION SYSTEMS IN SOUTH BRAZIL.

<u>á. Aldaz</u><sup>1</sup>, D. Tres<sup>2</sup>, D. Veit<sup>2</sup>, K. Franz<sup>2</sup>, V. Fernandes<sup>2</sup> <sup>1</sup>Zoetis Inc, Parsippany, NJ, USA <sup>2</sup>Zoetis Inc

# **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is the primary causative agent of enzootic pneumonia in pigs. The infection and chronic respiratory disease caused by Mhyo affects swine production systems worldwide. In Brazil studies show important production losses on farms and slaughterhouses due to injuries caused by this agent. Therefore, the porpoise of the present study was to assess the prevalence of M. hyopneumoniae in five big production systems in the mainly production region in Brazil.

# **Material and Methods**

The study was carried out between August 2022 and September 2023 in five pig production systems in Brazil (A, B, C, D and E). To determine the prevalence in each system, pigs were sampled at 4, 9, 13, 17 and 23 weeks of age on the same day. Tonsil samples were collected by the introduction of sterile rayon swabs (Jiangsu Huida Medical Instruments Co., LTD, YJ. China) into the mouth cavity until reaching the tonsil and rotated and counter clockwise. A total of 758 samples were tested for Mhyo DNA by PCR

# Results

Mhyo DNA was present in the five production systems sampled with variable prevalence patterns by system at the five ages sampled. Farm A: 33%, 33%, 63%, 40%, and 70%. Farm B: 3%, 10%, 50%, 20%, and 30%. Farm C: 10%, 5%, 10%, 10%, and 5%. Farm D: 7%, 0%, 12%, 0%, and 0%. Farm E: 40%, 42%, 30%, 28%, and 34%.

## **Discussion and Conclusion**

Piglets that become infected with Mhyo in the suckling period are the main source of the spread of infection within the growing and fattening units. The results of this study confirm that Mhyo is highly prevalent in Brazilian herds, especially in the early phases, with prevalences of 33% and 40% in some pig flows at 4 weeks of age. In the same production systems, the prevalence in subsequent ages remained high when compared to other flows that had lower positivity at 4 weeks of age.

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# TONSILLAR SCRAPPING AS AN ALTERNATIVE TOOL FOR APP MONITORING IN LIVE PIGS: A CASE REPORT.

D. Never 1, V. Buntenkötter 1, I. Hennig-Pauka 2, P. Könighoff 3

<sup>1</sup>Tierärztliche Gemeinschaftspraxis An der Maiburg, Bippen, Germany <sup>2</sup>Field Station for Epidemiology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany <sup>3</sup>Intervet Deutschland GmbH; MSD Animal Health, Unterschleißheim, Germany

## **Background and Objectives**

Lung tissue from dead pigs is usually used for Actinobacillus pleuropneumoniae (APP) diagnostic, but sometimes with negative results in subclinical infection APP detection in living pigs is difficult and serological examination can be false negative prior to seroconversion after infection. For APP monitoring in positive herds tonsillar scrapings are appropriate samples, because tonsils are frequently colonized by APP. The aim of this case was to verify if the pigs were colonized with APP already in the nursery of a farm with mortality due to APP.

# **Material and Methods**

In 2023, several fattening pigs died in a German fattening farm due to App Serotype (ST) 2. To clarify, if nursery pigs were the source of infection, lungs of dead nursery piglets were examined for APP with negative results. To clarify whether nursery piglets were already colonized with APP, tonsillar scrapings were sampled cross-sectionally from 20 piglets from two age groups ((4-weeks (A); 10-weeks (B)). Tonsillar swaps were pooled in groups of 5 (Pool A1, A2, B1, B2) and analyzed by rt-PCR. Pool B2 was further analyzed by capsular gene multiplex PCR (serotype 1-19) for direct serotyping.

#### Results

All pools of tonsillar swaps were positive by PCR (ct-value): A1 = 37.4; A2 = 37.8; B1 = 28.1; B2 = 24.0. The capsular gene multiplex PCR resulted in APP ST 12.

# **Discussion and Conclusion**

Under the conditions of this clinical case, tonsillar scraping was a useful tool for monitoring APP in living pigs and assessing colonization after weaning. APP ST 12 found in tonsillar swaps of nursery pigs could have been involved in APP outbreaks. It has not been found in dead fattening pigs. This suggests that the infection of the fattening pigs with ST 2 could not be deduced so far to an infection in the nursery period, although the sample size is too small to decline the assumption.

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# AN AUTOMATED ASSAY FOR THE MEASUREMENT OF AMMONIA IN PIG SALIVA

<u>E. Llamas-Amor</u><sup>1</sup>, M.J. Lopez-Martínez<sup>2</sup>, A. González Bulnes<sup>3</sup>, E. Garcia Manzanilla<sup>4</sup>, J. Ceron<sup>5</sup>, A. Muñoz-Prieto<sup>6</sup> <sup>1</sup>Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum, University of Murcia, Espinardo, Murcia, 30100, Spain.

<sup>2</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Campus de Espinardo s/n, 30100 Murcia, Spain <sup>3</sup>Departamento de Producción y Sanidad Animal, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, C/Tirant lo Blanc, 7, Alfara del Patriarca, Valencia, 46115, Spain.

<sup>4</sup>Pig Development Department, Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland & School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland

<sup>5</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia

<sup>e</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (INTERLAB-UMU), Department of Animal Medicine and Surgery, Veterinary School, Regional Campus of International Excellence Mare Nostrum, University of Murcia, 30100 Murcia, Spain

## **Background and Objectives**

Ammonia (NH3) has been shown to be a key biomarker for a wide variety of diseases, such as hepatic and chronic kidney diseases (CKD) and cancers in human beings. In addition, hyperammonemia can develop in situations of systemic inflammation. This study aimed to validate a spectrophotometric assay for ammonia in pigs using saliva as a non-invasive sample. Additionally, variations of this analyte in the saliva of pigs tested positive on PCR analysis for S. suis were investigated.

## Material and Methods

Ammonia was determined using a commercially available immunoturbidimetric assay (Spinreact, Girona, Spain) adapted to an automated Olympus AU400 system. The method was analytically validated by determining precision and accuracy (recovery study) for porcine saliva samples. The stability of the analyte at 24 and 48h at different temperatures (room temperature, 4°C and -80°C) was investigated in ten saliva samples. In addition, ammonia levels of 12 saliva samples of pigs with meningitis tested positive for s. suis on PCR analysis was compared with 12 healthy pigs.

The Wilcoxon-rank test assessed differences between groups. Results were expressed as median and interquartile range. A P<0.05 was considered significant.

## Results

The assay showed an imprecision lower than 10% and a good linearity under dilution. Ammonia was stable in porcine saliva samples at room temperature for 24 h, showing a significant increase after 48 h, but was stable when samples were refrigerated or frozen at both times.

Median ammonia levels were significantly higher in pigs with meningitis due to S. suis (median= 5.98 g/mL, 25-75% percentile= 2.65 – 9.48) (p=0.003) compared to healthy pigs (median= 1.22 g/mL, percentile= 0.24 – 1.65).

# **Discussion and Conclusion**

This study indicated that ammonia can be measured in the saliva of pigs, and the levels were stable when samples were refrigerated or frozen for 48 hours. In addition, this analyte increases in the saliva of pigs with S. suis infections, indicating its possible role in systemic inflammation, as reported in human medicine. Further investigations are warranted to evaluate ammonia as a biomarker in the saliva of pigs.

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# ANTIMICROBIAL RESISTANCE IN STREPTOCOCCUS SUIS FROM PIGS: SHOULD WE BE CONCERNED?

<u>S. Chagas</u><sup>1</sup>, E. Paladino<sup>2</sup>, R. Singer<sup>3</sup>, L. Mendonça Pascoal<sup>4</sup>, F. Vannucci<sup>5</sup> <sup>1</sup>PhD student at Universidade Federal de Goiás and University of Minnesota <sup>2</sup>University of Minnesota <sup>3</sup>Department of Veterinany and Biomedical Sciences, College of Veterinany Mi

<sup>3</sup>Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

<sup>4</sup>Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Goiânia, Brazil <sup>5</sup>Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA

## **Background and Objectives**

Antimicrobials used to control Streptococcus suis in pig farms might accelerate the development of antimicrobial resistance (AMR) (1). Since the bacterium is zoonotic (2,3), monitoring AMR is also important from a public health standpoint. This study was conducted to investigate the AMR pattern of S. suis isolated from pigs in the US over the last 10 years.

# **Material and Methods**

suis data isolated from clinical cases were obtained from the Minnesota Veterinary Diagnostic Laboratory, from January 2014 and June 2023. 5,697 isolates were tested for antimicrobial susceptibility (Sensititre ™, Thermo Scientific) to assess the minimal inhibitory concentration (MIC). Antimicrobials with MIC breakpoints established by the CLSI (Vet01S, 2023) (4) were included: ampicillin and penicillin (penicillins), ceftiofur (cephalosporins), enrofloxacin (fluoroquinolones), florfenicol (phenicols), and oxytetracycline (tetracyclines). An isolate was considered multi-drug resistant (MDR) when resistant to at least three different classes.

#### Results

Overall, AMR rates were: oxytetracycline (95.59%), penicillin (10.8%), enrofloxacin (3.34%), ceftiofur (2.4%), florfenicol (1.38%), and ampicillin (1.21%). 100% were resistant to at least one class, and 3.78% were MDR. Across the years, an ascendent trend in MDR was observed up until 2018, when 5.20% were MDR. Enrofloxacin showed an increasing resistance trend from 2020 (2.95%) to 2023 (8.88%), as well as ampicillin from 2022 (1.06%) to 2023 (3.47%). A decreasing trend for penicillin from 2018 (13.65%) to 2021 (7.18%) was observed, followed by an increase from 2021 (7.18%) to 2023 (10.42%).

### **Discussion and Conclusion**

Penicillins and tetracyclines are the most used classes in pig production worldwide (5), and it was observed as the highest resistance rates. Similar findings were observed in the US (6) and France (7). The use of two classes might be related to its high resistance rate. Besides the low overall resistance rate of enrofloxacin, the value tripled throughout the years, thus it is important to keep monitoring this antimicrobial. MDR is considered by the WHO of highest concern and should be monitored closely in food animals (8), especially due to the occupational risk that S. suis represents. There are options to treat S. suis in pigs with antimicrobials commonly used in the field. However, the presence of AMR highlights the importance of monitoring.

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# ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF MYCOPLASMA HYOSYNOVIAE STRAINS ISOLATED FROM SWINE ACROSS EUROPE BETWEEN 2018 AND 2023

D. Földi <sup>1</sup>, U. Klein <sup>2</sup>, E.Z. Nagy <sup>1</sup>, L. Tóth <sup>1</sup>, N. Belecz <sup>1</sup>, K. Költö <sup>1</sup>, M. Merenda <sup>3</sup>, S. Catania <sup>3</sup>, J. Spergser <sup>4</sup>, U. Siesenop <sup>5</sup>, P. Vyt <sup>6</sup>, Z. Kreizinger <sup>1</sup>, M. Gyuranecz <sup>1</sup>

<sup>1</sup>HUN-REN, Veterinary Medical Research Institute, Budapest, Hungary
<sup>2</sup>Huvepharma NV, Antwerp, Belgium
<sup>3</sup>Mycoplasma Unit, SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Buttapietra (VR), Italy
<sup>4</sup>Institute of Microbiology, University of Veterinary Medicine Vienna, Austria
<sup>5</sup>University of Veterinary Medicine Hannover, Institute for Microbiology, Hanover, Germany
<sup>6</sup>Dialab, Belsele, Belgium

#### **Background and Objectives**

Mycoplasma hyosynoviae is a facultative pathogenic bacterium present in pig farms world-wide. The bacterium colonizes the tonsils, is shed via nasal secretions and is transmitted through close contact. M. hyosynoviae causes arthritis in pigs mainly older than 10-weeks of age. The economic losses due to the infection can be reduced by targeted antibiotic treatment. The aim of this study was to determine minimal inhibitory concentrations (MIC) of antibiotics potentially used in cases of M. hyosynoviae infection treatment in swine production.

#### **Material and Methods**

One hundred and six M. hyosynoviae isolates (20 Austrian, 20 Belgian, 25 German, 21 Hungarian and 20 Italian) collected between 2018 and 2023 from various tissues and the type strain (NCTC10167) were examined by broth micro-dilution tests for ten antimicrobial agents. Clinical breakpoints for MIC result interpretation on Mycoplasma spp. are currently not available.

## Results

Low concentrations of the examined antibiotics inhibited the growth of the clinical isolates with four exceptions. Enrofloxacin, oxytetracycline and florfenicol inhibited the growth of the clinical isolates at moderate concentrations (enrofloxacin MIC<sub>90</sub> 0.625  $\mu$ g/ml, oxytetracycline MIC<sub>90</sub> 2  $\mu$ g/ml and florfenicol MIC<sub>90</sub> 2  $\mu$ g/ml) and tulathromycin could inhibit these isolates at high concentrations (MIC<sub>90</sub> 64  $\mu$ g/ml). For the other six examined antibiotics the following low MIC<sub>90</sub> values were obtained: tiamulin <0.039  $\mu$ g/ml, tylosin 0.5  $\mu$ g/ml, tilmicosin 1  $\mu$ g/ml, tylvalosin <0.039  $\mu$ g/ml, lincomycin <0.25  $\mu$ g/ml and doxycycline 0.312  $\mu$ g/ml. The MIC distributions for all tested antibiotics were monomodal, except for tulathromycin where the MIC values showed a bimodal right shift MIC distribution.

# **Discussion and Conclusion**

The study results show variable antimicrobial susceptibility patterns among European M. hyosynoviae isolates against different antibiotics. The data underline the importance to continue susceptibility monitoring on pan-European level and to use antibiotics responsibly for treatment purposes. Based on our results, tiamulin, tylosin, tylvalosin and lincomycin are the most effective compounds against European M. hyosynoviae isolates in vitro.

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# AWARENESS OF THE RELATIONSHIP BETWEEN POST-WEANING DIARRHEA AND SUBSEQUENT S. SUIS MENINGITIS: MARKET RESEARCH ANALYSIS

#### F. Vangroenweghe 1

<sup>1</sup>BU Swine & Ruminants, Elanco Benelux, Elanco Animal Health

#### **Background and Objectives**

Post-weaning Escherichia coli diarrhea (PWD) remains a major cause of economic losses for the pig industry. Besides enterotoxigenic E. coli (ETEC) diarrhea post-weaning, Streptococcus suis (S. suis) meningitis is the second most important reason for antimicrobial treatment post-weaning. This market research study aimed to evaluate the awareness of swine producers about the relationship between PWD and subsequent S. suis meningitis during the post-weaning period based on a questionnaire.

# **Material and Methods**

We aimed to collect data from at least 50 swine producers in both Belgium and the Netherlands. Therefore, a total of 647 swine producers were contacted in Belgium (n = 505) and the Netherlands (n = 142). Response rate was 10% in Belgium and 39% in the Netherlands. Data were collected through telephone interviews and registered in Excel for further processing and analysis.

### Results

In total, 48% of all swine producers had a high willingness to take specific actions to control S. suis on their farm, with several types of management and treatment measures applied. In 77% of the farms, S. suis outbreaks were diagnosed regularly during the previous year. In 55% of the farms, problems with S. suis already occurred immediately post-weaning. Awareness of the intestine as a potential route of entry for S. suis into the blood circulation was overall high with 75%. Therefore, several specific actions were taken to control PWD. Overall, 93% of the swine producers implemented at least one specific measure to prevent PWD.

#### **Discussion and Conclusion**

Streptococcus suis remains one of the major disease problems post-weaning in many swine farms in Belgium and the Netherlands. Although many swine producers are aware of the potential route of entry of S. suis through the intestine, control of PWD to reduce this mechanism remains an issue to be tackled. As many as 31% of the swine producers do not take any measures to control PWD and therefore continue to suffer from S. suis outbreaks which are difficult to control. The overall willingness to take supplementary measures to reduce the impact of S. suis on their swine farm is high.

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# CAN BACTERIN-AUTOGENOUS VACCINES PROVIDE A SOLUTION FOR POST-WEANING DIARRHEA? AN EFFICACY EVALUATION ON TWO DUTCH FARMS

W. Van Herten <sup>1</sup>, R. Aerts <sup>2</sup>, <u>J. Schumans</u> <sup>3</sup>, S. Van Colen <sup>4</sup>, P. De Backer <sup>5</sup>, H. Gantelet <sup>6</sup> <sup>1</sup>DAC Zuid-Oost, Deurne, Netherlands <sup>2</sup>Lintjeshof, Nederweert, The Netherlands <sup>3</sup>Ceva Santé Animale, Naaldwijk, the Netherlands <sup>4</sup>Ceva Santé Animale Benelux, Tiendweg 8-C, 2671 SB Naaldwijk, The Netherlands <sup>5</sup>Ceva Santé Animale, Brussels, Belgium <sup>6</sup>Ceva Biovac, 6 Rue Olivier de Serres, 49070 BeaucouzE, France

# **Background and Objectives**

Post-weaning diarrhea (PWD) due to Escherichia coli (E. coli) is known as one of the biggest problems in nursery piglets. Treatment with antibiotics and prevention with commercial oral vaccines appeared to be insufficient in some cases and antibiotic use is under pressure. The objective of this field observation was to assess the efficacy of autogenous vaccines to control PWD.

# **Material and Methods**

For this field observation, 3 types of bacterin-autogenous vaccines were developed (1 mL/dose): a one-shot aluminum hydroxide vaccine (product A1), a two-shot aluminum hydroxide vaccine (product A2) and a one-shot oily vaccine (product A3). Each vaccine contained farm specific strains and was administered to three subsequent weaning groups in two Dutch farms. Products A1 and A3 were administered to piglets 8-10 days of age, product A2 to piglets 8-10 and 21-24 days of age. Mortality rate and the antibiotic use were monitored from weaning until three weeks after weaning and compared with the initial situation.

## Results

To observe the antibiotic use, the DDD (Defined Daily Dose Animal per farm per year) was determined before and during the period of vaccination. In farm 1 (1700 sows) the DDD decreased from 1.48 to 0. Mortality rate was low (0.56% before vaccination) because using a standard two-week antibiotic treatment starting the first day of weaning. In the vaccinated piglets that were not treated with antibiotics, it declined to 0.11% (product A1), 0.20% (product A3) and was 0.61% (product A2). In farm 2 (700 sows) the DDD increased from 1.3 to 2.39 due to an influenza virus and Streptococcus suis outbreak. The mortality rate decreased from 5% to 0.27% (product A1), 0.63% (product A2) and 0.00033% (product A3).

### **Discussion and Conclusion**

PWD due to E. coli in the nursery unit can be controlled using bacterin-autogenous vaccines. This preventive method can provide reduction in antibiotic use and mortality rate in nursery piglets. Nevertheless, the cascade system should always be respected. Implementation of this type of product is therefore a last option. Besides that, good management in the farm is necessary as well. This to increase the chances of success.

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# CASE REPORT: SUCCESSFUL MYCOPLASMA HYOPNEUMONIAE ERADICATION USING TRACHEA-BRONCHIAL SWAB MONITORING IN A FARROW-TO-WEAN SOW HERD WITH COMBINED VACCINATION AND TREATMENT PROTOCOL

<u>F. Vangroenweghe</u><sup>1</sup>, H. Neleman <sup>2</sup>, K. Koenders <sup>2</sup> <sup>1</sup>Elanco - BU Food Animals - Benelux <sup>2</sup>Lintjeshof Vet Group, The Netherlands

## **Background and Objectives**

Mycoplasma hyopneumoniae (M. hyo), the primary pathogen of Enzootic Pneumonia, occurs worldwide and causes major economic losses to the pig industry. Affected pigs show chronic coughing, are more susceptible to other respiratory infections and have a reduced performance. Piglets become infected with M. hyo during the suckling period and have been shown positive from weaning onwards. Moreover, once infected with M. hyo, animals can excrete the pathogen over a long period of time (254 days post-infection). Therefore, dedicated programs to monitor for freedom of M. hyo has been developed consisting of serological screening and further confirmation tests using PCR. The objective of the current case report was to rerport a successful M. hyo eradication in a farrow-to-wean sow herd with a M. hyo-positive status using a combined program of vaccination and treatment while monitoring progress and freedom of disease with TBS.

## **Material and Methods**

A high health breeding farm in the Netherlands (1000-sow herd with internal breeding gilt facility) had been negative for M. hyo for 10 years using a standard serological monitoring schedule. Following initial confirmation of M. hyo-positivity using TBS (n = 120 samples), an eradication program was established combining an additional double M. hyo one-shot vaccination (4-week interval) with a double individual injection of pigs present on-farm with tulathromycin (2-week interval). Furthermore, a 2-week stop was introduced for breeding gilt replacement. Monitoring was carried out using TBS at 8 weeks after the second antimicrobial treatment and 12 weeks later to confirm M. hyo eradication in the herd.

#### Results

The first TBS sampling revealed several pools as M. hyo-positive in both breedings sows (6/16 pools) and post-weaned piglets (2/24 pools). Following the eradication protocol, including vaccination and treatment, no M. hyo-positive TBS samples could be detected at both 8 weeks and 20 weeks post-treatment

### **Discussion and Conclusion**

The current case report demonstrated that M. hyo eradication is possible in farrow-to-wean sow herd situated in a pigdense region under Dutch field conditions. Monitoring of M. hyo infection using TBS sampling is a reliable technique to confirm infection and follow-up the evolution towards freedom of disease.

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# COINFECTIONS BETWEEN VIRULENCE FACTORS OF ESCHERICHIA COLI, CLOSTRIDIUM PERFRINGENS AND CLOSTRIDIODES DIFFICILE IN THAILAND DURING 2023.

J. Miguel <sup>1</sup>, D. Giralt <sup>1</sup>, A. Buakhiew <sup>2</sup>, C. Ananratanakul <sup>2</sup>, S. Krasaesub <sup>2</sup>, <u>S. Traiyarach <sup>1</sup></u> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA Thailand, Bangkok

## **Background and Objectives**

Neonatal diarrhoea (ND) in piglets is a multifactorial disease and its control should consider the differential diagnosis of the multiple agents involved. This study describes the prevalence of bacterial agents in piglets suffering from diarrhoea in Thailand.

## Material and Methods

During 2023, a total of 184 pooled faecal samples from litters (age ranging from 0-28 days old) from 49 farms (3,000 sows on average, ranging from 150 – 12,000) were analysed in HIPRA Diagnos Thailand. Samples were collected using FTA® Elute cards (Whatman). Only 6 farms were vaccinating against bacterial neonatal diarrhoea with vaccines containing E. coli and Clostridium perfringens type C antigens. A PCR test was performed to detect F4, F5, F6 adhesion factor, the heat-labile toxin (LT) of Enterotoxigenic E. coli, A and B toxins of C. difficile, and  $\alpha$ ,  $\beta$ ,  $\varepsilon$ -toxins of C. perfringens.

### Results

97.3% of the samples were positive for the  $\alpha$ -toxin of C. perfringens, 54.9% of them with a high load (Ct <26).  $\beta$  and  $\epsilon$  toxins were negative in all samples. In relation to C. difficile, in 39.7% of the cards A-toxin was detected, and in 24.5% B-toxin was also detected. Regarding ETEC, F4 was detected in 43.5% of samples, 8.2% F5, and 26.6% to LT. Regarding coinfections, 42.39% of samples were positive to 3 or more of the virulence factors.

# **Discussion and Conclusion**

The research shows that bacterial agents are highly observed in piglet diarrhoea. In most cases, a multidisciplinary approach is needed as more than one agent is co-infecting the piglet, which in turn complicates the control of the farrowing unit's health. Nevertheless, further investigation and complete assessment of other variables like specific management practices, vaccination protocols or antibiotic usage would be needed to identify specific reasons for such an observation.

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# DETECTION OF IMMUNOGENIC PROTEINS OF GLAESSERELLA PARASUIS USING SERUM FROM PROTECTED ANIMALS

<u>S. Hau</u><sup>1</sup>, K. Eberle<sup>2</sup>, J. Nally<sup>1</sup>, J. Lippolis<sup>1</sup>, S. Brockmeier<sup>1</sup> <sup>1</sup>Agricultural Research Service, USDA <sup>2</sup>Agricultural Research Service, USDA / Oak Ridge Institute for Science and Education, ORAU

### **Background and Objectives**

Glaesserella parasuis is a Gram-negative pathogen and the causative agent of Glässer's disease, which is characterized by arthritis, meningitis, polyserositis, and septicemia. G. parasuis isolates are characterized by their capsular polysaccharide into 15 serovars that vary in virulence both within and between groups. Glässer's disease has been difficult to control due to the development of serovar or strain specific immunity with the use of inactivated vaccines. Because of this, recent research has focused on novel vaccine platforms, including subunit vaccines.

# **Material and Methods**

In this study, we used serum from pigs immunized with bacterin vaccines with a known capacity for heterologous protection against G. parasuis to identify immunogenic proteins specifically associated with a cross protective immune response. To detect proteins of interest, we used two comparative methods: 2-dimensional gel electrophoresis (2DGE) and immunoprecipitation.

# Results

We identified 50 unique proteins associated with heterologous protection using 2DGE and 46 unique proteins with immunoprecipitation.

#### **Discussion and Conclusion**

Some of the identified proteins are known virulence factors or have been previously identified as vaccine candidates for G. parasuis or other Gram-negative bacteria, indicating these screening methods are capable of identifying proteins that have potential for vaccine development. Future investigations will be directed at testing selected proteins associated with the cross-protective immune response to determine their potential as candidates for a novel subunit vaccine.

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# DIAGNOSTIC SPECIFICITY ASSESSMENT OF AN INDIRECT ELISA FOR MYCOPLASMA HYOPNEUMONIAE UNDER FIELD CONDITIONS

<u>G.M.R. Simão</u><sup>1</sup>, E. Tapia<sup>2</sup>, S. Zimmerman<sup>2</sup>, S.S. Kuchiishi<sup>3</sup>, L.H.G. Saraiva<sup>1</sup>, T.D.F. Freitas<sup>1</sup>, K.L.D. Carmo<sup>1</sup>, R. Pigozzo <sup>1</sup>, J. Ferreiro<sup>4</sup>, J.P. Sato<sup>5</sup>, C. Pissetti<sup>3</sup>, D. Linhares<sup>6</sup> <sup>1</sup>Agroceres PIC, Rio Claro, SP, Brazil <sup>2</sup>IDEXX Laboratories Inc., Westbrook, ME, USA <sup>3</sup>Centro de Diagnóstico de Saúde Animal (CEDISA), Concórdia, SC, Brazil <sup>4</sup>Agroceres PIC Argentina, Buenos Ayres, Estado de Buenos Ayres, Argentina <sup>5</sup>Dr. Bata, Chapecó. SC, Brazil <sup>6</sup>Veterinary Diagnostic and Population Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

### **Background and Objectives**

The monitoring of Mycoplasma hyopneumoniae (Mhyo) infections by indirect ELISA is an important tool for swine genetic companies and multipliers that need to prove Mhyo-free herds for customers. Therefore, monitoring herd status over the years generates serological history providing absolute security of the source's health status, even if there are unexpected results. This study assessed the diagnostic specificity of IDEXX Mhyo antibody ELISA in Mhyo-free herds in Brazil based on routine testing, observation of clinical signs, and qPCR testing of tracheal swab samples from unexpected ELISA-positive results.

#### **Material and Methods**

Serum samples (n = 18,170) were collected over 7 years (Jan 2017 to Sept 2023) from twelve Agroceres PIC multipliers farms in Brazil that had been Mhyo-negative until current date. Samples were tested on the IDEXX Mhyo antibody ELISA in CEDISA, an accredited laboratory by Brazil's official service. The mean S/P, standard deviation, and percent of presumed false positive results were calculated monthly and annually. Swine populations testing ELISA-positive were followed-up with extensive respiratory clinical signs evaluation and qPCR testing of tracheal swabs.

# Results

Serum sample testing produced 17,478 negative (96.2%), 355 positive (1.9%), and 337 suspect (1.8%) results. Diagnostic specificity was estimated at 96.2% based on negatives or 98.0% based on negatives and suspect results. The overall mean S/P value was 0.052, 8 times lower than the test cutoff (0.400). The overall S/P standard deviation was 0.105 and ranged from 0.110–0.169 among farms. Over the 7 years in which data were collected, the aggregate rate of false reactions was 1.95%.

# **Discussion and Conclusion**

The data analyses show that the mean and standard deviation are similar year after year, which indicates consistency in the results and, consequently, the efficacy of this health monitoring tool. In addition to serology, monthly clinical assessment and confirmatory qPCR significantly increase the accuracy for detecting truly positive pig herds, providing more assurance to customer farms regarding the health status of the genetic replacement source.

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# EVALUATION OF THE ANTIBACTERIAL EFFICACY OF MEDIUM-CHAIN FATTY ACIDS AGAINST ESCHERICHIA COLI AND SALMONELLA SPP.

H. Kuo<sup>1</sup>, W.C. Chiu<sup>2</sup>, H. Tseng<sup>1</sup>, L.Y. Fong<sup>1</sup>, C. Wang<sup>1</sup>, S. Huang<sup>1</sup>, <u>C. Wu<sup>1</sup></u> <sup>1</sup>Department of Veterinary Medicine, National Chiayi University, Chiayi, Taiwan <sup>2</sup>Ecolex Animal Nutrition, Kuala Lumpur 42920, Malaysia

# **Background and Objectives**

Medium-chain fatty acids (MCFAs), naturally occurring compounds long utilized in animal nutrition, have emerged as promising alternatives to in-feed antibiotics due to the growing concern over antibiotic resistance. This study aims to evaluate the antimicrobial efficacy of MCFAs containing C8 to C14 fatty acids against strains of Escherichia coli and Salmonella spp.

# **Material and Methods**

A total of 20 strains of both E. coli and Salmonella spp. were isolated. The antimicrobial activity of six different products containing C8 to C14 fatty acids (FAs) was evaluated against 20 strains of both E. coli and Salmonella spp. The tested products were: Product A: calcium laurate and calcium myristate, Product B: calcium laurate, Product C: C8:0 and C10:0 fatty acid with calcium, Product D: C8:0, C10:0, C12:0 FA and C14:0 FA mixed with free fatty acids, Product E: C8:0, C10:0 and C12:0 FA mixed with free fatty acids, and Product F: monolaurin. Bacterial susceptibility to the MCFAs was determined using a microbroth dilution assay. The minimum inhibitory concentration (MIC) was determined after 18-20 hours of incubation at 37°C, with 4000 mg/L as the maximum concentration and 62.5 mg/L as the minimum concentration.

### Results

All 20 test strains had an MIC value of 1000  $\mu$ g/mL for Product A. Product B yielded identical results to Product A. The MIC values of Products D, E, and F were comparable in both E. coli and Salmonella spp.. The MIC<sub>50</sub> and MIC<sub>90</sub> values for these products were both around 500 mg/L, placing them lower than Products A and B. In contrast, Product C exhibited the highest MIC values, with MIC<sub>50</sub> and MIC<sub>90</sub> values of 4000 mg/L.

# **Discussion and Conclusion**

Based on the MIC values, Products D, E, and F appear to be more effective against E. coli and Salmonella spp., with most isolates exhibiting an MIC value of 500 mg/L. The common MCFA component among these three products is C8:0 FA. This suggests that C8:0 FA may play a crucial role in the antimicrobial activity of these products.

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# EVALUATION OF THE DIVERSITY OF HOUSEKEEPING GENES ATPD AND G3PD FROM GLAESSERELLA PARASUIS ISOLATED IN BRAZIL

T. Viana Fialho Martins <sup>1</sup>, M. Maria Cavalcante Granja <sup>1</sup>, J. Viktor De Paula Barros Baêta <sup>1</sup>, G. Pereira Paschoalini <sup>1</sup>, W. Vieira Guimarães <sup>1</sup>, D. Lúcio Dos Santos <sup>1</sup>, <u>L. Fernando Dos Santos <sup>1</sup></u>, J. Lúcio Dos Santos <sup>1</sup> <sup>1</sup>*MICROVET - Microbiologia Veterinária Especial* 

## **Background and Objectives**

Glaesserella parasuis, responsible for Glässer's disease in pigs, has been traditionally characterized by serotyping. However, Multilocus Sequence Typing (MLST) is gaining importance for its superior strain differentiation using internal fragments of housekeeping genes like atpD, infB, mdh, rpoB, 6pgd, g3pd, and frdB. Genetic variations in these genes can influence the pathogen's growth and its ability to colonize host cells. This study aims to evaluate the genetic diversity of these housekeeping genes in Brazilian G. parasuis isolates and compare them to samples in the PubMLST database.

# **Material and Methods**

Thirteen samples of G. parasuis were collected from the lungs of diseased pigs in Brazil's nursery phase. The DNA was meticulously processed, serotyped, and prepared for next-generation sequencing (NGS). Subsequently, these sequences were compared with the G. parasuis MLST database, supplemented by an additional 70 sequences from the PubMLST database representing isolates from the USA, China, and Mexico. The sequences were then aligned, and haplotypes were defined using DNAsp and Network softwares.

# Results

Serotyping evaluation indicated that the G. parasuis isolates belonged to various serotypes. Notably, the atpD gene was predominant in 12 of the sequenced G. parasuis samples, while the g3pd gene was linked to just one isolate. Analysis of genetic diversity within the atpD gene among 47 isolates revealed 21 distinct haplotypes, indicating a high level of haplotypic diversity (Hd) of 0.9241, even when considering representatives from the USA, China and Mexico. Conversely, the g3pd gene, associated with one isolate and 35 PubMLST representatives, yielded four distinct haplotypes with an Hd of 0.5429.

## **Discussion and Conclusion**

The Brazilian G. parasuis isolate displayed significant genetic divergence from those in China and the USA in the PubMLST database. Surprisingly, no correlation emerged between serotypes and haplotypes. The atpD gene exhibited a high Hd value, indicating substantial genotypic variability, while the g3pd gene had an average Hd value, suggesting a lower mutation rate. These genetic variations could impact essential cellular processes and herd health and potentially contribute to antibiotic resistance due to overuse. An alternative disease prevention strategy may involve customized autogenous vaccines targeting specific G. parasuis strains in circulation.

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# EVALUATION OF THE EFFICACY OF ORAL VACCINATION AGAINST SALMONELLA BY GEL

<u>J. Nerem</u><sup>1</sup>, A. Sponheim<sup>3</sup>, D. Hanson<sup>2</sup>, E. Little<sup>2</sup>, J. Rustvold<sup>3</sup>, R. Edler<sup>2</sup>, F. Leite<sup>3</sup> <sup>1</sup>Pipestone Veterinary Services <sup>2</sup>Pipestone Research, Pipestone, MN <sup>3</sup>Boehringer Ingelheim Animal Health USA Inc.

# **Background and Objectives**

Salmonella can present a significant challenge to swine health and serve as a foodborne human pathogen. Vaccination is an important tool to help prevent this pathogen. In this study, we evaluated a novel method of Salmonella vaccination using an edible gel. Advantageously, the gel allows pigs to be administered the oral vaccine without being individually handled, saving time and labor. This study had the objective of evaluating the efficacy of the oral administration of a Salmonella vaccine (Enterisol Salmonella T/C®) by gel and to compare its efficacy with traditional vaccination by oral drench.

# **Material and Methods**

A randomized, controlled trial was conducted with three different treatment groups containing 24 pigs each: 1) non-vaccinated; 2) Salmonella vaccinated by gel; 3) Salmonella vaccinated by oral drench. Pigs in groups 2 and 3 and were vaccinated at 14-16 days of age. For the administration by gel, the live attenuated Salmonella vaccine was mixed with a gel (Underline® Gel) and applied to the farrowing crate mat to allow for its consumption. All pigs were individually weighed and challenged with a virulent strain of Salmonella Typhimurium at 28 days post vaccination. The study ended ten days post challenge when intestinal lesions were evaluated, and pigs were individually weighed.

### Results

Treatment group 1 (non-vaccinated) had a significantly higher level of colon lesions with a mean of 0.40 than both group 2 (0.9) and 3 (0.04) (p<0.001). The average daily weight gain measured during the ten-day challenge period was significantly lower in group 1 (122g) compared to group 2 (372g) and 3 (376g) (p<0.01). While both vaccinated groups performed significantly superior to the non-vaccinated challenged group, no significant differences were observed between the gel and oral drench vaccinated groups.

# **Discussion and Conclusion**

This study demonstrates that the administration of Salmonella vaccine can be successfully performed with the use of an edible gel. This importantly saves time, labor and minimizes any stress associated with individual piglet handling.

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# GENETIC DIVERSITY OF BRACHYSPIRA HYODYSENTERIAE IN SPANISH SWINE FARMS: IMPLICATIONS FOR DISEASE CONTROL

J.L. Arnal<sup>1</sup>, M. Ubieto<sup>1</sup>, A. Fernandez<sup>1</sup>, E. Martinez<sup>1</sup>, A.á. Benito Zuñiga<sup>1</sup>, M. Melero<sup>1</sup>, C. Sanz<sup>1</sup>, S. Lázaro<sup>1</sup> <sup>7</sup>EXOPOL SL

#### **Background and Objectives**

Brachyspira hyodysenteriae, the causative agent of swine dysentery, poses a significant challenge to the swine industry. The objective of this study is to outline the epidemiologic situation of B. hyodysenteriae in Spain through MLST characterization. This aims to provide insights into disease transmission, guide epidemiological interventions, and support the development of immunological strategies and vaccines.

## Material and Methods

A total of 260 strains, isolated between 2017 and 2023 through specific microbiological culture from pig feces that initially tested positive via qPCR, were included. These samples originated from a minimum of 187 distinct farms across 23 Spanish provinces. While only one isolate per outbreak was chosen, some farms contributed up to five strains from different time points. The MLST methodology, as described by Råsbäck et al. (2007), was employed. Data analysis utilized goeBURST to explore relationships between acquired Sequence Types (STs) and clonal complexes.

#### Results

Following MLST analysis, 31 distinct STs were identified, with 21 newly described and submitted to the PUBMLST database. Forty-seven isolates lacked alp gene amplification product but were tentatively assigned to ST71\* and ST71\*\* based on alleles from other loci, assumed to belong to CC71. The most dominant STs were ST70 (23%), ST87 (19%), ST71\* (9%), ST237 (6%), ST71\*\* (6%), and ST289 (5%). Sixteen distinct clonal complexes were found, with CC87 (28%), CC70 (23%), and CC71 (18%) comprising 70% of the total. Geographical distribution showed associations with specific STs; for example, ST70 and ST289 were predominant in the southern regions, while ST87 was confined to Aragon, and ST289 was specific to Barcelona. However, the distribution of CC71 did not exhibit a clear pattern.

# **Discussion and Conclusion**

Our study reveals a genetically diverse landscape in Spain. While some findings align with previous reports in our country (ST52, ST70, and ST71), other significant strains, such as ST87, ST237, and ST289, were newly identified. This underscores the need to consider genetic diversity in future control measures, especially immunization programs. Moreover, this technology has proven effective in tracing the origin of certain outbreaks, emphasizing the importance of understanding this diversity for effective swine dysentery management within the industry.

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# GENOTYPIC AND PHENOTYPIC ANTIMICROBIAL RESISTANCE OF STREPTOCOCCUS SUIS STRAINS FROM NORTH AMERICAN SWINE CLINICAL CASES

<u>R. Mugabi</u>, X. Hu<sup>2</sup>, N. Macedo<sup>3</sup>, O. Sahin<sup>1</sup>, G. Li<sup>1</sup>, P. Harms<sup>5</sup>, R. Rupasinghe<sup>4</sup>, B. Martínez-López<sup>4</sup>, M.J. Clavijo<sup>6</sup> <sup>1</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

<sup>2</sup>Iowa State University Veterinary Diagnostic & Production Animal Medicine

<sup>3</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

<sup>4</sup>University of California, Davis

<sup>5</sup>PIC North America, Hendersonville, TN 37075

<sup>e</sup>Veterinary Diagnostic and Population Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, Pig Improvement Company, PIC®, Hendersonville, TN, USA

# **Background and Objectives**

Streptococcus suis is a Gram-positive coccus that causes various infections in pigs, including meningitis, arthritis, endocarditis, septicemia, and pneumonia, leading to significant economic losses in the swine industry, especially in young pigs. The objective of this study was to the describe the genotypic and phenotypic antimicrobial susceptibility patterns of clinical S. suis isolates from North America.

#### **Material and Methods**

MICs for 19 different antibiotics were determined for 400 whole-genome sequenced isolates using the broth microdilution method. Out of the 19 antibiotics, only 6 have recommended CLSI breakpoints for S. suis and those were the main focus for this study. Isolates were whole-genome sequenced in-house using the Illumina MiSeq.

## Results

A total of 388/400 (97%) isolates were resistant to tetracycline. Ampicillin, ceftiofur, penicillin, florfenicol, and enrofloxacin resistance rates ranged from 0% to 7.8%. Genotypically, numerous AMR genes were detected in the dataset but mostly in low proportions, with the exception of tetO (89%) and the ermB (80.1%) genes that were detected in the majority of isolates. Other detected genes included: tetM (7%), tet44 (5%), tetS (5%), ant6-la (5.5%), lnuC (4.5%), linB (4%), tet32 (2.5%), and tetL (2.5%) were among the most prevalent detected genes.  $\beta$ -lactam resistance genes were not detected. A total of 95.1% of tetracycline resistant isolates were positive for at least one gene conferring resistance to tetracycline. This trend was not observed for other drugs evaluated.

#### **Discussion and Conclusion**

The high prevalence of tetO and ermB genes is consistent with previous studies. Phenotypic and genotypic correlation was not evident for  $\beta$ -lactams as there was an absence of known AMR genes in phenotypically resistant isolates. This could be attributed to alternative resistance mechanisms or unidentified mechanisms not captured by the currently studied AMR genes. The majority (71%) of the penicillin-resistant isolates are likely opportunistic/commensal. Only two penicillin-resistant isolates belonged to ST1 or ST28, the most common pathogenic strains in US herds. This suggests that florfenicol, enrofloxacin, and  $\beta$ -lactam drug resistances are likely very low within pathogenic populations. Continued research is crucial for advancing the understanding and effective utilization of whole-genome sequencing data in the context of antimicrobial stewardship programs targeting S. suis.

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# LAWSONIA INTRACELLULARIS INFECTION ALTERS ILEAL MICROBIOME COMPOSITION IN NATURAL FIELD CONDITIONS

<u>F. Leite</u><sup>1</sup>, I. Declerck <sup>2</sup>, E. Vandekerckhove <sup>2</sup>, S. Leenknegt <sup>2</sup>, R. Jansen <sup>3</sup> <sup>1</sup>Boehringer Ingelheim Animal Health USA Inc. <sup>2</sup>Boehringer Ingelheim Animal Health Belgium SA <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH

### **Background and Objectives**

Porcine proliferative enteropathy is a disease caused by Lawsonia intracellularis, a bacterium that is endemic in swine herds worldwide. L. intracellularis infects intestinal epithelial cells primarily of the ileum and leads to disease which can vary from subclinical to acute, including death. The importance of the microbiome and the presence of indigenous microbiota in the establishment of disease has been established in gnotobiotic swine models and we have previously characterized microbiome changes that occur during experimental infection. However, the interaction of L. intracellularis with the microbiome in natural field conditions, and importantly at the ileal site of infection, remains unknown. This study had the objective of characterizing the ileal microbiome of pigs naturally infected with L. intracellularis.

# **Material and Methods**

Thirty-eight groups of pigs raised in commercial farm conditions were included in this study, representing a total of 389 samples. Ileal samples were collected when pigs were harvested at slaughter and tested for the presence of L. intracellularis by PCR and histopathology, microbiome investigation was performed by 16S rRNA gene sequencing.

#### Results

Infection status among all samples was significantly associated with community composition (PERMANOVA, p=0.006). This finding is particularly interesting since farming conditions, including feed composition, can vary substantially across farms and suggests that L. intracellularis infection leads to a similar ileal microbiome community during infection, despite differences across farms. Network analysis revealed a significant association of L. intracellularis with other members of the microbial community. These associations varied across different positive farms with some genera being common among them.

#### **Discussion and Conclusion**

This work sheds important light into pathogen host microbiome interactions. L. intracellularis infection was confirmed to be associated with a significant change in ileal microbial community composition demonstrating a consequence of infection under field conditions. This likely contributes to disease and the promotion of other pathogens.

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# MAPPING OF ANTIMICROBIAL RESISTANCE GENES IN SALMONELLA ENTERICA ISOLATED FROM SWINE

T. Viana Fialho Martins <sup>1</sup>, M. Maria Cavalcante Granja <sup>1</sup>, J. Viktor De Paula Barros Baêta <sup>1</sup>, W. Vieira Guimarães <sup>1</sup>, <u>L.</u> <u>Fernando Dos Santos <sup>1</sup></u>, D. Lúcio Dos Santos <sup>1</sup>, J. Lúcio Dos Santos <sup>1</sup> *<sup>1</sup>MICROVET - Microbiologia Veterinária Especial* 

#### **Background and Objectives**

Many bacterial pathogens have become resistant to antibiotics, increasing morbidity and mortality. This is particularly concerning in intensive pork meat production, where antimicrobials are used irrationally to treat sick animals. Some Salmonella strains are pathogenic in pigs and can cause severe diseases. This study aimed to compare Whole Genome Sequencing (WGS) with phenotypic testing methods to detect antibiotic resistance in strains of S. enterica Choleraesuis and S. enterica Typhimurium isolated from sick animals.

# **Material and Methods**

Samples of S. enterica Choleraesuis and S. enterica Typhimurium were isolated from pigs and then submitted to antimicrobial susceptibility testing to determine their resistance to antimicrobial: sulfa/trimethoprim; tilmicosin; enrofloxacin and ciprofloxacin; ceftiofur; gentamicin and neomycin; doxycycline and tetracycline; penicillin, ampicillin and amoxicillin; florfenicol; lincomycin; norfloxacin and marbofloxacin. Ten isolates (AM1 to AM6 from S. Choleraesuis and AM7 to AM10 from S. Typhimurium) underwent DNA extraction and NGS for resistance profiling through whole-genome sequencing. Their sequences were then compared in the ResFinder 4.1 database, which captures antimicrobial resistance genes from whole-genome datasets on BLAST. The ResFinder results were compared with phenotypic antimicrobial susceptibility tests using EUCAST cutoff values.

### Results

In the genotypic analysis of the isolates, all sequenced samples showed resistance genes to various antimicrobial classes. The number of resistance genes varied within the same sample, with high coverage and similarity (above 98%) to gene sequences in the BLAST database. In antimicrobial sensitivity tests, among the evaluated Choleraesuis isolates, 100% exhibited resistance to ampicillin, 83% to tetracycline, and 66% to penicillin, while 50% of the isolates showed resistance to all three mentioned antimicrobials. In Typhimurium serovar isolates, 100% exhibited sensitivity to ampicillin, and 75% to tetracycline. Resistance to penicillin was recorded in 75% of the isolates. A reported concern is the emergence of extended-spectrum antimicrobial resistance, such as Ceftiofur. Variable resistance levels of 75% and 33% were observed for the Typhimurium and Choleraesuis serovars, respectively.

### **Discussion and Conclusion**

Antimicrobial resistance determined through WGS partially supports phenotypic test results for important isolates. The study suggests that the excessive use of antibiotics in production animals contributes to antimicrobial resistance. Autogenous vaccines are a promising alternative to antimicrobials, promoting the animal breeding industry.

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# PREVALENCE OF LESIONS COMPATIBLE WITH ENZOOTIC PNEUMONIA AND PLEUROPNEUMONIA IN CHILE PIG FARMS ON LUNG LESIONS SCORING

A. Vera <sup>1</sup>, F. Silva <sup>1</sup>, <u>R. Krejci <sup>2</sup></u>, J. Calveyra <sup>2</sup> <sup>1</sup>Ceva Animal Health, Chile <sup>2</sup>CEVA ANIMAL HEALTH

# **Background and Objectives**

Lung lesions scoring is a useful tool for assessing the status of herd lung health. Ceva Lung Program (CLP) was confirmed as a valuable tool to establish the prevalence and severity of Enzootic Pneumonia (EP) and pleuropneumonia, with A.p-like lesions. The objective of this study was to evaluate the level of lung lesions in herds in Chile.

# **Material and Methods**

During March 2022 and October 2023, a total of 45551 lungs, from 373 batches and 32 different farms in Chile were scored, excluding those with the Mhyo SPF Status. Lungs were scored using the CLP application, a methodology described previously. Bronchopneumonia lesions, cranioventral pleurisy and scarring associated with older EP-like lesions were recorded and scored. Dorsocaudal pleurisy suggestive for previous pleuropneumonia was scored to describe A.p-like lesions and A.p Index (APPI) was calculated.

### Results

The prevalence of 29% of Bronchopneumonia Lesions was found. The area of affected surface of lung parenchyma in pneumonic lungs reached 3.9%. Old lesions, in the form of scars, were evident in 4% of the lungs evaluated. Cranio ventral pleurisy was recorded in 11% of total number of lungs. As for pleuropneumonia 14% of lungs were affected by A.p-like lesions with the APPI index 0.36. All values are expressed as median.

# **Discussion and Conclusion**

The results show a relative challenge of EP-like lesions, although slightly lower compared to other Latin American countries. On the other hand, Ap-like lesions in Chile were quite significant and larger when compared to other countries in the region, demonstrating that agents associated with pleuropneumonia, such as A.p. are challenging the herds in Chile. Information like this can help in decision-making to establish the best programs for controlling and preventing respiratory diseases on farms.

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# PREVALENCE OF MYCOPLASMA HYOPNEUMONIAE INFECTION IN BACKYARD PIGS IN PARANÁ STATE, BRAZIL

A.C.A.D. Lima <sup>1</sup>, E.R. Braga <sup>1</sup>, A.K. Panneitz <sup>1</sup>, F.M. Petri <sup>1</sup>, J.H.T. Castro <sup>2</sup>, <u>L.G. De Oliveira <sup>1</sup></u> <sup>1</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil <sup>2</sup>Paraná Agribusiness Defense Agency (ADAPAR), Curitiba, Paraná, Brazil

## **Background and Objectives**

The bacterium Mycoplasma hyopneumoniae (Mhyo) is the etiological agent of porcine enzootic pneumonia and one of the main pathogens involved in the porcine respiratory disease complex (PRDC). It is known that over 90% of Brazilian commercial herds are infected with Mhyo; however, its prevalence in backyard pig farming has not been described. This research aimed to elucidate the prevalence of Mhyo in backyard pig farming in the state of Paraná, Brazil.

## **Material and Methods**

In collaboration with the Paraná Agribusiness Defense Agency (ADAPAR), we sampled 161 herds classified as backyard pig production from 122 municipalities in the state of Paraná, Brazil. A total of 500 serum samples were collected from pigs without vaccination of different ages and sexes. The detection of anti-Mhyo antibodies was performed using the ELISA method with a commercial kit. Prevalences were calculated using Epi Info 7.2.5.0 software.

## Results

The presence of anti-Mhyo antibodies was detected in 32.4% (162/500) of the sampled animals. Of the 160 properties evaluated, in 100 of them, at least one tested animal showed anti-Mhyo IgG antibodies (62.5%).

## **Discussion and Conclusion**

Since these animals were not subjected to immunization protocols, we can assert that the detected antibodies result from infection, indicating the circulation of Mhyo in a significant portion of pigs included in this study. The substantial prevalence suggests that backyard pig farming should be considered potential reservoirs and sources of propagation for this agent. The identification of anti-Mhyo antibodies in this group of animals also holds relevance in the epidemiology of the pathogen for commercial pig farming, considering the location of these farms and their possible proximity to commercial herds. As the state of Paraná is one of the largest commercial pig producers in Brazil, understanding data and occurrences of diseases in the vicinity of farms provides excellent guidance for control, prevention, and disease eradication plans.

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# PREVALENCE OF VT2E TOXIN GENE AND ASSOCIATED RISK FACTORS IN GREEK SWINE FARMS

P. Tassis <sup>1</sup>, G. Labronikou <sup>2</sup>, A. Gelasakis <sup>3</sup>, D. Floros <sup>1</sup>, E. Tzika <sup>1</sup>, D. Llopart <sup>4</sup>, S. Bruguera <sup>4</sup> <sup>1</sup>Farm Animals Clinic, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece <sup>2</sup>HIPRA Hellas, Athens, Greece <sup>3</sup>Laboratory of Anatomy and Physiology of Farm Animals, Department of Animal Science, School of Animal Biosciences, Agricultural University of Athens, Athens

⁴HIPRA HQ, Amer (Ĝirona), Spain

# **Background and Objectives**

Oedema disease (OD) caused by Verotoxin-producing strains of Escherichia Coli (VTEC) is a significant health menace for pigs, observed predominantly at post-weaning age, either as a clinical form with neurological signs, edema at various tissues and sudden death, or as subclinical or chronic form. The aim of the present study was to assess the prevalence of VT2e toxin gene in Greek swine farms, as well as the effect of major risk factors on the occurrence of OD.

## **Material and Methods**

In total, 109 oral fluid samples were collected with cotton ropes from 28 farrow to finish swine farms which represented almost 30% of the Greek pig industry. Ropes were suspended for 25 minutes at pigs' shoulder height at minimum two randomly selected pens (animals were sampled between 28 - 150 days of age). Collected fluids were applied on FTA cards and qPCR was performed for the detection of VT2e toxin gene of E. coli, (Verocheck<sup>a</sup> oral fluids sampling collection system). Moreover, data regarding major risk factors which could affect OD prevalence (disinfection procedures, antibiotic usage, vaccinations, etc.) were collected. Statistical analysis included mixed binary regression model [SPSS software (version 23)].

#### Results

In total, 22 out of 28 farms had at least one positive sample (78.6%) (reference qPCR values VT2e (Ct): POS< 38.5). Moreover, 42 out of 109 samples (38.53%) were positive (Ct range 26.7-38.4). Interestingly, only 12 positive samples (28.6%) from six positive farms, were collected from pens with clinical signs compatible with OD, whereas the rest 30 positive samples (71.4%) from 16 positive farms, were received from pens without clinical signs compatible with OD. As regards the risk factors, disinfection procedures had a significant effect (P=0.016) on OD prevalence. Farms applying proper disinfection programs showed 15 times less probability of having positive Ct values (P=0.006).

#### **Discussion and Conclusion**

The presence and circulation of VTEC strains among different age pig groups in Greek swine farms is significant. Detection of positive samples in farms without clinical signs compatible with OD supports a possible presence of the subclinical form of OD, which needs further assessment, especially under the prism of ZnO usage restrictions in the European Union.

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# PREVALENCE OF VIRULENCE FACTORS AND PATHOTYPES OF ESCHERICHIA COLI ISOLATED FROM PIGLETS WITH POST-WEANING DIARRHEA

#### F. Vangroenweghe 1

<sup>1</sup>BU Swine & Ruminants, Elanco Benelux, Elanco Animal Health

#### **Background and Objectives**

Post-weaning Escherichia coli diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic Escherichia coli (ETEC). The most common adhesins found in ETEC from pigs with PWD are fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are LT, STa and STb. The objective of the present study was to describe the evolution in the prevalence different pathotypes related to E. coli pathology during the postweaning period in Belgium and The Netherlands.

# **Material and Methods**

A total of 547 pig herds showing clinical signs of PWD were sampled between January 2018 and September 2023. Rectal swab samples (n=5) from diarrheic pigs were collected and submitted to IZSLER (Brescia, Italy) to analyze the presence of virulence factors - adhesins (F4, F5, F6, F18 and F41) and toxins (LT, STa, STb, Stx2e). Results were categorized according to virulence factors and pathotypes.

# Results

In 38.7% of the sampled farms (n = 212) no E. coli could be isolated. From the 335 E. coli-positive farms, 286 (85.4%) were categorized as ETEC, 42 (12.5%) had a combined ETEC-STEC pathotype and 7 (2.1%) had a STEC pathotype. The prevalence of the different ETEC subtypes was as follows: F4-ETEC (28.4%), F18-ETEC (29.3%) and F4-F18-ETEC (22.1%). Besides ETEC, 27 isolates (8.1%) were classified as Shiga toxin-producing E. coli (STEC) of which were 20 (6.0%) were combined ETEC-STEC pathotypes and 7 (2.1%) were STEC only

### **Discussion and Conclusion**

This study confirms that fimbriae type F4 and F18 had a similar prevalence among E. coli isolates from PWD cases in Belgium and The Netherlands. Compared to previous data, there was an increase in the proportion of combined F4-F18 prevalence. The F18-STEC pathotype remained around 2% as previously observed. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of E. coli in PWD outbreaks and initiate appropriate preventive and control measures such as a live oral vaccination.

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# PREVALENCE OF VIRULENCE FACTORS OF ESCHERICHIA COLI ISOLATED FROM PIGLETS WITH POST-WEANING DIARRHEA IN BRAZIL

<u>M.E. Ferraz</u><sup>1</sup>, I.M. Pasian <sup>1</sup>, P.E. Sbardella <sup>1</sup> <sup>1</sup>Elanco Animal Health - Brazil

#### **Background and Objectives**

Post-weaning diarrhea (PWD) caused by enterotoxigenic Escherichia coli (ETEC) with fimbriae F4 or F18 has been recognized as an economically important disease worldwide. PWD typically causes mild to severe watery diarrhea after weaning and the most common adhesins found in ETEC from pigs with PWD are fimbriae F4 (also called K88) and F18, while the predominant enterotoxins are LT, STa and STb. The objective of the present study was to determine the prevalence ETEC subtypes causing PWD in Brazil.

# **Material and Methods**

A total of 275 piglets from 21 to 40 days of life from 47 farms distributed in Brazil showing clinical signs of PWD were sampled from May 2022 to October 2023. For each analysis, five rectal swabs samples per farm were collected and submitted to reference laboratories to analyze the presence of virulence factors – adhesins (F4, F18) and toxins (LT, Sta, Stb, Stx2e) by PCR. The samples were classified as ETEC when present at least one fimbriae (F4 and/or F18) and one toxin (Sta, Stb, Lt), and classified as STEC when present the fimbriae F18 and the toxin Stx2e.

## Results

ETEC was identified in 62% (n=34/47) of farms and ETEC F4/F18 was the most prevalent subtypes of E. coli detected in 35,3% (n=12/34), followed by 32,4% ETEC F4 (n=11/34) and 32,4% ETEC F18 (n=11/34). In the analysis isolates from 7 farms were classified as STEC being only 12,7%. All the isolates classified as STEC also had positivity to ETEC.

### **Discussion and Conclusion**

This study confirmed that ETEC is the most prevalent E. coli identified from PWD cases in Brazil. An association between Edema disease and PWD was found in all the isolates classified as STEC. The characterization of virulence factors is essential to understand the role of bacteria in PWD outbreaks and initiate appropriate preventive and control measures such as live oral vaccination.

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# PREVALENCE OF VIRULENCE FACTORS OF ESCHERICHIA COLI ISOLATED FROM PIGLETS WITH POST-WEANING DIARRHEA IN POLAND

D. Plawska<sup>2</sup>, R. Jedryczko<sup>1</sup>, <u>M. Kocik<sup>2</sup></u> <sup>1</sup>Weterynaryjne Laboratorium Diagnostyczne Vet Lab Group, Gietrzwałd, Poland <sup>2</sup>Elanco Animal Health, Poland

# **Background and Objectives**

Post-weaning diarrhea (PWD) is a common problem in pig production worldwide and is usually observed during the first two weeks post-weaning of the piglet. Enterotoxigenic Escherichia coli (ETEC) is considered the most important cause of PWD in piglets. The fimbriae most found in ETEC cases from pigs suffering PWD are F4 and F18. The main enterotoxins detected in porcine ETEC include heat-labile toxin (LT) and heat-stable toxins a and b (STa and STb). ETEC strains can produce different combinations of these toxins. The objective of the present study is to investigate the prevalence of ETEC virulence factors involved in cases of PWD in Poland.

#### **Material and Methods**

The study was conducted between 2018-2022, and a total of 387 samples from Polish farms with a recent history of PWD in nursery pigs were included in the study. Fecal samples were collected within 48 hours of the start of the clinical signs and submitted to the laboratory for diagnostic. The study considered routine bacteriology for E. coli, followed by DNA extraction and PCR testing for the presence of genes encoding virulence factors: adhesins (F4, F18) and toxins (LT, Sta, STb, Stx2e). Analysis was focused on isolates carrying at least one fimbria with at least one enterotoxin. Isolates encoding at least one of the investigated enterotoxins together with Stx2e and F18 fimbriae were classified as ETEC. Isolates carrying genes for fimbriae F18 and Stx2e toxin were classified as STEC.

#### Results

The study shows that fimbriae type F18 was significantly more prevalent than F4 among E. coli isolates from PWD cases in Poland. F4-ETEC F18-ETEC F4-ETEC & F18-ETEC E. coli isolates were recovered from 52 herds from 116 submitted. ETEC and STEC isolates were found in 110 samples. ETEC was the most frequent pathotype, 93.6% of isolates, while isolates classified as STEC (F18, Stx2e) 6.4%.

# **Discussion and Conclusion**

Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of E. coli in PWD outbreaks and initiate appropriate preventive and control measures such as live oral vaccination.

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# PREVALENCE STUDY OF GLAESERELLA PARASUIS IN POLYSEROSITIS CASES ON ARGENTINIAN SWINE FARMS

S. Fernandez <sup>1</sup>, M. Colitto <sup>1</sup>, E. Daffunchio <sup>1</sup>, R. Manzano <sup>1</sup>, <u>L. De Lucas</u><sup>2</sup>, L. Nodar <sup>2</sup> <sup>1</sup>*Hipra Argentina, S.A.* <sup>2</sup>*HIPRA, Amer (Girona), Spain* 

### **Background and Objectives**

Glaesserella parasuis, as an early commensal colonizer in the upper respiratory tract of weaning pigs, is a key pathogen in the polyserositis complex and a major bacterial threat to pig populations, causing Glässer's disease. This disease results in systemic infections, including polyserositis, meningitis, and arthritis, often leading to high mortality and morbidity, with substantial economic losses for pig producers. To assess the overall prevalence of G.parasuis in polyserositis cases in swine herds in Argentina, we conducted an analysis using PCR diagnostic results.

# **Material and Methods**

Between 2021 and 2022, 51 pig farms were monitored in 11 Argentinian provinces, where a total of 262 piglets exhibiting clinical signs compatible with polyserositis were sampled between 5 and 70 days of age.Necropsy samples were collected with cotton swabs from pleura, pericardium, peritoneum, joints, and brain, transferred to FTA cards and send to Diagnos Brasil for PCR analysis to detect G. parasuis genetic material (Glassercheck®, Hipra). Positive results were expressed by age and semi-quantitatively, with '+' indicating a low amount, '++' a medium amount, and '+++' a high amount of genetic material in the sample.

# Results

The prevalence of Glässer was 88.2% at the farm level, where 45 farms tested positive for G. parasuis in at least one animal of the 51 sampled farms. Of the 262 animals sampled, 154 were positive (58.78%); moreover, 35.02% of the animals tested in positive farms, were negative.

### **Discussion and Conclusion**

The analysis highlights the ubiquitous presence of Glaeserella parasuis in polyserositis cases in Argentina, emphasizing the need for effective control measures. Interestingly, the study also revealed instances where animals in positive farms tested negative for G. parasuis, potentially attributed to antibiotic therapy during the monitoring period. This emphasizes the importance of sample size when screening specially in farm with antibioterapy. Further investigation into this phenomenon is warranted.

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# PROBIOTIC BACILLUS INHIBITORY CAPABILITIES AGAINST STREPTOCOCCUS SUIS

L.H.B. Hansen <sup>1</sup>, D. Sandvang <sup>1</sup>, L. Capern <sup>1</sup> <sup>1</sup>Chr. Hansen A/S, Animal and Plant Health & Nutrition, Bøge Alle 10–12, 2970 Hørsholm, Denmark.

### **Background and Objectives**

Streptococcus suis (S. suis) infections are considered a major problem in the pig industry causing septicemia, meningitis, and arthritis mostly during the nursery phase. The objective of this study was to investigate the inhibitory potential of Bacillus strains and commercial products on different S. suis serotypes in vitro.

### **Material and Methods**

The inhibitory potential of the probiotic strains and products were evaluated in a supernatant assay. The supernatant of the probiotic strains was inoculated with the S. suis suspension. Growth (OD600nM) of S. suis was measured for 8h at 37°C. In the first evaluation testing inhibition of S. suis serotype 2 SVS 321, ten probiotic strains of the species B. subtilis, B. amyloliquefaciens, B. licheniformis, B. pumilus, and B. velezensis were included. In the second evaluation, four different S. suis were investigated: Serotype 2 (DSM28762), R; serovar 2 (DSM9682), S; serovar 1 (DSM9683), and T (DSM9684). The three tested products were 1) SolPreme® (consisting of B. subtilis – 541 and B. amyloliquefaciens – 516), 2) Commercial B. subtilis, and 3) Commercial B. licheniformis.

#### Results

All strains except one of the two B. pumilus strains tested inhibited the growth of S. suis by >85%. Four B. subtilis strains were tested and one showed reduced inhibition capabilities by inhibiting the growth of S. suis by 85% compared with >98%. Both B. subtilis – 541 and B. amyloliquefaciens – 516 inhibited S. suis by >98%. The three commercial Bacillus-based probiotic products inhibited the four S. suis serotypes to different extents. The SolPreme® probiotic inhibited the growth of all S. suis serotypes, whereas the commercial B. licheniformis product inhibited the growth of S. suis ranging from 5-40% with the highest capability towards the serovar 2 strain. The commercial B. subtilis product inhibited the S; serovar 1 strain by 100% whereas inhibition of the other serotypes only was approximately 35%.

# **Discussion and Conclusion**

Results demonstrate that inhibitory capabilities of Bacillus probiotics against S. suis are species and strain specific. Various S. suis serotypes are inhibited to different extents by commercially available Bacillus-based probiotic products. In conclusion, these findings may indicate a potential of the dual-strain Bacillus-based probiotic to mitigate S. suis infections in pigs.

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# SALMONELLA SPP. SERUM ELISA TEST PERFORMANCE IS BETTER THAN ORAL FLUID PCR TEST

<u>J. Hulzing</u><sup>1</sup>, H. Prüst<sup>2</sup>, M. Steenaert<sup>2</sup> <sup>1</sup>Coöperatie Varkensartsen, the Netherlands <sup>2</sup>Boehringer Ingelheim Animal Health Netherlands bv

## **Background and Objectives**

To confirm Salmonella spp. (Ss) infection in a production system and to estimate the right timing for vaccination several diagnostic testing methods are available. The objective of this study: Is Ss Oral Fluid testing by PCR as reliable as is Ss serum sample ELISA testing.

### Material and Methods

In a Dutch farrow to finish herd 3 different batches of finishing pigs were included. Each batch was housed in 2 rooms of which 4 pens per room were tested. Per pen at 3 different timepoints 2 to 3 Serum samples (Sero) and 1 Oral Fluid sample (OF) were taken at 0 and 4 weeks (t+0, t+4) after placement in the finishing barn respectively. Experimental unit was a pen. A pen was considered ELISA positive when per event at least one serum sample tested above cut-off OD value 10, and PCR positive when the OF sample was below cut-off ct value 40. All results were compared in a contingency table: 66 events of samples taken on the same day and in 21 events the Serum Samples were taken 4 weeks after the Oral Fluid samples.

### Results

Sensitivity of testing OF PCR compared to Sero ELISA was 8% and 14% at t+0 and t+4 respectively. Specificity of testing OF PCR compared to Sero ELISA was 90% and 86% at t+0 and t+4 respectively.

#### **Discussion and Conclusion**

The sensitivity of detecting Ss in OF PCR compared to Sero ELISA was very low, resulting in false negative results. The specificity of detecting Ss in OF PCR compared to Sero ELISA was slightly low, resulting in false positive results. This study shows that the results from both methods are hard to compare and that for Salmonella spp. detection within a herd serum-ELISA is more reliable than Oral Fluid-PCR.

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# SURVEY ON POLYSEROSITIS LESIONS IN NURSERY PIGS FROM SPANISH AND BRAZILIAN FARMS

M. Sibila 1, V. Aragón 1, L.G. De Oliveira 2, J. Segalés 3, F. Correa-Fiz 1

<sup>1</sup>IRTA Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Spain

<sup>2</sup>São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil.

<sup>3</sup>Unitat Mixta d'Investigació IRTA-ÜAB en Sanitat Ànimal, Centre de Recerca en Sanitat Animal (CReSA), and Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Spain

## **Background and Objectives**

Fibrinous/fibrous polyserositis is a lesion frequently seen in nursery pigs caused mainly by Glaesserella parasuis (Gp), Streptococcus suis (Ss) and/or Mycoplasma hyorhinis (Mhr). The objective of this survey was to gather information regarding this lesion in Spain and Brazil.

# **Material and Methods**

Information was obtained by a questionnaire consisting of 14 questions focused on the frequency of observation, clinical manifestation, diagnosis, and control strategies. A total of 20 and 39 veterinarians from Spain and Brazil responded the survey.

### Results

In Spain, 18 veterinarians (representing 583 farms, average of 1,316 sows/farm) reported to have herds with recurrent cases of polyserositis (mainly in 5-7 weeks-old piglets). Twelve of them had >25% of their farms with cases in the last 12 months, representing 1-10% of mortality. The most prevalent lesions observed in in-farm necropsies were pericarditis and arthritis. From these 18 responders, 6 reported triple infections (Gp, Ss and Mhr), 9 double co-infections and 3 mono-infections, being Gp the pathogen most frequently detected. The main control strategy was medication followed by its combination with autogenous and/or commercial vaccines. Only 2/17 responders considered that the medication used was inefficient. In Brazil, 29 veterinarians (representing 2,135 farms, average of 1,244 sows/farm) reported recurrent cases of polyserositis (mainly in 5-7 weeks-old piglets). Fifteen of them diagnosed an outbreak in >25% of their farms in the last 12 months with a 1-5% of associated mortality. The most prevalent lesions observed in in-farm necropsies were pericarditis and pleuritis. From the 29 veterinarians, 4 reported triple, 14 double and 11 mono-infections, being Gp also the main pathogen. The main control strategy was the combination of autogenous vaccines with medication. Among veterinarians, 10/29 (34%) considered that the used antibiotic treatments were inefficient.

# **Discussion and Conclusion**

The epidemiology and clinical outcome of polyserositis was similar in both countries. Interestingly the average mortality reported in Brazil was lower than that of Spain, which might be attributed to the absence of PRRSV in the former country. While in Spain medication is frequently used alone, in Brazil was normally combined with autogenous vaccines. Finally, the number of reported antibiotic failures was higher in Brazil than in Spain.

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# THE EFFECT OF DIFFERENT COMBINATIONS OF VACCINES AGAINST MYCOPLASMA HYOPNEUMONIAE IN PIGS' LUNG HEALTH AND PRODUCTION PARAMETERS

P. Filsner <sup>1</sup>, J. Calveyra <sup>2</sup>, F. Betiolo <sup>1</sup>, M. Walter <sup>1</sup>, C. Postal <sup>1</sup>, C. Sartori <sup>1</sup>, A. Coldebella <sup>3</sup>, I. Dalpozzo <sup>4</sup>, A. Holdefer <sup>4</sup>, <u>R. Krejci</u> <sup>2</sup>

<sup>1</sup>Ceva Animal Health, Brazil <sup>2</sup>CEVA ANIMAL HEALTH <sup>3</sup>Brazilian Agricultural Research Corporation (EMBRAPA), Concórdia, Brazil ⁴Cooperativa de Produção e Consumo Concórdia-Coperdia, Brazil

### **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is a common agent on farms, causing major economic impacts. Efficient control requires long-lasting protection, which can be assessed through on-farm performance and lung scores. The objective of this study was to evaluate the effectiveness of different vaccination protocols against Mhyo on a commercial farm in Brazil.

# Material and Methods

The study was conducted on a Cooperative in southern Brazil, where one farm cluster was selected to compare a mycoplasma vaccine against their usual vaccine protocol in the rest of the production sites. The Group 1 (G1) with 7504 animals from 10 batches received 2ml, IM Hyogen® at weaning. The Group 2 (G2) with 67995 animals from 83 batches received 1 ml IM vaccine(Mycoflex®) or 0.2 ml intradermal vaccine (MSD IDAL®) at 21 days of age during the weaning stage. Performance parameters were analysed, such as DWG, FC, mortality rate and carcass weight, health parameters such and lung lesions at slaughter.Zootechnical data were evaluated using analysis of variance of the model containing treatment effects. Logistic regression analysis was applied to farm mortality, culling and % of pigs identified with lung lesions. Whenever overdispersion was detected in the data, the dispersion factor of the logistic regression analysis was corrected using Pearson's chi-squared statistic.

#### Results

The DWG difference was 22g (923g vs 901g). The live weight at slaughter was higher by 2.8 kg in the G1 = 133.2 kg vs G2 = 130.4 kg. The carcass weight of G2 was 96,48 Kg compared to 98.57 kg in G1 (P $\leq$ 0.05). Lung lesions related to Enzootic Pneumonia evaluated at the slaughterhouse showed, a 3.4 % percentage of Affected Lung Surface on G1, while G2 it was 6.1%. Lesions related to mycoplasma infection was measured by the Enzootic Pneumonia index, which the G1 reached 2.3 and G2 3.9. A difference in favour of G1 group. The weight discarded at the slaughterhouse per animal was also evaluated. In group G1 0.328g were discarded per animal, while in group G2 0.648g. Other indices did not show any statistically significant difference (P>0.05).

# **Discussion and Conclusion**

Under the conditions of this study, G1 animals showed greater benefit in production parameters and respiratory health compared to the other vaccine protocols.

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# THE PREVALENCE STUDY OF PORCINE GASTRIC ULCER AND HELICOBACTER SUIS IN TAIWAN

P.J. Lin<sup>1</sup>, C.W. Liao<sup>1</sup>, H.H. Chiang<sup>1</sup>, D.Y. Lo<sup>1</sup>, H.C. Kuo<sup>1</sup>, C.F. Wu<sup>1</sup> <sup>1</sup>Department of Veterinary Medicine, National Chiayi University, Chiayi city, Taiwan

#### **Background and Objectives**

Porcine gastric ulcer causes death in the pig population during the growth and fattening stages. To date, prevalence of Helicobacter suis (H. suis) resulting in porcine gastric ulcer in Taiwan has not been investigated.

## **Material and Methods**

We collected 360 pig stomachs from the slaughterhouse, and stomach tissues from the 88 diseased pigs that were submitted for necropsy were divided into symptomatic (N=44) and asymptomatic (N=44) groups. Scoring of gastric lesions and molecular biology tests were used to determine the occurrence of gastric ulcer and the prevalence of H. suis in pigs.

# Results

The positive rate of H. suis in the samples from slaughtered pigs was 49.7%, and infection of H. suis and presence of gastric lesions were prone to occur in autumn. The positive rates of H. suis infection in the symptomatic and asymptomatic groups pigs were 59.1% and 31.8%, respectively. Additionally, proportion of the samples with gastroesophageal ulcer in the symptomatic group was 68.2%, which was mostly observed in the pigs at the growing stage. In the samples sent for necropsy, the incidence of gastroesophageal erosion to ulceration was higher in H. suis-infected pigs than in H. suis-uninfected ones without significant difference; however, in the samples from the slaughterhouse, the incidence in H. suis-infected pigs was higher than uninfected ones and revealed a significant difference (p<0.05).

## **Discussion and Conclusion**

H. suis infection was associated with gastric ulcer in slaughtered pigs, but not the main cause of gastroesophageal ulcer in the diseased pigs with clinical symptoms. This is the first study that reports epidemiological analysis of H. suis in the clinical cases and slaughtered pigs in Taiwan. The epidemiological investigation contributes to a better understanding of the association of H. suis infection and porcine gastric ulcer.

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# DETECTION OF COLONIZATION AND VIRULENCE FACTORS OF E. COLI IN GROWING-FINISHING PIGS

L. Czanderlova<sup>1</sup>, M. Zizlavsky<sup>1</sup>, M. Slanikova<sup>1</sup>, B. Rackova<sup>1</sup> Sevaron, Czech Republic

#### **Background and Objectives**

Edema disease and post weaning diarrhea can also occur in pigs in the grow-finish age category. The aim of our study was to map the distribution of E. coli strains with virulence factors in pig farms in the Czech Republic in the growing-finishing pigs.

## **Material and Methods**

Samples were taken either from faeces and rectal swabs from animals with visible diarrhoea or swabs of small intestines and mesenteric lymph nodes from dead pigs with enteritis. Samples collected from July 1, 2022 to September 30, 2023 were included in the study. A total of 57 strains were isolated from growing-finishing pigs (age category 5 weeks after weaning until slaughter). PCR detection for the presence of fimbrial adhesins F4, F5, F6, F41, F18, non-fimbrial adhesins AIDA1 and eaeA and determination of genes for the production of toxins STa, STb, LT, stx1 and stx2 were performed in all strains.

### Results

Non-pathogenic strains of E. coli predominate in this age category - 37 strains (64.9%). Of the fimbrial adhesins, F18 was the most common, which was detected in 7 strains (12.3%), F4 was detected in 3 cases (5.3%). Nonfimbrial adhesin AIDA1 was detected in 1 strain (1.8%). Of the toxins, STa was the most frequently detected in 10 strains (17.5%), followed by stx2 in 7 strains (12.3%) and STb in 6 cases (10.5%). LT was detected in 3 strains (5,3%). Other factors have not been identified.

# **Discussion and Conclusion**

In growing-finishing pigs, there is a significant decrease in the detection of pathogenic strains of E. coli. The strains causing Edema disease predominate. Relatively frequent was the detection of strains without adhesin and with the production of toxins Sta, STb and LT, the significance of which is unclear. These strains are unlikely to cause clinically significant problems in this age group of pigs. Even in this category, stx1, which is very pathogenic for humans, was not detected. Some strains of E. coli with stx1 may also occur sporadically in pigs as part of the normal microflora, and the pig can be a source of infection for humans. However, it was not detected in our study.

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# AN AUTOMATED ASSAY FOR THE MEASUREMENT OF CYSTATIN C IN PIG SALIVA

E. Llamas-Amor<sup>1</sup>, M.J. López-Martínez<sup>2</sup>, G. Ramis<sup>3</sup>, A. González Bulnes<sup>4</sup>, A. Miralles-Chorro<sup>5</sup>, E. Garcia Manzanilla<sup>6</sup>, J. Ceron<sup>7</sup>, A. Muñoz Prieto<sup>8</sup>

<sup>1</sup>Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum, University of Murcia, Espinardo, Murcia, 30100, Spain.

<sup>2</sup>University of Murcia

<sup>3</sup>Departamento de Producción Animal. Universidad de Murcia

<sup>4</sup>Departamento de Producción y Sanidad Animal, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, C/Tirant lo Blanc, 7, Alfara del Patriarca, Valencia, 46115, Spain.

<sup>5</sup>CefuSA, 30840, Alhama de Murcia, Murcia, Spain.

<sup>e</sup>Pig Development Department, Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland & School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland

<sup>7</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia

<sup>8</sup>1Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence "Campus Mare Nostrum", University of 9 Murcia, 30100, Espinardo, Murcia, Spain

# **Background and Objectives**

Cystatin C is considered a biomarker for inflammatory conditions <sup>1</sup> and sepsis <sup>2</sup> in humans. This study aimed to validate an immunoturbidimetric assay for Cystatin C in pigs using saliva as a non-invasive sample <sup>3</sup>. Additionally, variations of this analyte in saliva in Streptococcus suis and Escherichia coli infections that cause substantial mortality and morbidity in swine production were investigated.

## Material and Methods

Cystatin C was determined using a commercially available immunoturbidimetric assay (Gentian diagnostics) adapted to an automated Olympus AU400 system. The method was analytically validated by determining precision (intra- and interassay determinations) and accuracy (recovery study) for porcine saliva samples. In addition, cystatin C levels were measured in a total of 51 porcine saliva samples divided into three different groups: (1) pigs with meningitis tested positive on PCR analysis for S. suis (n=17), pigs with diarrhoea tested positive for E. coli in rectal swabs (n=17), and (3) healthy pigs as controls (n=17).

The Wilcoxon-rank test assessed differences between groups. Results were expressed as median and interquartile range. A P<0.05 was considered significant.

# Results

Validation data showed inter and intra-assay imprecision levels lower than 6.1% and recovery rates between 88% and 94%.

Median cystatin C levels were significantly higher in pigs with meningitis due to S. suis (median= 0.76mg/mL, 25-75% percentile= 0.52 - 1.05) (p<0.001)) and in pigs with diarrhoea due to E. coli (median= 0.65 mg/mL, 25-75% percentile= 0.49 - 0.91) (p=0.01) compared to healthy pigs (median= 0.41 mg/mL, percentile= 0.29 - 0.48).

## **Discussion and Conclusion**

This study indicated that cystatin C can be measured in the saliva of pigs. In addition, this analyte increases in the saliva of pigs with S. suis and E. coli infections. In this line, elevated levels of cystatin C in human serum have been associated with immunity and inflammation processes in viral infections being closely related to worsening <sup>4</sup>. Further investigations are warranted to evaluate cystatin C utility as a biomarker in the saliva of pigs.
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# ANTIMICROBIAL EFFECT OF CLOVE ESSENTIAL OIL (EUGENIA CARYOPHYLLATA) ON ENTEROBACTERIA ISOLATED FROM SWINE

I.D.S. Lima <sup>1</sup>, C.D. Segovia <sup>1</sup>, <u>S. Rodrigues Chagas <sup>2</sup></u>, D. Guedes Barbosa De Lima <sup>1</sup>, M.C. Pereira Silva <sup>1</sup>, L. Mendonça Pascoal <sup>3</sup>, C. Silva Minafra E Rezende <sup>1</sup>

<sup>1</sup>Universidade Federal de Goiás - Goiânia - Brasil

<sup>2</sup>Universidade Federal de Goiás and University of Minnesota

<sup>3</sup>Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Goiânia, Brazil

# **Background and Objectives**

Antimicrobial resistance rates are growing in pig farming (1). Therefore, the search for new antimicrobial agents is important. Thus, this study aimed to investigate the antimicrobial effect of clove essential oil (EO) (Eugenia caryophyllata) on enterobacteria isolated from swine in Brazil.

# **Material and Methods**

We selected strains of enterotoxigenic Escherichia coli and Salmonella enterica serovar Heidelberg isolated on disease outbreaks on a Brazilian pig farm. The strains were identified using biochemical and molecular tests.

To assess the antimicrobial effect of the EO, we used the Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) assays (CLSI M7-A6) (2,3). MIC determines the lowest concentration of the EO that visibly inhibits bacterial growth, while the MBC determines the lowest concentration that results in the complete eradication of the bacteria in the sample. Triphenyl tetrazolium was used to detect the presence of live cells/bacteria in the sample (4).

#### Results

MIC values for E.coli and S. Heidelberg ranged from 2 to 1 mg/mL. MBC values for E.coli and S. Heidelberg ranged from 2 to 1 mg/mL.

#### **Discussion and Conclusion**

Clove EO demonstrated an antimicrobial effect on both E. coli and S. Heidelberg.

The main component of clove EO is eugenol, a phenolic compound recognized for its antimicrobial properties (5). Previous studies also reported the efficacy of eugenol in inhibiting bacterial growth, corroborating our findings (6,7,8).

To our knowledge, this is the first study to assess MIC and MBC for clove EO on E.coli and S. Heidelberg strains isolated from pigs. Nada et al (2021) also tested the antimicrobial effect of clove EO on E. coli isolated from humans in food-borne infections (9); they reported a MIC of 1.5 mg/mL.

Overall, clove EO can be considered a potential alternative to conventional antibiotics, thus contributing to the reduction of antimicrobial resistance in both pig production and public health scenarios.

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# ANTIMICROBIAL RESISTANCE PROFILE OF BRAZILIAN CLINICAL STRAINS OF STREPTOCOCCUS SUIS SEROTYPE 9

K. Rodeghiero <sup>1</sup>, <u>M. Tamanini</u><sup>2</sup>, A.P. Mori <sup>2</sup>, K.J. Will <sup>1</sup>, R. Budzisz Petri <sup>2</sup>, R. Da Rosa Ulguim <sup>1</sup>, F. Bortolozzo <sup>1</sup>, S. Prigol <sup>3</sup>, E. Bresolin <sup>3</sup>, R. Frandoloso <sup>4</sup>, L.C. Kreutz <sup>4</sup>, A.P. Mellagi <sup>1</sup>

<sup>1</sup>Setor de Suínos, Departament of Animal Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil <sup>2</sup>Vaxxinova, Vargem Grande Paulista, Brazil

<sup>3</sup>AFK Imunotech, Passo Fundo, Brazil

<sup>4</sup>University of Passo Fundo & AFK Imunotech, Passo Fundo, Brazil

# **Background and Objectives**

Streptococcus suis (Ss) is a Gram-positive bacterium that infects piglets early in life. Recently, in Brazil, Ss serotype 9 has been associated with outbreaks of neurological disease in nursey piglets with mortality rates reaching up to 20%. Assertive management combined with the use of vaccines are central to control the bacteria spread. Here we evaluated the sensitivity profile of 57 clinical isolates of Ss serotype 9 to different antimicrobials frequently used in Brazil.

# Material and Methods

A total of 57 clinical strains of Ss serotype 9 isolated from South Brazilian pig farms throughout 2023 were evaluated. The strains were isolated from piglets and molecularly classified according to their capsular type. The antimicrobial susceptibility testing (AST) was performed following CLSI guidelines. The AST was carried out using the disk diffusion method and 12 antimicrobials (amoxicillin, ceftiofur, doxycycline, florfenicol, fosfomycin, lincomycin, marbofloxacin, norfloxacin, sulfamethoxazole-trimethoprim, tilmicosin, tulathromycin, and tetracycline) were assessed.

#### Results

The Ss serotype 9 clinical isolates were 100% resistant to lincomycin and sulfamethoxazole-trimethoprim. The resistance percentage to others antimicrobial were: 94.74% to Tilmocosin; 92.8% to tulathromycin and norfloxacin; 91.07% to tetracycline; 83.93% to marbofloxacin; 72.73% to doxycycline; 14.04% to florfenicol; 12.3% to ceftiofur; 10.5% to amoxicillin, 1.8% to fosfomycin.

## **Discussion and Conclusion**

The sensitivity profile of Brazilian clinical strains of Ss serotype 9 to antimicrobials is extremely worrying. Of the 12 molecules tested, only Fosfomycin, amoxicillin, ceftiofur and florfenicol were effective in more than 85% of the strains evaluated. Thus, AST of isolates prior to medication and the strategic use of effective vaccines are necessary to prevent the spread of Ss serotype 9 in Brazil. Due to the high antigenic diversity of Streptococcus suis, autogenous vaccines are the first choice to control this microorganism. In Brazil, these vaccines are widely used with satisfactory results in reducing mortality and cost rates.

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# CHARACTERIZING ENTEROTOXIGENIC E. COLI ISOLATES RECOVERED FROM HEALTHY AND DIARRHEIC POST-WEANING PIGS

Y. Lin<sup>2</sup>, C. Schut<sup>2</sup>, J.M. Fairbrother<sup>1</sup>, B.N. Lillie<sup>2</sup>, R.M. Friendship<sup>2</sup>, <u>V. Farzan<sup>2</sup></u> <sup>1</sup>University of Montreal, Canada <sup>2</sup>University of Guelph, Canada

## **Background and Objectives**

Post-weaning diarrhea (PWD) poses a significant challenge to the swine industry due to associated economic losses, reduction in animal welfare, and increased antimicrobial use and resistance. While PWD is a multifactorial disease, enterotoxigenic E. coli (ETEC) have been implicated as an important infectious agent. The objective of this study was to identify virulence factor genes in E. coli isolates recovered from pigs with post-weaning diarrhea and healthy pigs.

# **Material and Methods**

Fecal swabs from healthy and diarrheic pigs were cultured on Columbia Blood and MacConkey agar. The E. coli isolates were tested by PCR for fimbriae (F4, F18, F5, F41, and F6), adhesins (AIDA, EAE, and Paa), and enterotoxins (LT, Stx1, Stx2, STa, STb, EAST-1) genes. A multi-level mixed effects logistic regression modelling method with farm as a random effect was used to compare the shedding of virulent E. coli (defined as an isolate that carried at least one virulence gene) and the virulence factor genes detected in healthy and diarrheic pigs.

### Results

Of 225 pigs (75 healthy, 150 diarrheic) collected from 15 farms, 122 (54.2%) pigs were shedding virulent E. coli. The most frequently detected fimbriae genes were for F4 (6.7% healthy and 10% diarrheic pigs) and F18 (5.3% healthy and 8.7% diarrheic pigs). The most frequently detected enterotoxin genes were for EAST-1 (30.7% healthy and 39.3% diarrheic pigs), LT (17.3% healthy versus 31.3% diarrheic pigs; p=0.010), and STb (17.3% healthy compared to 30.0% diarrheic pigs; p=0.010). Overall, the pigs with clinical signs of PWD were more likely to shed virulent E. coli than healthy pigs (p=0.019). Moreover, the pigs tested during the spring (p=0.045) and autumn (p=0.043) were more likely to shed virulent E. coli compared to the pigs tested during the winter.

## **Discussion and Conclusion**

The findings indicated that the limited success of current vaccination programs targeting F4/F18 may be in part due to the variation of E. coli virotypes from farm to farm, with many strains not expressing any tested fimbriae or adhesins. It is also possible that genetically resistant pigs shed ETEC but did not develop the clinical signs of PWD, which need to be further investigated.

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# FIELD TRIALS OF MEDIUM-CHAIN FATTY ACID FEED ADDITIVES AGAINST BRACHYSPIRA HYODYSENTERIAE IN A PIG FARM

H. Kuo<sup>1</sup>, W.C. Chiu<sup>2</sup>, H. Tseng<sup>1</sup>, L.Y. Fong<sup>1</sup>, C. Wang<sup>1</sup>, S. Huang<sup>1</sup>, <u>C. Wu<sup>1</sup></u> <sup>1</sup>Department of Veterinary Medicine, National Chiayi University, Chiayi, Taiwan <sup>2</sup>Ecolex Animal Nutrition, Kuala Lumpur 42920, Malaysia

## **Background and Objectives**

Swine dysentery, an economically significant disease caused by Brachyspira hyodysenteriae (B. hyodysenteriae), primarily affects fattening pigs. While mortality rates are relatively low, the disease severely hampers growth performance and feed conversion efficiency. Conventional in vitro screening methods for antibiotics and alternative antimicrobials are ineffective due to B. hyodysenteriae's fastidious nature. This inadequacy contributes to the indiscriminate use of antibiotics, exacerbating the growing concern of antibiotic resistance. Medium-chain fatty acids (MCFAs) have demonstrated antimicrobial properties and, when employed as feed additives in pig production, promote gut health and enhance growth. Given their promising potential in controlling B. hyodysenteriae, this study aims to evaluate the effectiveness of MCFAs in a pig farm.

# **Material and Methods**

During the trial, C12:0 and C14:0 MCFAs feed additives were added to the feed at a concentration of 2 kilograms per ton of feed. The additives were administered from 12 weeks of age until the pigs were marketed. To assess the effectiveness of the MCFAs, fecal samples were collected from B. hyodysenteriae-positive pigs at 10, 15, 20, and 24 weeks of age. These samples were subjected to real-time quantitative polymerase chain reaction (qPCR) to quantify B. hyodysenteriae bacterial nucleic acid levels.

#### Results

The Ct values of B. hyodysenteriae detected at 10, 15, 20, and 24 weeks of age were 27.2, 29.6, 40.0, and 40.0, respectively. These results indicate an increasing trend in Ct values over time. Additionally, the positivity rates were 100% (10/10), 90% (9/10), 0% (0/10), and 0% (0/10) for the corresponding weeks.

# **Discussion and Conclusion**

These findings suggest that the MCFAs feed additives were effective in reducing the shedding of B. hyodysenteriae.

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# FIRST DETECTION OF CLOSTRIDIODES DIFFICILE IN CASES OF NEONATAL DIARRHOEA IN THE PHILIPPINES

J. Miguel <sup>1</sup>, <u>J. Rubico</u><sup>2</sup>, S. Calixtro <sup>2</sup>, A. Salazar <sup>2</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA Philippines, Manila

#### **Background and Objectives**

Neonatal diarrhoea (ND) in pigs is multifactorial. ND control should consider the differential diagnosis of the multiple agents involved. Fresh faeces can be a problematic sample for this purpose, as results may be affected by storage and transport conditions. Other factors, such as the limited capacity of bacterial culture growth of agents like C. difficile can impair its proper diagnosis. Currently, there is no information related to the incidence of C. difficile in The Philippines because of a lack of availability of diagnosis services in local animal health laboratories. The aim of this study was to evaluate the presence of C. difficile in cases of neonatal diarrhoea in The Philippines by using a novel PCR.

# **Material and Methods**

During 2023, 8 farms located in high-density producing areas in the Philippines suffering from neonatal diarrhoea were sampled, although they were vaccinated with a combo vaccine for E. coli and C. perfringens type C. On each farm, three rectal swab samples from 3 piglets per litter were collected and placed onto FTA® ELUTE cards (Whatman Inc., Florham Park, NJ). The samples were delivered to HIPRA Diagnos Philippines, and a PCR to detect C. difficile A and B toxins was used.

#### Results

On 3 out of the 8 farms sampled, positive results for C. difficile were detected. In terms of positivity by litter, 60% were positive to toxin A and 47% to toxin B. These farms were negative for Rotavirus type A & C and PED virus.

# **Discussion and Conclusion**

C. difficile is considered a ubiquitous pathogen and an early colonizer of the intestinal tract of piglets. Its implication in cases of ND could have gone unnoticed because of the limited availability of tools for its proper diagnosis. These results provide the first insights of the presence of the bacterium in The Philippines, and although more research is needed, it must be considered as an etiological agent of neonatal diarrhoea.

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# FIRST MOLECULAR DETECTION OF BRACHSPIRA SUANATINA IN POLAND

P. Cybulski 1, K. Strutzberg-Minder 2, E. Michalik 1, R. Kondratiuk 1, A. Jablonski 3

<sup>1</sup>Goodvalley Agro S.A., Przechlewo, Poland

<sup>2</sup> IVD GmbH, Innovative Veterinary Diagnostics, Seelze, Germany

<sup>3</sup>Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences—SGGW, Warsaw, Poland

### **Background and Objectives**

Swine dysentery (SD) is a severe enteric disease substantially affecting the swine sector worldwide. SD was first described in the 1920s in the USA. Historically, SD is a name of a specific disease associated with Brachyspira hyodysenteriae, Gram-negative, strongly beta-haemolytic, motile species of the anaerobic spirochaetal genus Brachyspira. Recently, B. hampsonii and B. suanatina have been recognised as a new pathogenic species causing SD symptoms. Prevalence of these species in European farms has not been adequately investigated yet. Therefore, this study aimed to identify the presence of all the aforementioned Brachyspira species in faecal samples collected from diarrhoeic finishers reared under conditions of modern swine farms in Poland.

# **Material and Methods**

The investigation was carried out in November 2022 in 9 Polish herds with 6000 - 18000 finishers. At every finisher farm one fresh pooled faecal sample was collected by a veterinarian from 40 individuals (60 - 110 kg) defecating abnormal, loose stools. Each sample containing approximately 120 ml of faecal matter was collected into a sterile screw-cap specimen jar using a plastic spoon and then allowed to cool down. All the samples were transported overnight to IVD lab (Seelze-Letter, Germany) and processed on the following day using multiplex PCR.

#### Results

The genetic material of B. suanatina was detected in samples collected from 7 out of 9 finisher farms. All the collected samples were B. hyodysenteriae and B. hampsonii negative.

# **Discussion and Conclusion**

Official reports describing infections with B. suanatina in pig farms are exceptionally rare and still limited to a few countries located in Northern and Western Europe. To the best of the authors' knowledge, the described investigation was the very first to prove the occurrence of this pathogen in Poland. Moreover, our results clearly highlight a considerable need for unified diagnostics programs across the EU regions.

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## GENOMIC INSIGHTS OF PASTEURELLA MULTOCIDA FROM SWINE IN AUSTRIA

L. Barta<sup>1</sup>, D. Polzer<sup>1</sup>, A. Stoeger<sup>2</sup>, W. Ruppitzsch<sup>2</sup>, T. Sattler<sup>3</sup>, F. Schmoll<sup>1</sup> <sup>1</sup>Austrian Agency for Health and Food Safety, Mödling, Austria <sup>2</sup>AGES MED, Vienna, Austria <sup>3</sup>Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Leipzig University

## **Background and Objectives**

Pasteurella multocida can be the causative agent for progressive rhinitis atrophicans. Despite their toxigenic potential they are opportunistic pathogens and play a major role in the porcine respiratory disease complex. Pasteurella multocida is classified into different Serotypes (A, B, D, E, F) and harbours a variety of virulence associated genes. Aim of the study was to investigate the capsule type predominant in Pasteurella multocida isolated from Austrian pig farms and the variety of virulence genes that occur. In times of rising antimicrobial resistant pathogens, these isolates were also analysed in respect of resistance genes.

#### **Material and Methods**

96 isolates of Pasteurella multocida from Austrian pig farms associated with respiratory diseases were analysed. For a genomic comprehension and eventually research of outbreaks we used next generation sequencing. Capsular typing and detection of 12 virulence associated genes were performed using in silico PCR. The resistance genes were detected by AMR Finder Plus.

#### Results

In 59 Isolates, capsule type A and in 35 capsule type D was found. Two isolates showed no capsule type. Genes encoding for the virulence factors ExbB-ExbD-TonB-Lokus, Typ-4-Fimbrie, HgbA, SodA, NanH, NanB, SodC, Oma87 were present in all cases. 87 isolates were harbouring genes encoding OmpH and 75 HgbB. In only 15 isolates, genes encoding PfhaB and just five were positive for ToxA genes. Resistant genes against kanamycin, streptomycin, lincosamide, sulfonamide, trimethoprim and tetracycline were found in nine Pasteurella multocida isolates. Seven of them showed resistance to two or more antimicrobials.

## **Discussion and Conclusion**

To our knowledge this is the first study about the genomic variety of Pasteurella multocida from swine in Austria. Our results regarding to the virulence associated genes are similar with those of previous studies from other European countries like Italy or Germany. The prevalence of toxigenic Pasteurella multocida is low but still occurs in Austrian swine flocks. In contrast to studies from Asia our isolates are sensitive to most antimicrobials. Nevertheless, practitioners should keep in mind that we found strains which are resistant to commonly used antibiotics like tetracycline and sulfonamide/trimethoprim.

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# HISTOPATHOLOGICAL RESEARCH ON THE RELATION BETWEEN PORCINE PULMONARY CONSOLIDATION AND MYCOPLASMA HYOPNEUMONIAE INFECTION

P. Nueangphuet <sup>1</sup>, N. Fuke <sup>1</sup>, R. Uemura <sup>2</sup>, T. Fujiwara <sup>3</sup>, C. Maeda <sup>3</sup>, M. Hosokawa <sup>4</sup>, T. Hirai <sup>1</sup>, R. Yamaguchi <sup>1</sup>, <u>T. Ueda <sup>4</sup></u> <sup>1</sup>Department of Veterinary Pathology, Faculty of Agriculture, University of Miyazaki, Japan

<sup>2</sup>Laboratory of Animal Health, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Japan <sup>3</sup>Fujiwara Animal Hospital, Kagoshima, Japan

<sup>4</sup>Boehringer Ingelheim Animal Health Japan Co., Ltd.

## **Background and Objectives**

Mycoplasma hyopneumoniae (Mhp) is one of the major pathogens involved in porcine respiratory complex disease (PRDC), and its typical gross lesion has been described as consolidation of cranioventral lung areas. In this study, we report some new findings obtained from histopathological research on pulmonary consolidation in slaughter-aged pigs.

## **Material and Methods**

Lung samples were collected at a slaughterhouse from a total of 32 pigs shipped from two SPF pig farms in Japan and scored for lung lesions. We also attempted to detect some major pathogens of porcine pneumonia by conventional PCR, and the DNA levels of Mhp-positive samples were determined by quantitative PCR. Histopathology and immunohistochemistry with several inflammatory markers were also performed for further investigation. All indicators were scored based on intensity and distribution.

#### Results

Pulmonary consolidation were observed in 75% of the lungs collected, of which 45.8% were positive for Mhp-PCR. Quantitative PCR and histopathology showed that the amount of Mhp DNA correlated with lesion severity score and immunohistochemistry score. Double immunohistochemistry showed that Caspase3, an indicator of apoptosis, was expressed in Mhp-associated airway epithelial cells, which tended to be stronger in specimens with higher Mhp-DNA levels and histological scores.

### **Discussion and Conclusion**

Less than half of the consolidated lungs were Mhp-PCR positive. This suggests that while Mhp is still a major cause of pneumonia in some SPF farms in Japan, not all pulmonary consolidation are necessarily associated with Mhp infection. Therefore, a gross assessment of lung lesions at slaughterhouse is not a sufficient indicator of the extent of mycoplasmal pneumonia on the farm. In this study, however, no pathogens other than Mhp were detected in most of the specimens, and the causes of the consolidation in Mhp-negative lungs are unknown. On the other hand, immunohistochemistry results suggest the severity of Mhp-positive lung lesion may be due to the amount of its infection, indicating that Mhp may induce cellular apoptosis in the respiratory tract.

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# METAGENOMIC ANALYSES OF STREPTOCOCCUS SUIS STRAINS IDENTIFIED DIFFERENT PROPHAGES USEFUL FOR GENOTYPING.

D. Martínez-Gómez <sup>1</sup>, J.J. López Islas <sup>1</sup>, E.T. Méndez Olvera <sup>1</sup>, A. Romero-Flores <sup>2</sup>, J.I. Sánchez-Betancourt <sup>3</sup>, L. Hernandez-Andrade <sup>4</sup>, A. Ciprian-Carrasco <sup>2</sup>, <u>S.E. Mendoza Elvira <sup>5</sup></u> <sup>1</sup>UAM-Xochimilco. México <sup>2</sup>UNAM-FESC, México <sup>3</sup>UNAM-FMVZ <sup>4</sup>Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias <sup>5</sup>Facultad de Estudios Superiores Cuautitán. UNAM.

### **Background and Objectives**

Streptococcus suis is an important pathogen of pigs, causing arthritis, endocarditis, meningitis, pneumonia, and septicemia. For this microorganism serotypes have been associated with virulence. Serotypes 2 and 1/2 are the most virulent and the most frequently isolated from diseased animals. However, the correlation between virulence and serotype is not decisive, and a genetic diversity of S. suis isolates between and within serotypes has been shown. Metagenome analysis is considered a powerful method to investigate the genetic diversity in S. suis strains. In this study a metagenome analyses showed different prophages in 23 S. suis strains. Comparison of prophage DNA sequences showed differences between strains associated with clinical features.

#### **Material and Methods**

Sixty-three samples of organs were obtained from pigs with clinical signs of S. suis infection from different regions in Mexico. The samples were processed by standard bacteriological techniques. DNA extraction was performed using a commercial DNA purification kit (Promega, USA). Serotyping was done using the two-step multiplex PCR (Okura et al. 2014). Metagenome sequencing was performed in Illumina HiSeq 2500 platform. The data was analyzed using different bioinformatic software.

### Results

The metagenome analysis identified 29 different prophages in the 23 strains analyzed. Prophages identified includes Streptococcus prophage 315.5, Streptococcus phage P9, Streptococcus phage PH10, Streptococcus phage DCC1738 and Streptococcus pneumoniae bacteriophage MM1. Serotypes were similar between strains isolated from distinct organs, however, sequence analysis of 614 phage proteins showed differences between S. suis strains. Also, different phage proteins were identified showed a genetic divergence suggesting evolutive events.

# **Discussion and Conclusion**

Many studies used serotypes to describe the virulence of S. suis strains, however this method is limited to identify genetic variations. In our study, analyses of phage proteins showed differences between strains isolated from distinct organs. Whole-Genome Sequencing Approaches is a powerful method to identify variations between strains. Also, in this study a genome analyses offered useful information like prophage lysins proteins used like therapeutic agent.

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# MICROBIOTA ANAEROBIC BACTERIA VS BRACHYSPIRA HYODYSENTERIAE: THE BREAKTHROUGH OF NEW PROBIOTICS.

S. Gómez-Martínez <sup>1</sup>, <u>H. Arguello</u> <sup>1</sup>, L. Perez-Perez <sup>1</sup>, H. Puente <sup>1</sup>, P. Rubio <sup>1</sup>, A.M. Carvajal <sup>1</sup> <sup>1</sup>DIGESPORC research group, Facultad de Veterinaria, Campus de Vegazana, 24007, España

#### **Background and Objectives**

Brachyspira hyodysenteriae is the main etiological agent of swine dysentery (SD) a disease characterized by severe mucohaemorragic diarrhoea. SD generates important losses due to mortality, detrimental performance, and treatment costs. The antimicrobial resistance to commonly used antibiotics encourages research in new strategies like probiotics as control alternatives. In this study, we explore the in vitro competition of anaerobic commensals against B. hyodysenteriae.

## **Material and Methods**

Seven anaerobic commensals including Megasphaera elsdenii C6/22-ME7 (swine), Faecalibacterium prausnitzii CC-F21 (culture collection strain), Mitsuokella multacida C2/23-EG2 (swine), Acidaminococcus fermentans C6/22-A15 (swine), Roseburia spp C6/22-RB1 (swine), Megasphaera indica C2/23-CM15 (swine), Intestinibaculum porci J1/23-CM6 (wild pig) were co- cultured with B. hyodysenteriae (B204 strain) in broth culture, under anaerobic conditions at 37° C for 96h. Cell free supernatant (CFS) from commensals exhibiting anti-B. hyo activity were used in another experiment to evaluate their antibacterial activity. Antagonistic effects over B204 strain growth were all measured through quantitative PCR at 24, 48 and 96h.

#### Results

Four out of seven anaerobic isolates limited or even inhibited the growth in B204 in broth. The strains C6/22-ME7 and J1/23-CM6 limited B204 growth by two log-units and C2/23-CM15 and C2/23-EG2 limited B204 by one log-unit in the co-cultures. C6/22-ME7 and J1/23-CM6 CFS also limited B204 by co-culture with reductions between one and two log-units.

## **Discussion and Conclusion**

The results of this study reveal the potential competitive activity of certain anaerobic gut commensals against B. hyodysenteriae, directly by nutrient competition and also potentially by the antimicrobial activity of their metabolites as demonstrated by the activity of the CFS. Further detailed characterisation of strains with competitive activity against B. hyodysenteriae and or their metabolites both in vitro and in vivo animal models will provide further details of theior potential as new probiotics which may help to replace antibiotics in the control of swine dysentery.

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# MINIMUM INHIBITORY CONCENTRATIONS OF TIAMULIN AGAINST MYCOPLASMA HYOPNEUMONIAE ISOLATED ACROSS THAILAND OVER A 6-YEAR PERIOD (2017-2022)

<u>T. Sujit</u><sup>1</sup>, W. Ketphan<sup>1</sup>, S. Simjee<sup>2</sup>, S. Talummuk<sup>3</sup>, A. Ratanamongkolmas<sup>3</sup>, W. Deeampai<sup>3</sup> <sup>1</sup>National Institute of Animal Health, Department of Livestock Development, Ministry of Agriculture and Cooperative, Bangkok, Thailand <sup>2</sup>Elanco Animal Health, Greenfield, IN, USA <sup>3</sup>Elanco (Thailand) Ltd., Bangkok, Thailand.

## **Background and Objectives**

Mycoplasma hyopneumoniae is the causative agent of porcine enzootic pneumonia, a chronic respiratory disease, causing significant economic losses. Tiamulin is the standard treatment option for M. hyopneumoniae infections and therefore essential that they are used responsibly to maintain long term efficacy. Results from a 6-year susceptibility monitoring survey of hyopneumoniae against tiamulin across Thailand are presented.

#### **Material and Methods**

Lung samples were collected from pigs across 8 provinces in Thailand showing clinical signs of porcine enzootic pneumonia between 2017-2022.

Following isolation and identification of M. hyopneumoniae using standard methods, MIC against innovator tiamulin was conducted as described by Hannan (2000) with BHL broth, incubated at  $35 \pm 1$  °C for 5–12 days. M. hyopneumoniae strain J was used as Quality Control isolate. The MIC data was interpreted using the following interpretive criteria Susceptible = <16 µg/ml and Resistance = >32 µg/ml.

#### Results

In total 32 isolates of M. hyopneumoniae were recovered (2017 n=2; 2018 n=3; 2019 n=4; 2020 n=4; 2021 n=14; 2022 n=5) across 8 provinces in Thailand. Tiamulin MIC distribution ranged between  $\leq 0.008-0.5 \mu g/ml$ . MIC distribution ( $\mu g/ml$ ) was as follows:  $\leq 0.008 n=5$ ; 0.016 n=4; 0.032 n=5; 0.0625 n=6; 0.125 n=5; 0.25 n=6; 0.5 n=1. There was a range of MIC values across all regions. No isolates displayed resistance to tiamulin, MIC<sub>50</sub>=0.0625  $\mu g/ml$ ; MIC<sub>90</sub>=0.25  $\mu g/ml$ . The QC isolate was in MIC range, validating the test system.

# **Discussion and Conclusion**

In total 32 M. hyopneumoniae isolates across 8 provinces over a 6-year period were recovered in Thailand. The antibiogram displayed a narrow unimodal distribution with no resistant isolates. The data indicates that despite over a decade of responsible use of tiamulin in Thailand resistance has not developed in M. hyopneumoniae. The data does suggest, however, standardised laboratory methods and interpretive criteria for MIC testing of veterinary mycoplasmas are clearly needed; there are currently no clinical breakpoints available to facilitate data interpretation and correlation of MICs with in vivo efficacy.

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# MINIMUM INHIBITORY CONCENTRATIONS OF TIAMULIN AGAINST MYCOPLASMA HYORHINIS ISOLATED ACROSS THAILAND OVER A 3-YEAR PERIOD (2020-2022)

<u>T. Sujit</u><sup>1</sup>, W. Ketphan<sup>1</sup>, S. Simjee<sup>2</sup>, S. Talummuk<sup>3</sup>, A. Ratanamongkolmas<sup>3</sup>, W. Deeampai<sup>3</sup> <sup>1</sup>National Institute of Animal Health, Department of Livestock Development, Ministry of Agriculture and Cooperative, Bangkok, Thailand <sup>2</sup>Elanco Animal Health, Greenfield, IN, USA <sup>3</sup>Elanco (Thailand) Ltd., Bangkok, Thailand.

# **Background and Objectives**

Mycoplasma hyorhinis, a swine pathogen of concern in weaners, commonly found in the upper respiratory tract of swine and shed through nasal secretion or oral fluids and transmitted through direct contact of sows and pigs. The pathogen is globally distributed, generating significant economic losses. Currently no commercially vaccine is available and therefore, besides preventative measures such as improved biosecurity, antibiotic therapy is the only option. Results from a 3-year susceptibility monitoring survey of M. hyorhinis against tiamulin across Thailand are presented.

# Material and Methods

Lung and tonsil samples were collected from pigs across 10 provinces in Thailand showing clinical signs associated with M. hyorhinis infections between 2020-2022.

Following isolation and identification of M. hyorhinis using standard methods, MIC against innovator tiamulin was conducted as described by Hannan (2000) with modified Hayflick's broth, incubated at  $35 \pm 1$  °C for 5–12 days. M. hyorhinis strain BTS7 was used as Quality Control isolate. Interpretive criteria for MIC were Susceptible =  $\leq 16 \mu g/ml$  and Resistance =  $\geq 32 \mu g/ml$ .

### Results

In total 30 isolates of M. hyorhinis were recovered (2020 n=5; 2021 n=9; 2022 n=16) across 10 provinces in Thailand. MIC distribution ranged between  $0.032-0.25 \mu g/ml$ . MIC distribution was as follows:  $0.032 \mu g/ml$  n=4;  $0.0625 \mu g/ml$  n=6;  $0.125 \mu g/ml$  n=13;  $0.25 \mu g/ml$  n=7. Apart from one region where a wider MIC distribution was observed ( $0.0625-0.25 \mu g/ml$ ) all other regions showed a tight MIC range to tiamulin indicating potential regional clonal spread. No isolates displayed resistance to tiamulin, MIC<sub>50</sub>=0.125  $\mu g/ml$ ; MIC<sub>50</sub>=0.25  $\mu g/ml$ . The QC isolate was in MIC range, validating the test system.

#### **Discussion and Conclusion**

In total 30 M. hyorhinis isolates across 10 provinces over a 3-year period were recovered in Thailand. Antibiogram demonstrated a tight unimodal distribution with no isolates displaying resistance. The data does suggest, however, standardised laboratory methods and interpretive criteria for MIC testing of veterinary mycoplasmas are clearly needed; there are currently no clinical breakpoints available to facilitate data interpretation and correlation of MICs with in vivo efficacy.

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# NEONATAL DIARRHEA - RESULTS OF DIAGNOSTICS PERFORMED ON DUTCH COMMERCIAL FARMS OVER A LONG PERIOD OF TIME.

<u>B. Cornelis</u><sup>1</sup>, P. De Backer <sup>2</sup>, J. Schumans <sup>3</sup>, S. Van Colen <sup>4</sup> <sup>1</sup>DVM Ceva Santé Animale <sup>2</sup>Ceva Santé Animale Benelux <sup>3</sup>Ceva Santé Animale, Naaldwijk, the Netherlands <sup>4</sup>Ceva Santé Animale, Brussels, Belgium

# **Background and Objectives**

In case of neonatal diarrhea (ND) in piglets, different pathogens have been recognized as potential pathogenic such as Clostridium perfringens type A (CpA), Clostridium perfringens type C (CpC), Clostridioides difficile (C.diff), Escherichia (E.) coli and Rotavirus type A (RVA) and type C (RVC). In this study, presence of these pathogens in cases of ND on Dutch commercial farms over a period of 18 months was evaluated.

## **Material and Methods**

Between January 2022 and June 2023, diagnostics were performed on faecal samples and swabs of 78 cases of ND on Dutch commercial farms. Techniques used are bacterial culturing for detection of CpA, CpC, C.diff and E. coli and PCR testing for presence of Porcine RVA and RVC. For CpA/C and C.diff a semi-quantitative indication of the bacterial load is reported and classified as low, moderate or high concentration. Typing of CpA/C was performed by identification of toxin genes by multiplex PCR-analysis and E. coli pathotyping was performed by multiplex PCR-analysis.

## Results

In the samples 64% (139:219) identified positive as CpA/C, 44% (96:218) C.diff, and 23% (50:215) E. coli pathotype. Investigated CpA/C isolates (n=110) identified as 96% type A ß2-toxin positive, 3% type A ß2-toxin negative and 1% type C. The CpA/C samples (139) bacterial load was 28% with a high, 71% moderate and 1% low bacterial concentration, and for C.diff this was respectively 1%, 39% and 60%. In 69% (141:204) of the samples RVA and in 38% (80:202) RVC was found.

# **Discussion and Conclusion**

In this study on samples from farms with ND, CpA with ß2-toxin producing capacity is the most frequently detected bacterial pathogen and RVA is the most detected viral pathogen. For the evaluation of the potential role of some of the pathogens, estimation of bacterial load is critical. Isolates of CpA/C from clinical cases of ND were characteristic by moderate to high growth on cultivation media, suggesting its role in clinical cases, on the opposite, C.diff isolates grew in low concentration in majority of the cases. ND is often a multifactorial disease. Diagnostics to identify the involved pathogens can help to decide on the preventive measures to be taken.

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# PHARMACOKINETICS OF CEFTIOFUR CRYSTALLINE FREE ACID: ONSET AND DURATION OF THERAPEUTIC LEVELS AFTER A SINGLE INTRAMUSCULAR DOSE IN SWINE

<u>M. Jansen</u><sup>1</sup>, E. Jablonski<sup>1</sup> <sup>7</sup>Zoetis Inc, Parsippany, NJ, USA

#### **Background and Objectives**

Swine practitioners consider many factors when implementing antibiotic regimens, including judicious use and good veterinary practice (GVP) guidelines, labeled indications, sensitivity profiles, minimum inhibitory concentrations (MIC), and withdrawal times. Pharmacokinetics (PK) is another consideration. Two PK studies were completed with ceftiofur crystalline free acid (CCFA, Excede for Swine®, Zoetis) which is labeled in the U.S. for the treatment and control of swine respiratory disease.1,2 To understand how quickly and how long CCFA reaches concentrations at or above MIC values for targeted bacteria, data from both studies were collated.

# **Material and Methods**

In both studies, pigs were administered a single dose of CCFA at 5 mg/kg intramuscularly. Blood samples were collected before treatment and at specified timepoints post-administration. The concentration of ceftiofur and its active metabolite, desfuroylceftiofur, in plasma was determined using validated high-performance liquid chromatography. The full PK profile was charted with MIC90 reference lines overlaid to determine how quickly and how long MIC levels are achieved for the following labeled pathogens: Actinobacillus pleuropneumoniae (APP), Pasteurella multocida (PM), and Streptococcus suis (SS). MIC90 reference lines were based on recently published in vitro broth microdilution susceptibility testing.3

# Results

Data from both studies was compiled into a single PK curve. MIC90 values utilized were ≤0.03 ug/mL for APP and PM and 2.0 ug/ml for SS.3 For APP and PM, CCFA reached therapeutic plasma concentrations within 5 minutes post-injection. For SS, CCFA reached therapeutic plasma concentrations within 30 minutes post-injection. Plasma CCFA concentrations remained above MIC90 for 168 hours for APP and PM, and 96 hours for SS.

# **Discussion and Conclusion**

This PK data demonstrates that CCFA reaches therapeutic levels rapidly (5 minutes), and also has a long duration of therapy (168 hours). CCFA must be used in a manner within the present standards of judicious use of antibiotics, which differ by country, to maximize therapeutic efficacy and minimize development of resistance.

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# PREVALENCE OF GLAESSERELLA PARASUIS IN CHINA, VIETNAM, THE PHILIPPINES AND THAILAND

S. Romero <sup>1</sup>, W. Fei <sup>2</sup>, T. Yang <sup>2</sup>, N. Duong <sup>3</sup>, H. Vu <sup>3</sup>, J. Rubico <sup>4</sup>, C. Ananratanakul <sup>5</sup>, <u>M. Hsun Wu <sup>1</sup></u> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA China, Beijing <sup>3</sup>HIPRA Vietnam, Ho Chi Minh City <sup>4</sup>HIPRA Philippines, Manila <sup>5</sup>HIPRA Thailand, Bangkok

### **Background and Objectives**

Glaesserella parasuis (GP) is a commensal bacterium of the upper respiratory tract of pigs. Some strains of this bacterium can cause severe systemic disease (also called Glässer's disease), especially in nursery pigs. Antimicrobials have been commonly used to treat this bacterial disease in farm animals, but the emergence of antimicrobial resistance needs the implementation of alternatives for disease control. Vaccination of sows and piglets is capable of significantly reducing mortality, proving to be a good alternative for disease control. The objective of this study was to determine the prevalence of Glaesserella parasuis in pigs with symptoms on swine farms in China, Vietnam, Thailand and The Philippines

# **Material and Methods**

For the analytical procedure, samples were obtained from piglets with symptoms compatible with Glässer's disease (polyserositis, polyarthritis, meningitis and bacterial pneumonia) that had not received antibiotic treatment. The animals were necropsied and sampled with a sterile swab, the contents of which were transferred to FTA cards and the cards sent to HIPRA DIAGNOS for performance of a qPCR analysis of GP. From January 2022 to October 2023, 479 samples were collected from farms in different countries: 277 animals from 46 farms in China, 87 animals from 43 farms in Vietnam, 59 animals from 26 farms in The Philippines and 14 animals from 5 farms in Thailand. A farm was considered positive when at least one of the animals tested positive.

#### Results

The presence of GP was detected on 78.3% (94 positive of 120) of the farms. Prevalence varied according to the different countries: China 80% (37+/46), Vietnam 83.7% (36+/43), Philippines 61.5% (16+/26) and Thailand 100% (5+/5). Regarding individual animal results, the presence of GP was detected in 65% of the sampled animals. China 64.3% (178+/277), Vietnam 72.4% (63+/87), Philippines 62.7% (37+/59) and Thailand 71.4% (10+/14).

#### **Discussion and Conclusion**

Based on these results and as previously reported, we can conclude that GP is one of the main pathogens involved in polyserositis cases, with an average prevalence on swine farms of 78.3% in China, Vietnam, The Philippines and Thailand

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# PREVALENCE OF ILEITIS IN MAJOR SWINE FARMING REGIONS OF CHINA

X. Mei <sup>1</sup>, H. Xiao <sup>1</sup>, <u>T. Tan <sup>1</sup></u> <sup>1</sup>*Elanco China* 

### **Background and Objectives**

Swine lleitis is a gastrointestinal infectious disease caused by Lawsonia intracellularis (Li) in swine, which is widely distributed globally and has a high infection rate, resulting in economic losses<sup>[1]</sup>. This survey covers China's major swine farming regions and aims to investigate the infection status of Li in different pig populations. The result can provide a scientific basis for the prevention and control of lleitis and a customized approach to treatment and control of lleitis.

### Material and Methods

Technical team of Elanco China collected 1,667 fecal samples from 169 pig farms across 26 provinces in China from September 2022 to July 2023. A PCR kit (Intracellularis Thermo VetMax) was used for testing the fecal samples. These samples included 1,099 loose feces samples, 452 normal feces samples, 67 bloody feces samples, 39 watery feces samples and 10 samples with indistinguishable forms. The samples were tested as a part of a diagnostic service to Chinese farmers supported by Elanco Animal health.

## Results

Among the 1667 samples, 1,433 were positive, resulting in a positive rate of 86% for Lawsonia intracellularis. For all the 169 pig farms, 163 farms tested positive, with a farm-level positivity of 96.4%. Among all these 1,667 fecal samples, the positive rate of detecting Li in normal feces was lower than in watery, loose feces, and bloody feces. For loose feces samples, the positivity reached 98%, the positive rate of bloody feces reached 100%, while the positivity for normal feces was only 56%.

# **Discussion and Conclusion**

This survey indicates that there is a high positive rate of ileitis in Chinese pig farms despite the current use of medication programs. Therefore, it is necessary for Chinese pig farms to take corresponding measures to solve ileitis such as customized treatment programs under the antibiotic responsible use policy. This study suggests also that in vitro activity of selected molecules should be analyzed to determine the best candidate for treatment of the clinical condition.

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# RELATIONSHIP BETWEEN LAWSONIA INTRACELLULARIS FECAL LOAD AND GROWTH PERFORMANCE IN 25 FRENCH PIG FARMS.

D. Duivon <sup>1</sup>, L. Eon <sup>1</sup>, L. Daluzeau <sup>1</sup>, S. Lopez <sup>2</sup>, J. Sevin <sup>3</sup>, <u>F. Voisin <sup>1</sup></u> <sup>1</sup>MSD Animal Health, 49 Beaucouzé, France <sup>2</sup>UNIVET SANTE ELEVAGE, rue Monge, 22600 Loudéac, France <sup>3</sup>Socavet, 75 boulevard de penthièvre, 22600 Loudéac, France

#### **Background and Objectives**

Lawsonia intracellularis (LI) is an important enteric pathogen in pigs with a worldwide endemic prevalence. Several recent publications have reported a correlation between LI fecal load and growth performance in pig-farms, even in the absence of clinical signs. The aim of this study was to evaluate the relationship between LI fecal load and technical performances during fattening in French pig farms.

# **Material and Methods**

Two panels of farms with poor (INF group; n=11) or good (SUP group; n=14) growth performance were set up based on ADG and FCR. These farms were required to have no systematic antibiotic treatment, no history of ileitis nor vaccination against it. In each farm, 2 cross-sectional salivary samples were taken from 3 consecutive batches at age 1, 2 and 3. In total, 150 saliva samples were tested by qPCR for LI (BactoReal Lawsonia kit, Ingenetix, Cut-Off=50). A mixed model was adjusted to estimate the impact of group and age on qPCR quantification value (Cq, with farm as a random effect). In addition, a two factor (group and fattening period) ANOVA model was adjusted to compare the Cq, using two periods: "start" (15 weeks and less) and "end" (20 weeks and more).

### Results

Eleven farms were enrolled in the INF group with average ADG and FCR of 681g/d and 2.57, respectively. Fourteen farms were included in the SUP group with average ADG and FCR of 754g/d and 2.39, respectively. Mean of ages 1, 2 and 3 were 95, 121 and 146 days respectively. The interaction between age and group had an effect on Cq: in group INF, Cq increased during fattening whereas it decreased in group SUP (p=0.017). At the beginning of fattening, mean Cq was 39 and 46 for groups INF and SUP, respectively. At the end of fattening, it was 47 and 43, respectively (p=0.014).

# **Discussion and Conclusion**

Under the conditions of our study, farms with poor technical performance had high levels of LI fecal load at the start of fattening, which then fell significantly. On the other hand, farms with good technical performance had low fecal load in the early fattening part, which then increased slightly.

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# RNA-SEQ ANALYSES TO DECIPHER THE IMMUNE CONTEXT IN ACUTE SWINE DYSENTERY

<u>H. Arguello</u><sup>1</sup>, L. Perez-Perez <sup>1</sup>, M. De Toro <sup>2</sup>, S. Zaldivar-Lopez <sup>3</sup>, P. Rubio <sup>1</sup>, A.M. Carvajal <sup>1</sup> <sup>1</sup>DIGESPORC research group, Facultad de Veterinaria, Campus de Vegazana, 24007, España <sup>2</sup>Centro de Investigación Biomédica de La Rioja (CIBIR), Spain. <sup>3</sup>Departamento de Genética, Facultad de Veterinaria, Universidad de Córdoba, Córdoba, Spain.

## **Background and Objectives**

Swine dysentery is a major enteric disease in swine which usually affects pigs in growing and finishing stages. The disease is characterized by an insidious muco-hemorrhagic diarrhoea which ends in fatal death if an effective antibiotherapy is not established on time. Despite the relevance and severity of the disease, the information about its pathogenesis is scarce. In ordert to shed light in the pathogen-host cross-talk, the present study evaluates the host response against Brachyspira hyodysenteriae in pigs during the acute stage of infection by transcriptomic analyses.

# **Material and Methods**

Sixteen 7-week-old pigs were randomly allocated into control (n=8) and infected (n=8) groups, the last orally challenged in three consecutive days with 1E+08 B. hyodysenteriae. Pigs were humanely euthanised at the acute stage of infection (2 consecutive days with mucohaemorrhagic diarrhoea and large B. hyodysenteriae shedding in faeces). RNA from the colon mucosa from animals was extracted and the mRNA was sequenced by RNA-seq. Sequences were cleaned, annotated and analyses were performed to obtain differential expression genes and enriched pathways.

#### Results

DESeq and EdgerR analytical tools revealed 3470 and 2903 differentially expressed (DE) genes between acute-infected and control pigs. A mean number of 115 genes were upregulated (fold-change FC >2) in infected pigs while 145 genes were downregulated (FC<2). Data were enriched with different tools. Enrichment analyses revealed a global downregulation of immune and metabolic functions in the mucosa. Indeed, from the 35 routes identified with FC>2, 33 were downregulated. Upregulated routes were associated to extracellular matrisome while downregulated routes were associated to the lower expression of cytokines and chemokines (CXCL9- CXCL10-CXCL-11-CXCL13) with impairment of neutrophil recruiting or intestinal network for IgA production.

# **Discussion and Conclusion**

The results of the study reveal the usefulness of RNA-seq transcriptomics to decipher the immunological context in acute infection in swine dysentery with clear disruption of physiological and immunological processes in the colonic mucosa. The results demonstrate the impairment of the host response in acutely infected pigs which may limit the host defence and explains the fatal outcome of the infection if an effective treatment is not established on time.

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# SEROCONVERSION DYNAMICS AFTER VACCINATION AGAINST ERYSIPELAS AND AUJESZKY'S DISEASE VIRUS WITH DIFFERENT VACCINATION PROTOCOLS IN FINISHING PIGS IN SPAIN

E. Sanchez <sup>1</sup>, <u>G. Abella</u><sup>1</sup>, C. Terrones <sup>1</sup>, D. Arroyave <sup>1</sup>, J. Llorens <sup>1</sup>, R. Garcia <sup>1</sup>, T. Coll <sup>1</sup>, X. Bernaus <sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health España, S.A.U. <sup>2</sup>Agropecuaria d'Artesa de Segre SCCL

## **Background and Objectives**

Although sows are vaccinated, finishing periods still lack protection against Erysipelas due to minimal antibiotic treatments. In countries where Aujeszky's Disease virus (AD) vaccine is still mandatory in the finishing phase, farmers tend to vaccinate the same day against Erysipelas for convenience. The objective of this study was to better understand seroconversion dynamics using two different Erysipelas vaccines and vaccination regimes (1 dose vs 2 doses) while the standard AD vaccination protocol was applied the same day.

# **Material and Methods**

The study was performed in 2000 pig finishing site in Spain where AD vaccination is performed at 12 and 16 weeks of age (woa). A total of 80 animals were vaccinated against AD virus and against Erysipelas using Ingelvac Ery® in 40 animals (test) and another Erysipelas vaccine with an aqueous adjuvant (control) on the rest. After 4 weeks, 20 animals of each group were revaccinated with the same Erysipelas vaccine they received in the first dose. The blood samples were analyzed using a commercial ELISA.

### Results

Regarding AD, 90% (test) and 74% (control) were found positive 4 weeks after AD vaccination (16 woa). After that point, all animals remained positive. Regarding Erysipelas, four weeks after vaccination, 90% of animals did seroconvert in the test group while 2,6% did in the control. At 23 woa, 90% and 95% were positive in the vaccine test groups (1 dose and 2 doses) and 0% and 11% were positive in the controls. At the final sampling (27woa), the % of Erysipela positives remained at 42% and 71% for the 1 dose or 2 doses of the test group and was 0% in the remaining groups.

# **Discussion and Conclusion**

Vaccination against Erysipelas showed no impact on AD immunization (total Antibodies expected), suggesting no interference on vaccine replication and presumably not on protection. Ingelvac Ery® showed an excellent seroconversion after 4 weeks of vaccination even with one dose in comparison to the control. Moreover, a booster effect was shown with the second dose until 27 woa, especially important in outdoor production with higher challenge and longer finishing periods.

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# SEROPREVALENCE OF PORCINE PROLIFERATIVE ENTEROPATHY IN KOREA

<u>Y. Oh</u><sup>1</sup>, S. Shin <sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Korea Ltd, South Korea <sup>2</sup>Optipharm, Korea

#### **Background and Objectives**

Porcine Proliferative Enteropathy (PPE) leads to considerable economic losses, due to diarrhea, weight loss and subclinical illness in growing pigs. An easy to use and cost-effective blocking Enzyme Linked Immunosorbent Assay (ELISA) was developed for detection antibody levels against PPE and the ELISA have been utilized as a valuable way for investigation of seroprevalence of PPE in Korea. The objective of this study is to investigate the seroprevalence and epidemiology of ileitis in Korea in 2022.

# **Material and Methods**

From October to November 2022, total 1,000 serum samples were collected from 43 farms nationwide. The sampling scheme included sows, weaned piglets (30-40 days old), nursery (70-80 days old), grower (100-120 days old) and finisher (over 160 days old). An ELISA was performed according to the manufacturer's instructions (SVANOVIR® L. intracellularis/Ileitis-Ab). The OD values were determined at 450 nm after developing. According to the manual of the ELISA PI values >30 was considered as positive for antibodies against PPE.

### Results

All farms seropositive animals were detected, and 318 (31.8%) samples were seropositive. As highest 76.4% of samples from sows showed seropositive whereas 0.5% of weaning piglet. 2.9% from nursery were seropositive, 20.5% in grower, 68.1% in finisher. The average percentage of seropositive pigs against PPE in different provinces was varied. The positive rates of individual pigs in Gyeonggi-Do, Chungcheongbuk-Do, Chungcheongnam-Do, Jeollabuk-Do, Jeollabuk-Do, GyeongSangbuk-Do, GyeongSangnam-Do and JeJu-Do was 25.2%, 34.8%, 27.9%, 37.6%, 35.2%, 32.7%, 28.7% and 31.8%, respectively.

### **Discussion and Conclusion**

Compared to the survey done 2005, 2006, 2008 and 2012, sow and nursery seropositive rate is decreasing but grower and finisher's is increasing. The seroconversion in grower and finisher suggests the risk of acute hemorrhagic form of PPE. To mitigate losses from PPE, methods to increase immunity and reduction of exposure in pigs such as use vaccine or strategic antibiotic treatment are recommended.

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# STREPTOCOCCUS SUIS CAPSULAR TYPES 2, 4 AND 9: IMPLEMENTATION OF A FARM SPECIFIC VACCINE – A STORY OF SUCCESS

<u>M. Viehmann</u><sup>1</sup>, K. Lillie-Jaschniski<sup>2</sup> <sup>7</sup>Tierarztpraxis Cappel, Germany <sup>2</sup>Ceva Santé Animale, Libourne, France

## **Background and Objectives**

The gram-positive, opportunistic porcine pathogen Streptococcus (S.) suis is globally distributed and responsible for great economic losses. To date, 29 different serotypes (cps) are recognized. Pigs are regularly colonized, but not all animals are clinically affected. Whether infected pigs show clinical signs depends on a variety of factors, like age, health status, circulating serotypes and/or co-infections. They can range from asymptomatic pigs to severe pathologies such as meningitis, polyarthritis, septicaemia, endocarditis and peracute deaths. Diseased animals should be treated with antimicrobials; in Germany a commercial vaccine is not available. In general, S. suis has zoonotic potential causing meningitis, fever, and central nervous symptoms, being considered as a potential threat to the farmer. In 2016 a farrow-finish farm in south Germany, experienced a sudden surge of peracute deaths in nursery pigs.

# Material and Methods

Deceased pigs were sent for necropsy. Main pathological findings were endocarditis, polyserositis and meningitis. S. suis capsular polysaccharide (cps) types 4 and 9 were isolated (IVD GmbH). These clinical isolates were cultured, and an autogenous vaccine was generated (Ceva Bestvac Dr. Felgenträger & Co., Dessau). End of 2016 the vaccine was implemented in the sow herd, five- and three-weeks ante partum. Piglets of unvaccinated sows were treated with amoxicillin. In 2019 and 2021 new S. suis outbreaks appeared and S. suis cps4 and cps2 were isolated and added to the vaccine. Mortality and antimicrobial usage were documented.

#### Results

After the implementation of the vaccine, clinical signs of meningitis and rapid breathing due to polyserositis were reduced. The overall mortality in weaners was lowered from 4.5% to 1.5%. Also, the use of antimicrobials could be reduced by 50%.

# **Discussion and Conclusion**

Higher use of antimicrobials due to S.suis outbreaks, increases the risk of resistance development – also in the accompanying bacterial flora. To counteract this, autogenous vaccines are of great help where no commercial vaccine is available. This case report shows that by using an autogenous vaccine against S. suis clinical signs and antimicrobial use could be reduced and animal welfare improved. However, piglets can be affected by different S. suis cps types leading to the need of an update of the used autogenous vaccines.

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# TACKLING ANTIMICROBIAL RESISTANCE IN THAI SWINE: AN IN-VITRO TRI-DRUG STRATEGY AGAINST COLISTIN-RESISTANT AND ENTEROTOXIGENIC ESCHERICHIA COLI.

W. Niyomtham <sup>1</sup>, J. Yindee <sup>1</sup>, N.O. Khine <sup>1</sup>, I. Sankankhum <sup>1</sup>, S. Simjee <sup>2</sup>, W. Dee-Ampai <sup>2</sup>, <u>N. Prapasarakul <sup>3</sup></u> <sup>1</sup>Faculty of Veterinary Science, Chulalongkorn University. Bangkok, 10330, Thailand. <sup>2</sup>Elanco (Thailand) Ltd., Bangkok, Thailand.

<sup>3</sup>Center of Excellence in Diagnosis and Monitoring of Animal Pathogen, Faculty of Veterinary Science, Chulalongkorn University. Thailand

# **Background and Objectives**

Antimicrobial resistance (AMR) represents a significant challenge to the veterinary and public health sectors across the globe. In particular, the emergence of resistance to colistin, a last-resort antibiotic, underscores the urgency for alternative antimicrobial strategies. Thai pig farms are a critical focus for tackling AMR to ensure both food safety and animal welfare [1]. This in-vitro study evaluates the potential of a combination antimicrobial activity involving Tilmicosin, Avilamycin, and Haquilnol against colistin-resistant Escherichia coli (CR-Ec) and Enterotoxigenic Escherichia coli (ETEC) frequently implicated in porcine gastrointestinal diseases.

# **Material and Methods**

Ten strains of colistin-resistant E. coli, 10 strains of enterotoxigenic E. coli, and 10 strains of Clostridium perfringens isolated from multiple pig farms in Thailand were assessed. Using MALDI-TOF mass spectrometry and PCR, all strains were identified and characterized. Using in-vitro methods the study evaluated the minimum inhibitory concentration (MIC) of antimicrobial drugs using recognized microbiological techniques in line with the CLSI Vet 01 standard [2] and a checkerboard method employing a broth microdilution test [3].

#### Results

Susceptibility testing revealed that the MIC<sub>50</sub> values for Tilmicosin (256 mg/mL), Avilamycin (512 mg/mL), and Halquinol (8 mg/mL) were distinct. In combination with Avilamycin and Halquinol, the MIC of Tilmicosin was lowered to 16 mg/mL. MIC<sub>50</sub> values of Halquinol and Avilamycin were lowered to 2 and 64 mg/mL, respectively. E. coli examined had a 60 % synergistic, 35 % indifferent, and 5 % antagonistic response to the combination of Tilmicosin and Halquinol. Tilmicosin and Avilamycin had a synergistic effect on 60% E. coli and 40% were indifferent. One of the noteworthy findings of the in-vitro study is the synergistic effect. The combination displayed a synergistic response in 60%, indicating this combination has the potential to enhance antimicrobial activity and combat bacterial resistance effectively.

# **Discussion and Conclusion**

The combination of Tilmicosin, Avilamycin, and Halquinol demonstrates promising potential in combating antimicrobial resistance in Thai pig farms, especially against CR-Ec, and ETEC. The significant reduction in MIC values suggests a viable alternative antimicrobial strategy. Further research and in-vivo field trials are encouraged to validate these findings and explore broader applications in tackling antimicrobial resistance in animal husbandry.

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# A RETROSPECTIVE STUDY OF THE INCIDENCE OF ESCHERICHIA COLI ADHESION FACTORS SINCE THE BAN OF ZINC OXIDE AS A VETERINARY MEDICINAL PRODUCT

L. Pérez Esteruelas<sup>1</sup>, M. Claver Mateos<sup>1</sup>, J. Bringas<sup>1</sup> <sup>1</sup>Elanco Animal Health, Alcobendas, Spain

#### **Background and Objectives**

The use of zinc oxide (ZnO) as a veterinary medicinal product in the EU in weaned piglet feed was withdrawn by the European Commission in 2022<sup>1</sup>. It was the main tool together with antibiotics to fight against postweaning diarrhoea (PWD). This study aimed to investigate if the withdrawal of ZnO is related to an increase of the incidence of PWD caused by Escherichia coli (E. coli) in the postweaning period.

## **Material and Methods**

A total of 733 Spanish pig farms were included into the study. The farms were divided into two groups based on the use of ZnO as a medicinal product in feed resulting in 423 farms with ZnO and 310 farms without it. Animals were selected based on PWD clinical signs. Three rectal swabs were collected from four- to eight-week-old pigs. Swabs with fecal matter were submitted to the laboratory (Exopol; Zaragoza, Spain) for diagnosis. PCR was performed to detect adhesion factor including F4 (K88), F18 and adhesin involved in diffuse adherence (AIDA-1). The statistical analysis was carried out using the statistical software package STATA 17.0 (STATA, USA). A homogeneity test using contingency tables was used to detect statistical associations.

#### Results

In the test of homogeneity, the results showed that the incidence of AIDA-1 was statistically higher ( $X^2$ =14.45; p=0.000) on farms where ZnO was not used anymore (78.39% vs 65.48%). Related to F4, it did not exist a statistical relation ( $X^2$ =2.74; p=0.097), although numerically an increase of this fimbria was observed on farms without ZnO (60.97% vs 54.85%). For F18, the results did not show any statistical association ( $X^2$ =0.91; p=0.339) resulting in 75.48% on farms without ZnO and 72.34% with it.

### **Discussion and Conclusion**

This study demonstrated that a higher amount of E. coli's virulence factors is present on farms since the ban of ZnO as a medicinal product. The non statistical association between ZnO and F18 can be explained as the percentage is considered as high for the two groups of farms. In conclusion, other tools are needed to control the increased incidence of postweaning diarrhoea caused by E. coli since ZnO as a medicinal product is not allowed and the use of antibiotics is being limited<sup>2</sup>.

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# ANTIBIOTIC RESISTANCE AND SEROTYPE PREVALENCE OF STREPTOCOCCUS SUIS FROM 2016 TO 2022 IN CHINA

J. Zhang <sup>1</sup>, L. Wang <sup>2</sup>, <u>D. Sperling</u> <sup>3</sup>, J. Huang <sup>2</sup>, X. Cao <sup>1</sup>, D. Ye <sup>1</sup> <sup>1</sup>Ceva Animal Health China <sup>2</sup>Nanjing Agricultural University <sup>3</sup>Ceva Animal Health France

### **Background and Objectives**

Streptococcus suis is a ubiquitous pathogen in piglets, causing significant economic losses to the pig industry worldwide. This study was conducted to investigate antibiotic resistance and serotype prevalence of Streptococcus suis isolated on commercial farms over the past 7 years in China.

# **Material and Methods**

From 2016-2022, 1,032 clinical samples were collected from piglets with suspected S.suis infection on different farms in China. The strains of S. suis were isolated and identified. Minimum Inhibition Concentration (MIC) was determined for 7 antibiotics representing different classes, frequently used in practice. Microbroth dilution method and interpretation of MIC based on established breakpoints (CLSI M100-ED33) were used. Serotype characteristic was identified by multiplex PCR on wzy-gene of CPS cluster.

### Results

In total, 184 strains of Streptococcus suis were isolated. Amoxicillin had the lowest resistance rate recorded (19.02%). The highest resistance rates were recorded for tilmicosin (86.96%), tetracycline (92.62%) and lincomycin (98.91%). Florfenicol (38.04%), penicillin (46.74%) and enrofloxacin (46.74%) showed moderate resistance rates. The majority of the strains (95.3% (178/184)) showed a multi-drug resistance pattern, mainly resistant to 3 different classes of antibiotics (ATB), reaching 42.9% (79/184). 15 different serotypes were detected, of which the highest prevalence after non-typeable type (39.58%) was serotype 2 (18.75%) followed by serotype 9 (8.33%) and serotype 27(6.25%). Serotype 3 (2.08%), 15 (2.08%), 16 (4.17%), 21 (3.13%), 24 (2.08%), 29 (4.17%) and 30 (4.17%) were isolated less frequently. Other serotypes were rare (serotype 5, 11,13, 28 and 31).

#### **Discussion and Conclusion**

Antibiotic resistance for S. suis was common and high attention should be paid in Chinese industrial farms. Amoxicillin, classified in category D by EMA 2019 as first line treatment, showed lowest resistance rate in the study. When implementing a treatment option, selection of the ATB should consider both the results of MIC testing and the classification of the antibiotic. According to our study, serotype 2 and 9 were the most frequent isolates detected, which are therefore considered as main virulence serotypes.

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# ANTIMICROBIAL RESISTANCE IN E. COLI ISOLATED FROM PIG HERDS WITH POST-WEANING DIARRHEA IN GERMANY (2019 – 2023)

<u>A. Luehrs</u><sup>1</sup>, R. Bauerfeind<sup>2</sup>, C. Ewers<sup>2</sup>, E. Prenger-Berninghoff<sup>2</sup>, P. Münster<sup>1</sup> <sup>1</sup>Elanco Deutschland GmbH, Rathausplatz 12, 61348 Bad Homburg, Germany <sup>2</sup>Institute for Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Giessen, Germany

## **Background and Objectives**

Post-weaning diarrhea (PWD) due to infection with enterotoxigenic E. coli, primarily F4- and F18-ETEC, is a significant contributor to the global use of antibiotics in pig production. This study aims to explore variations in antimicrobial resistance (AMR) among E. coli strains with different pathotypes isolated from PWD outbreaks in Germany.

## Material and Methods

Rectal swabs were collected from 573 piglets with PWD on 191 farms and examined for the presence of E. coli by culture methods. Coliform isolates were classified according to their virulence gene patterns into pathotypes ETEC, edema disease E. coli (EDEC), enterotoxigenic and Shiga-toxin encoding E. coli (ETEC/STEC hybrids), and enteropathogenic E. coli (EPEC). Antimicrobial susceptibility testing was carried out for one isolate per farm (n=138) or in cases where two (n=52) or three (n=1) different pathotypes were present, one isolate per pathotype and farm. In total, 245 isolates were assessed for minimum inhibitory concentrations (MICs) of 16 antimicrobials. Susceptibility categories intermediate and resistant were combined for statistical analysis (program JMP 15.0).

#### Results

F4-ETEC showed significantly higher level of AMR (p<0.001) being resistant on average to 63.3% of antibiotics tested (all other pathotypes: 52%). F4-ETEC was the pathotype with the highest resistance rates for eight antibiotics: ampicillin (F4-ETEC 85.1% vs. all isolates 67.3%), tetracycline (72.3% vs. 58%), trimethoprim/sulfonamide (67% vs. 52.2%), spectinomycin (65.9% vs. 48.5%), cephalothin (58.8% vs. 42.3%), enrofloxacin (28.7% vs. 13.9%), amoxicillin/clavulanic acid (11.7% vs. 8.2%) and gentamicin (8.5% vs. 4.9%). Ceftiofur resistance was highest in EPEC (7.7% vs. 3.7%). F18-ETEC/STEC hybrids showed highest resistance rate for colistin (40.7% vs. 21.2%).

## **Discussion and Conclusion**

This study demonstrated substantial AMR in various E. coli pathotypes associated with PWD. To reduce selective pressure, fewer antibiotics should be used, while promoting alternative control strategies, such as administering live oral E. coli vaccinations to piglets. We also detected significant differences of AMR frequencies between distinct E. coli pathotypes. These findings may indicate that pathotypes are differentially exposed to antimicrobials and/or go through different processes of selection for AMR in their habitats. Elucidating the underlying mechanisms may help to develop new strategies of prudent antimicrobial use.

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# ANTIMICROBIAL RESISTANCE PROFILES OF STREPTOCOCCUS SUIS ISOLATED FROM DISEASED PIGS IN THAILAND

T. Kaminsonsakul <sup>1</sup>, S. Kankuntod <sup>2</sup>, M. Suwunwong <sup>2</sup>, <u>Y. Woonwong <sup>1</sup></u>, A. Boonsoongnern <sup>1</sup>, P. Jirawattanapong <sup>1</sup>, N. Thanantong <sup>1</sup>

<sup>1</sup>Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

<sup>2</sup>Kamphaengsaen Veterinary Diagnostic Center, Faculty of Veterinary Medicine, Kasetsart University, Thailand

## Background and Objectives

Streptococcus suis (S. suis) is a bacterium that commonly resides in healthy pigs but can occasionally cause severe symptoms under certain conditions. It remains a significant cause of economic losses in the global pig industry and is an important zoonotic pathogen. The frequent exposure of S. suis to antimicrobials due to their widespread use on pig farms can lead to the development of antimicrobial resistance (AMR). Therefore, it is essential to continuously monitor and maintain a comprehensive understanding of antimicrobial resistance trends in S. suis among pig populations.

#### **Material and Methods**

Clinical samples were collected from diseased pigs exhibiting S. suis-related clinical signs and lesions. The pigs were from farms located in central and western parts of Thailand. The tissue samples were retrieved from necropsied pigs at the Kamphaengsaen Veterinary Diagnostic Center, Faculty of Veterinary Medicine, Kasetsart University, from January to October 2023. The clinical samples were cultured for bacteria by routine methods and identified for S. suis using either conventional biochemical characterization or MALDI-TOF Mass Spectrometry. Subsequently, each S. suis isolate was tested for antimicrobial susceptibility via a disk diffusion method.

#### Results

A total of 63 isolates of S. suis were obtained. Resistance rates were high for lincomycin (93.65%), followed by oxytetracycline (84.13%) and sulfamethoxazole (76.19%). Moderate resistance rates were observed for penicillin G (44.44%), ceftriaxone (41.27%), cephalexin (39.68%), florfenicol (37.29%), enrofloxacin (36.51%), and doxycycline (30.16%). S. suis exhibited low AMR rate for amoxicillin (22.22%), ampicillin (20.63%), amoxicillin + clavulanic acid (12.70%). Fifty-four isolates demonstrated multidrug resistance, with the predominant resistotypes being LIN-OXY-SXT and LIN-OXY out of a total of 52 resistotypes.

# **Discussion and Conclusion**

Due to their moderate to low resistance levels, drugs in beta-lactams and doxycycline remain the drugs of choice for the treatment of streptococcosis in pigs. These AMR rates are similar to those reported in previous studies. Interestingly, all isolates in this study exhibited resistance to at least one antimicrobial class, with 85.71% of the isolates showing resistance to three or more drug classes, indicating multidrug resistance. The establishment of antimicrobial susceptibility surveillance programs is crucial for selecting appropriate treatment options.

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## APXI-III POTENCY IN THREE COMMERCIAL A. PLEUROPNEUMONIAE VACCINES

M. Tenk <sup>1</sup>, N. Palmai <sup>2</sup>, M. Ivok <sup>2</sup>, I. Alapi <sup>2</sup>, H. Smits <sup>3</sup>, <u>P. Mortensen <sup>3</sup></u> <sup>1</sup>University of Veterinary Medicine Budapest, Hungary <sup>2</sup>Ceva Phylaxia, Budapest, Hungary <sup>3</sup>Ceva Corporate Swine, Libourne, France

# **Background and Objectives**

The Actinobacillus pleuropneumoniae (A.p.) RTX toxins, ApxI, ApxII, and ApxIII, are major virulence factors, hence vaccine antigens of major importance to protection, too. Together these three antigens are responsible for the potential, serotype-independent protectivity of a given A.p. vaccine. Therefore, the presence, and immune inductive potency of these three toxins are imperative to the power of any A.p. cross-protective vaccine. The objective of this study was to assess the ApxI, ApxII, and ApxIII potencies of two different batches of an A.p. whole-cell vaccine based on A.p.1 and A.p.2 strains expressing ApxI-III, and two other ApxI-III containing vaccines.

#### **Material and Methods**

Forty Hycole rabbits were individually ear-tagged and randomly allocated to 4 groups of 10 (G1-G4). G1 and G2: vaccinated twice in 14 days interval with either one of two different batches of Coglapix® (Ceva, France), G3: with a subunit A.p. vaccine based on ApxI-III and OMP, and G4: with an A.p.2,9,11 based whole-cell and ApxI-III. Dosages according to recommendations. All animals' serum sampled 14 days following 2<sup>nd</sup> vaccination and tested using Ceva Phylaxia-ApxI, - ApxII, and -ApxIII in-house, specific monoclonal antibody-based ELISAs. The assays measure the antibodies produced against the universal antigenic structure of the toxins, therefore the result is vaccine-independent. The animals enrolled to this study were seronegative to all 3 Apx's on D0. This study followed Directive 2010/63/EU, Hungarian Act XXVIII/1998 and Hungarian Governmental Decree No. 40/2013.

#### Results

The respective average ApxI, ApxII, and ApxIII potency titres in the vaccine groups were, G1: 204.8-29.4-21.1, G2: 171.0-30.6-12.5, G3: 239.4-0-0, G4: 0-0-0. ApxI, ApxII, and ApxIII potencies of G1 and G2 were well within product release limits of 28.9, 16.7, and 6.8 ELISA units/ml, respectively. G3 did only induce a measurable ApxI potency titre, and G4 produced no measurable ApxI-III titres using Apx-specific in-house ELISAs.

# **Discussion and Conclusion**

The ApxI-III potency relationship between the three vaccines in this study G1+G2>G3>G4. Since the validated ELISAs measure the native anti-Apx titres, i.e. potency, the observed titre differences between vaccines may not only be attributed to vaccine antigen concentration or immune stimulation, but likely also to differences in vaccine antigen design.

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# BACTERIAL MENINGITIS IN PIGS - WHICH PATHOGENS DO PLAY A ROLE?

<u>M. Beumer</u>, S. Freytag <sup>1</sup>, I. Spiekermeier <sup>1</sup>, C. Helmer <sup>1</sup> <sup>1</sup>SAN Group Biotech Germany GmbH, Hoeltinghausen, Germany

### **Background and Objectives**

Meningitis symptoms are one of many disease patterns that can be observed in pigs. Meningitis itself is often associated with decreased animal welfare in terms of suffering or dying as well as economic losses. In many cases, bacterial pathogens can be isolated by laboratory diagnostics and identified as cause of the disease. Streptococcus suis and Escherichia coli are most commonly found. But there are other agents that should be kept in mind when dealing with bacterial meningitis in pigs. Affected animals are often treated with antibiotics, but due to minimisation strategies, this should be prevented. The following analyses aim to provide an overview of bacterial findings in the brain of pigs showing clinical signs of meningitis and to address the question to which extent vaccination can provide assistance in dealing with such problems.

# **Material and Methods**

344 brain swabs gained in 2022 from pigs with pre-reported clinical signs of meningitis were evaluated regarding the prevalence of various bacterial agents in bacterial cultural diagnostics using standard bacteriological as well as molecular biological methods. Further diagnostics as serotyping, detection of virulence factors and others were performed, if available.

#### Results

As expected, Streptococcus suis (66%) and Escherichia coli (13%) predominate in culture with positive pathogen detection. However, other pathogens are also found in individual cases. Mainly to be mentioned are Streptococcus dysgalactiae (4%), Staphylococcus aureus (3%) and Glaesserella parasuis (2%). Regarding the findings of Streptococcus suis, serotype 2 or ½ (25%), serotype 9 (25%) and serotype 7 (18%) were predominantly found during further PCR diagnostics.

### **Discussion and Conclusion**

21% of the samples analysed in bacterial culture revealed in bacteria different from Streptococcus suis and Escherichia coli. Therefore, it makes sense to use a broad, non-directional cultural approach rather than a specific PCR test to also detect less common pathogens. Depending on the pathogen found, the cause of entry must be clarified. For recurring stock problems, in addition to antibiotic measures, the implementation of a prophylactic vaccination can be considered.

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# BENEFITS OF VACCINATION AGAINST SWINE OEDEMA DISEASE USING A RECOMBINANT VEROTOXIN 2E (VT2E) VACCINE ON A COMMERCIAL SWINE FARM IN SOUTH KOREA

H. Noh <sup>1</sup>, J. Byun <sup>1</sup>, S. Noh <sup>1</sup>, M. Choi <sup>2</sup>, D. Giralt <sup>3</sup>, G. Alvarez <sup>3</sup>, <u>I.B. Orozco <sup>4</sup></u> <sup>1</sup>HIPRA Korea <sup>2</sup>Kwanghee Animal Hospital (Wanju, Korea) <sup>3</sup>HIPRA, Amer (Girona), Spain <sup>4</sup>Corporate Brand Manager, HIPRA, Amer, Spain

## **Background and Objectives**

The objective of this study was to demonstrate the benefits offered by a recombinant Vt2e vaccine on a farm infected with swine oedema disease.

# **Material and Methods**

Swine oedema disease was confirmed on a Korean commercial farm with 400 sows by the detection of verotoxin-producing Escherichia coli (VTEC) by real-time PCR assay. To control the swine oedema disease, a vaccine containing recombinant verotoxin (Vt2e) antigen (Vepured®) was applied according to the manufacturer's instruction from January 2021, four weeks after the first outbreak. From December 2020 to February 2021, newly born animals from 12 different batches were tracked and information was collected on productivity parameters such as number born alive, number of dead or culled piglets per batch, body weight at 70 days of age, market weight, days to slaughter and amount of totally consumed feed. Mortality, average daily gain (ADG) and feed conversion rate (FCR) for each batch were calculated accordingly. Information on animals from different batches was collected and divided into two groups, "Non-vaccinated" group (927 animals) referring to the first 4 batches after the outbreak but without vaccination (NV-1 to 4) and "Vaccinated" group (1.898 animals) referring to the 8 batches after applying the vaccine (V-1 to 8), respectively. The average productivity parameters for each group were calculated and compared to demonstrate the efficacy of the recombinant Vt2e vaccine. A linear regression model was performed for analysis of body weight at 70 days of age, days to slaughter, mortality, market weight, ADG and FCR.

### Results

Compared to the "Non-vaccinated" group, all the parameters in the "Vaccinated" group were improved with a statistically significant difference. The "Vaccinated" group showed a 2.7kg higher average body weight at 70 days of age (p<0.001), 16.8% lower mortality(p<0.001), 1.8kg higher market weight (p<0.001), 5.3 fewer days to slaughter (p<0.001), 36.4g greater average daily gain (p<0.001) and 0.09 lower feed conversion rate (p<0.001).

# **Discussion and Conclusion**

As swine oedema disease not only causes increased mortality but also various negative impacts on productivity, the results of this study mean that additional benefits apart from reduced mortality can be expected from controlling swine oedema disease.

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## CALPROTECTIN AND PROCALCITONIN: SIMILAR NAMES, DIFFERENT BIOMARKERS

M.J. Lopez-Martínez <sup>1</sup>, S. Martínez Subiela <sup>2</sup>, G. Ramis <sup>3</sup>, J. Ceron <sup>4</sup>, A. Muñoz Prieto <sup>2</sup>

<sup>1</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Campus de Espinardo s/n, 30100 Murcia, Spain <sup>2</sup>Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence "Campus Mare Nostrum", University of 9 Murcia, 30100, Espinardo, Murcia, Spain

<sup>3</sup>Dpto. Producción Animal, Facultad de Veterinaria., Universidad de Murcia, Spain

<sup>4</sup>Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia

## **Background and Objectives**

Biomarkers can provide helpful information to detect inflammatory and septic conditions early. Calprotectin (CALP) is the heterodimer complex of two calgranulins, S100A8 and S100A9. It has shown a high performance in detecting gastrointestinal diseases like colitis in pig and could also increase inflammatory and septic conditions. Procalcitonin (PCT) is a widely used analyte in human sepsis detection and can help make a faster diagnosis and guide antibiotic stewardship in this condition. In addition, measuring these biomarkers in saliva ensures animal welfare and allows for serial sample collections by non-trained personnel. This study aimed to compare how CALP and PCT behave in experimental models of septic and non-septic inflammation to better understand the interpretation of these biomarkers in the saliva of pigs.

#### **Material and Methods**

In this study, CALP and PCT were measured in the saliva of pigs in a model of sepsis and a model of aseptic inflammation. For this purpose, a group of five 14-week old large white pigs in which Lipopolysaccharide of Escherichia coli was administered to induce septic inflammation, and a group of another five pigs in which turpentine-oil was injected to induce non-septic inflammation was used.

#### Results

CALP showed a significant major 8.5-fold increase after 24 hours of the LPS administration (median=1.26 mg/L) compared with pre-LPS sample (median=0.18 mg/L) (p=0.01), being reduced to nearly basal levels after 48 h. A tendency to increase was observed after the inoculation of turpentine-oil, but it was not significant. PCT showed a 2.7-fold increase after 24 h in the animals injected with LPS (mean=5790  $\mu$ g/L) compared to the pre-treatment sample (mean=916  $\mu$ g/L), remaining high after 48 h. No significant changes were observed in the turpentine-oil group.

# **Discussion and Conclusion**

CALP and PCT showed significant increases in the sepsis model of this trial, higher in CALP but, in this case, also with a tendency to increase in non-septic conditions. Further studies should be performed to clarify the potential application of these biomarkers to detect inflammatory and septic conditions in pigs.

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# CEFTIOFUR AND TULATHROMYCIN ADMINISTRATION ON SOWS ALTERS THE COMPOSITION OF THE NASAL MICROBIOTA OF THEIR OFFSPRING

L. Bonillo-Lopez <sup>1</sup>, <u>P. Obregon-Gutierrez</u> <sup>1</sup>, E. Huerta <sup>1</sup>, F. Correa-Fiz <sup>1</sup>, M. Sibila <sup>1</sup>, V. Aragón <sup>1</sup> <sup>1</sup>IRTA Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Spain

## **Background and Objectives**

The nasal microbiota plays an important role in animal health as the first barrier respiratory pathogens need to overcome for host infection. Its composition is affected by several factors, being antibiotics a concerning one. This study aimed to compare the effect of two intensive antibiotic treatments on sows (ceftiofur alone or together with tulathromycin) on the nasal microbiota of their piglets, including the pathobionts Glaesserella parasuis, Mycoplasma hyorhinis and Streptococcus suis. We also assessed whether the effect of the double antibiotic treatment in sows was potentiated by an additional treatment of ceftiofur on piglets.

#### **Material and Methods**

Four pregnant sows received ceftiofur and tulathromycin treatment ( $CT_{sows}$ ) while two others received ceftiofur ( $C_{sows}$ ). Ceftiofur was given at D-4, D3, D10 and D17 (D0=birth), while tulathromycin treatment was performed at D-3, D4 and D11. Additionally, half of the piglets born to  $CT_{sows}$  were treated at D1 with ceftiofur. Nasal swabs were taken from sows before and after the first dose of antibiotic and from piglets at 22-24 days of age. Bacterial load and nasal microbiota composition were defined by 16s rRNA gene qPCR and amplicon sequencing, respectively. Microbiota analysis was performed using qiime2. Furthermore, the transmission of the pathobionts from sows to piglets was assessed by PCR.

### Results

The intensive antibiotic treatment reduced the bacterial load in the nasal cavities of sows and their offspring. Piglets' nasal microbiota composition exhibited signs of dysbiosis, showing unusual taxa, but a reduced presence of pathobionts. The addition of tulathromycin to ceftiofur treatment enhanced the deleterious effect on microbiota diversity by diminishing some bacteria commonly found in the piglets' nasal cavity, such as Glaesserella, Streptococcus, Prevotella, Staphylococcus and several members of Ruminococcaceae and Lachnospiraceae families. However, the additional treatment of piglets with ceftiofur had no further effect beyond the treatment of sows.

### **Discussion and Conclusion**

In farms, sows that are treated with antibiotics to control pathogen transmission may present an offspring with altered early colonization. Our results suggest that intensive antibiotic treatments of sows disrupt the nasal microbiota of piglets. This highlights the importance of sow-to-piglet microbiota transmission.

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# FIELD COMPARISON OF LAWSONIA INTRACELLULARIS PCR AND ELISA RESULTS, AT FARM LEVEL

<u>J. Sevin</u><sup>1</sup>, L. Daluzeau <sup>2</sup>, L. Eon <sup>2</sup>, S. Lopez <sup>3</sup>, F. Voisin <sup>2</sup>, D. Duivon <sup>2</sup> <sup>1</sup>Socavet, 75 boulevard de penthièvre, 22600 Loudéac, France <sup>2</sup>MSD Animal Health, 49 Beaucouzé, France <sup>3</sup>UNIVET SANTE ELEVAGE, rue Monge, 22600 Loudéac, France

## **Background and Objectives**

Lawsonia intracellularis (LI) is still causing significant damage in pigs. Pooled faeces or saliva samples tested by qPCR are a tool to determine the bacterial load and infection pressure. Blood samples are very common, usable for a wide variety of tests and LI serologic tests are available. The aim of this study is to evaluate the correlation between qPCR and ELISA serology at farm level.

# Material and Methods

Cross-sectional profiles were carried out on 35 farms, on three successive batches of fatteners, at 3 successive ages (age1, 2 and 3). At each age, we took 10 individual blood samples and two chewing ropes were offered to the same pigs to sample two oral fluids. Finally, CDS (Boxmeer, The Netherlands) tested 210 saliva samples by qPCR for LI (BactoReal Lawsonia kit, Ingenetix) and 1050 sera by ELISA for LI (Svanovir Lawsonia kit, Svanova). PCR Cut-off was Cq=50. Quantitative results were Cq and inhibition percentage (Inh%) for PCR and ELISA respectively. They were compared using Principal Component Analysis and linear regression analysis.

#### Results

Mean age of the age groups 1, 2 and 3 was 95, 121 and 146 days, respectively. At farm level, PCR Cq at age1 and ELISA Inh% at age2 were significantly correlated (p=0.008;  $R^2=21\%$ ). PCR Cq at age2 and ELISA Inh% at age3 were also significantly correlated (p<0.001;  $R^2=31\%$ )

## **Discussion and Conclusion**

Under the conditions of this study, LI PCR results of a production batch are correlated with ELISA results of the previous one. To our knowledge, this is the first time that such a correlation at farm level is reported. These two tools should appear to be complementary to practitioners, as the persistence of antibodies is longer than the excretion of LI by pigs.

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# FIELD EFFICACY COMPARISON OF A READY-TO-USE AND A FRESHLY MIXED COMBINATION PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES

<u>J. Wu</u><sup>1</sup>, K. Wang<sup>1</sup>, Y. Lu<sup>1</sup>, P. Chen<sup>1</sup>, Y. Zhao<sup>1</sup> <sup>1</sup>Swine Business Unit, Zoetis China, Shanghai, P.R. China

#### **Background and Objectives**

Porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhp) are two economically important pathogens in the pig industry. The aim of this study was to compare the effectiveness of a ready-to-use PCV2 and Mhp bivalent vaccine and a freshly mix of monovalent PCV2 and Mhp vaccines under field conditions.

# Material and Methods

Data collection took place between October 2022 and May 2023 on a 10,000-sow farm with PRRSV-negative and a weanto-finish farm with PRRSV-positive. In the sow farm, 150 litters of piglets were selected, 6 piglets in each litter were used for this study, one barrow and one female (1B1F) received ready-to-use 2ml Fostera PCV MH vaccine (T01), 1B1F received freshly mixed 2 ml of monovalent PCV2 and Mhp vaccines (T02), and 1B1F received 2ml PBS (T03), all the groups received either vaccines or PBS at 3 weeks old. These pigs were ear-tagged and then transferred to the wean-tofinish farm and randomly assigned to pens. Blood samples were collected at several time points for qPCR and ELISA tests. Individual body weights were collected at weaning, nursery and finishing phase. Mortality and clinical observation results were daily recorded. Lung lesion scores were performed at slaughterhouse.

#### Results

There was no difference in PCV2 and PRRSV viremia among three groups in the nursery and fattening phases, T01 has numerically less PCV2 and PRRSV viremia than other groups. The average daily gain (ADG) from weaning to end finishing of T01, T02 and T03 was 761 $\pm$ 87 g/d, 749 $\pm$ 85g/d, 727 $\pm$ 89g/d respectively. Among them, the ADG of T01 was significantly higher than that of T03 (p<0.01). There was no significant difference between other groups. From wean to finish, the survival rate of pigs inT01, T02 and T03 was 97.0%, 97.3% and 93.0% respectively. The Mhp-like lung lesion scores in T01 were significantly lower than those in T02 (p<0.05) and T03 (p<0.001), and there was no significant difference between T02 and T03.

#### **Discussion and Conclusion**

Based on production parameters and lung lesion scores, under the conditions of this farm, pigs in T01 showed better clinical and growth performance.

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# GEOGRAPHIC DISTRIBUTION AND GENOMIC CHARACTERIZATION OF STREPTOCOCCUS SUIS AND GLAESSERELLA PARASUIS ISOLATES IN MEXICO

<u>J.M. Hernandez</u><sup>1</sup>, M.J. Clavijo<sup>2</sup>, A. Flores<sup>3</sup>, A. Medina<sup>1</sup>, S. Medina<sup>1</sup>, V. Orozco<sup>1</sup>, M. Torrez<sup>3</sup>, A. Chavez<sup>3</sup> <sup>1</sup>Lapisa Laboratory Mexico <sup>2</sup>lowa State University <sup>3</sup>Lapisa diagnostic Laboratory

# **Background and Objectives**

Endemic swine systemic bacterial agents, such as Streptococcus suis and Glaesserella parasuis, have taken on important relevance in recent years. Being able to control these agents through specialized diagnostic methodologies and different management strategies such as medications and/or vaccinations is a global challenge for swine production. The objective of this study was to investigate the geographical distribution and genetic diversity of bacterial isolates from samples sent to the Lapisa diagnostic laboratory from different pig farms in Mexico.

#### **Material and Methods**

Between August 2021 and October 2023, samples were sent from animals with clinical signs consistent with these diseases and were referred for isolation; the identity was confirmed by qPCR tests. 158 isolates for Streptococcus suis and 12 isolates for Glaesserella parasuis were obtained and sent to the ISU-VDL for serotyping. 93 selected samples (81 Streptococcus suis and 12 Glaesserella parasuis) were submitted for genomic characterization by next-generation sequencing (NGS) using the Illumina MiSeq; to determine the serotype diversity of all isolates and for the multilocus Sequence Type (ST) of the selected samples, the SRT2 program was used in the raw runs.

#### Results

16 serotypes of S. suis and 5 serotypes of G. parasuis were identified. This study indicates that the highest incidence in different regions of Mexico for S. suis were serotypes 9 (42%) and 2 (12%). 8% of the isolates were non-typeable. 8 STs were detected; the most predominant being ST 28 (26%) and 94 (15%). 14% of the STs were novel and 15% were non-typeable. For G. parasuis isolates, the highest incidence was serotype 7 (42%). 4 STs were detected, the most predominant being ST 624 (33%). 8% STs were reported as novel.

## **Discussion and Conclusion**

Currently, Mexico does not have enough information to know the current status, serotypes and STs currently present in the country. NGS is a tool that allows us to identify, type and characterize bacteria with a specific technical criterion of virulence and resistance of each of these agents. In the event of changes in prevalence or the appearance of new outbreaks, it could give us great diagnostic certainty and success in the development of control programs according to the objectives of the production system.

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# GLAESSERELLA PARASUIS: FIELD-CASE OF USING A DIAGNOSTIC TOOL TO OBTAIN OPTIMAL VACCINATION RESULTS.

H. Van Loocke <sup>1</sup>, <u>A. Dereu</u> <sup>1</sup>, E. Seesing <sup>1</sup>, K. Verduyn <sup>2</sup>, V. Geurts <sup>3</sup> <sup>1</sup>Kernfarm B.V., Breukelen, The Netherlands <sup>2</sup>DAP Verduyn, Gistel, Belgium <sup>3</sup>BioChek BV, Smart Veterinary Diagnostics

### **Background and Objectives**

A 350 Danish genetic sow farm had a history of weak and wasting piglets just after weaning caused by Glaesserella parasuis, diagnosed via individual necropsy. Glaesser's disease resulted in higher antibiotic use to avoid further problems. This field study describes the use of a diagnostic tool to assess the prevalence of infection in the herd and secondly, the results of sow-vaccination to prevent Glaesser's disease in their piglets.

# **Material and Methods**

Prior to sow-vaccination with a commercially available Glaesserella-Erysipelas vaccine (FIXR HP Ery), sows and their offspring (3 piglets per sow) were blood sampled 3 days before farrowing and respectively 1 week before weaning. Identical sampling was repeated after implementing sow-vaccination according to leaflet, to protect their piglets beyond weaning. Testing was performed with the BioChek®HPS Antibody ELISA (test cut off: S/P 0.5). This OppA based ELISA is non-serotype-specific and detects only clinically infected or vaccinated pigs, while it provides negative results in clinical healthy carrier-pigs. Furthermore, the piglets were clinically monitored.

#### Results

Non vaccinated sows had negative to very low ELISA titers (S/P range 0.3-0.6), while a substantial proportion (41%) of the piglets showed higher titers compared to their mother, indicating active infections. Vaccinated sows showed higher titers (S/P range 0.7-1.3), which were also found on a lower or equal level in their offspring in 3 out of the 4 sows. Piglets of sow 4 were negative without clinical signs.Clinical signs were sharply reduced with no or less antibiotic use.

## **Discussion and Conclusion**

The use of the protocol with the OppA based HPS ELISA supported the diagnosis of the clinical problems and facilitated monitoring the effect of sow-vaccination via comparing titers of sows and their piglets. In this case, implementing sow-vaccination led to sharply reduced clinical problems and substantial reduced use of antibiotics. Healthy piglets of one serological positive sow, were serological negative. A possible explanation could be the absence of sufficient colostrum intake. It remains unclear if those piglets were not challenged by the sow and/or had lower protective level at a younger age.

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# HIGH INCIDENCE OF MULTIDRUG-RESISTANT CLINICAL SALMONELLOSIS FROM HOG FARMS BETWEEN 2017 TO 2022 IN BRAZIL BY PRODUCTION PHASE

L. Fiorin Hernig<sup>2</sup>, F. De Quadros<sup>2</sup>, C. Pisseti<sup>1</sup>, E. De Conte<sup>2</sup>, J. Deon Kich<sup>3</sup>, A. Lino Fiuza<sup>2</sup>, A. Paula Teixeira<sup>2</sup>, <u>R. Tesche</u> Lippke<sup>2</sup>

<sup>1</sup>Centro de Diagnóstico de Sanidade Animal (CEDISA), Brazil <sup>2</sup>Boehringer Ingelheim Animal Health Brazil <sup>3</sup>EMBRAPA Suínos e Aves, Concórdia, Brazil

# **Background and Objectives**

Antimicrobial resistance is a relevant issue both in animal production and for public health. As salmonellosis is a reemerging disease in Brazil, this study aimed to characterize phenotypic antimicrobial resistance in clinical salmonellosis cases from hog farms in different Brazilian states

# **Material and Methods**

Two hundred twenty-eight clinical salmonellosis events diagnosed in farms from nine Brazilian states from 2017 to 2022 were studied. The CEDISA laboratory carried out Salmonella bacteriological isolation. One representative isolate of each clinical occurrence was characterized by serovar and antimicrobial susceptibility testing (AST). The AST was carried out using the disk diffusion method against 15 antimicrobials: amoxicillin (10µg), ceftiofur (30µg), ciprofloxacin (5µg), doxycycline (30µg), enrofloxacin (5µg), streptomycin (10µg); florfenicol (30µg), fosfomycin (200µg); gentamicin (10µg), lincomycin-spectinomycin (9µg and 100µg), marbofloxacin (5µg), neomycin (30µg), norfloxacin (10µg), sulfamethoxazole-trimethoprim (23.75µg and 1.25µg), and tetracycline (30µg). The E. Coli ATCC 25922 strain was used as a control test.

#### Results

Results from the serotyping method revealed that 42.54% (97/228) of the strains were monophasic variant of Salmonella Typhimurium (4,[5],12:i:-), followed by 33.33% (76/228) of Choleraesuis, and then 13.15% (30/228) of Typhimurium. The other 10.96% (25/228) were from other serovars. Regarding the AST, out of 228 isolates, 1.31% (3/228) were susceptive to all antimicrobial tested, and 96.05% (219/228) showed resistance to three or more antimicrobial classes and were classified as multidrug-resistant (MDR). All monophasic variant of S. Typhimurium (4,[5],12:i-) serovar isolates were MDR, with frequencies more than 90% against amoxicillin, doxycycline, gentamicin, and tetracycline; 39.18% (38/97). Both serovar Choleraesuis and Typhimurium showed high resistance against amoxicillin, tetracycline, doxycycline, and florfenicol, between 70 and 100%. Of the total MDR strains, 4.8% were from the farrowing phase, 62.7% were from the nursery phase, 24.1% were from the growth/finishing phase, 3.9% from the reproduction phase, and 4.4% of positive strains for Salmonella had the phase not informed.

# **Discussion and Conclusion**

Salmonella isolated from clinical cases between 2017 and 2022 showed a high percentage of multidrug resistance. These data show that alternatives other than antimicrobial medication should be considered in cases of salmonellosis in Brazil. As an alternative, vaccination can be used to prevent clinical salmonellosis.
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# IDENTIFICATION OF ACTINOBACILLUS PLEUROPNEUMONIAE SEROVARS IN PLEUROPNEUMONIA OUTBREAKS IN BRAZILIAN SWINE HERDS

J. Calveyra <sup>1</sup>, Y. Li <sup>2</sup>, J.T. Bossé <sup>3</sup>, P.R. Langford <sup>2</sup>, <u>P. Mortensen</u> <sup>4</sup> <sup>1</sup>Ceva Saúde Animal, Brazil <sup>2</sup>Department of Infectious Disease, Imperial College London, London, UK <sup>3</sup>Section of Paediatrics, Department of Medicine, Imperial College London, London, UK <sup>4</sup>Ceva Corporate Swine, Libourne, France

# **Background and Objectives**

Pleuropneumonia remains an important challenge to swine production worldwide. Country A. pleuropneumoniae (App) prevalence can change over time. In a 1993-2006 slaughterhouse investigation in Brazil, Kuchiishi et al. (2007) identified serovars 1=1.8%, 3=13.8%, 4=3.2%, 5=14.8%, 6=6.5%, 7=7.3%, 8=3.0%, 10=7.3%, 11=0.5%, 12=1.3% and 42.9% being non-typable by capsular polysaccharide 1-12-based agar-gel immunodiffusion. Sample sizes and serovar prevalence clearly varied between years. The objective of this study was to identify the acute clinically relevant serovars of App currently present in Brazil.

# **Material and Methods**

Forty-five samples of lung lesions typical of App infection were collected from 22 pig herds in 17 conglomerates of ownership during clinical outbreaks of swine pleuropneumonia between August-2019 and November-2020. Important swine producing states were well-represented: Santa Catarina, Paraná, São Paulo, Minas Gerais, Goiás, and Mato Grosso. App isolation was performed at the CEDISA Animal Health Diagnostic Center, Brazil, transferred to FTA-cards, and shipped to Imperial College London. Serotyping, based on capsular loci, was carried out by multiplex-PCR as described by Stringer et al. (2021).

#### Results

Forty-eight isolates were recovered from the 45 samples as three were dual-serovar infected, all App8+2. Five serovars were detected from the 48 App isolates: Serovar 1 (1=2.1%), 2 (7=14.6%), 3 (1=2.1%), 5 (7=14.6%), 6 (5=10.4%), 7 (7=14.6%), and 8 (20=41.7%).

#### **Discussion and Conclusion**

During 2019-20, App8 was predominant followed by equally prevalent App2, App5, App6, and App7 with only one each of App1 and App3. App13 to App19 serovars were not detected, as in several other countries. Compared to previous findings, the investigational period was 16 months vs 14 years, and sampling subjects and identification methodologies were different. This most likely explains differences between the studies. The substantial reduction in non-typeable and the change towards App8 from App3 is most likely explained using PCR-rather than immune-based diagnostics which, due to cross-reactivity, is known to overestimate App3 over App8 as seen in other countries like the UK. The data obtained from PCR-capsular-based serotyping is unequivocal, so the identification of App serovars 1, 2, 3, 5, 6, 7, and 8 as causes of clinical App in Brazil reflects the current situation.

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# PREVALENCE OF NEONATAL BACTERIAL DIARRHOEA AGENTS IN ARGENTINA

M. Colitto <sup>1</sup>, E. Daffunchio <sup>1</sup>, S. Fernandez <sup>1</sup>, R. Manzano <sup>1</sup>, D. Giralt <sup>2</sup>, <u>O. Boix <sup>2</sup></u> <sup>1</sup>*Hipra Argentina* <sup>2</sup>*HIPRA, Amer (Girona), Spain* 

#### **Background and Objectives**

Neonatal diarrhoea (ND) is a complex disease with various contributing factors, requiring a thorough examination of the different agents involved. While using fresh faeces can be challenging due to potential issues related to storage and transportation conditions, the FTA Elute card offers a practical solution. This support system provides several advantages over fresh samples, as they can be safely sent to the laboratory via postal mail without posing a biological risk. In this study, the prevalence of bacterial neonatal diarrhoea pathogens in piglet faeces from Argentina using FTA Elute cards was investigated

# **Material and Methods**

A total of 148 faecal samples collected from 42 farms in Argentina underwent analysis. These samples were collected between February 2021 and October 2023 and sent to the DIAGNOS Laboratory using FTA® ELUTE cards (Whatman Inc., Florham Park, NJ). Employing a multiplex PCR test, adapted from prior studies, the genes responsible for F4, F5, and F6 adhesion factors, the heat-labile toxin (LT) of ETEC, A and B toxins of C. difficile, and  $\alpha$ ,  $\beta$ , and  $\epsilon$ -toxins of C. perfringens were detected. Results were split into "High" and "Low" bacterial loads. "High loads" comprehended Ct values below 30 for C. difficile and E. coli and below 26 for C. perfringens. Differences regarding genetics, farm size and the replacement source were also evaluated.

#### Results

Of the total samples, 98.6% were positive for  $\alpha$ -toxin of C. perfringens, and 49.3% with a high load. None of the cards tested positive for  $\beta$ -toxin and  $\varepsilon$ -toxin. On 62.8% of the cards C. difficile A-toxin was detected (26.3% with a high load), and on 35.1% C. difficile B-toxin was also detected (0.68% with a high load). F4 and LT toxin showed 26.3% and 20.3% positivity rates, respectively. No statistically significant differences were seen between genetics, farm size and the origin of replacement.

# **Discussion and Conclusion**

Earlier research has emphasized the importance of differentiating between high and low concentrations of bacteria in faecal samples. The current investigation reveals the proportion of Argentine samples in which various bacterial agents could be deemed causative factors for neonatal diarrhoea.

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# COINFECTIONS BETWEEN BACTERIAL NEONATAL DIARRHOEA AGENTS IN DANISH FARMS

<u>A.M. Strunz</u><sup>1</sup>, O. Boix <sup>2</sup> <sup>1</sup>Hipra Nordic Aps <sup>2</sup>HIPRA, Amer (Girona), Spain

### **Background and Objectives**

Neonatal diarrhoea (ND) in piglets is a multifactorial disease, therefore, a differential diagnosis of the multiple agents involved should be considered. Differentiating the bacterial load of a sample has been described as a valuable indicator to distinguish between a mere positivity from a commensal bacterium from a presumptive diagnosis due to an intestinal dysbiosis. This study describes the prevalence of high & low concentrations of bacterial neonatal diarrhoea pathogens in piglet faeces from Danish farms.

# **Material and Methods**

A total of 44 Danish farms and 158 faecal samples were included in the present study. Samples were obtained between May 2021 and October 2023 and subsequently sent to the DIAGNOS Laboratory using FTA® ELUTE cards (Whatman Inc., Florham Park, NJ). A multiplex PCR test adapted from previous studies was performed to detect genes encoding F4, F5, F6 adhesion factors, the heat-labile toxin (LT) of ETEC, A and B toxins of C. difficile and  $\alpha$ ,  $\beta$ ,  $\varepsilon$ -toxins of C. perfringens. Regarding the interpretation of the results, samples were considered as "High load" when the Ct values were lower than 30 for C. difficile and E. coli and lower than 26 for C. perfringens  $\alpha$ ,  $\beta$ , or  $\varepsilon$  toxins.

#### Results

Of the total samples, 99.4% were positive for  $\alpha$ -toxin of C. perfringens, 58.6% with a high load.  $\beta$ -toxin was only positive on 1.3% of the cards and none of the cards tested positive for  $\epsilon$ -toxin. C. difficile A-toxin was detected in 78.5% of the cards (52.4% with high load), and in 52.5% C. difficile B-toxin was also detected (15.7% with high load). It is worth noting that 47.5% of the samples were positive for ETEC antigen F4 and 25.9% were positive for LT toxin. ETEC antigens F5 and F6 were only found in 10.8% and 10.8% respectively.

# **Discussion and Conclusion**

Pathogenic bacterial agents can be commonly found in piglet diarrhoea without any clinical interpretation. The present study shows the percentage of Danish samples where different bacterial agents might be considered etiological agents of neonatal diarrhoea.

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# SALMONELLA PREVALENCE IN VIETNAM FROM 2020 TO 2023

G. Pham <sup>1</sup>, <u>H. Nguyen</u> <sup>1</sup>, P. Nguyen <sup>1</sup>, V. Tran <sup>1</sup>, R. Jansen <sup>2</sup>, C. Maala <sup>3</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Viet Nam <sup>2</sup>Boehringer Ingelheim Animal Health GmbH <sup>3</sup>Boehringer Ingelheim Animal Health ROPU ASKAN

# **Background and Objectives**

Nontyphoid Salmonella infections are an important cause of foodborne disease in South-East-Asia To monitor the prevalence of several serotypes in Vietnam a prevalence study was conducted from 2020 to 2023 in both pig farms as in meat markets.

#### Material and Methods

In total 58 farms (12 farms in 2020 and 46 farms in 2023) were selected spread all over Vietnam. On all farms various age groups were sampled: Post weaning (PW) at 5 weeks of age (WOA), at start (SF; 10 WOA), mid (MF; 18 WOA) and the end of finishing (EF; 26 WOA). Per age group 5 swabs were taken which were pooled for analysis by age group. Pooled samples were analyzed for presence of Salmonella by fist an 24h enrichment culture and were subsequent tested for presence of Salmonella serotypes by PCR.

For the Salmonella prevalence survey on meat markets, in total 60 markets throughout various regions for Vietnam were visited. Per market 2-5 meat shops were tested. Per meat shop 1 carcass was swabbed and the swab was stored in a Salmonella enrichment culture media. Samples were pooled per market and were tested for presence and serotype of Salmonella.

#### Results

In 2020 total 39% of the pooled samples were positive for Salmonella. The prevalence in 2023 was higher and increased to 57% positive samples. There was no clear difference between the age groups and distribution of the positive samples amongst the different age groups were: PW18%, SF 29%, MF 23%, EF 29%. In total 97 Salmonella isolates could be obtained out of which the majority was Salmonella Typhimurium (58 isolates; 60%). From the meat markets, 25% of the sampled markets were positive for Salmonella spp, 40% of the isolates belonged to Salmonella Typhimurium.

# **Discussion and Conclusion**

These results show that the prevalence of Salmonella is increasing with the dominant serotype S.Typhimurium. This serotype is associated with a high degree of food born infections and are resistant to various classes of antimicrobials. The current routine use of antimicrobials in pig production in Vietnam can predispose the spread of this zoonotic and therefore other control measures are needed, like for example, improved hygiene, biosecurity practices & vaccination.

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# STUDY OF THE BEST SAMPLING PERIODS FOR THE DETECTION OF THE VEROTOXIN (VT2E) ON SWINE FARMS WORLDWIDE BY ORAL FLUIDS

<u>R. Jordà 1</u>, G. García 1, I. Bernal 1, L. Nodar 1, G. Alvarez 1 1HIPRA, Amer (Girona), Spain

### **Background and Objectives**

Oedema disease (OD) is an enterotoxaemia caused by Escherichia coli, which possesses adhesion factors enabling the bacteria to colonise the small intestine and produce the verotoxin 2e (VT2e). VT2e is absorbed into the blood circulation and causes vascular damage, growth problems, nervous disorders, and mortality. The aim of this study was to determine which are the best periods for detection of the VT2e on swine farms throughout the world.

### **Material and Methods**

Verocheck is a sampling test (qPCR) that checks the E.coli gene that codes for the VT2e in oral fluids which are transferred to FTA Elute cards (Ct value: POS <38,5). A total of 3,785 farms from 47 different countries, with a total of 19,633 samples, were analysed from 2017 to September 2023. The different ranked ages were from less than 4 weeks (w), 4-8 w, 8-12 w, 12-16 w, and more than 16 w. A farm was considered positive when at least one of the samples was positive.

### Results

The age with the highest percentage positivity for VT2e was 12-16 w (64.1%) (a), followed by >16 weeks (63.3 %) (a), then 8-12 weeks (54.8%) (b), and finally, 4-8 weeks (48 %) (c), and < 4 weeks (34.2 %) (c). Different letters indicate statistically significant differences on a logistic regression model. However, the highest percentage of VT2e-positive farms with a high amount of the VT2e gene detected (+++) was in the 4-8 weeks age group.

#### **Discussion and Conclusion**

Based on the bibliography, the expression of fimbria 18 (F18) in the small intestine occurs from 3 weeks onwards. This factor, together with the high use of antibiotics and additives during the first weeks of life and in the nursery period, may play a role in the low prevalence of VT2e observed in the earliest age groups (especially in those countries where the use of ZnO is not prohibited). These results are relevant for field veterinarians because they may suggest high economic losses if the VT2e is present at later ages (>12 weeks), as this could indicate clinical or subclinical losses directly related to the VT2e effects, and co-infections with other pathogens.

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# SURVEY OF LUNG LESION AT SLAUGHTERHOUSES ACROSS CHINA

J. Fang <sup>1</sup>, H. Xiao <sup>1</sup>, <u>T. Tan <sup>1</sup></u> <sup>1</sup>*Elanco China* 

### **Background and Objectives**

Respiratory diseases are among the critical health issues affecting swine production. Detecting lung lesions in slaughterhouses provides valuable insights into the threat of respiratory diseases. Elanco China has implemented LLS services since 2022 to help pig farms with severe respiratory diseases. This survey presents the lung lesion status of slaughterhouse examination in China by LLS.

# Material and Methods

From January 2022 to July 2023, 134 LLS sessions were conducted across 26 provinces in China, examining 4,308 pig lungs from slaughterhouses. Consolidations are assessed by the Straw method, while SPES assessed pleuritis. Abscesses, scars, pericarditis, edema, atelectasis, and the diaphragmatic lobe diffuse lesions as bleeding, congestion, and consolidation were recorded.

# Results

**General Lung Health:** Lung consolidation was detected in all 134(100%) farms, with 4,251(98.68%) affected lungs. The lung consolidation index ranged from 0.87 to 5.93, with lower values indicating better lung health. **Suspected Lesions Related to Mycoplasma hyopneumoniae Infection:** 4,241(98.44%) lungs showed characteristic consolidation in cranioventral lobes (apical, cardiac, middle).**Suspected Lesions Related to Viral Infections:** Pulmonary edema, atelectasis, and the diaphragmatic lobe diffuse lesions (36.74%) observed in the slaughterhouses might be associated with viral infections and secondary infections. Regression analysis of lesion severity indicated that suspected viral lesions were positively correlated with lung consolidation and suspected mycoplasma pneumonia lesions.**Pleuritis-like Lesions:** Pleuritis was present in 116(86.57%) farms with 1,120(26%) affected lungs. There was no linear relationship between pleuritis and consolidated or suspected viral lesions.

### **Discussion and Conclusion**

Results showed that severe and complex lung lesions were observed in pig herds. Systematic measures should be taken for multiple respiratory pathogens control based on long-term LLS.

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# THE EFFECT OF ORAL LIVE LAWSONIA INTRACELLULARIS VACCINATION TO ENHANCE PIG PERFORMANCE IN THAILAND UNDER FIELD CONDITION

K. Samatiwat <sup>1</sup>, N. Young <sup>1</sup>, T. Paphavasit <sup>1</sup>, N. Bundee <sup>1</sup>, P. Jitplodprong <sup>1</sup>, S. Aiyimaphan <sup>1</sup>, J. Jittidecha <sup>1</sup>, <u>C. Maala</u> <sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health (Thailand) Ltd. <sup>2</sup>Strategic Business Unit Swine, Boehringer Ingelheim Animal Health,

#### **Background and Objectives**

One prevalent pathogen associated with porcine proliferative enteritis (PPE) is Lawsonia intracellularis. Clinical signs of PPE vary including bloody or diarrhea, weakness, and a slowing growth rate until no symptoms. PPE mostly affects the economy since it reduces pig performance. However, most farmers are unaware of PPE's financial benefits. The goal of the study is to enhance pig performance using oral live Lawsonia intracellularis vaccine.

# **Material and Methods**

Samples of blood were collected from two farms located in the central and eastern regions of Thailand. Using a commercial blocking antibody ELISA (SVANOVIR®), 80 serum samples were tested. Every farm has two groups in the non-vaccination and vaccination groups. For vaccine group, the oral live vaccine (Enterisol Ileitis®) is given at the time of weaning. Pig performance metrics such weight in, weight out, percentage of total loss, ADG, and FCR were compared.

#### Results

The Lawsonia intracellularis antibody is seropositive on both farms, despite their differing locales. Seroconversion occurs between 16 and 20 weeks of age, with a larger seroprevalence in the east 55% than in the Central 25%. The vaccination group in both regions had a reduced total loss, a greater ADG, and a lower FCR in the nursery and finisher phases. Additionally, the vaccine group's FCR in the finisher phase is 2.7 and 2.6 in the central and eastern regions, respectively, which is lower than the control group's FCR of 2.8 and 2.9 in both regions.

#### **Discussion and Conclusion**

PPE-causing Lawsonia intracellularis has a negative impact on pig performance. Oral live vaccination, on the other hand, improves performance in seropositive farms, particularly by reducing FCR in the finisher phase.

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# USE OF ENTERISOL® ILEITIS IN A GROW-FINISHING SITE WITH SUBCLINICAL ILEITIS IN NORTH CHINA

C. Sun <sup>1</sup>, S. Fang <sup>1</sup>, L. Huang <sup>1</sup>, <u>R. Jansen</u><sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health (Shanghai) Co. Ltd., Shanghai, China; <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH, Germany

# **Background and Objectives**

Ileitis is widely present in farms all over the world with different clinical symptoms, impacting growth and FCR of growing pigs. The objective is to investigate the use of Enterisol ileitis for improving ADG and mortality in finishers with subclinical ileitis in China.

#### **Material and Methods**

The trial was performed in a finishing site of North China in 2020. Before the study, sectional sampling was conducted to detect the exposure of ileitis. The trial included 2 groups, a vaccinated group (n=246) and a control group (n=245). Vaccinated pigs and control pigs were mixed in pens. The trial pigs were tagged and weighed individually at trial day 0. Vaccinated pigs were orally vaccinated at 6 weeks of age. Groups were evaluated for ADG from weaned to finishing stage and for mortality. Finisher pig weight variation was also evaluated. Data were analyzed by ANOVA using Minitab. Sera and oral fluids were collected from 10 weeks to 27 weeks of age, with 4 week intervals. Serum was checked for presence of antibodies by an ileitis ELISA test. Oral fluids were tested for the presence of Lawsonia intracellularis (Li) by PCR test.

#### Results

Ileitis antibody positive animals gradually increased from 10% at 10 weeks of age to 55% at 27 weeks of age. PCR test results of oral fluids confirmed the active infections with Li during the grow-finish period. Oral vaccination against ileitis resulted in a significant higher ADG when compared to the control group (ADG 859 vs 834g; market weight 137.5 vs 133.7kg;  $p \le 0.05$ ). Vaccinated animals showed better market weight uniformity with a smaller standard deviation (15.2 vs 17.43). Vaccinated animals had numerically lower mortality (6.10% vs 7.76%).

#### **Discussion and Conclusion**

Subclinical ileitis is the main manifestations of infections of Lawsonia intracellularis, causing negative impact on growth performance. In this Chinese subclinical infected ileitis finishing herd, oral vaccination against ileitis improved the ADG significant. Live weight variation together with mortality were reduced numerically.

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# AN UPDATE OF THE PREVALENCE OF POSTWEANING DIARRHEA CAUSED BY ESCHERICHIA COLI IN SPAIN

L. Pérez Esteruelas <sup>1</sup>, M. Claver Mateos <sup>1</sup>, J. Bringas <sup>1</sup> <sup>1</sup>Elanco Animal Health, Alcobendas, Spain

#### **Background and Objectives**

Post-weaning diarrhea (PWD) is a multifactorial disease that is becoming increasingly important for the animal health<sup>1</sup>. Since the ban of the use of zinc oxide in the EU in weaned piglet feed and the regulation of the responsible use of antibiotics, the disease is ever more difficult to control<sup>2</sup>. This study aimed to show the most prevalent Escherichia coli (E. coli) virulence factors that are contributing to the postweaning diarrhea.

#### **Material and Methods**

A total of 733 Spanish pig farms with acute cases of PWD were sampled between 2020 and 2023. Animals were selected based on PWD clinical signs. An average of three rectal swabs were collected from three different pigs on each farm from four- to eight-week-old pigs within the first 24 hours of the acute phase of the disease. Swabs with fecal matter were submitted to the laboratory (Exopol; Zaragoza, Spain) for diagnosis. PCR was performed to detect adhesion factor and toxin genes, including F4 (K88), F18, F41, F5 and F6 fimbriae, adhesin involved in diffuse adherence (AIDA-1), heat-stable and heat-labile enterotoxins (STa, STb, LT), shigatoxin (STx2e), enteroaggregative heat-stable toxin (EAST-1) and, E. coli attaching and effacing (eae). Rotavirus A and Epidemic diarrhea (PEDv) were analyzed as viruses due to the importance for the nursery period.

#### Results

Only 0.68% (5 out of 733) of farms were negative for all the E.coli's virulence factor genes. F18 gene was the most found (71.3%) followed by AIDA-1 (67.7%) and F4 (53.8%). The most prevalent toxin was EAST-1 (94.0%) followed by STb (88.1%) and STa (83.2%). Rotavirus type A was found in 61.9% of the PWD cases and PEDV in 2.8%.

# **Discussion and Conclusion**

The present study shows F18 E. coli as the main fimbria involved in postweaning diarrhea. There is a high degree of variability in the E. coli virulence factors within country as well as differences between countries<sup>3,4,5</sup>. It is necessary to conduct a rigorous diagnosis in order to characterize the E.coli's virulence factors and find the most appropriate solution in each PWD case, since it is described that an outbreak of F18+ E. coli persists in nursery pigs despite antibiotic treatment in 58% of the herds studied<sup>6</sup>.

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# ANTIBIOTIC SENSITIVITY ANALYSIS OF MYCOPLASMA HYOPNEUMONIAE TO TIAMULIN

H. Xiao <sup>1</sup>, J. Li <sup>2</sup>, <u>T. Tan <sup>1</sup></u> <sup>1</sup>Elanco China <sup>2</sup>Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, Nanjing, China

#### **Background and Objectives**

Mycoplasma pneumonia is a wasting disease due to Mycoplasma hyopneumoniae(M. hyo) and related pathogens infections. In most Chinese swine farms, antimicrobial agents remain crucial for the clinical treatment of mycoplasma pneumonia. The selective pressure of antimicrobial drugs may lead to the development of drug-resistant pathogens. This study aimed to evaluate the drug sensitivity of clinical isolates of Mhyo from China to tiamulin, one of the most frequently used clinical drugs for mycoplasma pneumonia.

# **Material and Methods**

Ten strains of M. hyo isolated between 2010 and 2017 from clinical samples of coughing pigs in Jiangsu and Anhui provinces in China were selected for the antimicrobial sensitivity testing towards tiamulin. Tiamulin (Denagard®), with a purity of 80%, was provided by Elanco (Shanghai) Animal Health Co., Ltd. Following established protocols<sup>[1]</sup>, the antibiotic was double diluted from 256 to 0.015  $\mu$ g/mL with 0.1 mL of each concentration added sequentially to an equal volume of M. hyo suspension (10<sup>4</sup>~10<sup>5</sup> CCU/mL) in the 96-well cell culture microplate incubated at 37°C. Each MIC test was performed in triplicate, and the color changes were observed daily. M. hyo strain J was used as reference control strain to validate the test. The lowest drug concentration at which no visible color change occurred in the test well, was considered the MIC for that antibiotic against the bacterium. The initial and final MIC value were recorded.

#### Results

According to the results, the MIC of tiamulin to M. hyo strain J was 0.06  $\mu$ g/mL as in other reports <sup>[2]</sup>. The MIC range of tiamulin against the ten strains was 0.015 ~ 0.25  $\mu$ g/mL. The MIC<sub>50</sub> was 0.06  $\mu$ g/mL, and the MIC<sub>90</sub> was 0.12  $\mu$ g/mL.

#### **Discussion and Conclusion**

The results of this study indicate that clinical pathogenic isolates of M. hyo from China are relatively sensitive to tiamulin. This indicates that tiamulin is an effective drug for the clinical treatment of mycoplasma pneumonia due to M. hyo.

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# COMPARATIVE FIELD EFFICACY STUDY BETWEEN TWO COMMERCIAL E. COLI VACCINES IN THAILAND

N. Bundee <sup>1</sup>, T. Paphavasit <sup>1</sup>, K. Samatiwat <sup>1</sup>, <u>C. Maala</u><sup>2</sup>, N. Kaewchomphu <sup>3</sup>, L. Yimpanfai <sup>3</sup>, R. Arharat <sup>3</sup> <sup>1</sup>Boehringer Ingelheim Animal Health (Thailand) Ltd. <sup>2</sup>Strategic Business Unit Swine, Boehringer Ingelheim Animal Health <sup>3</sup>Thai Foods Swine Farm Ltd.

# **Background and Objectives**

Piglet diarrhea is a major cause of preweaning loss. Several types of pathogens cause diarrhea including PEDV, Clostridium perfringens and E. coli. The objective of this study was to compare the efficacy of two E. coli vaccines in a breeder farm in Thailand.

#### **Material and Methods**

The 2,400 sow farm was a PRRS negative herd located in Eastern part of Thailand. E. coli vaccine ;Suiseng® was previously administered in two doses to replacement gilts in quarantine houses and once as a booster to 13-week-old pregnant pigs. The control group used Suiseng®. The treatment group used Entericolix®. 220 sows in both groups were vaccinated intramuscularly at 13-week-old pregnancy. Adverse reactions after vaccination were observed. Performance data was collected including total born, born alive, %still born, %mummification, birth weight, %preweaning mortality, weaning weight and ADLWG. For fecal score<sup>3</sup> indicates diarrhea: 0, normal; 1, pasty; 2, semi-liquid; and 3, liquid (Liu et al.,2010). Statistic was tested by Student's t-test.

#### Results

According to the study, there is no significant difference in sow performance between the two groups in various parameters. On the other hand, the birth weight was  $1.32^{\circ}$  Kg significant lower (p<0.01) in the treatment group;  $1.44^{\circ}$  Kg. For diarrhea scores, there are no difference at one and three weeks old suckling piglets. Whereas at 2 weeks of age, the fecal score of control group was  $2.41^{\circ}$  which is higher than the treatment group;  $1.65^{\circ}$  significantly (p<0.01). Furthermore, both groups were found to effectively protect preweaning loss based on sow performance.

### **Discussion and Conclusion**

Although the birth weight in the treatment group is lower, there is no significant difference between both groups regarding the weaning weight. Evidently, the treatment group reduced the diarrhea score at two weeks of age. Furthermore, this study is based on the farm's vaccination program which administers the vaccine once at 13 weeks of pregnancy while Entericolix®'s recommendation of two doses at 10 and 12 weeks.

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# COMPARISON OF PLEURISY IN SLAUGHTERED PIGS USING LLS APPLICATION IN COMMERCIAL SWINE FARMS IN ASIA

<u>K.A. Fabros</u><sup>1</sup>, R.F. Cudal <sup>2</sup>, M.G. Alba <sup>3</sup>, W. Dee-Ampai <sup>4</sup>, A. Ratanamongkolmas <sup>4</sup>, C.N.A. Nguyen <sup>5</sup>, P.H. Nguyen <sup>5</sup>, R. Chompuming <sup>4</sup>, J. Fang <sup>6</sup> <sup>1</sup>Elanco Animal Health, Asia <sup>2</sup>Food Animal Business, Elanco Philippines <sup>3</sup>Elanco Philippines <sup>4</sup>Elanco (Thailand) Ltd., Bangkok, Thailand. <sup>5</sup>Elanco Animal Health Vietnam <sup>6</sup>Elanco Animal Health

#### **Background and Objectives**

Pleurisy is a chronic lesion observed in the lungs due to pathogens like Actinobacillus pleuropneumonia, Pasturella multocida, and Glaesserella parasuis [1]. These lesions can be assessed by lung lesion scoring (LLS), providing valuable information on the farms' incidence and severity of respiratory diseases. This study compares the pleurisy status of slaughtered pigs in several Asian countries.

# **Material and Methods**

The study covered 177 lung lesion reports in 92 Asian swine farms over eight years (2016-2023). The scoring system used in the Elanco Lung Lesion Scoring application was the Slaughterhouse Pleurisy Evaluation System (SPES) [2]. Pleurisy scores, mean prevalence, and dorsal pleurisy index were compared across countries (MY, PH, TH, VN, and CN).

#### Results

A total of 8,021 lungs were evaluated. The mean pleurisy prevalence of MY, PH, VN, TH, and CN is 49.9%, 54.7%, 24.5%, 30.1%, and 37.2%, while the mean dorsal pleurisy prevalence is 37.2%, 36.2%, 14.3%, 17.8%, and 18.6% respectively. The respective countries' dorsal pleurisy index (DPI) is 1.116 for MY, 1.074 for PH, 0.362 for TH, 0.573 for VN, and 0.460 for CN. Pleurisy lesions have a high prevalence in pig lungs investigated at slaughter. PH has the highest prevalence of pleurisy, while MY has the highest prevalence of dorso-caudal pleurisy and dorsal pleurisy index, which is highly associated with Actinobacillus pleuropneumonia. Interestingly, TH has the lowest pleurisy prevalence and DPI among the countries.

# **Discussion and Conclusion**

This study showed that pleurisy lesions were high in Asian countries. Lung lesion scoring is a valuable tool in monitoring and evaluating the respiratory health of swine farms. The lung lesion application can benchmark countries and farm performances.

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# COMPARISON OF THE BENEFITS OF DIFFERENT VACCINATION CONCEPTS AGAINST ILEITIS UNDER PRACTICAL CONDITIONS

N. Rudolph <sup>1</sup>, R. Jansen <sup>2</sup>, M. Genzow <sup>2</sup>, <u>R. Deitmer <sup>2</sup></u> <sup>1</sup>*Tierarztpraxis Pfannes, Wabern, Germany* <sup>2</sup>*Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany* 

# **Background and Objectives**

L. intracellularis is known to cause subclinical and clinical ileitis leading to a reduction in production parameters and animal welfare. Vaccines are successfully used to prevent ileitis. This field experience compares the benefits that can be achieved with the two different vaccination concepts.

### **Material and Methods**

In a finishing farm managing a barn with 1,200 places pigs were vaccinated within the first week after placement. For the analysis, three consecutive fattening groups of 1,200 pigs were compared: first unvaccinated, after that vaccinated with the licensed intramuscular inactivated vaccine (killed i.m.) and then with the licensed live oral vaccine by water (oral live). Subclinical ileitis was confirmed by PCR and antibody test. Production parameters such as average daily gain (ADG), weight gain, mortality and duration of fattening were recorded and analysed on an individual animal basis.

#### Results

With the introduction of ileitis prophylaxis, mortality fell from 3.1% (unvaccinated) to 1.3% (killed i.m.) and 1.2% (oral live), respectively. In contrast to the unvaccinated pigs, the vaccinated animals continued to gain weight as the duration of fattening increased. The total weight gain for the vaccinated pigs (i.m. 71.8 kg, oral live 71.1 kg) was higher than for the unvaccinated pigs (67.0 kg). Comparing the two vaccination concepts, the oral live vaccinated pigs had a significantly shorter duration of fattening (106 days) than the unvaccinated (112 days) and the killed i.m. (118 days) vaccinated pigs ( $p\leq0.05$ ). ADG was significantly higher ( $p\leq0.05$ ) for the oral live group (884.5 g/d) compared to the other two groups, the unvaccinated controls (810.4 g/d) and the killed i.m. (813.3 g/d).

# **Discussion and Conclusion**

As shown previously, lleitis vaccination proved to be an effective tool to prevent ileitis. Additionally, this field experience confirms previous studies showing that the oral route of application tended to generate a more comprehensive protection than i.m. vaccination route resulting in a superior increase in zootechnical parameters. Based on these economically relevant results as well as the very animal- and user-friendly oral administration, the oral vaccine proved to be an effective and sustainable tool in ileitis prevention.

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# DETECTION OF CLOSTRIDIUM NOVYI BY PCR IN SOWS SUFFERING FROM SUDDEN DEATH IN CHINA

J. Miguel <sup>1</sup>, T. Yang <sup>2</sup>, <u>W. Fei <sup>2</sup></u>, M. Wu <sup>1</sup> <sup>1</sup>*HIPRA, Amer (Girona), Spain* <sup>2</sup>*HIPRA China, Beijing* 

#### **Background and Objectives**

Sow mortality is a growing concern in the swine industry worldwide. Many causes contribute to sow mortality such as lameness, reproductive disorders, infection, and sudden deaths, amongst others. Clostridium novyi Type B, which causes sudden death (SD), is an anaerobic and opportunistic bacterium, affecting sows that are metabolically stressed. Most deaths due to C. novyi occur in late gestation or early lactation, causing significant economic losses for the farmers. The  $\alpha$ -toxin produced by C. novyi is its main pathogenic factor. Different cases of sow sudden death have been reported in America, Europe, and Asia where  $\alpha$ -toxin was detected in the liver. The aim of this study was to present the first data on C. novyi implication in sows suffering SD on Chinese farms.

# **Material and Methods**

A total of 118 sows from different parities (0-6) from 48 farms (sized 1,200-10,000) distributed in 15 provinces were included from 2020 to 2023. Only sows suffering from SD within a known time range of 16 hours were included in the study. Samples were taken from the liver using a swab, transferred to an FTA card to avoid disease transmission, and then sent to HIPRA Diagnos China. The qPCR previously validated was used to detect the presence of the gene encoding  $\alpha$  toxin of C. novyi. Previous studies demonstrated the absence of the toxin in fresh livers of healthy sows.

#### Results

45% of sows were positive for C. novyi, and on 67% of the farms analysed there was at least one positive animal. Positivity was similar in the different parities, but 67% of the samples received, belonged to sows from parity 0 to 2, compared to 33% from parity 3 to 6.

#### **Discussion and Conclusion**

The results of this study demonstrate that C. novyi is highly implicated in sows suffering from SD. This data would be in accordance with previous studies about the seroprevalence of C. novyi on Chinese farms. Considering that it is not a guided study, but samples were collected whenever cases of SD were observed, first parities were more affected by SD than older ones.

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# FIRST REPORT OF LUNG LESION IN DANISH SLAUGHTER PIGS BY CEVA LUNG PROGRAM

H. Bak <sup>1</sup>, C.S. Kristensen <sup>4</sup>, A.S. Juel <sup>2</sup>, M. Albin <sup>4</sup>, <u>P. Mortensen <sup>3</sup></u> <sup>1</sup>SEGES Innovation P/S, Aarhus, Denmark <sup>2</sup>3L&F Veterinary Lab, Kjellerup, Denmark <sup>3</sup>Ceva Corporate Swine, Libourne, France <sup>4</sup>Ceva Animal Health A/S, Vejle, Denmark

# Background and Objectives

Post-mortem lung scoring is an acknowledged tool to assess the respiratory health status of a pig farm. With the Ceva Lung Program (CLP), estimated prevalence and severity of Enzootic Pneumonia (EP) and pleuropneumonia, the manifestation of Actinobacillus pleuropneumonia (APP), is recorded systematically for comparative evaluation of respiratory health, vaccine efficacy and economic impact of the lesions. The aim of this study is to evaluate the level of EP- and APP-like lesions and their possible correlation, in a random survey on Danish pig farms in November 2022.

#### **Material and Methods**

The November 2022 campaign, "Lungvember", was performed to increase the interest for respiratory health monitoring. Participating herds had lungs collected at slaughter from intentionally 30 pigs/herd. Bronchopneumonic lesions (BP) and dorso-caudal pleurisy (DCP) were scored with the CLP method at the Veterinary Laboratory in Kjellerup and categorized as EP-like and APP-like lesions, respectively. Based on the lung lesions, farm specific EP- and APP-indexes (APPI) were calculated, and their correlation investigated by simple plots.

#### Results

A total of 1145 lungs from 39 farms (<28 lungs/farm>) were scored using the CLP method. In these farms, 29 vaccinated against EP, 1 against APP and 3 against both. BP lesions was found in 33 of 39 farms with an average farm prevalence of 41% (max=89%), with 7% (max=12%) affected surface of lung parenchyma, providing an EP-index of 2.00 (max=5.03); 28 of these vaccinated against EP. In 34 of 39 farms DCP was found with an average farm prevalence of 37% (max=97%), providing an APP-Index of 1.07 (max=3.06) in the 34 herds with APP-lesions; 4 of these vaccinated against APP. No correlation was found between EP and APP-index at farm level (r<sup>2</sup>=0.0299)

#### **Discussion and Conclusion**

Although 80% of the herds with BP-lesions vaccinated against EP, the prevalence and severity of BP was evident. The even more evident DCP prevalence and severity and the low APP-vaccination rate indicates room for further improvement by vaccination. The lack of correlation between EP- and APP-indexes may be biased by very different prevalence of EP- and APP-vaccination and potential differences in BP control between EP-vaccines.

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# FREQUENCY OF INFECTION WITH MYCOPLASMA SUIS IN SWINE USING REAL-TIME PCR IN VIETNAM

<u>T.T.H. Le</u><sup>1</sup>, T.H. Do<sup>1</sup>, T.B.N. Nguyen <sup>2</sup> <sup>1</sup>Vetlatech Joint Stock Company, Vietnam <sup>2</sup>Vetlatech Joint Stock Company, Vietnam, Institute of Biology, Vietnam Academy Science and Technology

#### **Background and Objectives**

Mycoplasma suis (Eperythrozoon suis), the causative agent of histoplasmosis in pigs, is a pathogen distributed worldwide and causes significant economic losses in the swine industry. For a long time, direct reading of stained blood smears was the only diagnostic method available is limited. The real-time PCR technique was used, which demonstrates higher specificity and sensitivity when compared to the conventional PCR assays. Therefore, real-time PCR testing can be considered the gold standard for blood, tissue, and organ detection in swine herds. The objective of our study was to assess the frequency of infection with Mycoplasma suis infection in some provinces in Vietnam.

# **Material and Methods**

A total of 315 blood and 09 organ samples (lung, liver, kidney, lymph nodes) were collected from Hanoi, Hung Yen, Bac Giang, Bac Ninh, Thai Binh, Vinh Phuc, Phu Tho, Thanh Hoa, Nghe An, Other provinces in 2023. Pooled testing of samples (pool 5) by Real-time PCR was tested for Mycoplasma suis using real-time PCR detection by Kylt® (Art – No 31543) (Anicon, Germany).

#### Results

All swine blood and organ samples were in real-time PCR of sample pools collected from ten provinces of Vietnam. The frequency of infection of Mycoplasma suis in this study ranged from 0 to 66.7%. The percentage of positives in this study is 33.33%. Among the samplers, the samples from 03 provinces had free Mycoplasma. suis and the highest positive sample rate is the Bac Ninh province (66.67%).

#### **Discussion and Conclusion**

In recent years, M. suis has been detected in pig farms in Vietnam. This first study uses real-time PCR kit detection of M. suis in the swine population in ten provinces in Vietnam. The percentage of positives in this study is 33.33% was similar to that found in pig farms in Thailand (37,1%), China (86%), Germany (30 -76,2%), France (53%), and Brazil (5 - 95%) M. suis 86% in pigs. This study has shown, that real-time PCR could be an appropriate tool for a sufficiently coherent identification of M. suis in latent carrier animals in view of introducing effective treatment and disease control measures.

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# PK/PD AND CLINICAL RELATIONSHIPS OF VETMULIN PREMIX (TIAMULIN HYDROGEN FUMARATE) ADMINISTERED TO PIGS FOR THE TREATMENT OF SWINE DYSENTERY

<u>U. Klein</u><sup>1</sup>, M. Anderson <sup>2</sup>, L. Claerhout <sup>1</sup>, W. Depondt <sup>1</sup> <sup>1</sup>Huvepharma NV Antwerp, Belgium <sup>2</sup>Fermenta Animal Health Company, Kansas City, Missouri, USA

### **Background and Objectives**

The pharmacokinetics (PK) of tiamulin hydrogen fumarate (THF) (Vetmulin<sup>®</sup> - Huvepharma NV) colon content concentration (CCC) based on feed medication was related to tiamulin MICs against Brachyspira hydrysenteriae (Bhyo) and Brachyspira hampsonii (Bhamp) pharmacodynamics (PD).

# Material and Methods

In a PK study the tiamulin CCC was determined in groups of five pigs (average body weight 16.9kg), which were medicated in doses of 1.98mg/kg bw (38.5ppm), 6.6mg/kg bw (110ppm) and 13.2mg/kg bw (220ppm). Application period medicated feed: 14 days. On the last day of treatment the pigs were euthanized and colon mucosa, colon content, lung and blood samples were collected for microbiological assay analysis.

Tiamulin MIC data were generated in a EU study based on susceptibility testing of Brachyspira hyodysenteriae (n=38) strains from Germany and UK isolated 2019-2020. In a susceptibility study conducted in the US tiamulin MIC data were generated on Brachyspira hyodysenteriae (n=83) and Brachspira hampsonii (n=53) strains isolated 2013-2020. Tested MIC dilutions:  $0.015-256\mu$ g/ml. MIC<sub>50</sub>, MIC<sub>90</sub> and MIC ranges were determined.

#### Results

A tiamulin CCC of <1.62 $\mu$ g/g (LOQ) at 1.98mg THF/kg bw dose was determined. At 6.6mg THF/kg bw and at 13.2 THF/kg bw dose levels, tiamulin CCC of 2.84 $\mu$ g/g and of 8.05 $\mu$ g/g were recorded after feed medication, respectively. In the EU study MIC<sub>90</sub> values of 0.5 $\mu$ g/ml and an MIC range of 0.015-16 $\mu$ g/ml were measured for the tested Bhyo strains. A tiamulin MIC<sub>90</sub> value of <0.06 $\mu$ g/ml and MIC range of 0.06-4 $\mu$ g/ml (Bhyo) and of 0.06-0.25 $\mu$ g/ml (Bhamp) were determined for the US Brachyspira strains. PK/PD relationships show that tiamulin CCC`s achieved at the three tested dosages are high and exceed the tiamulin MIC values of all US Bhyo and Bhamp strains and 98% of the tested Bhyo strains from Europe.

# **Discussion and Conclusion**

Excellent therapeutic and metaphylactic effect of tiamulin feed medication in the case of swine dysentery diseased pigs at registered treatment dose of 5-10mg/kg bw can be expected based on available PK/PD data. The use of PK/PD data help to optimize treatment procedure according to prudent use policies and avoid treatment failures against swine dysentery.

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# PK/PD RELATIONSHIPS OF TILMICOSIN (TILMOVET) ORAL ADMINISTRATION FOR THE TREATMENT OF BACTERIAL RESPIRATORY DISEASES

<u>U. Klein</u><sup>1</sup>, T. Fangman<sup>2</sup>, D. Nolan<sup>2</sup>, L. Claerhout<sup>1</sup>, W. Depondt<sup>1</sup> <sup>1</sup>Huvepharma NV Antwerp, Belgium <sup>2</sup>Huvepharma Inc. Peachtree City, GA, USA

# **Background and Objectives**

The paper describes pharmacokinetic (PK) properties of tilmicosin (Tilmovet<sup>®</sup>- Huvepharma NV) and relates tilmicosin MIC results generated on US and EU respiratory pathogens (M.hyopneumoniae-Mhyo, A.pleuropneumoniae-App, P.multocida-Pm, G.parasuis-Gp, B.bronchiseptica-Bb, S.suis-Ss) to the pharmacokinetics of tilmicosin.

### Material and Methods

In an App challenge/PK study (study 1) tilmicosin plasma and lung concentrations were determined (HPLC) following 15 consecutive days oral medication (dose 16mg tilmicosin/kg bw). In a PK study (study 2) tilmicosin distribution in serum, trachea epithelium, lung and macrophage samples was determined (HPLC) (14 days feed medication; dose 19.4mg tilmicosin/kg bw).

Antimicrobial susceptibility to tilmicosin were determined in a US study (App, Pm, Bb, Ss; isolates from 2019/2020) and a EU study (App, Pm, Bb, Ss, Mhyo<sup>\*</sup>; isolates from 2019/2020, from 2015/2016<sup>\*</sup>). MICs were determined using broth microdilution method (CLSI).

# Results

In study 1 higher tilmicosin lung versus plasma concentrations (lung:serum ratio 34:1) were determined. Lung concentrations increased to peak lung level of 2.712µg/g 24 hours post-medication. Tilmicosin accumulation in alveolar macrophages in study 2 led to 4.3 times higher tilmicosin concentrations than in lung tissue. 20-times higher tilmicosin concentrations in trachea epithelium and lung tissue vs. serum were determined.

US MIC data show high levels of susceptibility for App, Pm and lower susceptibilities for Bb and Ss. US tilmicosin  $MIC_{90}$  values (App 2019 = 16µg/ml and 2020 = 8µg/ml; Pm 2019 = 16µg/ml and 2020 = 4µg/ml) correspond well with the approved CLSI clinical breakpoints established for App and Pm.

In the EU study very high susceptibility to tilmicosin were determined for Mhyo, Gp and high susceptibility for Pm and App isolates. Susceptibility for Bb and Ss were lower.

Tilmicosin MIC values for M.hyopneumoniae isolates (MIC<sub>90</sub> =0.5 $\mu$ g/ml) and for G.parasuis (MIC<sub>90</sub> = 4.0 $\mu$ g/ml) are below tilmicosin concentrations achieved in lung macrophages. Tilmicosin MICs for Pm (MIC<sub>50'90</sub> = 4.0/8.0 $\mu$ g/ml) and App MIC<sub>50'90</sub> = 16.0/32.0 $\mu$ g/ml) correspond with the approved CLSI clinical breakpoints.

#### **Discussion and Conclusion**

The Mhyo-, Gp-, Pm- and App-specific tilmicosin PK/PD relationship data predict therapeutic success of tilmicosin use at registered treatment dosage and administration time (16mg/kg bw; 15 consecutive days) in cases of respiratory infections caused by these pathogens.

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# PREVALENCE STUDY OF POLYSEROSITIS CASES ON MEXICAN FARMS

D.B. Garcia <sup>1</sup>, Z.A. Gutierrez <sup>1</sup>, C. Cruz <sup>1</sup>, A.D.J. Gonzalez <sup>1</sup>, <u>L. De Lucas</u> <sup>2</sup>, D. Giralt <sup>2</sup> <sup>1</sup>*Hipra México Laboratorio Veterinario, S.A. de C.V.* <sup>2</sup>*HIPRA, Amer (Girona), Spain* 

#### **Background and Objectives**

Polyserositis complex is a very common disease in the post-weaning phase, with nonspecific signs such as fever, discharge, anorexia, dyspnoea and finally death. Results indicate that the aetiology of polyserositis is an intricate puzzle of pathogens. The objective of this study was to analyse the prevalence of Glaesserella parasuis, Streptococcus suis, Mycoplasma hyorhinis and Mycoplasma hyosinoviae in polyserositis cases on Mexican pig farms.

### **Material and Methods**

To estimate the prevalence of pathogens on Mexican swine farms, a total of 83 samples were analyzed in the HIPRA Diagnos Mexico Laboratory (Jalisco). All the samples were obtained through active screening on the farms by HIPRA Technical Services during the period from January 2023 to November 2023. The samples were collected with a sterile swab from different body locations (pleura, pericardium, peritoneum, joints, and brain), during the necropsy of piglets from 4-8 weeks old that presented clinical signs compatible with polyserositis and were then transferred to an FTA card. The prevalence of pathogens was analyzed by multiplex real-time PCR to detect G.parasuis, S. suis, M. hyorhinis and M. hyosinoviae genetic material (Glässer check PLUS, Hipra).

#### Results

With regard to the positivity for each of the pathogens, 77 samples (92.8 %) were positive for G. parasuis and only 6 (7.2%) reports were negative. 69 samples (83.1%) were positive for M. hyorhinis, 0 samples for M. hyosinoviae and 77 samples (92.8 %) for S. suis. When we consider the combination of pathogens on the farms, of the 92.8 % positive reports for G. parasuis, 62 samples (75%) were also positive for S. suis and M. hyorhinis.

#### **Discussion and Conclusion**

The results obtained show a high presence of G.parasuis on Mexican farms with polyserositis signs and are in line with the previously published literature. Combination of G.parasuis, S. suis, M. hyorhinis was also high, which highlights the fact that the correct diagnosis of polyserositis is crucial and can be really challenging, often making the implementation of therapeutic and control strategies frustrating. This is especially important in the case of G.parasuis, where alternative control methods such as prevention by vaccination will help farms to reduce the use of antibiotics for the prevention or control of polyserositis cases.

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# SEASONAL SURVEY OF LUNG LESION SCORING IN SLAUGHTER PIGS OF CHINA

J. Zhang <sup>1</sup>, Z. Zhang <sup>1</sup>, C. Liu <sup>1</sup>, <u>R. Krejci</u><sup>2</sup>, P. Mazerolles <sup>2</sup> <sup>1</sup>Ceva Animal Health China <sup>2</sup>Ceva Animal Health France

#### **Background and Objectives**

Porcine Enzootic Pneumonia (Ep) is a major health concern for pig farms around the world. The aim of this survey was to analyze the seasonal variation of the lung lesions scores in China by Ceva Lung Program Scoring Methodology.

# **Material and Methods**

Total 40,286 lungs from 1,203 batches were scored at the time of slaughter from October 2022 to September 2023 in different regions of China, representing the most important areas of swine production. EP-like lesions were scored and calculated considering the proportion of each lobe of lungs. The season was selected as a risk factor and divided into 4 clusters: Q1(1<sup>st</sup> Jan-31<sup>st</sup> Mar 2023, total 312 batches), Q2(1<sup>st</sup> Apr-30<sup>st</sup> Jun 2023, total 323 batches), Q3(1<sup>st</sup> Jul-30<sup>st</sup> Sept 2023, total 324 batches) and Q4(1<sup>st</sup> Oct-31<sup>st</sup> Dec 2022, total 244 batches). Lung lesions were described by: -% of lungs with EP-like lesions (broncho-pneumonic lesions BP).

-% of average affected area of all lungs.

-Enzootic pneumonia Index (EP index).

#### Results

The median value of %BP was, 56.3% (Q1), 45.5% (Q2), 53.1% (Q3) and 58.2% (Q4) respectively. The median of affected area of all lungs was, 4.5% (Q1), 2.2% (Q2), 2.1% (Q3) and 2.6% (Q4) respectively. The median of EP index was 1.54, 1.42, 1.43 and 1.74 respectively in those periods. Q2 showed better lung health in terms of %BP and EP index (p<0.05 vs other 3 quarters, except vs Q3 in Ep index). On the contrary, lung lesion scores in Q4 were at the worst level (p<0.05 vs other 3 quarters, except vs Q1 in BP).

#### **Discussion and Conclusion**

This survey showed that Chinese pig farms had suffered from the intensive Mhyo infections in those areas. The seasonal variation of lung lesion scoring is most probably due to the changes in climate and corresponding farm management practices such as ventilation.

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# THE FIRST REPORT OF SALMONELLA CHOLERESUIS IN SERBIA

<u>N. Zdravković</u><sup>1</sup>, B. Savic<sup>1</sup>, O. Radanovic<sup>1</sup> <sup>1</sup>Scientific Veterinary Institute of Serbia, Belgrade, Serbia

#### **Background and Objectives**

So far the Salmonella Choleraesuis is not marked as a predominant serovar. In the Serbia, the most common pig S. enterica serovars are: Typhimurium, Derby, Infantis, and Enteritidis. This report discourse the first report finding of S. Choleraesuis in Serbia.

# **Material and Methods**

The problem arose at single site continuous-flow farm inhabited with 1300 sows with farrow to finish orientation. The farm reported weaned pigs mortality in up to 5%. During previous period, the farm experienced porcine epidemic diarrhea virus (PEDV) and mycotoxicosis, Salmonella outbreak haven't been recorded on farm earlier. In the last 2 years, the farm imported about 200 gilts and 7 boars from different EU countries and traded pigs with other swine farms within Serbia.

# Results

Pneumonia indicative clinical signs, lethargy, inappetence, and ears cyanosis, extremities, and abdomen started in nursery building housing 900 piglets 8–10wk old. Approximately 120 of 900 piglets affected while 50 piglets were febrile, temperatures  $39.5-41.5^{\circ}$ C. In following day the disease spread in 2 nursery buildings with cca. 460 out of 9000 piglets affected. The disease lasted for 3 weeks and mortality increased for ~5%. Of the 460 diseased piglets, 152 died -a fatality rate of 33%. Postmortem examinations found resilient lungs, failed to collapse, firm and red fluid separated lobules; lesions affected the cranial lobes. Samples of 47 piglets were sent for microbiology examination. Samples were cultured for primary pneumonia pathogens revealed tentative diagnosis for Salmonella. Further serotyping, biochemical and molecular analysis were done. Discovered antigenic formula was 6,7:c:1,5 with the biochemical features: mucate, dulcitol and H<sub>2</sub>S indicated: S. Choleresuis var. Kunzedorf. All isolates had specific PCR product for fliC gene, sequenced and submitted to Gen-Bank (MN563751).

### **Discussion and Conclusion**

The most likely source of infection were carrier pigs, although other sources cannot be ruled out.

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# THE IN-VITRO SYNERGISTIC EFFECTS OF TIAMULIN IN COMBINATION WITH AVILAMYCIN AND HALQUINOL AGAINST COLISTIN RESISTANCE ESCHERICHIA COLI AND ENTEROTOXIGENIC ESCHERICHIA COLI ISOLATED FROM THAI PIG FARMS

J. Yindee <sup>1</sup>, W. Niyomtham <sup>1</sup>, N.O. Khine <sup>1</sup>, W. Dee-Ampai <sup>2</sup>, S. Simjee <sup>2</sup>, <u>N. Prapasarakul <sup>3</sup></u> <sup>1</sup>Faculty of Veterinary Science, Chulalongkorn University. Bangkok, 10330, Thailand. <sup>2</sup>Elanco (Thailand) Ltd., Bangkok, Thailand. <sup>3</sup>Center of Excellence in Diagnosis and Monitoring of Animal Pathogen, Faculty of Veterinary Science, Chulalongkorn University. Thailand

#### **Background and Objectives**

The rise of colistin-resistant E. coli bacteria in swine production, notably the plasmid-mediated mcr-1 variety, has created substantial public health and animal husbandry concerns [1]. The purpose of this study was to investigate the in-vitro synergistic effects of tiamulin, avilamycin, and haquilnol against Thai pig farm-derived resistant strains.

# Material and Methods

Multiple pig farms in Thailand were sampled extensively for the presence of 10 colistin-resistant E. coli and 10 enterotoxigenic E. coli (ETEC). The MALDI-TOF mass spectrometry and PCR techniques were used to identify and characterize all strains. The study utilized established microbiological techniques to evaluate the minimal inhibitory concentration (MIC) of antimicrobial agents in accordance with the CLSI Vet 01 standard [3] and a checkerboard method employing a broth microdilution test [2].

#### Results

Susceptibility testing revealed that the MIC50 values for Tiamulin (512 mg/mL), Avilamycin (512 mg/mL), and Halquinol (8 mg/mL) were distinct. In combination with Avilamycin and Halquinol, the MIC of Tiamulin was lowered to 8-16 mg/mL. Avilamycin and Haquilnol's MIC50 values were lowered to 2 and 64 mg/mL, respectively, in the presence of Tiamulin, indicating a synergistic effect of over 75%. Halquinol distinguished itself by exhibiting the highest efficiency against all E. coli strains. This in-vitro study demonstrated no antagonistic effects with the employed drug combination. This triad's ability to combat resistant bacterial strains is supported by the fall in MIC values observed when the combination was employed. This in-vitro study is especially significant in view of the increasing global trends of antibiotic resistance. The combination of these findings highlights the necessity for ongoing research in this field, with a focus on the long-term consequences and potential resistance of the combinations themselves.

# **Discussion and Conclusion**

The in-vitro synergistic effect found with the combination of tiamulin, avilamycin, and halquinol against colistin-resistant E. coli bacteria suggests promising options for treatment. Such combinations could potentially reach new levels of antimicrobial treatments in swine production, mitigating the challenges posed by resistant bacteria. Further research and in-vivo field trials are encouraged to validate these findings.

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# ANTIBACTERIAL ACTIVITY OF THYME ESSENTIAL OIL (THYMUS VULGARIS) AGAINST MULTIDRUG-RESISTANT ENTEROBACTERIA ISOLATED FROM PIGS

S. Rodrigues Chagas <sup>1</sup>, C.D. Segovia <sup>1</sup>, I.D.S. Lima <sup>1</sup>, J. De Moraes Reis <sup>1</sup>, F. Ferreira Taka <sup>1</sup>, C. Silva Minafra E Rezende <sup>1</sup>, L. Mendonça Pascoal <sup>1</sup>

<sup>1</sup>Universidade Federal de Goiás - Goiânia - Brasil

#### **Background and Objectives**

Bacteria such as Escherichia coli and Salmonella sp. have shown varying degrees of resistance to antimicrobials (1-3). In this scenario, essential oils (EOs) are being explored due to their antimicrobial potential (4,5). This study aimed to determine the antimicrobial effect thyme (Thymus vulgaris) EO on enterobacteria isolated from pigs.

### Material and Methods

We selected an enterotoxigenic E. coli (ETEC) and multi-drug resistant (MDR) Salmonella enterica serovar Heidelberg strain (the strain was classified as MDR by de Paula Nascente et al. 2022(6)), isolated from pigs in diarrhea outbreaks. Thyme EO was used and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using previously described methods (7, 8). Triphenyltetrazolium was used as a colorimetric indicator to read the results after incubation (9). The tests were carried out in three replicates.

#### Results

E. coli ETEC: MIC ranged from 0.5 to 1 mg/ml and the MBC ranged from 0.5 to 1 mg/ml.

S. Heidelberg: MIC ranged from 0.25 to 0.5 mg/ml and the MBC ranged from 0.25 to 0.5 mg/ml.

# **Discussion and Conclusion**

The results show the antimicrobial effect of thyme EO even on bacterium classified as MDR, a positive result in a scenario where the antimicrobial resistance rate is increasing in several countries (10,11).

Other studies carried out with thyme EO on E. coli ETEC and Salmonella spp. isolated from humans reported similar MIC and MBC values (5, 12) thus demonstrating the efficacy of this EO. This study contributes to the possibility of using EOs as an alternative to antibiotics in pig farming.

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# ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND DETECTION OF AMR GENES IN STREPTOCOCCUS SUIS ISOLATED FROM PIGS WITH AND WITHOUT MENINGITIS

J. Santos Streauslin <sup>1</sup>, A.P. Silva <sup>2</sup>, M. Hakimi <sup>3</sup>, R. Main <sup>2</sup>, O. Sahin <sup>2</sup>, B. Arruda <sup>4</sup>, D. Nielsen <sup>5</sup>, N. Macedo <sup>6</sup>

<sup>1</sup> Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>2</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

<sup>3</sup>Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

<sup>4</sup>Agricultural Research Service United States Department of Agriculture

<sup>5</sup>Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, United States Department of Agriculture, Agricultural Research Service, Ames, Iowa, USA

<sup>e</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

# Background and Objectives

Streptococcus suis is a commensal opportunistic pathogen of swine worldwide, causing pneumonia, sepsis, and meningitis. S. suis is also a significant zoonotic disease. Antimicrobials are the standard treatment for S. suis and are widely used since vaccine efficacy is limited due to poor cross-protection among serotypes. The susceptibility patterns and molecular epidemiology of S. suis isolates causing meningitis in pigs are poorly understood. Studies report higher MICs for S. suis, emphasizing the need for surveillance, particularly for isolates linked to meningitis. This study assessed antimicrobial susceptibility patterns, genetic diversity, and penicillin-binding proteins (PBP) sequence variations in S. suis isolates associated with bacterial meningitis, comparing them to non-disease-associated isolates.

#### **Material and Methods**

The minimum inhibitory concentrations (MIC) were determined by the broth microdilution method, using the BOPO7F Sensititre panel and CLSI standards, conducted on 141 disease-associated (DA) S. suis isolates from the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) between 2015 and 2019. Additionally, 49 non-disease-associated (NDA) S. suis isolates were included. Whole-genome sequencing identified antimicrobial resistance genes using the NCBI database through ABRICATE.

#### Results

Both DA and NDA isolates exhibited resistance to tetracycline and clindamycin (>85%). Notably, NDA isolates showed higher resistance to penicillin (28%) and Ceftiofur (14%) compared to DA isolates (3.5% and 1.4%, respectively). A high proportion of DA (94%) and NDA (96%) isolates carried antimicrobial resistance (AMR) genes associated with the tetracycline class, and 81% of DA and 96% of NDA harbored AMR genes related to the macrolide-lincosamide-streptogramin (MLS) class.

# **Discussion and Conclusion**

In conclusion, both DA and NDA isolates demonstrated high rates of phenotypic resistance to tetracycline and clindamycin, along with the presence of associated AMR genes, as previously reported. Notably, NDA isolates display decreased susceptibility to beta-lactams, which are the major drugs for treating S. suis infections. Ongoing PBP sequence analysis has detected amino acid replacements previously associated with resistance. These findings underscore the urgent need for continued surveillance and effective preventive strategies in the swine industry. The study emphasizes the importance of exploring alternative treatments and promoting responsible antimicrobial use.

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# ASSESSING THE EFFECTS OF CLOSTRIDIOIDES DIFFICILE AND CLOSTRIDIUM PERFRINGENS TYPE A VACCINATION ON PIGLET PERFORMANCE

<u>T. Vraeghe</u><sup>1</sup>, A. Michiels <sup>1</sup>, E. Claeyé<sup>1</sup>, L. Nodar <sup>2</sup>, F. Roggeman <sup>3</sup>, O. Boix <sup>2</sup> <sup>1</sup>HIPRA Benelux, Ghent, Belgium <sup>2</sup>HIPRA HQ, Amer, Spain <sup>3</sup>Provet DAP, Kortemark, Belgium

#### **Background and Objectives**

Clostridioides difficile (C. difficile) and Clostridium perfringens type A (CpA) are known to cause diarrhoea in piglets between 1 and 10 days of age. Next to this, C. difficile has also been associated with increased pre-weaning mortality. The aim of the study was to determine the impact of adding a commercially available vaccine for C. difficile and CpA to the existing Escherichia coli and Clostridium perfringens type C vaccination schedule and its effects on diarrhoetic piglets, weaning mortality and the number of weaned pigs.

# Material and Methods

Six farms with recurrent neonatal diarrhoea already implementing preventive measures were included. Before the study, a molecular diagnosis (PCR) for the most relevant bacterial agents causing neonatal diarrhoea was performed on each farm to evaluate if additional vaccination against C. difficile and CpA was necessary. On each farm, the sows were divided in two groups: the regular neonatal diarrhoea vaccination schedule, control group (Con) and the group vaccinated with SUISENG® Diff/A (Diff/A) and the regular neonatal diarrhoea vaccination schedule.

#### Results

In total, 48 litters of primiparous and 106 litters of multiparous sows were included in the Con group, whereas 27 primiparous and 140 litters of multiparous sows were included in the Diff/A group. The number of weaned pigs was  $13.01 \pm 2.34$  and  $12.85 \pm 2.68$  in the Con and Diff/A groups respectively (p>0.05). The percentage of litters with diarrhoea was significantly reduced in the primiparous sows in the Diff/A (18.5%) vs. Con (50%) (p=0.006), and when all parities were analysed (28.1% vs. 33.1%). Mortality was reduced in Diff/A groups when all parities were included and significantly (p=0.014) in the multiparous sows (9.92% vs 13.39%).

# **Discussion and Conclusion**

This study demonstrated that including SUISENG<sup>®</sup> Diff/A in the vaccination schedule of sows having piglets suffering from recurrent problems of scours, can reduce the occurrence of diarrhoea and mortality in those piglets and thus have a positive impact on the future piglet performance.

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# DETERMINING THE PREVALENCE OF BRONCHOPNEUMONIA IN SWINE IN VIETNAM 2022-2023 USING LUNG LESION SCORING APPLICATION

H. Nguyen Pham <sup>1</sup>, T. Kieu Minh <sup>1</sup>, R. Chompuming <sup>2</sup>, <u>K.A. Fabros</u> <sup>3</sup> <sup>1</sup>Elanco Vietnam <sup>2</sup>Elanco (Thailand) Ltd., Bangkok, Thailand. <sup>3</sup>Elanco Animal Health Asia

### **Background and Objectives**

Cranio-ventral consolidation or bronchopneumonia is one of the most common and important lung lesions in pig farming (Bojkovski et al., 2022). This kind of damage in the lung affects swine respiratory health and performance, thereby driving economic loss for pig breeding (Straw et al., 1989). The objective of this study is to determine prevalence of bronchopneumonia lesions in slaughtered pigs in Vietnam.

# **Material and Methods**

Elanco Lung Lesion Scoring (LLS) application is based on the Straw method. In this study, LLS was used to determine the prevalence of bronchopneumonia infection status of 2,722 lung samples at slaughterhouses from forty-four commercial farms across Vietnam from January 2022 to October 2023. The mean scores showed the prevalence rate of bronchopneumonia.

#### Results

The results showed the prevalence rate of bronchopneumonia in Vietnam was 57.75%. Of these, the total of pneumonia and severe pneumonia was 22.4%. Although most pigs had been vaccinated and administrated antibiotic programs against Mycoplasma and other respiratory diseases, the prevalence of cranio-ventral consolidation in the lung remained high.

# **Discussion and Conclusion**

The LLS application showed that pneumonia and severe pneumonia rates in Vietnam remained high (22.4%). Therefore, it was necessary to assess and provide an appropriate antibiotic and vaccination program with good farm management practices to control respiratory diseases, especially pathogens causing bronchopneumonia.

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# DIVERSITY OF VIRULENCE FACTORS OF PASTEURELLA MULTOCIDA STRAINS ISOLATED FROM DISEASED PIGS FROM A PIG FARM IN THE STATE OF SãO PAULO, BRAZIL.

M. Maria Cavalcante Granja <sup>1</sup>, G. Pereira Paschoalini <sup>1</sup>, T. Viana Fialho Martins <sup>1</sup>, J. Viktor De Paula Barros Baêta <sup>1</sup>, <u>L.</u> <u>Fernando Dos Santos <sup>1</sup></u>, D. Lúcio Dos Santos <sup>1</sup>, W. Vieira Guimarães <sup>1</sup>, J. Lúcio Dos Santos <sup>1</sup> *MICROVET - Microbiologia Veterinária Especial* 

### **Background and Objectives**

Pasteurella multocida typically inhabits the respiratory tract of healthy animals but can cause respiratory diseases, such as progressive atrophic rhinitis in pigs and pneumonia. The most common serogroups in pigs are A and D, and the isolates exhibit a variety of virulence genes categorized based on their function in pathogenicity. This study assesses the distribution of genes by category in P. multocida strains isolated from pigs with signs of pasteurellosis on a farm in the state of São Paulo.

# **Material and Methods**

Seven isolates of P. multocida from diseased pigs originating from a pig farm in the state of São Paulo, Brazil were evaluated. Their DNAs were sequenced using NGS based on WGS to identify virulence factors. The sequences were compared with relevant P. multocida genes to classify pathogenicity and compare virulence profiles. The sequences were mapped to relevant P. multocida virulence genes, and the genes were categorized to assess pathogenicity and compare the virulence profiles.

#### Results

The identified genes were able to classify into three main categories: immune modulation, adhesion, and metabolism, with the absence of toxin-related genes. These three categories were present in 100% of the evaluated isolates. The immune modulation category was most prominent in 71% of the isolates, followed by adhesion and metabolism. Genes related to immune modulation were more frequently observed in isolates Am3 and Am7, while adhesion-related genes were more prevalent in isolates Am1, Am2, and Am5. Isolates Am2 and Am7 of P. multocida exhibited the highest number of relevant virulence genes.

# **Discussion and Conclusion**

NGS technology is a valuable tool for identifying the virulence profile of bacterial isolates. Through sequencing, it was possible to characterize and differentiate P. multocida isolates from a pig farm in São Paulo. Iron metabolism proteins are important in pathogenicity and are highly prevalent in P. multocida isolates from pigs in Brazil, which was corroborated in this study. Genetic variability among the isolates in relation to virulence genes was observed, enabling the exploration of each strain's specificities and aiding in the decision-making process for selecting samples for more accurate autogenous vaccines on the farm. The use of autogenous vaccines provides greater efficiency in the prevention of diseases on farms.

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# EVOLUTION OF THE ASSESSMENT OF LUNG LESION SCORE IN THE SLAUGHTERHOUSE IN SPAIN DURING 2023

<u>J. Abadias</u><sup>1</sup>, C. Farre<sup>1</sup>, J. Hernández Martín<sup>1</sup>, E. Maiqués<sup>1</sup>, Y. Lostao<sup>2</sup> <sup>1</sup>Zoetis Spain SLU <sup>2</sup>CUARTE S.L.

# **Background and Objectives**

The post-mortem evaluation carried out in the slaughterhouse allows veterinarians to assess the swine respiratory complex thanks to the measurement of lesions associated with enzootic pneumonia and pleuritis, and correlating this with productive parameters [1]. The objective of this study was to compare the outcome of assessments during 2023 with previous years (vs. 2021+2022).

# **Material and Methods**

The researches established a system for evaluating 90 lungs from each batch of pigs slaughtered in Grupo Jorge company. An average of 220 batches have been evaluated weekly since 2021 representing a total of 1.080.000 lungs in 2023 vs. 1.659.371 lungs during 2021 and 2022. Of the different methods for assessing enzootic pneumonia [2], the Mesons 0-5 system was used (0 = absence and 5 = maximum % of lesion), obtaining a disease index for each batch where a result greater than 1.0 is suspicious for disease. Pleuritis was determined as the absence or presence of lesion in each lung, obtaining the % of affected lungs in each batch.

#### Results

The average annual disease index in 2023 increased to 0,60 vs. 0,51 (p-value<0,001); this increase starting in week 22 with a maximum of 0,95 (week 35) and a minimum of 0,36 (week 12). 19% (vs. 15%) of the batches have a disease index > 1,0, and this increased to more than 29% from week 28 onwards. Regarding pleuritis, the results have been grouped by the percentage of injured lungs in each batch; between 0 and 5% (43% vs. 63%; p value<0,001), between 5 and 20% (51% vs. 34%; p value<0,001) and >20% (5% vs. 3% of batches).

# **Discussion and Conclusion**

The average enzootic pneumonia index during 2023 increased during the second semester, and an increase in pulmonary pleuritis has been observed since the last quarter of 2022, and both statistically significant increases. The causes of this increase may be several, although the introduction of high virulent variant of PRRS (Rosalia PRRSv) along with the reduction of use of antibiotics against bacterial co-infections may be the most likely factors. Implementation of this type of monitoring complement health information systems to better understanding of respiratory disease complex dynamics in production systems.

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# EVOLUTION OF THE PREVALENCE AND SEVERITY OF LUNG LESIONS ASSESSED IN SLAUGHTERHOUSES IN SPAIN

M. Lasierra <sup>1</sup>, M. Carmona <sup>1</sup>, <u>D. Espigares</u> <sup>1</sup> <sup>1</sup>Ceva Salud Animal, Barcelona, Spain

### **Background and Objectives**

Assessment of lung lesions in slaughterhouses is a very useful tool to estimate the incidence of respiratory disease. The most frequently observed lesions in slaughterhouses are cranioventral bronchopneumonia, associated with Mesomycoplasma hyopneumoniae, the main agent of Enzootic Pneumonia (Ep); and dorso-caudal pleuritis, strongly associated with subacute and/or chronic Actinobacillus pleuropneumoniae (Ap). The aim of this study was to analyse the evolution of prevalence and severity of Ep-like lesions and Ap-like lesions over the years 2016-2023 in Spain.

# **Material and Methods**

A total of 986,203 lungs from 6,200 batches from different farms located around Spain were evaluated between 2016 and October 2023 using the Ceva Lung Program (CLP) score methodology, which assists lung scoring for Ep-like lesions using modified Madec grid, and dorso-caudal pleurisy (DCP) to evaluate Ap-like lesions. The following parameters were calculated at batch level: EP index Broncho-pneumonic lungs (%) (BPL) Affected surface out of all lungs (%) (ASAL) Dorso-caudal pleuritis (%) (DCP) AP index. Results were compared through Kruskal-Wallis test with pairwise comparisons and p-value was set at 0.05.

#### Results

Ep-like lesions indexes over time decrease from 2016 to 2023 (p<0.01), except for an increase during 2021 and 2022, both in terms of prevalence and severity (EP\_index 2016\_2.16; 2017\_1.81; 2018\_1.64; 2019\_1.45; 2020\_1.22; 2021\_1.32; 2022\_1.32; 2023\_1.05).DCP prevalence over time increases from 2016 to 2023 (p<0.01), while App index improves 2022-2023, indicating that, despite the increase in prevalence, the severity of DCP decreases in these years.

#### **Discussion and Conclusion**

There has been a positive evolution in the control of EP, possibly due to the improved effectiveness of the prevention plans implemented. The rebound of EP-like lesions in 2021-2022 may be associated with the severe cases of PRRS, Rosalia strain, which severely affected farms in the North-East of Spain, where the highest percentage of pigs are concentrated. In relation to DCP, no clear trend is observed. One of the reasons may be due to the large number of Ap serotypes present with high virulence diversity. The severity of DCP seems to be improving in the last two years, but not the prevalence. This trend should be monitored over time.

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# IMPACT OF VT2E VACCINATION ON OEDEMA DISEASE ON A COLOMBIAN SWINE FARM

R.D.C. Hinestrosa <sup>1</sup>, <u>G. García</u><sup>2</sup>, L. Andrés <sup>1</sup>, M. Tomàs <sup>2</sup> <sup>1</sup>HIPRA Colombia <sup>2</sup>HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

Oedema disease (OD) poses a significant threat to swine populations, attributed to Escherichia coli-induced enterotoxaemia. The pathogen's possession of adhesion factors facilitates colonisation of the small intestine, leading to the production of the verotoxin 2e (VT2e). This toxin, when absorbed into the bloodstream, induces vascular damage, growth impediments, neurological disorders, and elevated mortality rates. The aim of this study was to assess the efficacy of VT2E vaccination in mitigating OD-related challenges on a farm situated in Colombia.

# **Material and Methods**

The farm, exhibiting clinical signs of VT2E infection, was confirmed positive by VEROCHECK analysis (PCR on oral fluids targeting the VT2E gene). Two distinct groups were sequentially established: the non-vaccinated (NON-VAC) group, comprising 2488 piglets, and the vaccinated (VAC) group, which received VEPURED® (HIPRA, Spain) at 3 days of age, with 2457 piglets. Comprehensive productivity data for both groups were systematically recorded and subjected to comparative analysis using the ANOVA test.

For the economic analysis, the cost of the feed consumed, and the cost of the vaccine and treatments were compared in both groups.

# Results

In the VAC group, all production parameters exhibited progressive improvement. Notably, the lower mortality rate achieved statistical significance (p=0.038; ANOVA test), recorded at 7.43% (185 animals) for the NON-VAC group and 2.52% (62 animals) for the VAC group. Economic analysis revealed a substantial saving of \$23,747 (Colombian pesos) in the VAC group.

### **Discussion and Conclusion**

In line with previous studies, VT2E vaccination yielded substantial enhancements in key production parameters, particularly a significant reduction in mortality rates during the nursery period. This outcome is of paramount importance given the elevated value of piglets on the international market. The study scores the economic and physiological benefits of VT2E vaccination as a strategic measure in swine herd management, emphasising its potential to enhance both animal welfare and farm profitability.

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# PK/PD AND CLINICAL RELATIONSHIPS OF VETMULIN WATER SOLUBLE (TIAMULIN HYDROGEN FUMARATE) ADMINISTERED TO PIGS FOR THE TREATMENT OF SWINE DYSENTERY

<u>U. Klein</u><sup>1</sup>, M. Anderson <sup>2</sup>, L. Claerhout <sup>1</sup>, W. Depondt <sup>1</sup> <sup>1</sup>Huvepharma NV Antwerp, Belgium <sup>2</sup>Fermenta Animal Health Company, Kansas City, Missouri, USA

#### **Background and Objectives**

The pharmacokinetics (PK) of tiamulin hydrogen fumarate (THF) (Vetmulin<sup>®</sup> - Huvepharma NV) colon content concentration (CCC) based on water medication was related to tiamulin MICs against Brachyspira hydrogenteriae (Bhyo) and Brachyspira hampsonii (Bhamp) pharmacodynamics (PD).

# Material and Methods

In a PK study the tiamulin CCC was determined in groups of five pigs (average body weight 20.1kg) which were medicated in doses of 6.16mg/kg (60ppm), 13.2mg/kg (120ppm) and 20.9mg/kg bw (180ppm). Medicated water was offered ad libitum (5 consecutive days). On day 5 of the study pigs were euthanized and colon mucosa, colon content, lung and blood samples were collected for microbiological assay analysis.

Tiamulin MIC data were generated in a EU study based on susceptibility testing of Brachyspira hyodysenteriae (n=38) strains from Germany and UK isolated 2019-2020. In a susceptibility testing study conducted in the US tiamulin MIC data were generated on Brachyspira hyodysenteriae (n=83) and Brachyspira hampsonii (n=53) strains isolated 2013-2020. Tested MIC dilutions: 0.015 - 256µg/ml. MIC<sub>50</sub>, MIC<sub>90</sub> and MIC ranges were determined.

#### Results

A tiamulin CCC of 2.16µg/g at 6.16mg THF/kg bw dose was determined. At 13.2mg THF/kg bw and at 20.9mg THF/kg bw dose levels, tiamulin CCC of  $5.59\mu$ g/g and of  $18.58\mu$ g/g were recorded after water medication, respectively. In the EU MIC study tiamulin MIC<sub>90</sub> values of  $0.5\mu$ g/ml and an MIC range of  $0.015-16\mu$ g/ml were measured for the tested Bhyo strains. A tiamulin MIC<sub>90</sub> value of  $<0.06\mu$ g/ml and MIC range of  $0.06-4.0\mu$ g/ml (Bhyo) and of  $0.06-0.25\mu$ g/ml (Bhamp) were determined for the US Brachyspira strains. PK/PD relationships show that tiamulin CCC's achieved at the three tested dosages are high and exceed the tiamulin MIC values of all US Bhyo and B.hamp strains and 98% of the tested Bhyo strains from Europe.

# **Discussion and Conclusion**

On the basis of the available PK/PD data an excellent therapeutic effect of tiamulin water medication in the case of swine dysentery infection treatment at treatment dose of 8.8mg/kg bw can be predicted. The use of PK/PD data help to optimize treatment procedure according to prudent use policies and avoid treatment failures against swine dysentery.

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#### RECOMBINANT VEROTOXIN 2E(VT2E) VACCINE IMPROVES UNIFORMITY OF SLAUGHTERED PIGS.

H. Noh <sup>1</sup>, J. Byun <sup>1</sup>, S. Noh <sup>1</sup>, M. Choi <sup>2</sup>, D. Giralt <sup>3</sup>, G. Alvarez <sup>3</sup>, <u>I.B. Orozco <sup>4</sup></u> <sup>1</sup>HIPRA Korea <sup>2</sup>Kwanghee Animal Hospital (Wanju, Korea) <sup>3</sup>HIPRA, Amer (Girona), Spain <sup>4</sup>Corporate Brand Manager, HIPRA, Amer, Spain

# Background and Objectives

Subclinical swine oedema disease, caused by verotoxin 2e (Vt2e), only shows non-specific manifestations such as delayed growth and decreased uniformity. The objective of this study was to assess the benefit provided by a recombinant Vt2e vaccine by analysing the uniformity of slaughtered pigs before and after vaccination on a farm with subclinical swine oedema disease.

# **Material and Methods**

Swine oedema disease was confirmed on a Korean commercial farm with 600 sows (farrow to finish) by the detection of verotoxin-producing Escherichia coli (VTEC) by real-time PCR assay. The type was classified as subclinical due to the lack of specific symptoms. A vaccine containing recombinant Vt2e antigen (Vepured®) was applied. Data on individual slaughtered animals, such as the date of slaughter, carcass weight, thickness of backfat and grade of carcass, were collected at the slaughterhouse. Considering the average days to market in this farm, 12,544 animals which were slaughtered from January 2020 to May 2021 were defined as "Non-vaccinated" group and 8,419 animals which were slaughtered from June 2021 to September 2023 were defined as "Vaccinated" group. To estimate uniformity by group, the coefficient of variation of carcass weight was calculated in both groups. Distribution of carcass quality grade for both groups was also analysed. For the statistical analysis, linear regression model was performed with group as a factor for coefficient of variation and logistic regression with group as a factor was done for carcass quality grade.

#### Results

The coefficient of variation of carcass weight calculated for the "vaccinated" group (6.82%) was lower than for the "Non-vaccinated" group (7.45%), with a statistically significant difference (P-value<0.0001). Regarding the distribution of carcass quality grade, the "Vaccinated" group showed a higher proportion of Grade 1+ and 1(69.79%) compared to the "Non-vaccinated" group (61.76%), with a statistically significant difference (P-value=0.001).

#### **Discussion and Conclusion**

Benefits from the control of subclinical oedema disease can be estimated by using various parameters such as average daily gain and feed conversion ratio. The results of this study show that other parameters that have not usually been estimated, such as uniformity of animals or grade of carcass, can be also increased.

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# RISK FACTORS AND PREVALENCE OF COMMON BACTERIAL PATHOGENS CAUSING DIARRHEA IN NEONATAL PIGLETS IN VIETNAM

T. Phan <sup>1</sup>, T. Ngo <sup>1</sup>, K. Tran <sup>1</sup>, J. Miguel <sup>2</sup>, S. Traiyarach <sup>2</sup>, H. Vu <sup>3</sup>, N. Duong <sup>3</sup>, <u>D. Do <sup>1</sup></u> <sup>1</sup>Faculty of Animal Science and Veterinary Medicine, Nong Lam University, HCMC, Thu Duc city, Ho Chi Minh city, Vietnam <sup>2</sup>HIPRA, Amer (Girona), Spain <sup>3</sup>HIPRA Vietnam, Ho Chi Minh City, Vietnam

# **Background and Objectives**

Neonatal diarrhea is one of the most frequent diseases in modern swine production. It can be associated with high mortality, decreased growth rates and increase of antibiotic treatment. Several potential pathogens have been reported as causing agents, including Escherichia coli (E. coli), Clostridium perfringens (C. perfringens), Clostridioides difficile (C. difficile). The objective of this study was to investigate the prevalence of E. coli (F4, F5, F6 and LT toxin), C. perfringens (-toxin,  $\beta$ -toxin, -toxin) and C. difficile (A and B toxins) in neonatal piglets with diarrhea, as well as the related risk factors.

#### **Material and Methods**

A total of 40 commercial pig farms located in the North, Central and South of Vietnam were included in the study. 33 out of 40 farms had a vaccination scheme against neonatal diarrhoea in sow. Risk factors were investigated to understand their association with the diarrhea identified pathogens. Pooled fecal samples were collected from 3 piglets in 114 litters (1-14 days of age), using rectal swabs. Samples were inoculated in FTA Elute cards. The DNA extraction process, as well as the one-step multiplex PCR technique to detect target genes, were carried out using specific probes according to HIPRA Diagnos, Vietnam.

# Results

In farm level, C. perfringens was the most frequently detected pathogen (40/40, 100%) followed by E. coli (27/40, 67.5%) and C. difficile (24/40, 60%) (P<0.001). Co-infection with three pathogens (E. coli, C. perfringens and C. difficile) had a rate of 35% (14/40), when two pathogens (E. coli and C. perfringens) had a rate of 32.5% (13/40), and with other remaining two pathogens (C. perfringens and C. difficile) had a rate of 25% (10/40). Risk factors that play a role in relation to the surveyed pathogens and neonatal diarrhea are also clarified, including vaccination and parities of sows.

# **Discussion and Conclusion**

The results of the study confirmed that E. coli, C. perfringens and C. difficile are enteric pathogen of concern in neonatal piglets diarrhoea on swine farms. This study presents novel insights into the prevalence of these pathogens in Vietnam and emphasizes the need for ongoing vaccine research in neonatal piglets.

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# DEVELOPMENT OF AN ACCESSIBLE, PRACTITIONER-FOCUSED OUTREACH TOOL FOR MYCOPLASMA HYOPNEUMONIAE ERADICATION

A. Sponheim <sup>1</sup>, E. Fano <sup>2</sup>, M. Pieters <sup>3</sup>

<sup>1</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA

<sup>2</sup>Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA; Current affiliation: Pipestone Veterinary Services, Pipestone, MN, USA

<sup>3</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

#### **Background and Objectives**

Mycoplasma hyopneumoniae is by far the most important respiratory bacterial agent affecting pigs in the US<sup>1</sup>. Eradication of M. hyopneumoniae not only supports pig welfare, reduces antibiotic use, and improves production performance, but also provides a competitive advantage to pork producers, thereby promoting sustainability of the industry. To date, various M. hyopneumoniae eradication methods have been described and disease elimination efforts are growing. Here, we describe the development of a scientifically curated web-based outreach tool for M. hyopneumoniae eradication in the field, as an accessible, efficient, comprehensive resource for the swine practitioner who would like to be current with the mechanics of M. hyopneumoniae eradication programs.

#### **Material and Methods**

A framework for five M. hyopneumoniae eradication methods, including herd closure and medication, whole herd medication, depopulation/repopulation with and without off-site breeding projects, and partial depopulation, was outlined and organized into topics, with relevant literature, podcasts, and newly developed resources collected for each subject. Swine practitioners, professionals, and researchers who authored proceedings abstracts or peer-reviewed papers relevant to each topic were invited to review content, providing their most recent experiences, specific to their area of expertise. The web-based platform was then built to allow the information to be updated as new advances are made.

#### Results

Content was first developed for herd closure and medication and whole herd medication eradication methods over the course of one year, encompassing approximately 200 man-hours. Twenty-six of the 27 (96.3%) invited experts collaborated on the project, with levels of participation ranging from content review to development of new resources. Sixteen topics were summarized, composing 62 publications, 17 resources, and 16 recordings. Development of the remaining three methods is underway.

#### **Discussion and Conclusion**

A deep understanding of successes, best practices for application, potential limitations and causes of failure, are key for proper implementation of M. hyopneumoniae eradication programs and development of contingency plans. Providing the most relevant information in a single resource allows for efficient use by practitioners and facilitates the application of scientific literature. The willingness of swine practitioners, professionals, and researchers to share successes and failures through the web-based outreach resource is an example of true industry collaboration towards eradication efforts.

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# THE GUT MICROBIOME PROFILES OF POST-WEANING PIGS

P. Tansarawut <sup>1</sup>, P. Boonsoongnern <sup>2</sup>, A. Kamlangdee <sup>2</sup>, <u>Y. Woonwong</u> <sup>2</sup>, A. Boonsoongnern <sup>2</sup> <sup>1</sup>Veterinary Clinical Study Program, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, 73140

<sup>2</sup>Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, 10900

# **Background and Objectives**

Gut health has a significant impact on the overall health and growth performance of the pigs. Roles of gut microbiomes is crucial for the development of immunopathology and tolerance in host health and disease. One important strategy for improving gut health is understanding the profiles of gut microbiomes that play a crucial role in health, growth, development, and preparing the pig's intestine for the early stage of weaning. Identify the gut microbiome profile of the 5-6 weeks old nursery pigs and comparing the gut microbiome profile of the 5-week-olds to the 6-week-olds nursery pigs.

# **Material and Methods**

Total of 16 cross-bred piglets were observed in this experimental. Fresh fecal samples were collected at 5-week-olds (n=9) and 6-week-olds (n=7). Fecal genomic DNA was extracted from the sample by using ZymoBIOMICS<sup>™</sup> DNA Microprep Kit (Zymo Research, Irvine, CA, USA). The samples were stored at -20 °C until Next-Generation Sequencing. The sequence was performed using an Illumina iSeq Sequencing system (Illumina, San Diego, CA, USA).

### Results

Alpha microbial diversity indices, including chao1, shannon, and simpson indices, we found that the microbial biodiversity in the 6-week fecal sample was significantly higher compared to the 5-week (p < 0.05). Firmicutes and Bacteroidetes were the major phyla in the two groups, which together make up for over 90% of the entire piglet core gut microbiota. The relative abundance of the phylum Firmicutes, Patescibacteria and F/B ratio was significantly higher in younger pig than older pig (p < 0.05). On the other hand, the relative abundance of the phylum Bacteroidetes was significantly higher in older pig than younger pig (p < 0.05). In 5-week-olds, the predominant family was Lactobacillaceae, whereas in 6-week-olds, the predominant family shifted to Prevotellaceae.

#### **Discussion and Conclusion**

As the result indicate, the diversity of the gut microbiome in pigs increases as they grow older. Additionally, the types of bacteria also undergo changes, consistent with previous studies. Understanding the gut microbiome composition of young pigs contributes to enhancing their digestive health, ultimately leading to more efficient pig farming practices.

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# ANTIBODY RESPONSE DYNAMICS OF PIGS VACCINATED AND CHALLENGED AGAINST STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS

H. Burlet <sup>1</sup>, <u>A. Jeffery</u> <sup>1</sup>, A. Norte <sup>1</sup>, R. Nosach <sup>1</sup>, A. Finatto <sup>1</sup>, D. Gutierrez Montiel <sup>2</sup>, S. Kiorala <sup>1</sup>, S. Stubert <sup>3</sup>, M. De Oliveira Costa <sup>4</sup>

<sup>1</sup>University of Saskatchewan

<sup>2</sup>Universidad Autónoma de Aguascalientes

<sup>3</sup>University of Paraná

<sup>4</sup>University of Saskatchewan / Utrecht University

#### **Background and Objectives**

Streptococcus equi subsp. zooepidemicus (S. zoo) is an emerging pathogen of swine worldwide. It causes septicaemia, sudden death in pigs of any age, and abortion in sows. Outbreaks associated with S. zoo can be extremely costly, and antimicrobials are the only control method available as commercial vaccines are not available and autogenous do not elicit protection. Enzyme-linked immunosorbent assay (ELISA) is a fast and sensitive test that allows for relative measurement of antibody levels. The most common ELISA tests measure antibody levels against a single antigen. However, the isolation of a specific antigen for plate coating is costly and time consuming. The use of whole bacterial cells as an antigen is an alternative that maintains specificity of the test. The objective of the study was to develop and test an ELISA to characterize antibody response following vaccination and challenge of pigs with Strep zoo.

#### **Material and Methods**

Four piglets (sourced from a S. zoo negative farm) received 2 doses of an oral, live attenuated vaccine, 21 days apart. Fourteen days after the second dose, piglets were challenged orally with virulent S. zoo strains. Serum samples were collected prior to each vaccine dose, before challenge and at euthanasia (7 days post-challenge with S. zoo). Whole cellbacterial ELISA was used to measure total Ig antibody response. Ninety six-wells plates were coated with the inactivated vaccine strains grown to 10<sup>s</sup> CFU/mL. Serum from a positive (diseased animal) and negative (S. zoo negative piglet) were used as controls. Goat anti-swine conjugated IgG [IgG + IgM] was used for total immunoglobin (Ig) measurement. Data is presented as mean± standard deviation. Friedman test with multiple comparisons was performed to compare ranked means between sampling times.

# Results

Mean antibody titres for pre-vaccination, pre-challenge and at euthanasia were 731 ( $\pm$ 240.7), 2882.75 ( $\pm$ 1969.1), and 31904.75 ( $\pm$ 55544.1, respectively. A significant increase in antibody response was observed when comparing samples at pre-vaccination and euthanasia (P=0.04). There was no difference in antibody responses between pre- and post-vaccination.

# **Discussion and Conclusion**

An increased antibody levels was identified following challenge of vaccinated pigs. This evidences a systemic antibody response to the oral vaccine, providing further confirmation on the immunogenicity of the vaccination strategy used.
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# ASSOCIATION BETWEEN GROWTH AND FAECAL SHEDDING OF LAWSONIA INTRACELLULARIS ON PIGS VACCINATED AND NON-VACCINATED AGAINST ILEITIS

<u>R. Del Pozo Sacristán</u><sup>1</sup>, H. Swam<sup>2</sup>, S. Von Berg<sup>3</sup>, A.E. Taylor<sup>4</sup> <sup>1</sup>MSD Animal Health <sup>2</sup>Center for Diagnostic Solutions, MSD AH Boxmeer, The Netherlands <sup>3</sup>MSD Animal Health, Munich, Germany <sup>4</sup>University of Leeds, UK

# **Background and Objectives**

The aim of this clinical trial was to investigate the association between growth and faecal shedding of Lawsonia intracellularis on pigs vaccinated intramuscularly or not vaccinated against this bacterium.

# **Material and Methods**

A randomized, controlled, blind, side-by-side trial was performed in a herd with history of subclinical ileitis. At 4 weeks of age (woa), 240 piglets were allocated to Vaccination (V; n=120; vaccinated with Porcilis®Lawsonia mixed with Porcilis®PCV M Hyo) or Control (C; n=120; Porcilis®PCV M Hyo) group. Faecal samples (n=30/group) were taken (4, 7, 10, 13, 16, 19woa) from the same individual pigs directly from the anus at defecation. Bacterial load in faeces was assessed by qPCR (Ingenetix®). Daily feed intake and daily weights of the individual pigs were measured using pig performance testing technology (Pig Insight Asserva System for weaners/growers; Pig Performance Nedap ProSense system for fatteners). Average Daily Gain (ADG), Average Daily Feed Intake (ADI) and Feed Conversion (FCR) were calculated. The association between bacterial load and ADG, ADI and FCR for the 7 days prior to the peak of bacterial shedding was determined (linear regression). A multilevel mixed-effect linear model was also performed.

#### Results

No bacterial shedding was detected before 16woa. The average bacterial load (log10 copies/µl) was very low in both vaccinated and control pigs at 16woa (V:0.19±0.13; C:0.20±0.14; P>0.05) and 19wk (V:1.17±0.27; C:1.48±0.27; P>0.05). Average AUC from 4-19woa was numerically lower for vaccinates (14.96±20.64 log10 copies/µl) than for controls (17.53±19.85 log10 copies/µl) (P>0.05). There was a significant negative correlation between bacterial load in faeces and both ADG (R2=-0.193) and ADI (R2=-0.111) of controls, whereas there was no correlation in vaccinated pigs. There was a significant positive correlation between bacterial load and FCR (R2=0.136) of controls, whereas there was no correlation in vaccinated pigs.

#### **Discussion and Conclusion**

An increase in L. intracellularis bacteria/gram of faeces was associated with a reduction in ADG 7 days prior to sampling at the peak of shedding (19woa). However, this association was dependent on vaccination status and was only seen in unvaccinated pigs against L. intracellularis.

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# CHARACTERIZATION OF SEROVARES OF SALMONELLA ENTERICA ISOLATED FROM CLINICAL CASES IN PIGS IN BRAZIL

L. Fiorin Hernig<sup>1</sup>, F. De Quadros<sup>1</sup>, C. Pisseti<sup>2</sup>, E. De Conti<sup>1</sup>, J. Deon Kich<sup>3</sup>, A. Lino Fiúza<sup>1</sup>, A. Paula Teixeira<sup>1</sup>, <u>R. Tesche</u> Lippke<sup>1</sup>

<sup>1</sup>Boehringer Ingelheim Animal Health Brazil

<sup>2</sup>Centro de Diagnóstico de Sanidade Animal (CEDISA), Concórdia, Brazil <sup>3</sup>EMBRAPA Suínos e Aves, Concórdia, Brasil

# **Background and Objectives**

Salmonella spp. is an endemic agent in pig farms in Brazil. Between 2011 and 2017, the serovars Typhimurium monophasic variant (4,[5],12:i:-) and Choleraesuis showed higher prevalence in pig samples, referring to clinical cases in the field in Brazil. Therefore, this study aimed to identify Salmonella serovars isolated from pig farms in Brazil between 2017 and 2022.

#### Material and Methods

A total of 228 Salmonella isolates were studied, referring to clinical cases of salmonellosis occurring in nine Brazilian states from 2017 to 2022. Bacteriological isolation and typification of a representative isolate of each clinical case were run in the CEDISA laboratory through serum agglutination on a slide, according to the -Kauffmann- White classification for serovars Typhimurium and Choleraesuis. The other serovars were typed by using the microarray technique (Check & Trace Salmonella, Check Points, Wageningen, Netherlands).

#### Results

The results showed that among the 228 isolates analyzed, the monophasic variant of S. Typhimurium remains the most prevalent serovar (43%), followed by S. Choleraesuis (33%) and S. Typhimurium (13%). In addition, over the years analyzed, there was no considerable variation in the percentage of positive samples for the three mains isolated serovars.

#### **Discussion and Conclusion**

These data show that Salmonella remains a relevant agent in both enteric and septicemic clinical cases in pig herds in Brazil. The monophasic variant of S. Typhimurium was the most prevalent serovar. S. Choleraesuis, on the other hand, continues to be related to septicemic clinical conditions. These results alert us to the need to diagnose the involvement of Salmonella in clinical cases and seek forms of prevention, such as biosecurity and vaccination. Salmonella is present on farms in different states in Brazil and continues to cause clinical signs of salmonellosis, as well as being a risk to public health. Therefore, monitoring protocols and prevention strategies are essential to mitigate losses caused by these agents.

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# COMPARISON OF LUNG LESIONS AT SLAUGHTERHOUSE USING DIFFERENT VACCINES PROTOCOLS FOR MYCOPLASMA HYOPNEUMONIAE IN COLOMBIA'S PRODUCTION

E. Vargas <sup>1</sup>, J. Calveyra <sup>2</sup>, <u>R. Krejci</u> <sup>2</sup> <sup>1</sup>Ceva Animal Health, Colombia <sup>2</sup>CEVA ANIMAL HEALTH

### **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is the primary pathogen of Enzootic Pneumonia. Vaccination is a common tool used to reduce clinical signs, lung lesions and performance losses due to Mhyo infection. Evaluation of lungs at the slaughterhouse is a common method to assess the efficacy of vaccination. The aim of this study was to compare the efficacy of different vaccines for Mhyo, through evaluations of EP-like Lesions.

# **Material and Methods**

The evaluation was carried out in the 4 main swine producing regions in Colombia (Antióquia, Centro, Valle, Eje Cafetero), between January 2019 and July 2022. A total of 13.847 lungs of 163 herds were scored at the slaughterhouse for Enzootic pneumonia (EP)-like lesions using Ceva Lung Program (CLP) scoring methodology described previously. For each batch the indicators of EP-like lesions were calculated, using CLP application. The animals involved in this study were vaccinated with five different vaccination protocols: Group 1: Hyogen® & Circovac®, (N=711), Group 2 (Hyogen®) (N=6948), Group 3 (N=1529), Group 4 (N=3204), Group 5 (N=1455). The effect of the season was also evaluated: dry period (January-March and July-September) and rainy period (April-June and October-December). Comparative effects of lung lesions between treatments were calculated using ANOVA.

#### Results

Lungs from farms vaccinated in the Groups 1 and 2 showed statistically lower percentage of affected lungs with EP-like lesions than the other vaccines, 21,4% vs 68,7% (p<0.001). And less percentage of affected surface out of lungs with EP-like lesions than in the other groups 3,75% vs 8,9% (p<0,001). There was no difference in the effect of the season.

# **Discussion and Conclusion**

Vaccination of piglets in Groups 1 and 2 reduced the prevalence and severity of EP-like lesions compared to average of farms vaccinated with other vaccines. Lung lesion scoring at the slaughterhouse confirmed its relevancy in assessing respiratory health. Slaughter check results are increasingly accepted as valuable indicators of herd health by farmers and their veterinarians. These results can be used as basis for herd health improvement programs by farmers and their consulting veterinarians.

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# EFFECT OF THE VACCINATION AGAINST SHIGA TOXIN 2E E.COLI IN A PIG PRODUCTION FARM WITH HISTORY OF OEDEMA DISEASE: CASE STUDY

J. Calveyra <sup>1</sup>, J. Candeias <sup>2</sup>, V. Aguiar <sup>2</sup>, P. Filsner <sup>2</sup>, <u>D. Sperling</u> <sup>1</sup> <sup>1</sup>CEVA ANIMAL HEALTH <sup>2</sup>Ceva Animal Health, Brazil

# Background and Objectives

Oedema disease (OD) is one of the most important diseases that cause economic losses in the nursery phase. The objective of this study was to evaluate the effect of vaccination on a farm with a history of OD caused shigatoxigenic Escherichia coli (STEC) harbouring the stx<sub>2e</sub> gene and adhesins F18.

### Material and Methods

The study was conducted on a 1000 sow farm, in Minas Gerais, Brazil. The farm had confirmed history of OD and diagnosis of E.coli, with confirmed stx2e gene by isolation and PCR typing. The farm had a weekly batch management system and the observational period evaluated the effect of vaccination across 15 consecutive batches, with alternating vaccinated (G1) and unvaccinated groups (G2). In total, G1 included 8 batches, with 4177 piglets that received Ecoporc Shiga® (Ceva Animal Health), 1 mL IM at 3 DOA, and G2 included 7 batches, with 3784 piglets, unvaccinated. Numerous parameters were evaluated including mortality, weight gain (nursery phase 25,6 – 75,8 DOA) and isolation and characterization of STEC strains was also carried out during the study period. Zootechnical performance and mortality of the two treatments were analyzed using ANOVA.

#### Results

The history of OD was confirmed during the previous outbreaks with isolation of STEC E.coli (positive for F18 and stx2e toxin gene). No significant difference in initial age (G1 = 25.8 DOA; G2 = 26 DOA) and initial weight (G1 = 5.52 kg; G2 = 5.48 kg) between the two groups was found. Weight gain was higher in G1, with a difference in means of 1.034kg (G2-G1). Mortality in the nursery period was significantly lower in G1 (2.07%) vs. G2 (3.64%). (P<0.0001). During the study, neurological clinical signs were observed in three batches from G2, with confirmation of the agent by isolation of STEC E.coli and characterization by PCR.

#### **Discussion and Conclusion**

The OD challenge was evidenced on farm and supported by laboratory diagnosis confirmation. The benefit of vaccination against OD was proven by the reduction in mortality and increased weight gain at the end of nursery. Like other published studies, vaccination against OD can be a useful tool to reduce mortality and improve the zootechnical performance of animals.

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# EFFECTS OF DIETARY SUPPLEMENTATION WITH A PLANT-BASED PREMIX OF FEED ADDITIVES IN PIGLETS EXPERIMENTALLY CHALLENGED WITH BRACHYSPIRA HYODYSENTERIAE

M. Araujo <sup>1</sup>, M. Duarte <sup>1</sup>, J. Barbosa <sup>1</sup>, P. Correia <sup>1</sup>, M. Lourenço <sup>1</sup>, B. Oliveira <sup>1</sup>, L. Santos <sup>1</sup>, N. Oliveira <sup>1</sup>, D. Biasibette <sup>1</sup>, J. Bošnjak-Neumüller <sup>2</sup>, J. Raj <sup>2</sup>, M. Vasiljević <sup>2</sup>, <u>R. Guedes <sup>1</sup></u> <sup>1</sup>Departamento de Clinica e Cirurgia Veterinaria, Escola de Veterinaria, UFMG <sup>2</sup>PATENT CO DOO., Serbia

#### Background and Objectives

The most common control measurement of swine dysentery (SD) is antimicrobial administration, which has been progressively controlled<sup>1</sup>. Therefore, the aim of this study was to evaluate the effects of supplementation with a plant-based premix of feed additives (PPF) on controlling SD.

# Material and Methods

Forty 24-day-old piglets were randomly assigned to four groups: Negative Control (NC), not supplemented or challenged; Positive Control (PC), not supplemented and challenged with a pathogenic strain of B. hyodysenteriae; D1, supplemented with 1kg/ton of the PPF, and D2, supplemented with 2kg/ton of the PPF, both challenged with the same strain and supplemented for 18 days prior inoculation. Fourteen days after inoculation, all animals were humanly euthanized. Average weight gain and gross lesion were evaluated. Clinical diarrhea score was evaluated daily, based on the following scale (0 = normal, 1 = semi-solid, 2 = pasty, 3= watery and 4 = watery with mucus and/or blood). Animals were considered positive for diarrhea when presented a score 2 or greater, with the onset of diarrhea being the first experimental day were an animal for a given group was considered positive for diarrhea. The Skillings-Mack or Kruskal-Wallis test were performed for statistical analysis where applicable.

#### Results

No difference was observed on the weight gain or the clinical signs, however, the onset of diarrhea started at 11 days post inoculation (DPI) on the D2 group, while PC presented at 7 DPI. The gross lesions were significantly lower on D2 group in comparison with PC.

### **Discussion and Conclusion**

No previous report of PPF on the treatment and control of on live animals challenged with SD in experimental conditions was found. The reduction of gross lesions and delayed onset of diarrhea on D2 group indicate an action over B. hyodysenteriae infection, through unknow mechanism, that could potentially be associated with a direct action inhibiting the colonization of B. hyodysenteriae or a modulation of gut microbiome<sup>2</sup>, hindering it. Therefore, the PPF could potentially be an alternative for controlling SD in commercial farms.

#### References

- 1. Van-Boeckel et al, PNAS, 2015.
- 2. Qi, R. et al. Front Vet Sci 7, 1162 (2021).

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# EFFECTS OF ORAL VACCINATION AGAINST E. COLI F4/F18 ON PIG PERFORMANCE FROM POST-WEANING TO SLAUGHTER

<u>J. Sevin</u><sup>1</sup>, L. Faisant<sup>2</sup>, J. Fernand-Genty<sup>3</sup>, B. Fily<sup>3</sup>, S. Vigneron<sup>3</sup>, V. Burlot<sup>3</sup> <sup>1</sup>Socavet, 75 boulevard de penthièvre, 22600 Loudéac, France <sup>2</sup>Eureden, Z.A. de Lanjouan, BP 90251 - 22402 Lamballe Cedex, France <sup>3</sup>Elanco France SAS – Crisco Uno. Bâtiment C. 3-5 avenue de la Cristallerie. CS 80022 - 92317 Sèvres CEDEX

#### **Background and Objectives**

Limited data is available on effects of oral E. coli F4/F18 vaccination from post-weaning to slaughter. A farrow-to-finish farm wanted to improve the homogeneity and the percentage of pigs selected for slaughter at the first departure. First technical development was implemented for fattening. Then, the focus was placed on post-weaning where a E. coli F4 was detected. The farm presented with intermittent clinical episodes of post-weaning diarrhea. Oral vaccination with Coliprotec<sup>™</sup> F4/F18 (Elanco) was implemented on piglets at 18 days of age. The objective of this study was to evaluate effects of oral vaccination against E. coli F4/F18 on post-weaning, fattening and carcass quality.

# Material and Methods

Data from 22 batches between February 2021 and October 2022 were considered retrospectively in post-weaning, fattening and for carcass quality. Nine vaccinated batches alternated with 13 unvaccinated batches. Statistical analysis was performed with Student t-test and Mann-Whitney test.

#### Results

For the nine vaccinated batches, the weight at the end of post-weaning increased significantly by +2kg (p<0.01) and the ADG<sub>8-30</sub> by +42 g/d (p<0.01) compared to the 13 unvaccinated batches. The percentage of animals selected for slaughter at the first sending was significantly higher by +9% (p<0.05) on the vaccinated batches, which met the pig producer's objectives. In addition, carcass quality data showed a significant improvement of the lean meat content by +0.5% (p<0.05) and minimum muscle thickness by +1.2 mm (p<0.01) on the vaccinated batches.

# **Discussion and Conclusion**

This study showed significant improvement of 5 technical parameters on batches orally vaccinated with Coliprotec<sup>™</sup> F4/F18 (Elanco) in a farm with moderate and irregular post-weaning diarrhea. These results are consistent with the literature: oral vaccination against E. coli F4/F18 significantly improves growth performance in post-weaning and number of days in fattening unit. Furthermore, piglets with better performance in post-weaning preferentially deposit protein leading to an improvement in lean meat content. This data on carcass quality has never been demonstrated before. Moreover, this work shows the importance of managing colibacillosis in post-weaning, even without regular clinical signs, to improve technical and economic performance of farms from post-weaning to slaughter.

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# EVALUATION OF LUNG LESIONS AT SLAUGHTER AND COMPARISON OF ANIMALS BATCHES VACCINATED WITH DIFFERENT MYCOPLASMA HYOPNEUMONIAE VACCINES IN A CHINESE FARM

J. Zhang <sup>1</sup>, <u>R. Krejci</u><sup>2</sup>, P. Mazerolles <sup>2</sup> <sup>1</sup>Ceva Animal Health China <sup>2</sup>Ceva Animal Health France

#### **Background and Objectives**

Vaccination of pigs is a common practice to control Mycoplasma hyopneumoniae (Mhyo) infection, a primary cause of Enzootic pneumonia (EP). The objective of our study was to compare the prevalence and severity of EP-like lesions in slaughter pigs which were vaccinated with different Mhyo vaccines, in a Chinese swine farm.

#### Material and Methods

Evaluation of lung lesions at slaughter was performed in a conventional farm located in North China by using the Ceva Lung Program Scoring Methodology. Pigs involved in this evaluation received two different vaccines at weaning: Animals in Group1: a single dose of Hyogen®(Vaccine A), with 12 batches of 601 lungs evaluated from March 2022 to May 2022 and the Group 2: two shots of Mhyo IM (Vaccine B), with 24 batches of 1,435 lungs evaluated from December 2021 to February 2022. The prevalence of EP like lesions was recorded and statistically compared.

#### Results

The percentage of lungs with EP-like lesions was significantly lower in the vaccine A group than vaccine B (19.9% vs. 72.8%; p<0,01 with Student's t-test), as well as the average affected area of all lungs (0.9% in vaccine A vs. 5.0% in vaccine B; p<0,01). Animals vaccinated with vaccine A had an average of 0.6 of EP index, and animals vaccinated with vaccine B had 3.0, with highly significant difference (p<0.01).

### **Discussion and Conclusion**

In this study, lungs from pigs vaccinated with vaccine A showed superior lung health in terms of EP lesions compared to animals vaccinated with vaccine B.

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# FIELD ASSESSMENT OF EFFICACY OF TWO DIFFERENT AUTOGENOUS STREPTOCOCCUS SUIS BACTERINS FOR SOWS ON A DUTCH FARM

N. Auping <sup>1</sup>, <u>J. Schumans</u><sup>2</sup>, P. De Backer <sup>3</sup>, H. Gantelet <sup>4</sup> <sup>1</sup>Lintjeshof, Nederweert, The Netherlands <sup>2</sup>Ceva Santé Animale, Naaldwijk, the Netherlands <sup>3</sup>Ceva Santé Animale, Brussels, Belgium <sup>4</sup>Ceva Biovac, 6 Rue Olivier de Serres, 49070 BeaucouzE, France

### **Background and Objectives**

Streptococcus suis (S. suis) is an important cause of encephalopathy in pigs. In the Netherlands, serotypes 2, 7 and 9 are mainly linked to clinical signs in piglets. Antibiotics are the only treatment, a commercial preventive measure does not exist. The objective of this field observation is a comparison of the efficacy of two different S. suis autogenous vaccines.

# **Material and Methods**

The field observation was performed on a Dutch farm with 500 sows and working in a 4-week batch system. The sows and gilts were initially vaccinated with a standard autogenous vaccine (product A). The vaccine consisted out of two S. suis serotype 9 strains and a non-typable S. suis strain. Due to an ongoing high use of antibiotics and a high mortality rate, another autogenous vaccine (product B) was implemented. The composition of the vaccine remained the same but both S.suis serotype 9 strains were produced in a booster formulation. Next to this, probiotics were added to the piglet's feed. To compare the results of product A and B, the antibiotic use and mortality rate were monitored.

#### Results

In total 15 batches, 22,540 piglets, were monitored. The first group (G1), 8,844 piglets, was born out of sows vaccinated with product A. The second group (G2), 2,966 piglets, was born out of sows vaccinated with product A and probiotics were added to the feed. The third group (G3), 10,730 piglets, was born out of sows vaccinated with product B and probiotics were added as well. The mortality rate declined (G1 = 2.6%; G2 = 1.8%; G3 = 1.3%) and the same effect was visible in the antibiotic use. Therefore, the DDD (Defined Daily Dose Animal per farm per year) was determined for each group (G1 = 40.49; G2 = 18.73; G3 = 10.70).

# **Discussion and Conclusion**

Autogenous bacterins are a valuable tool in controlling S. suis outbreaks on a farm. They result in a reduction in mortality rate and antibiotic use. As every farm is different, a good follow up of the results of these vaccines is necessary. Therefore, a change in composition and production process are possible.

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# SCREENING FOR LAWSONIA INTRACELLULARIS IN DUTCH FINISHING FARMS WITH DIFFERENT CLINICAL SCENARIOS AND TECHNICAL RESULTS

W. Nico <sup>1</sup> MSD AH NL

#### **Background and Objectives**

The aim of this study is to get insight on the infection patterns of Lawsonia intracellularis (LI) and technical results of finishing pigs for the different clinical forms of lleitis in Dutch pig farms.

#### **Material and Methods**

In the period of February till August 2021, vets who submitted saliva samples for qPCR Lawsonia testing (in the BactoReal Lawsonia kit, Ingentix) to CDS in Boxmeer (The Netherlands), were asked to fill in a small questionnaire about that farm. The survey included questions about the clinical form of lleitis, performance data (ADG, FCR and mortality%), feeding system (dry/wet) and number of sows and finishing pigs on that farm site. The clinical form (acute, chronic, subclinical) was determined by the herd practitioner according to history, clinical evaluation and/or diagnostics (necropsy, histology, PCR). Collected data was combined with the pooled saliva (3 samples pooled per age groups results for detection LI (in Ct), with the four corresponding age group (10, 13, 16 and >19 weeks of age). Statistical analysis was done by Anova test.

#### Results

In total, 70 questionnaires and corresponding lab results were suitable for analysis. From the pooled saliva samples (n=195) analyzed, it was observed that 88,6 % of the farms had at least one positive pooled saliva sample and 82% had a Ct value below 34 (moderate load). Of the 70 farms, 21 (30%), 21 (30%) and 28 (40%) farms were classified by the attending vet as Acute, Chronic and Subclinical form, respectively. The corresponding ADG were 903<sup>a</sup>, 831<sup>b</sup> and 890<sup>a</sup> g/day, respectively; and mortality % 2,4<sup>a</sup> 2,7<sup>a</sup> and 1,8<sup>b</sup> (different superscript p<0,05). For FCR not enough data was present (n=16). No differences in prevalence of clinical forms of lleitis were found between dry or wet feeding system.

#### **Discussion and Conclusion**

LI is highly present on Dutch finishing farms. Significant differences in ADG were seen on farms presenting chronic vs acute and subclinical form of lleitis. High bacterial load (Ct<30) was detected on all 3 different forms of ileitis, in all age groups from 13 weeks and older, even in the absence of clinical signs or before the onset of clinical signs in case of acute ileitis.

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# EFFECT OF SEASONALITY ON THE INCIDENCE OF SLAUGHTERHOUSE-ASSESSED LUNG LESIONS IN SPAIN

M. Lasierra <sup>1</sup>, M. Carmona <sup>1</sup>, <u>D. Espigares</u> <sup>1</sup> <sup>1</sup>Ceva Salud Animal, Barcelona, Spain

#### **Background and Objectives**

Respiratory diseases are multifactorial. Assessment of lung lesions at slaughter is a useful tool for assessing the incidence and severity of Enzootic pneumonia (Ep) like lesions, and cranial and dorso-caudal pleurisy, associated with secondary bacterial pathogens and subacute or chronic Actinobacillus pleuropneumoniae (App) respectively. The aim of this study was to analyse the effect of seasonality on lung lesions assessed at slaughter.

### Material and Methods

6,200 batches and 986,203 lungs were assessed in slaughterhouses located around Spain between January-2016 and October-2023. Four periods are considered according to slaughtering season (spring-1152, summer-1209, autumn-2092 and winter-1744). Lungs were scored using Ceva Lung Program methodology. The following parameters were calculated at batch level: Enzootic pneumonia index (Ep\_index) Broncho-pneumonic lungs (BPL) (%) Affected surface out of all lungs (ASAL) (%) Affected surface of Bronco-pneumonic lungs (ASBL) (%) Cranial pleuritis (CP) (%) Dorso-caudal pleurisy (DCP) (%) Ap-index (App\_index) Results were compared through Kruskal-Wallis test with pairwise comparisons and p-value was set at 0.05.

#### Results

There is a statistical difference (EP\_index) between those slaughtered during autumn-summer and spring-winter. Specifically, pigs slaughtered in autumn show statistically more BPL than all other seasons. Considering ASAL, those slaughtered during spring-summer are statistically worse than those slaughtered during autumn-winter. Regarding ASBL, pigs slaughtered during summer are statistically worse than autumn, and these one worse than winter-spring. In relation to CP and DCP, pigs slaughtered during winter shows statistically worse than autumn -summer, and these one worse than spring. Considering APP\_index, pigs slaughtered during winter shows statistically worse than autumn, and these one worse than autumn, and these one worse than spring-summer.

# **Discussion and Conclusion**

There is higher prevalence of Ep-like lesions in batches fattened during summer. As for the severity of the lesions, batches fattened during spring-summer show more severity than those fattened in autumn-winter.Regarding the prevalence of CP and DCP, pigs fattened in autumn show more pleurisy than those fattened during spring-summer, and these one worse than those fattened during winter.Those fattened during winter show the least lung lesions, possibly contrary to what might be expected, perhaps because the colder season brings more attention to the control of environmental factors.

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# EVALUATION OF PERFORMANCE FROM WEANING TO SLAUGHTER BEFORE AND AFTER THE IMPLEMENTATION OF ORAL VACCINATION AGAINST E. COLI F4/F18

<u>T. Billy</u><sup>1</sup>, B. Monnerais<sup>2</sup>, J. Fernand-Genty<sup>3</sup>, B. Fily<sup>3</sup>, S. Vigneron<sup>3</sup>, V. Burlot<sup>3</sup> <sup>1</sup>HYOVET, SELAS de Surfonds, 72650 La Chapelle-Saint-Aubin, France <sup>2</sup>COOPERL, ZI Evre et Loire, 49600 Beaupréau-en-Mauges, France <sup>3</sup>Elanco France SAS – Crisco Uno. Bâtiment C. 3-5 avenue de la Cristallerie. CS 80022 - 92317 Sèvres CEDEX

#### **Background and Objectives**

Enterotoxigenic E.coli (ETEC) with attachment factors F4 and/or F18 produce enterotoxins which can induce diarrhea in piglets generally within two weeks following weaning. Even with moderate and irregular clinical signs of diarrhea after weaning, pig gut health and pig performance could be threatened by ETEC F4/F18. The aim of this retrospective study was to analyze performance results in fattening till slaughter before and after implementation of oral vaccination with Coliprotec<sup>™</sup>F4/F18 (Elanco).

#### Material and Methods

The present study was conducted in a farrow-to-finish farm in France. Piglets in post-weaning had moderate inconstant diarrhea and E.coli F4-F18-LT1-Sta-STb was detected. Slaughter data from 10 consecutive batches from August 2022 to May 2023 were analyzed retrospectively, 5 unvaccinated batches (2121 pigs) were followed by 5 vaccinated batches (2180 pigs). The batches compared received the same feed in post-weaning and in fattening. Wean-to-finish ADG was calculated by estimating the weight of the piglets at 6kg at 21 days of age. Statistical analysis was performed with the Mann-Whitney test.

#### Results

For the vaccinated batches, wean-to-finish ADG and lean meat content increased significantly respectively by +9g/d (p<0,001) and +0.3% (p<0,0001) compared to the unvaccinated batches. Age at slaughter, slaughter weight and minimum backfat thickness decreased significantly respectively by -3 days (p<0,0001), -1,4kg (p<0,0001) and -0,7mm (p<0,0001) on vaccinated batches.

# **Discussion and Conclusion**

Oral vaccination with Coliprotec<sup>™</sup>F4/F18 improved pig performance in a farm where piglets had occasionally moderate diarrhea in post-weaning. This study showed significant improvement of 4 technical parameters according to slaughter data: wean-to-finish ADG, age at slaughter, lean meat content and minimum backfat thickness. The results observed here in fattening are consistent with the literature. Piglets with accelerated growth in post-weaning had a higher lean meat content and lower backfat thickness at slaughter. Even if we cannot verify it here, improved growth in post-weaning after the implementation of oral E.coli F4/F18 vaccination, widely described in the bibliography, could be an explanation of the results observed on carcass quality. This study proved the need to manage colibacillosis in post-weaning, even without regular clinical signs, to improve performance of farms from weaning to slaughter.

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# ILEITIS VACCINATION STRATEGY AND OUTCOME ON CARCASS QUALITY

<u>R. Deitmer</u><sup>1</sup>, N. Rudolph<sup>2</sup>, R. Jansen<sup>1</sup> <sup>1</sup>Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany <sup>2</sup>Tierarztpraxis Pfannes, Wabern, Germany

#### **Background and Objectives**

In the German production system carcass payout per kg is based on a bonus malus system based on several carcass parameters established by ultrasonic measurements. Based on 32.000 data points per carcass, the different weights of meat cuts are determined and used to calculate the payout index. Objective of this study is to analyze the outcome of different lleitis vaccination strategies on carcass composition and payout index.

# **Material and Methods**

Subclinical ileitis (SI) was confirmed by PCR and antibody test in a German finishing pig production system during several timepoints. Two different ileitis vaccination strategies were evaluated on two different successive batches of grow-finish pigs: oral live vaccination (OL, n =1200; drinking water vaccination) & killed bacterin vaccination (KB, n =1200, intramuscular injection). Both vaccination strategies were applied 7 days after placement at the age of 10 weeks. Pigs were purchased from the same breeding herd. Pigs were housed in exact the same rooms and were fed the same commercial diets. Pigs were sent to a German slaughterhouse and carcasses were monitored by AUTOFOM. Data were compared and tested for differences in the meat cuts and their respective index points.

#### Results

Exposure to SI during the evaluation was confirmed with positive PCR results (faces). Mortality did not differ between both groups (#14 OI vs #15 KB; p=0.85). OL had higher index points compared to KB (gilts 1.015 vs 1.004 ; p<0,001; castrates 1.012 vs 0.997; p<0.001). Higher index points for OL compared to KB were due to heavier hams (gilts 20,3 vs 19.7 kg, adjusted to a uniform 96kg carcass weight; p<0.001) and higher lean meat percentages (gilts 63.3% vs 60.4%; p<0.001) and castrates 59.7% vs 58.4%; p<0.001).

### **Discussion and Conclusion**

The protein deposition in the OL group was higher resulting in heavier hams and higher lean meat percentages. Therefore, the index points of OL were higher. This is leading to a higher payout value of OL when compared to the KB. With a standard of 96 kg carcass weight and a baseline mean price of  $\epsilon$ 2.00/kg this resulted in a total of  $\epsilon$ 2.11 higher pay our per gilt carcass and  $\epsilon$ 2.88 per castrate carcass.

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# SALIVARY BIOMARKERS OF IMMUNE AND OXIDATIVE STATUS IN PIGLETS DURING AN OUTBREAK OF ACTINOBACILLUS PLEUROPNEUMONIAE.

<u>A.M. Gutiérrez</u><sup>1</sup>, F. Murciano<sup>2</sup>, B. Gonzalez-Guijarro<sup>3</sup>, A. Cuellar-Flores<sup>4</sup>, M. Matas-Quintanilla<sup>1</sup>, G. Ramis<sup>4</sup> <sup>1</sup>BioVetMed Research Group, Department of Animal Medicine and Surgery, Veterinary School, University of Murcia, 30100, Espinardo, Murcia, Spain <sup>2</sup>Facultad de Veterinaria, Universidad de Murcia, Spain <sup>3</sup>Departamento de Producción Animal. Universidad de Murcia

<sup>4</sup>Dpto. Producción Animal, Facultad de Veterinaria., Universidad de Murcia, Spain

#### **Background and Objectives**

Different pathogens produce variations in the health status of animals that can be evaluated in different matrices such as blood, saliva or faeces using biomarkers. In this work we have compared the level of salivary biomarkers in a batch of animals that suffered a clinical outbreak of pleuropneumonia with two other batches that did not suffer a clinical outbreak.

#### **Material and Methods**

Eighteen individual saliva samples were collected, according to PigMarkSaL guidelines, from pigs at 10<sup>th</sup> weeks of fattening in three different houses on the same farm. Sampling was performed on the day the animals in piggery 1 (P1) started to show symptoms of contagious pleuropneumonia, while no symptoms were observed in any of the other two piggeries (P2 and P3). Groups P1 and P3 were vaccinated against SwIAV in fattening, while group P2 was the unvaccinated control group. Clinical presumptive diagnosis was confirmed by necropsies and pathogen identification by PCR (Ct=30). C-reactive protein (CRP), haptoglobin (Hp), Pig-MAP, S100A12, Adenosine deaminase (ADA), Amylase, Cortisol, Total Antioxidant Capacity (TAC), total oxidant status (TOS) and Total Protein (TP) were determined in saliva. The presence of PRRSv, M. hyopneumoniae, SwIAV and M. hyorhinis was determined in oral fluid for the pens. The possible differences in levels of salivary biomarkers in the different groups of pigs (P1, P2 and P3) were analyzed using an Kruskal-Wallis non-parametric test.

#### Results

Concentration of CRP (p<0.001), Hp (p<0.001) and Pig-MAP(p<0.001) were found to be increased in the P1 group compared to P2 and P3. Differences were also observed in TAC (p<0.001), TOS (p<0.001) and TP (p<0.001), but these markers are increased in the P2 group in comparison to P3.

### **Discussion and Conclusion**

The clinical onset of contagious pleuroneupmonia results in an increase in innate immune markers such as CRP, Hp and Pig-MAP. On the other hand, clinically healthy pigs vaccinated against SwAIV showed a reduction in the levels of oxidative status in comparison to not vaccinated pigs. It is worth noting that the prevalence of PRRSv was 100% of the sampled blocks in P1, 67% in P2 and 39% in P3 that could influence the levels of salivary biomarkers.

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# ZOOTECHNICAL PERFORMANCE AND RETURN ON INVESTMENT IN ANIMALS VACCINATED AGAINST PROLIFERATIVE ENTEROPATHY ON A COMMERCIAL PIG FARM IN BRAZIL.

J. Borges Da Silva <sup>1</sup>, B. Maria Ferreira Passos E Prado Marques <sup>1</sup>, A. Gabrielle De Souza Daniel <sup>1</sup>, E. Raele De Oliveira <sup>2</sup>, L. Carlos Crestani <sup>2</sup>, A. Paula Pavaneli <sup>2</sup>, <u>S. Von Berg</u> <sup>3</sup> <sup>1</sup>*MSD Saúde Animal* <sup>2</sup>*InsideSui Pasarah Santiess, Insidesui, Patrocínia, MG, Brazil* 

<sup>2</sup>InsideSui Research Services, Insidesui, Patrocínio - MG, Brazil. <sup>3</sup>MSD Tiergesundheit

# **Background and Objectives**

Proliferative enteropathy is an endemic disease caused by Lawsonia intracellularis with a prevalence of 73% in Brazilian pig farms. This disease has economic impact and can reduce daily weight gain by 20.8% and increase feed conversion by 20.4%. The vaccination has been shown to be an important tool for control this disease. The aim of this study was to evaluate the effect of vaccination in zootechnical and economic performance compared with non-vaccinated pigs in a commercial farm.

# Material and Methods

Piglets from a positive farm for L. intracellularis were divided in two groups, negative control (n = 120) and vaccinated group (n = 120). The animals were randomized in block design and Porcilis® lleitis vaccine (2mL, IM at neck, Merck Animal Health, Madison, NJ, USA) was administered at 24 days old at vaccinated group. The animals were weighed individually at 63 (nursery exit), 120 (growing-finishing transition) and 156 days old (slaughter). Daily feed intake (DFI), daily weight gain (DWG), feed conversion ratio (FCR) and return on investment (ROI) were measured. Data were submitted to ANOVA using the Minitab 19 statistical program. Differences with p < 0.05 were considered statistically significant.

#### Results

Lower FCR were observed for vaccinated pigs considering the interval between 120 and 156 days old (2.143 vs. 2.341; p = 0.005), and the entire evaluation period (1.940 vs. 2.015; p = 0.011). In addition, vaccinated pigs were 2.52 kg heavier at slaughter (p = 0.454, not significant). About economic analysis, vaccination results in a gain of R\$ 37.50/pig and a ROI of 7.11 times the initial cost. The cost efficiency index (CEI, ratio between costs from both groups) shows an improvement of more than 26% for vaccinated pigs.

# **Discussion and Conclusion**

According to the results, vaccination results in greater productive and economic efficiency. One potential explanation for these results comes from the type of diarrhea caused by Lawsonia intracellularis, which is malabsorptive. The bacteria act on enterocytes, leading to cell proliferation and consequently an increase in immature cells with worse absorptive capacity. Vaccination is a proven tool for improving both clinical and subclinical proliferative enteropathy.

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# BENEFICIAL EFFECTS OF VACCINATION AGAINST OEDEMA DISEASE IN A VTEC-POSITIVE WEANER FARM IN POLAND

<u>P. Cybulski</u><sup>1</sup>, A. Formanowski<sup>2</sup>, R. Jorda<sup>3</sup> <sup>1</sup>Goodvalley Agro S.A., Przechlewo, Poland <sup>2</sup>HIPRA, Warsaw, Poland <sup>3</sup>HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

Highly prevalent in all swine rearing countries, verotoxin-producing Escherichia coli (VTEC) is a causative agent of porcine oedema disease (OD). The disease is a peracute enterotoxaemia of nursery pigs. Clinically affected animals are medicated with antibiotics, but their efficacy is usually severely limited by the toxin absorption and destruction of vascular endothelial cells. OD outbreaks are characterised by a high lethality; however, vaccination against the disease has been reported as an effective and safe tool in the reduction of significant financial losses. Therefore, the aim of our investigation was to evaluate the influence of OD vaccination on basic production parameters in a high-performing modern weaner farm located in Poland.

# **Material and Methods**

This study was performed in a Polish weaner farm with 8200 places. Before the trial the herd was diagnosed with OD based on clinical signs, autopsies, and VTEC detection in PCR tests. Group NON-VAC (weaned in 28 consecutive weekly batches) was not immunised against OD, while all the animals in Group VAC (weaned in another 28 weekly batches) received VEPURED injection (Hipra, Spain) at day 3. The total number of pigs weaned in Groups NON-VAC and VAC was 19686 and 20084, respectively. All the animals were born on a farm with 3600 DanBred sows and weaned after 4 weeks of suckling. Antimicrobial treatments and feeding programmes remained unchanged during the trial. Data regarding mortality rate (MR) and average daily weight gain (ADWG) were tracked.

#### Results

ADWG was numerically higher in Group VAC (496 g/day) compared to Group NON-VAC (486 g/day). MR was 4.43 % and 3.07 % for NON-VAC and VAC animals, respectively. The difference (1.36 percentage point) was found to be statistically significant (p<0.001; analysed using a logistic regression with a group as a factor).

# **Discussion and Conclusion**

The mortality of the animals was significantly reduced by vaccination with VEPURED. Our results corroborate data indicating that the vaccine can be considered a standard tool against high mortality caused by OD and its negative economic impact.

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# CASE REPORT: ERYSIPELOTHRIX RHUSIOPATHIAE - A REEMERGING OLD DISEASE?

<u>K. Gerdes</u><sup>1</sup>, E. Grosse Beilage<sup>1</sup>, L. Moorkamp<sup>2</sup>, S. Trittmacher<sup>1</sup>, I. Hennig-Pauka<sup>1</sup> <sup>1</sup>Field Station for Epidemiology, Bakum, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany <sup>2</sup>Tierärztliche Praxis Peheim, Peheim, Germany

#### **Background and Objectives**

Erysipelothrix (E.) rhusiopathiae is a bacterial agent causing erysipelas. The pathogen is ubiquitous, but disease became rare in recent decades likely due to consequent sow herd vaccinations. In winter 2022, a farmer reported piglets with erysipelas and an increase of sudden deaths in fattening pigs. In spring 2023, one sow showed erysipelas and the sow herd showed an increase of abortions and sudden deaths.

### **Material and Methods**

Pigs that died spontaneously and abortion materials were submitted for pathological examination and further diagnostics. Additionally, lithium-heparin blood and skin samples for bacteriological examination were collected from live piglets with clinical erysipelas. Affected piglets and sows were treated with amoxicillin and an anti-steroidal anti-inflammatory drug.

# Results

In spontaneously died fattening pigs, the main findings were endocarditis valvularis thromboticans and joint lesions; E. rhusiopathiae was detected by bacteriological culture. The pathogen was also detected in lithium-heparin blood and skin samples. In the necropsy the spontaneously died sows showed signs of sepsis and joint lesions. E. rhusiopathiae was detected in one sow. No pathogen was detected in the abortion material. Serotyping of four isolates resulted in serotype 2 in all of them.

#### **Discussion and Conclusion**

Although the sows have been regularly vaccinated against E. rhusiopathiae serotype 2, erysipelas occurred in the vaccinated animals as well as piglets and fattening pigs. There was no indication of deficits in vaccine storage or administration. The reduction of routine use of antibiotics in pig farming and/or inadequate protection of the animals by vaccination could be the reason. The pathogen is crucial in terms of food law due to its zoonotic potential and the risk of being infected is especially high among farmers, veterinarians and slaughterhouse employees.

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# COMPARATIVE ANALYSIS OF TWO MYCOPLASMA HYOPNEUMONIAE VACCINES ON LARGE-SCALE PIG FARMS IN CHINA

<u>W. Fei</u>, D. Giralt <sup>2</sup>, I. Bernal <sup>2</sup>, B. Wang <sup>1</sup>, P. Tu <sup>1</sup> <sup>1</sup>*HIPRA China, Beijing* <sup>2</sup>*HIPRA, Amer (Girona), Spain* 

### **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is a significant cause of swine enzootic pneumonia and poses substantial economic challenges for intensive swine producers. The aim of this study was to evaluate and compare the impact of two Mhyo vaccines on key production parameters.

### Material and Methods

Data were collected in 2023 from two large-scale pig farms in China housing 4,800 and 6,000 sows respectively. MYPRAVAC® SUIS (M) was administered to seven batches totalling 21,716 pigs, while another vaccine (Z) was given to eight batches totalling 22,466 pigs. Both vaccines followed an identical administration schedule: one dose administered at 7 days and another at 21 days of age. All weaned pigs originated from the same breeding farm and were subsequently sent to a secondary site for fattening.

# Results

Significant differences were noted in mortality rates, with the M group displaying a lower rate of 2.06% compared to the Z group's 3.22%, p < 0.001. However, no significant variations were observed in other production parameters such as Feed Conversion Ratio (FCR), Average Daily Gain (ADG), and Drug Cost. The M group showed an incremental gain of 5 RMB/pig in comparison to the Z group. While seemingly modest, these gains translate into substantial benefits for large-scale pig farms, resulting in annual savings exceeding 1,200,000 RMB.Logistic regression, treating group as a factor, was conducted to evaluate mortality. Linear regression, considering group and age at entry as factors, was employed for FCR and ADG analyses. A linear regression with group as a factor was used to assess drug cost. All analyses were conducted using R software v4.3.

### **Discussion and Conclusion**

Mhyo remains a significant economic burden for intensive swine producers. Vaccination has been proven to be effective in disease control and enhancing revenue. Based on our field data analysis, MYPRAVAC® SUIS demonstrates superior effectiveness in controlling Mhyo disease and offers a higher return on investment.

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# EFFECT OF VACCINATION AGAINST SHIGA TOXIN 2E IN A SOW FARM WITH THE HISTORY OF OEDEMA DISEASE

D. Krueger <sup>1</sup>, M. Schlegel <sup>1</sup>, C. Waehner <sup>2</sup> <sup>1</sup>Tierärztliche Praxis am Weinberg, Weinberge 39a, Jessen, Germany <sup>2</sup>Ceva Tiergesundheit GmbH Kanzlerstr. 4, 40472 Düsseldorf - Deutschland

### **Background and Objectives**

Escherichia coli (E. coli) are a major cause of disease and mortality in nursery. Clinical signs vary depending on the pathotype involved either resulting in diarrhoea or oedema disease (ED). ED is characterized by high losses and reduced performance, leading to reduction of daily weight gain and increased antimicrobial treatments. It is caused by Shiga-toxin (stx-2e) producing E. coli, which can harbour F18 fimbria for attachment. This case report describes a farm with an acute outbreak of ED monitored before and after implementation of vaccination against the disease.

# **Material and Methods**

The 3.500 sow farm located in Eastern Germany experienced first clinical signs of ED in animals in May 2023. Affected Animals were sent to the laboratory for necropsy and were tested positive for stx-2e producing E. coli. Additionally pooled faecal samples were tested and confirmed the results of the necropsy. The farm started vaccinating piglets with a commercial vaccine (ECOPORC SHIGA, Ceva Animal Health) at 4 days of age in July. The benefit of vaccination was evaluated by comparing average daily weight gain (ADWG), days in nursery and mortality of non-vaccinated animals positive for ED (G1) and vaccinated animals (G2).

# Results

The two groups did not differ significantly in weight at inclusion, however a tended to have a higher weight at the end of nursery (G1: 28,27kg, G2: 29,01kg, p=0.072). The ADWG from weaning to end of nursery was for G1 492,17gr and 517,06gr for G2 (p<0.001). Regarding the average days in nursery animals in G1 left nursery after 52,63days compared to 47,49days for G2. Mortality in both groups during nursery did not differ significantly with a 2.1% in G1 and 1.8% in G2.

# **Discussion and Conclusion**

The benefit of vaccination against ED on a farm with an acute outbreak involving of stx-2e E. coli strains was shown by a higher ADWG, reduced days spent in nursery and a lower mortality. Differences in antibiotic treatments could not be evaluated as precise data was not available for the evaluated time frame. Nevertheless, the data show the added value for the farm in terms of improved economy and higher animal welfare.

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# EVALUATION OF THE STATUS OF PLEURITIS LESIONS IN SWINE IN VIETNAM 2022-2023 USING LUNG LESION SCORING APPLICATION

C.N.A. Nguyen <sup>1</sup>, P.H. Nguyen <sup>1</sup>, M.T. Kieu <sup>1</sup>, R. Chompuming <sup>2</sup>, <u>K.A. Fabros</u> <sup>3</sup> <sup>1</sup>Elanco Animal Health Vietnam <sup>2</sup>Elanco Animal Health Thailand <sup>3</sup>Elanco Animal Health Asia

### Background and Objectives

Dorso-caudal pleurisy or pleuritis is a chronic lesion commonly associated with Actinobacillus pleuropneumoniae (APP). It has a significant impact on health and growth performance causing mortality outbreaks, reducing average daily gain, and increasing feed cost due to poor feed conversion ratio (Fittipaldi et al., 2005). The study aimed to monitor the pleurisy lesions in slaughtered pigs in Vietnam.

# **Material and Methods**

2,722 lung samples from forty-four commercial farms across Vietnam were evaluated at slaughterhouses from January 2022 to October 2023 to determine the prevalence of pleurisy infection status. The pleurisy scores were recorded using the Elanco Lung Lesion Scoring (LLS) application which is based on the SPES method (Dottori et al., 2007).

#### Results

In this result, 69.9% of lungs had no lesion. Lungs with cranial pleurisy (P1) were 26.8%. This lesion is often associated with Mycoplasma hyopneumoniae in combination with Pasteurella multocida. Pleurisy 2-4 represents dorsal pleurisy accounting for 17.8% and severe bilateral pleurisy (P4) accounts for 50% of the total pleurisy that is associated with APP. The results showed that chronic APP infection in Vietnam was serious.

This study provided an APPI (Actinobacillus Pleuropneumonia Index) range, a means of classifying the 44 farms. The best 25% of them had an APPI <0.269; the next best 25%, had an APPI between 0.269 and 0.444; the next 25%, had an APPI between 0.444 and 0.828; and the worst 25%, had an APPI >0.828. Farms with a high APPI range may consider a control program to improve the herd's respiratory health.

#### **Discussion and Conclusion**

The results showed that all the farms whose lungs were scored at the slaughterhouse had pleurisy lesions. Although there was a difference in the level of damage, the percentage prevalence remained high (17.8%). The LLS application helped monitor the infection status, thereby providing an appropriate antibiotic program to limit the impact of pleurisy.

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# GLOBAL MASS SCREENING FOR THE DETECTION OF THE VEROTOXIN (VT2E) ON SWINE FARMS WORLDWIDE BY ORAL FLUIDS

<u>G. García</u><sup>1</sup>, R. Jordà<sup>1</sup>, G. Alvarez<sup>1</sup>, I. Bernal<sup>1</sup>, L. Nodar<sup>1</sup> *HIPRA, Amer (Girona), Spain* 

#### **Background and Objectives**

Oedema disease (OD) is an enterotoxaemia caused by Escherichia coli, which possesses adhesion factors enabling the bacteria to colonise the small intestine and produce the verotoxin 2e (VT2e). VT2e is absorbed into the blood circulation and causes vascular damage, growth problems, nervous disorders and mortality. The aim of this study was to evaluate the presence of the gene coding for the verotoxin on farms where OD was suspected in a selection of countries worldwide.

### **Material and Methods**

For the analytical procedure, 19,633 oral fluid samples were obtained from 3,785 farms from 47 countries, over the last 6 years (from 2017 to September 2023). These oral fluids were then transferred to FTA Elute cards and samples were sent to HIPRA DIAGNOS for performance of a qPCR analysis targeting the gene coding for the verotoxin (Vt2e)<sup>2</sup>. A farm was considered positive when at least one of the samples was positive

#### Results

The mean prevalence of positive farms globally was reported to be 62.75% positive (2375 out of 3785 farms). When assessing the prevalence by region, we observed that the prevalence is high and very constant worldwide. In Europe, we analysed 2,224 farms, with 61.1% testing positive. In America, 64.1 % of 679 farms were positive. In Asia, 65.9% out of a total of 882 farms were positive.

Analysing the prevalence over the 6 years of the study, this remained stable over time: in 2017 it was 68.5% positive out of 54 farms analysed; in 2018: 56.5% out of 481 farms; in 2019: 63.6% out of 546; in 2020: 62.4% out of 583; in 2021 62.4% out of 726; in 2022: 64.7% out of 816 and finally during 2023: 64.6% out of 579 farms were positive.

#### **Discussion and Conclusion**

The results obtained are in line with findings from a previously published systematic literature review, where the Vt2e gene prevalence was estimated to be between 30 and 70%. It demonstrates that OD remains a major challenge for swine production worldwide, particularly in the context of sustainable production with limited use of antibiotics.

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# IN VITRO SUSCEPTIBILITY OF BRACHYSPIRA HYODYSENTERIAE TO PLANT-BASED ANIMAL HEALTH PREMIXES

Z. Jakovcevic<sup>1</sup>, J. Bošnjak-Neumüller<sup>1</sup>, S. Cujic<sup>1</sup>, H. Farkas<sup>1</sup>, J. Raj<sup>1</sup>, M. Vasiljevic<sup>1</sup> <sup>1</sup>PATENT CO DOO., Serbia

#### **Background and Objectives**

The genus Brachyspira represents a group of nine bacterial species, of which some are pathogenic to mammals and birds (1). For example, B. hyodysenteriae is associated with swine dysentery (SD), while B. pilosicoli and B. intermedia are known for spirochetal colitis in pigs, poultry, and humans (2). This study aimed to test in vitro antibacterial efficiency of the plant-based animal health premixes (PHP) with and without tannin addition.

#### Material and Methods

The minimal inhibitory concentration (MIC) of tested formulations from 0.0125% to 6,4% (2-fold dilutions), expressed in % (v / v), against ATCC 27164 B. hyodysenteriae strain, B. pilosicoli ATCC 51139 strain and Brachyspira intermedia (Collection of L'Istituto Zooprofilattico sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini") were determined using the broth microdilution susceptibility test following the procedures indicated by CLSI M31-A3. The tested mix PHP1 dominantly consisted of an essential oil blend of thyme, oregano, and coriander, with the addition of lysozyme and nicotinamide. The mix PHP2 has the same composition as PHP1 with the addition of tannins that originate from Castanea sativa extract. Both formulations PHP1 and PHP2 are the property of PATENT CO. company.

#### Results

Lab results showed that both formulations have antibrachyspiral activity. However, PHP2 formulation is achieving antibacterial activities in lower concentrations on all 3 strains. The MIC of PHP1 against B. hyodysenteriae, B. pilosicoli, and B. intermedia are all 0,25 %, while for PHP2 are 0,0625%, 0,125%, and 0,0625%, respectively.

#### **Discussion and Conclusion**

The results of our in vitro testing show us that tannins can play an important role in antibrachyspiral activity of the PHP2 formula, which already showed to control SD in in vivo conditions (3,4). Our future studies will be focused on understanding the antibacterial mechanism of active ingredients present in tannins and essential oils.

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# M.HYO ERADICATION: ANALYSIS OF PRODUCTIVE PARAMETERS

J. Gayán <sup>1</sup>, P. Sánchez <sup>1</sup>, C. Lasa <sup>2</sup>, J. Hernández <sup>2</sup>, <u>N. Casal <sup>2</sup></u> <sup>1</sup>Ars Alendi S.A., Spain <sup>2</sup>ECUPHAR SLU

#### **Background and Objectives**

Mycoplasma hyopneumoniae is considered the most important non-viral respiratory disease in swine. The economic impact of the disease is significant, affecting production costs, and increasing the susceptibility to other pathogens. For this reason, eradicating the disease typically offers significant advantages.

This study aims to assess the productive changes obtained in a sow farm where mycoplasma was eradicated.

### **Material and Methods**

The eradication program was conducted during summer 2021, in a 3000 productive sows farm (Danbred x Pietrain) located in Spain. Before the eradication, animals younger than 9 months were removed (except nursing piglets). Adult animals were medicated with Tylvalosin premix (2,125 mg/kg) for 32 days and animals showing a reduction in feed intake were injected with tulathromycin (2,5 mg/kg). Sucking piglets older than 3 days of age were injected with tulathromycin twice (2,5 mg/kg) separated by 7 days. The choice of antibiotics was influenced by the ineffectiveness against Mycoplasma spp. within the antibiotics categorized under group D (EMA category). Additionally, the decision was informed by the comparatively lower Minimum Inhibitory Concentrations (CMI) demonstrated for each antibiotic. Sentinel sows were analysed after 2 and 4 months in order to confirm the success of the eradication. Feed conversion rate (FCR), average daily gain (ADG), mortality during fattening period and medication cost per pig were compared before and after the eradication took place.

# Results

Medication costs presented a significant decrease in animals reared without mycoplasma (2,936  $\in$ /sold pig vs 1,293  $\in$ /sold pig, p <0,001). Furthermore, mortality during the fattening period presented a significant decrease after the eradication was concluded (6,57% vs 5,40%, p =0,014). No significant differences were observed in FCR and ADG.

### **Discussion and Conclusion**

Our results show a 56% reduction in drug costs, equivalent to €1,64 per sold pig. This reduction is probably attributed not only to the direct impact of Mycoplasma hyopneumoniae but also to the decrease in all secondary respiratory pathologies associated. This explanation may also account for the reduction in the mortality reported in this study. To further analyse possible differences in FCR and ADV, individual animals should be considered in future experiments instead of the entire fattening batch.

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# ASSESSING ETIOLOGICAL AND ECONOMIC CONSEQUENCES OF PLEURISY IN PIGS DURING THE FINISHING PHASE IN BRAZIL

C.S. Malcher<sup>1</sup>, F.M. Petri<sup>1</sup>, G.A. Aguiar<sup>1</sup>, L. Arruda<sup>1</sup>, G.Y. Storino<sup>1</sup>, L.T. Toledo<sup>2</sup>, K. Sonalio<sup>1</sup>, F. Hirose<sup>3</sup>, L.G. De Oliveira

<sup>1</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil <sup>2</sup>Federal University of Viçosa (UFV), Viçosa - MG, Brazil <sup>3</sup>New technologies strategy development Department, Ourofino Saude Animal, Cravinhos, Brazil

#### **Background and Objectives**

The evaluation of respiratory tract lesions emerges as a crucial approach to monitoring the health status of the herd. This study seeks to investigate the etiological and economic effects of pleurisy, focusing on the identification of associated pathogens and the evaluation of their financial impacts during the finishing phase in the Brazilian pig industry.

### **Material and Methods**

A total of 576 pigs from a single farm were placed in a shed, where they were weighed at the beginning of the finishing period and at the time of slaughter to determine daily weight gain. Of these animals, 165 pigs (approximately 28.6% of the total) were selected and examined individually due to the detection of lesions. Pleurisy severity was assessed by macroscopic examination using the Slaughterhouse Pleurisy Evaluation System (SPES), being classified on a scale of 0 to 4 according to the severity of pleural lesions. Simultaneously, lung tissue samples were collected to quantify the presence of pathogens, including Mycoplasma hyopneumoniae (Mhyo), Actinobacillus pleuropneumoniae (App), and Pasteurella multocida (Pm), using real-time PCR (qPCR). Subsequently, the lungs were submitted to pathological analysis, macroscopically and microscopically, to characterize the lesions of pneumonia and pleurisy. To correlate the findings, the Person test, using the software R. To determine the financial impact, a method was adopted that allocated the production costs and calculated the economic indicators, maintaining the principle "Ceteris paribus" to ensure a uniform cost for all pigs in the sample.

### Results

The impact on pig productivity showed an average loss of \$62.87 per carcass or \$0.766 per kilogram of carcass meat. The qPCR results indicated a positive correlation between three pathogens in pigs with grade 3 lesions, specifically the interaction of Mhyo, App, and Pm (r = 0.31 and p = 0.01). In addition, it is noteworthy that lung samples tested positive for Mhyo revealed statistically significant differences (p > 0.05) between grade 1 (p = 0.11) and grade 3 (p = 0.01).

#### **Discussion and Conclusion**

Our findings reveal the presence of coinfections involving diverse pathogens in commercial herds in Brazil, resulting in an economic impact of \$0.766 per kilogram of carcass meat.

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# COMPARISON OF MINIMUM INHIBITORY CONCENTRATIONS OF INNOVATOR TIAMULIN VERSUS GENERIC TIAMULIN AGAINST MYCOPLASMA HYOPNEUMONIAE ISOLATED ACROSS THAILAND

<u>W. Dee-Ampai</u>, A. Ratanamongkolmas <sup>1</sup>, S. Simjee <sup>2</sup>, S. Talummuk <sup>1</sup> <sup>1</sup>Elanco (Thailand) Ltd., Bangkok, Thailand. <sup>2</sup>Elanco Animal Health, Greenfield, IN, USA

#### **Background and Objectives**

Generic versions of antibiotics are not required to demonstrate therapeutic equivalence because therapeutic equivalence is assumed from pharmaceutical equivalence. Concerns have recently emerged about the potency and the quality of generic products based on experiments in neutropenic mouse thigh infection models. To test such assumptions, we studied innovator Elanco tiamulin (Denagard<sup>®</sup>) and generic tiamulin by microbiological assay against porcine Mycoplasma hyopneumoniae.

# Material and Methods

A total of 32 M. hyopneumoniae isolates from pigs exhibiting respiratory symptoms were collected across Thailand. MIC against innovator tiamulin and a generic tiamulin was determined as described by Hannan (2000) with BHL broth. All isolates incubated at  $35 \pm 1$  °C for 5-12 days. M. hyopneumoniae NCTC 10110 was used as Quality Control isolate. Interpretive criteria for MIC were Susceptible = <16µg/ml and Resistance = ≥32µg/ml.

#### Results

A total of 32 M. hyopneumoniae isolates of swine origin recovered across Thailand were susceptibility tested against innovator tiamulin (Denagard<sup>®</sup>) and generic tiamulin. MIC data indicated that 34.4% M. hyopneumoniae displayed higher MIC values against generic tiamulin compared to innovator tiamulin. Inferiority in MIC activity of generic compared to innovator tiamulin ranged from a 1to3 fold dilution difference.

#### **Discussion and Conclusion**

Failure of antibiotic therapies has always been a major problem and part of this failure maybe due to use of nonpharmacologically equivalent generic medications. With respect to antibiotic medications, non-equivalent generics may not only lead to treatment failure but also resistance. The prerequisite for successful antibiotic treatment is to achieve the MIC. The present study compared differences between innovator and generic forms of tiamulin. We assumed that innovator and non-equivalent generic differentially enrich resistance leading to therapeutic failure. A recent study estimated that use of generics in a scenario of 25% therapeutic non-equivalence would result in extra expenses approaching US\$1billion per year in the USA owing to selection of resistant microorganisms, greatly offsetting the savings gained from generic substitution.

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# COMPARISON OF TWO TIAMULIN SOURCES IN GROW-TO-FINISH PIGS IN A FARM IN CHINA

J. Ren <sup>1</sup>, X. Mei <sup>1</sup>, H. Wang <sup>1</sup>, H. Xiao <sup>1</sup>, <u>T. Tan <sup>1</sup></u> <sup>1</sup>Elanco China

#### **Background and Objectives**

In February 2023, respiratory and intestinal symptoms including coughing and diarrhea were found in a fattening pig farm in Hunan Province. Laboratory diagnosis showed the pigs were infected by Mycoplasma hyopneumoniae and Lawsonia Intracellularis. This trial compared the efficacy of two different tiamulin sources in this farm.

### **Material and Methods**

A field trial was designed to compare the original tiamulin (Denagard; Elanco AH – group 1) to a local generic competitor product (group 2). Within the affected group of animals (n = 5,821 pigs), the pigs were divided into two groups with 2767 pigs in group 1 and 3,054 pigs in group 2. Group 1 was treated with Denagard from Elanco and group 2 was treated a generic tiamulin from a local company. Both groups were medicated for 14 days at 75-89 days of age (Denagard 7.5 mg/kg bw and generic 10 mg/kg bw), and then were medicated again for 14 days at 125-139 days of age due to the recurrence of clinical signs (Denagard 5.4 mg/kg bw and generic 7.2 mg/kg bw). Both groups were housed in similar conditions and followed the same management practices. The mortality was recorded until the pigs were sent to slaughterhouses, and the difference was calculated using Chi-square tests. FCR and lung lesion in slaughterhouses were also calculated.

### Results

The pigs in group 1 showed a lower proportion of coughing, panting, and diarrhea than group 2. The overall health status of pigs in group 1 appeared better, with mortality of 5.71%, significantly(P<0.0001) lower than 11.26% in group 2. Group 1 showed a 0.14 lower in FCR than group 2. The two groups also conducted lung lesion scoring when the pigs were sent to the slaughterhouse, and the average lung lesion scoring in group 1 was 2.77, lower than 4.21 in group 2.

#### **Discussion and Conclusion**

This trial demonstrated that Denagard had a better efficacy in reducing symptoms and mortality than the local generic tiamulin product.

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### DIAGNOSTIC OF NEONATAL DIARRHEA IN PIGLETS IN TROPICAL COUNTRY: IT'S NOT AS EASY AS IT LOOKS

K. Kaeoket 1, K. Mungthisarn 2, K. Tuanpukdee 2, C. Chaionnom 2, S. Krasaesub 3, C. Serod 3, S. Traiyarach 3

<sup>1</sup>Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand

<sup>2</sup>Charoenchai Farm Group Co., Ltd., Wadsing, Chainat, Thailand <sup>3</sup>HIPRA Bangkok Thailand

### Background and Objectives

E. coli and Clostridium spp. are primarily pathogens causing neonatal diarrhea in piglets. Using antibiotics to control disease led to increasing of antibiotic-resistant bacteria. Vaccination against E. coli and/or Clostridium spp. for preventing diarrhea in piglets is alternative technique. However, in practice, it is difficult to diagnosis of primarily pathogen without sending fecal sample for bacterial isolation or molecular diagnosis. The correct diagnosis is not only benefit to pig farmer for cost saving of treatment in short term but also important for the prevention program and vaccination program for the long run. Thus, we performed rectal swab in neonatal diarrheal piglets during the first 4 day of life by using Enterocheck<sup>®</sup> (Hipra, Spain) and further analyses by molecular technique.

# Material and Methods

We selected 1,500-3,600-breeding sow herd (n=5), with 1-4 parity that had incident of neonatal. These farms have history of piglet dead during 4 days of life. Hence, we performed the test in piglets that showed yellowish diarrhea and/or nervous sign during 1-4 days of age. Three rectal swab samples from neonatal diarrheal piglets in each farm was taken from three different litters and smeared on ELUTE cards (FTA-like) (Enterocheck®, HIPRA, SPAIN) before sending to laboratory. The one-step real-time multiplex PCR reaction procedure for the identification of the portion of E. coli virulence genes (F4, F5, F6 and LT) and β toxin for the detection of ETEC, Clostridium perfringens type A, C and Clostridium difficile A, B using specific probes by following HIPRA Laboratories (HIPRA SPAIN) guideline was performed.

#### Results

For E. coli, a high prevalence was found for E. coli-F4 (4/15) and followed by E. coli-LT (3/15). For Clostridium perfringens, Clostridium perfringens type A was the dominant type (12/15) and Clostridium perfringens type C were not detected. Clostridium difficile A and B were equally found (5/15). Co-infection of E. coli and/or Clostridium perfringens and/or Clostridium difficile was found in 12/15 samples.

### **Discussion and Conclusion**

This prevalence is important for veterinarian in order to plan for a vaccination program for each farm.

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# RESISTANCE TO ANTIBIOTICS AND BIOFILM FORMATION BY STREPTOCOCCUS SUIS ISOLATED FROM PIGLETS

L. Hernández-Andrade<sup>1</sup>, S.E. Mendoza-Elvira<sup>2</sup>, A. Romero-Flores<sup>2</sup>, V. Quintero-Ramírez<sup>2</sup> <sup>1</sup>Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias, Department of Bacteriology, Mexico City <sup>2</sup>Universidad Nacional Autónoma de México. Facultad de Estudios Superiores Cuautitlán, Mexico City

# **Background and Objectives**

Streptococcus suis causes septicemia, encephalitis, endocarditis and bronchopneumonia in pigs and is a swine pathogen and also a zoonotic agent. The formation of biofilm allows to Streptococcus suis to become persistent colonizer. Biofilms are microbial sessile communities characterized by bacterium that are adhered to biotic or abiotic surfaces. Biofilms are the most common mode of bacterial growth in nature and are also important in clinical infections, especially due to the high antibiotic resistance associated with them. The objective of this study was to know the resistance to antibiotics and biofilm production in Streptococcus suis isolated from sick piglets.

#### **Material and Methods**

Samples of brain, heart, and lung were obtained from 16 animals with characteristic clinical signs of S. suis infection, the animals came from different states of the Mexican Republic (Veracruz, Jalisco and Puebla). The samples were macerated in sterile phosphate buffered, streaked onto 5% blood agar plate, and incubated at  $37^{\circ}$ C for 48 h. Colonies that were formed by  $\alpha$  - hemolytic, Gram possitive cocci, grouped in chains, catalase and oxidase negative were selected. The Kirby-Bauer technique was used to detect sensibility or resistance to Ampicillin, Cephalotin, Cefotoxime, Dicloxacillin, Ciprofloxacin, Clindamycin, Erythromycin, Oxacillin, Gentamicin, Ceftiofur and Tetracyclin. Biofilm formation was assessed in 96 well polystyrene tissue culture microplates. 200 µl were inoculated per well, the tissue culture plates were incubated at 37 °C. After incubation content of each well was gently removed by tapping the plates. The wells were washed three times with PBS pH 7.2 to remove free floating planktonic bacteria. Biofilm were stained with crystal violet 0.1%. Optical density of stained adherent bacteria was determined with a micro ELISA reader at 492 nm.

#### Results

Out of the 14/16 Streptococcus suis isolates 87.5% produced biofilms and 2/16 didnt produce biofilm. 100% of resistance was found to Ampicillin, Dicloxacillin, Cephalotin, Cefotoxime and Clindamycin

# **Discussion and Conclusion**

Bacterial adhesion and biofilm formation are very important concepts in bacterial disease and control and eventually lead to a better undestanding eradicate S. suis growing as biofilms with antibiotic therapy. More studies with a greater number of isolates from Mexico are necessary.

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# SURVEY ON LUNG LESION SCORING IN SLAUGHTER PIGS IN A CONVENTIONAL FARM

J. Zhang <sup>1</sup>, <u>R. Krejci</u><sup>2</sup>, P. Mazerolles <sup>2</sup> <sup>1</sup>Ceva Animal Health China <sup>2</sup>Ceva Animal Health France

#### **Background and Objectives**

Lung lesions scoring at slaughterhouses provide valuable information about the respiratory health in the pig population. Lesions suggestive for previous Mycoplasma hyopneumoniae (M.hyo) or Actinobacillus pleuropneumoniae (A.p.) infections and corresponding scoring methods had been widely reported. Scoring of the lesions can quantify the problems with enzootic pneumonia and pleuropneumonia. The aim of this survey was to collect and evaluate results of slaughter pigs' lung scoring in a traditional farm located in Beijing China after efforts implemented to improve respiratory health.

# **Material and Methods**

The farm had 3000 sows with farrow-to-finish production system. Serological gE positive rate of Pseudorabies virus (PRV) in fattening pigs reached 100% in 2021. Herds experienced suspected M.hyo infection (clinically presenting symptoms of dry cough) after 100 days of age. The pig farm replaced the traditional of M.hyo vaccination (two shots) with a single dose vaccine with water in oil adjuvant from Q1 2022, and started PRV eradication from 2022 onwards. Ceva Lung Program scoring methodology was implemented to score the lesions at the slaughterhouse. The results were collected from January 2021 until October 2023. The mean values and quartiles were calculated for % of lungs with bronchopneumonia (%BP), % of affected lung parenchyma out of sick lungs (% parenchyma), enzootic pneumonia Index (EP index), % of dorso-caudal pleurisy (%DP) and APP index (APPI).

#### Results

The total number of scored lungs was 2,758 from 47 reports with the average of 59 lungs per batch. The median values of %BP in years 2021, 2022 and 2023 were 79.2%, 67.8% and 31.9% respectively. The median of affected parenchyma was 8.8%, 5.5% and 5.0% respectively and median of EP index was 3.87, 2.50 and 0.84 respectively in those periods. For dorso-caudal pleurisy lesions the prevalence median values were 13.8% in 2021, 3.2% in 2022 and 2.9% in 2023 (until October) and for APPI the corresponding values were 0.28, 0.10 and 0.06 respectively.

#### **Discussion and Conclusion**

The results of this survey demonstrated a clear tendency for the improvements in the EP-like and A.p. like lesion scores, indicating efforts of PRV eradication and M.hyo control could contribute to these improvements.

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# UTILIZING PLEURISY LESIONS IN THE PHILIPPINES TO CALCULATE THE COST OF DISEASE AND CLASSIFY FARM RESPIRATORY STATUS USING A DORSAL PLEURISY INDEX

<u>R.F. Cudal</u><sup>1</sup>, M.G. Alba<sup>2</sup>, K.A. Fabros<sup>3</sup> <sup>1</sup>Food Animal Business, Elanco Philippines <sup>2</sup>Elanco Philippines <sup>3</sup>Elanco Animal Health Asia

#### **Background and Objectives**

Pleurisy is a chronic lesion seen at slaughter by examining the lungs. Observations of lung lesions can give incidence and severity of swine respiratory pathogens. Data may complete the health program assessment and provide guidance on treatment medication management.

# **Material and Methods**

A commercial farm in the Philippines used in-feed Pulmotil<sup>®</sup> (Tilmicosin) medication at 2 kg/ton for 21 days to address respiratory disease in the herd. Lung lesion scoring (LLS) activities were done across three years using the Slaughterhouse Pleurisy Evaluation System or SPES with scores ranging from P0 to P4. The Dorsal Pleurisy Index (DPI) was calculated as a frequency of pleurisy lesions with a SPES score of  $\geq$  P2 in the batch multiplied by the mean pleurisy score of animals with  $\geq$  P2 score. The DPI was put into quadrant groups providing a herd classification for swine farms describing their pleurisy status as Red (severe), Orange (high), Yellow (moderate) and Green (normal).

#### Results

Each LLS had a corresponding DPI correlated to the respiratory health shown by the pleurisy scores. The baseline LLS1 had a Red DPI, the subsequent LLS2 to 4 showed lowering from Red to Orange DPI when they used medication in the grower ration. Farm decided to change medication and the LLS5 showed a Red DPI. Back to Tilmicosin showed LLS6 to 7 with Yellow DPI. Addition to grower medication and in lactating feed showed LLS8 to 9 with Orange DPI. The LLS10 showed Red DPI with removal from grower ration. Farm opted for generic medication and LLS11 showed Red DPI. The farm was able to address the challenge with Tilmicosin eventually stopped grower use and continued in lactating feed, the LLS12 had a Yellow DPI.

Cost of pleurisy was calculated, considering mortalities from acute cases correlating with prevalence, the effect of pleurisy on ADG, slaughter weight and slaughter age was also calculated. Disease cost was below USD5 when Yellow DPI, between USD6 to USD10 at Orange and higher than USD10 when Red.

#### **Discussion and Conclusion**

Pleurisy lesions seen at LLS allowed evaluation of the farm's medication programs and calculation of the cost of respiratory disease incursions. DPI gave insights to farm respiratory status and alerted farms to the need to intervene.

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# "UNVACCINATED PIGS CHALLENGED WITH MYCOPLASMA HYOPNEUMONIAE AND TREATED WITH LIVE YEAST AND FRAGMENTED IN FEED"

J. Camacho-Medina <sup>1</sup>, L. Suárez-Vélez <sup>1</sup>, G. Bárcenas-Morales <sup>1</sup>, G. Vega-Munguía <sup>1</sup>, A. Ciprian-Carrasco <sup>1</sup>, <u>S.E. Mendoza</u> <u>Elvira <sup>2</sup></u>

<sup>1</sup>UNAM-FESC, México <sup>2</sup>Facultad de Estudios Superiores Cuautitlán, UNAM.

#### **Background and Objectives**

In this study, we describe the impact of treatments with two probiotics, one treatment based on live yeast, and another based on fragmented S. cerevisiae (beta-glucans) that were administered to piglets at birth and at prechallenge with Mycoplasma hyopneumoniae (M.hyo).

# **Material and Methods**

Thirty-two pigs were divided into four groups of eight animals each. The animals had free access to water and food. The groups were as follows: Group A, untreated negative control; Group B, inoculated by nebulization with M. hyopneumoniae positive control; Group C, first treated with disintegrated S. cerevisiae (disintegrated Sc) and inoculated by nebulization with M. hyopneumoniae; and Group D, treated with live S. cerevisiae yeast (live yeast) and inoculated by nebulization with M. hyopneumoniae. In a previous study, we found that on days 1 and 21 of blood sampling, nine proinflammatory cytokines.

#### Results

The pathological lung lesions found showed: Group, A, very mild pulmonary lesions were found in three lungs that ranged from 0.1 to 0.3% and were characterized by small areas of reddish consolidation on the lung surface. Group B challenged only with M. hyo had consolidated gray–reddish lesions that varied in extent from 9.2 to 29% in all animals. Group C treated with disintegrated S. cerevisiae and challenged with M. hyo presented reddish–gray consolidated areas with an extension of 0.1 to 1.0% in all pigs. Group D treated with live yeast and challenged with M. hyo showed reddish–gray consolidation areas of variable extension of 1.4 to 4.2% in all animals. The results of treated Groups C and D suggest that live yeast has an immunomodulatory effect in chronic proliferative M.hyo pneumonia characterized by Delayed-Type Hypersensitivity (DTH), which depends on the alteration or modulation of the respiratory immune response.

#### **Discussion and Conclusion**

These results suggest that live and disintegrated live yeast have an immunomodulatory effect in chronic proliferative pneumonia DTH, in the pathogenesis of M. hyopneumoniae, where it seems to depend on the alteration or modulation of the respiratory immune response. The data presented in this study showed that live yeast contributed to the innate resistance of infected pigs.

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# EPIDEMIOLOGICAL INVESTIGATION OF SWINE OEDEMA DISEASE IN CHINA

T. Yang <sup>1</sup>, L. Cong <sup>1</sup>, L. Juan <sup>1</sup>, T. Zhang <sup>1</sup>, <u>W. Fei <sup>1</sup></u>, M. Hsun Wu <sup>2</sup>, M. Solé <sup>2</sup>, G. Alvarez <sup>2</sup> <sup>1</sup>HIPRA China, Beijing <sup>2</sup>HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

Swine oedema disease, triggered by verotoxin 2e (Vt2e), induces degenerative angiopathy in pigs, leading to various manifestations such as sudden death, neurological signs, and restricted growth. The disease presents in three distinct forms based on clinical characteristics: clinical, chronic, and subclinical. This study utilizes PCR methodology to investigate the prevalence of oedema disease infection among pigs on Chinese farms.

### **Material and Methods**

Between November 2021 and August 2023, a total of 448 oral fluid samples were collected from 55 farms of varying sizes (Small <2000 sows, Medium-small 2000-5000 sows, Medium 5000-10000 sows, Large >10000 sows) and different age groups (4-6w, 7-9w, 10-13w, 14-16w, 17-20w, >20w, Gilts). These samples were processed at the HIPRA CHINA laboratory using RT-PCR (VEROCHECK®) to detect the presence and prevalence of the VT2e gene, with a single positive sample considered to be indicative of a positive farm.

#### Results

The farm-level positive rate for oedema disease in China stands particularly high at 72.73% (40/55), with a sample positivity rate of 40.18%. Large and medium-sized farms exhibited higher positivity rates—100% and 78.57%, respectively. Positivity increased gradually from weaning, particularly during the fattening stage, and showed a higher occurrence during hot seasons compared to cold seasons. It is essential to note that of the 55 farms investigated, only one displayed clinical symptoms, indicating that the remaining farms were considered subclinical, devoid of any observable oedema disease symptoms.

#### **Discussion and Conclusion**

This survey reveals the widespread prevalence of oedema disease in China, which primarily presents as subclinical. This significantly impacts growth performance during the fattening stage, necessitating the application of effective control methods within these farms.

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# SAFETY AND EFFICACY OF A NEW COMMERCIAL MHP VACCINE

<u>B. Seo</u><sup>1</sup>, S. Lee<sup>1</sup>, G. Park<sup>1</sup>, M.A. Jeong<sup>1</sup>, C. Kim<sup>1</sup> <sup>1</sup>Woogene B&G Co., LTD

### **Background and Objectives**

Mycoplasma hyopneumoniae (M. hyopneumoniae, Mhp) is a lethal and chronic respiratory disease that induces Mycoplasma pneumonia in the swine industry. Despite the porcine Mhp infections that may have been underestimated, however, the prevalence of Mhp has continuously increased in recent years. To control Mhp and secondary infection, vaccination is the primary option. In this study, we isolated the field strain and evaluated the vaccine candidate. To confirm its possibility, we developed the Mhp vaccine (IMMUNIS-MYCO).

### **Material and Methods**

A Mice experiment was conducted to confirm the safety and evaluated the anti-Mhp specific antibody levels of the vaccine. The anti-Mhp specific antibody levels were confirmed using a serum titer test produced in-house ELISA. Twenty-three-week-old piglets were purchased from PRRS, PCV, and Mhp seronegative farms and randomly distributed and housed; Group 1: Vaccine 1/10 dose, Group 2: vaccine 1 dose, Group3: Vaccine 10 dose, and Group 4: Positive control, Group5: Negative control. The vaccine groups were intramuscularly vaccinated at 0 days post-vaccination (0 DPV) and the Mhp challenge was performed at 21 DPV. The clinical signs and body weight were measured, and the blood was collected on designated days to evaluate the serum antibodies and immunological assays. For the pathological evaluations, all pigs were humanly euthanized and collected tissues. The statistical significance was determined with a one-way or two-way analysis of variance (ANOVA).

#### Results

The mice and pigs did not observe severe clinical symptoms after vaccination. After the Mhp challenge in pigs, the vaccine groups showed a significant (p<0.05) increase Mhp-specific antibodies and interferon- $\gamma$  secreting cells (IFN- $\gamma$ -SC) compared to the other groups after 42 DPV. Additionally, the vaccine groups observed significant (p<0.05) decreased nasal swab shedding. In histopathological evaluations, the vaccine groups showed mild symptoms of pneumoniae, however, the positive control showed severe symptoms at 63 DPV.

### **Discussion and Conclusion**

In this study the new commercialized M. hyopneumoniae vaccine was showed good efficacy and safety against Mhp infection. In addition, through the clinical trials, the profit of our vaccine was confirmed. The IMMUNIS-MYCO might be prevent respiratory diseases and secondary infection in swine industry.

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# ASSESSMENT OF PORCINE LUNG LESIONS AT SLAUGHTERHOUSE FROM BATCHES VACCINATED WITH DIFFERENT COMMERCIAL MYCOPLASMA HYOPNEUMONIAE & PCV2 VACCINES IN ECUADOR AND VENEZUELA'S FARMS.

J. Calveyra <sup>1</sup>, E. Vargas <sup>2</sup>, <u>R. Krejci</u> <sup>1</sup> <sup>1</sup>CEVA ANIMAL HEALTH <sup>2</sup>Ceva Animal Health, Colombia

#### **Background and Objectives**

Enzootic pneumonia, which is mainly caused by Mycoplasma hyopneumoniae (M. hyo), can be controlled by vaccination. One of the ways to evaluate vaccination processes is through the slaughterhouse scoring methods. The aim of this study was to examine the possible association of lung lesions at slaughter with two different commercial single-dose vaccines against M. hyo.

# **Material and Methods**

During 2022, 23865 lungs of 24 herds were evaluated at slaughterhouse according to the Ceva Lung Program methodology, in Ecuador and Venezuela. The animals underwent two different protocols at weaning. Animals in Group 1: vaccination with a single dose of Hyogen (2 mL) & Circovac (0.5 mL) IM, with 131 batches of 16,586 lungs and the Group 2: single dose vaccination of Mhyo + PCV2 (2 mL) IM, with 75 batches of 7289 lungs. The batches were contemporary and divided between the two treatment groups, on farms with the same management conditions, challenge, physical structure and location. Lung lesions scores were analyzed at the batch level. Lung lesions were statistically compared. The seasons of the year were also compared in the statistical model, being the dry season (January-March and July-September) and the rainy season (April-June and October-December). Comparative effects of lung lesions between treatments were calculated using ANOVA.

#### Results

The evaluated lungs of animals from Group 1 showed a BP frequency of 40.4% while that of Group 2 was 78% (p< 0.05). Regarding the severity of bronchopneumonic lesions, the percentage of affected area was 4.4% and 10% for the Groups 1 and 2, respectively (p< 0.05). Animals from Group 1 had an average of 19.4% of scar lesions, and animals from Group 2 had 26.3%. The rainy period had a BP of 62% and in the dry period it was 56% (p>0.05).

# **Discussion and Conclusion**

The results showed that the vaccination strategy in Group 1 was able to reduce the prevalence and severity of bronchopneumonia lesions when compared to the protocol of Group 2.

# HERD HEALTH MANAGEMENT

# HHM-PP-01

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#### WHAT ARE THE EXTERNAL BIOSECURITY MEASURES IN FRENCH OUTDOOR PIG FARMS?

M. Delsart <sup>1</sup>, <u>N. Rose</u><sup>2</sup>, B. Dufour <sup>1</sup>, E. Boudin <sup>2</sup>, V. Dorenlor <sup>2</sup>, F. Eono <sup>2</sup>, E. Eveno <sup>2</sup>, S. Kerphérique <sup>2</sup>, G. Poulain <sup>2</sup>, M. Souquière <sup>2</sup>, F. Pol <sup>3</sup>, C. Fablet <sup>2</sup>

<sup>1</sup>Anses, École Nationale Vétérinaire d'Alfort, Laboratoire de Santé Animale USC EPIMAI, 94700 Maisons-Alfort, France <sup>2</sup>Anses Ploufragan-Plouzané-Niort, Unité Épidémiologie, Santé et Bien-Être, 22440 Ploufragan, France <sup>3</sup>ONIRIS, 101 Rte de Gachet, 44300 Nantes, France

#### **Background and Objectives**

Outdoor pig farms are popular with consumers and citizens, particularly due to animal welfare concerns. Ensuring biosecurity is a significant challenge for these farms, but crucial to prevent the introduction of diseases relevant to both animal health and veterinary public health. The aim of this study is to provide an overview of the key external biosecurity measures, namely fencing and trapping, on 40 French outdoor pig farms.

# **Material and Methods**

External biosecurity measures were collected from 40 free-range pig farms with at least 30 animals. The presence and type of fencing and its level of maintenance and operation were recorded. Exposure and contact with wildlife were also documented during a farmer interview.

#### Results

As a whole, 59% of farms failed to fully comply with French regulations in terms of biosecurity and fencing to prevent wild boar contact. The level of non-compliance with national regulation differs according to the physiological stages: 54% of farms with outdoor farrowing, 29% of farms with outdoor mating and gestating, 45% of farms with outdoor quarantine and 25% of farms with finishing pigs. The integrity of the electric fences was deficient in 25% of the mating and pregnancy areas and 19% of the farrowing stages. Proper weeding around the electric fences was carried out in 69% and 55% of the farrowing and mating and pregnancy areas respectively. Wild boars were observed in the vicinity of 30 out of 40 farms, and more than twice a year on 43% of farms. Wild boar came into contact with pigs in 20% of farms, resulting in the birth of pig-boar hybrids in two of them. Other animals regularly seen on these farms include, in order of importance, foxes, hares, badgers, roe deer, cats, rabbits, martens and crows. Few farms (5%) set up protection systems against crows, while 28 out of 40 protect themselves against foxes by means of appropriate fencing and/or trapping.

#### **Discussion and Conclusion**

This study highlights the need to improve external biosecurity measures on outdoor farms, in order to prevent the introduction and spread of diseases from wildlife, foremost among which is African swine fever.

HHM-PP-02

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# ESTIMATION OF A BIRTH WEIGHT THRESHOLD ASSOCIATED WITH HIGHER MORTALITY IN PIGLETS SIRED BY DIFFERENT PIETRAIN GENETIC LINES

J.A. Calderon Diaz <sup>1</sup>, <u>M. Ros Fuertes</u> <sup>2</sup>, I. Huerta <sup>2</sup>, J. Garcia <sup>3</sup>, S. Badia <sup>3</sup>, J. Camp Montoro <sup>2</sup>, J. Lupi <sup>2</sup>, S. Shevchenko <sup>2</sup>, L. Zaragoza <sup>4</sup>

<sup>1</sup>Pig Improvement Company <sup>2</sup>Pig Improvement Company (PIC) <sup>3</sup>Piensos Costa S.A, Fraga, Huesca, Spain <sup>4</sup>Pig Improvement Company, Hendersonville Tennessee, USA

# **Background and Objectives**

Piglets weaned per sow per year is a key performance indicator in pig production systems. Hence, increased piglet survival during the pre-weaning period is an important driver for farm profitability and animal welfare. Thus, it is important to identify piglets at higher risk of death to implement management practices to improve their survivability. The objective of this study was to identify a birth weight (BW) threshold associated with reduced pre-weaning mortality in pigs sired by four different Pietrain genetic lines.

# **Material and Methods**

This study was conducted at a commercial farrow-to-wean sow farm with 3300-Landrace × Yorkshire hyperprolific sows. The farm was PRRSv and M. hyponeumoniae positive with no recent outbreaks. In total, 5241 piglets sired by four different Pietrain genetic lines (L-A=1398 piglets, L-B=1364 piglets, L-C=1156 piglets and L-D=1323 piglets) originating from 322 litters were used for this study. Within 24 h post-farrowing, piglets were individually identified and BW was recorded. Piglet mortality was recorded during the pre-weaning period. Segmented regression was used to estimate a threshold for BW associated with increased mortality risk for each of the four genetic lines. The probability of death associated with the estimated BW threshold was investigated using logistic regression models.

#### Results

Overall, piglets weighing  $\leq$ 1.12±0.08 kg at birth were at higher risk of death. Amongst the four genetic lines, 36.4% of piglets were born below this threshold. Different BW thresholds were identified for each genetic line: 1.16±0.21 kg for L-A, 1.00±0.12 kg for L-B, 1.25±0.18 kg for L-C, and 1.06±0.113 kg for L-D. For all genetic lines, the probability of death for piglets below BW threshold was higher compared with piglets with a BW above the threshold: 28.5 vs. 14.6% for L-A, 29.8 vs. 18.7% for L-B, 26.2 vs. 14.2% for L-C and 23.8 vs. 13.4% for L-D

# **Discussion and Conclusion**

Results indicate that variation exist for BW thresholds associated with lower pre-weaning mortality amongst genetic lines for the same breed. This is likely due to the different breeding goals amongst genetic companies. Identifying piglets below these BW thresholds could aid producers to implement management strategies during the lactation period to decrease neonatal mortality targeting at risk piglets.

### HHM-PP-03

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# SCREENING DNA MARKERS TO IMPROVE RESILIENCE TO PORCINE RESPIRATORY DISEASE COMPLEX OUTBREAKS

R. Pena <sup>1</sup>, <u>L. Fraile <sup>1</sup></u> <sup>1</sup>University of Lleida, Lleida, Spain

#### **Background and Objectives**

Porcine Respiratory Disease Complex (PRDC) results from a combination of infectious and non-infectious factors, affecting the pig's health, reducing its performance, and increasing medication costs and mortality. No genetic markers have been identified so far that could be used in the selection of pigs with a better predisposition to cope with PRDC. The aim of this study is to identify DNA markers associated with a better response to PRDC outbreaks under field conditions.

#### **Material and Methods**

Growing pigs (n=1049) from ten farms affected by PRDC outbreaks were sampled during the rearing period. Presence of PRRSV and Streptococcus suis or Actinobacillus pleuropneumoniae was diagnosed by microbiology and/or serology analysis. Animals dying or severely ill during the outbreak were considered CASES and those with the highest body condition scores at the end of the production phase (nursery of fattening) were considered CONTROLS. DNA was isolated from all the samples and genotyped for a panel of 16 polymorphisms in genes GBP1, GBP5, CD163, SGK1, TAP1, HDAC6, rs32803837, rs339180611, MX1 (3 variants), MRMN1, RNASEL (2 variants), PTGDR2 and MUC13. The association of the markers' genotype with the CASE/CONTROL groups was tested by logistic regression analysis including the farm as fixed effect.

# Results

The genotypes of GBP1, GPB5, HDAC6, SGK1, MRMN1, MX1 (2 variants) and RNASEL (2 variants) had a preferential distribution between CASE and CONTROL groups either in the farms affected by PRRSV and Streptococcus suis and/or Actinobacillus pleuropneumoniae (p<0.05). It is worth highlighting the consistent distribution of SGK1 rs338508371-AA animals preferentially in the CASE group in the 10 farms analyzed (p<0.01). On the contrary, the MX1 rs321643120-GG and RNASEL rs343351188-AA genotypes seemed to have a protective role for PRRSV and Streptococcus suis infection as these genotypes were more preferentially distributed in CONTROLS (78.1% and 60.3%, respectively) than in CASES (6.3% and 48%, respectively).

# **Discussion and Conclusion**

Our study paves the way to define panels of DNA markers that can contribute towards selecting pigs resilient to PRDC. Future work should aim to validate these results in other pig production systems and to study their interaction with other pathogens and environmental factors
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## A JOURNEY INTO ENHANCING PIG FARM INTERNAL BIOSECURITY THROUGH FARM STAFF MOVEMENTS

P.P. Pwint Thu 1, K. Wongrujira 2, N. Am-In 3

<sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>School of Engineering, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

<sup>3</sup>Center of Excellence in Swine Reproduction, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

## **Background and Objectives**

To maintain internal biosecurity, it is crucial to segregate various age cohorts inside a pig farm and adhere to designated pathways while accessing the barns. Presently, there is a dearth of study pertaining to the locomotion patterns of agricultural personnel within swine farm facilities. The primary aims of this observational study were to evaluate the mobility patterns of workers on pig farms, identify potentially hazardous movements, and examine potential variations in movements based on time (weekday versus weekend) and unit (farrowing, gestation/mating, wean to finish).

## Material and Methods

Two commercial pig farms were involved, with the installation of an tracking movement monitoring system on each farm. Throughout the farm, detection sites were strategically put, and workers were required to wear a personal track. Data on movement was gathered between August 1, 2022 and July 31, 2023. The following sequence of movements was deemed to be secure: (1) Dressing and disinfectant room, (2) farrowing, (3) gestation/mating, (4) wean to finish, (5) quarantine and replacement, and (7) Wasting and cleaning area.

#### Results

Any movements in the opposite way were deemed risky, unless one visited a Disinfectant and dressing room in between. The total number of movements according on the day of week, with the maximum number occurring during Monday to Wednesday and Friday. The proportion of hazardous actions was impacted by the day of the mating and the weaning period. The proportion of hazardous actions exhibited with a range of 12% to 42%. Weekdays had greater levels of activity in comparison to weekends. During the insemination and weaning, there was a higher frequency of movements towards the farrowing and gestation/mating unit compared to other day.

## **Discussion and Conclusion**

This study demonstrated a significant number of precarious movements occurring on pig farms, which exhibited variations based on the day of week in the 1-week batch system and the specific unit. This study aims to raise awareness, serving as an initial step towards optimizing work processes. Subsequent investigations should prioritize the examination of the underlying causes of certain hazardous actions and explore strategies to prevent them, thereby promoting enhanced biosecurity and improved health conditions within agricultural settings.

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## EFFECTIVENESS OF CLEANING AND DISINFECTION PROTOCOLS ON PIG FARMS IN NORTHERN-BELGIUM.

T. Vandersmissen <sup>1</sup>, C. Bonckaert <sup>1</sup>, C. Brossé <sup>1</sup>, C. Rigauts <sup>1</sup> <sup>1</sup>DGZ Vlaanderen, Hagenbroeksesteenweg 167, 2500 Lier, Belgium

## **Background and Objectives**

Manure-contaminated surfaces are the perfect source to perpetuate infection on a farm, given that this indirect transmission route has been demonstrated for many pathogens. To prevent these pathogens from surviving and infecting the next group, it is essential to clean and disinfect properly between batches. This project aimed to assess the effect of the farmer's cleaning and disinfection protocol using RODAC plates.

## Material and Methods

44 farms were included, all applying all-in/all-out with cleaning and/or disinfecting per department. After cleaning and/or disinfection, 13 surfaces in the farrowing unit (n=10), the nursery (n=20), or both (n=14) were sampled using RODAC plates. A hygiene score (0-5) was defined based on the number of bacteria grown on the plates, with a high number indicating poorer hygiene (0: zero CFU/plate; 1: 1-40 CFU/plate; 2: 41-120 CFU/plate; 3: 121-400 CFU/plate; 4: >400 CFU/plate; 5: uncountable). After receiving advice on improving their cleaning and disinfection protocol, 5 farrowing units and 7 nurseries were sampled a second time.

#### Results

The mean hygiene score of the farrowing unit was significantly higher compared to the nursery (2.6 versus 2.1, respectively). Surfaces at animal height had better hygiene scores than higher surfaces (2.19 versus 2.87, respectively). In the farrowing units a significantly lower hygiene score was achieved at the second sampling after receiving advice and improving their cleaning and disinfection protocol (2.07 versus 3.22 at first sampling).

## **Discussion and Conclusion**

The results show that farrowing units are not cleaned as effectively as nurseries. This may be due to insufficient time for cleaning and disinfecting between two batches. Additionally, the results show that more attention should be paid to higher places as they score worse than surfaces at animal height. As an improved hygiene score was obtained after receiving advice, the project proves the importance of informing the farmer of a proper cleaning and disinfection protocol.

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## FEWER CULTURABLE LACTOBACILLACEAE FOUND IN SOWS TREATED WITH NON-INFLAMMATORY ANTI-STEROIDAL DRUGS WITHIN A YEAR OF SAMPLING

<u>E. König 1,</u> M. Heinonen 1, A. Valros 1, S. Beasley 2 1Research Centre for Animal Welfare, Department of Production Animal Medicine, University of Helsinki, 00790 Helsinki, Finland

<sup>2</sup>Vetcare Ltd., 04600 Mäntsälä, Finland

## **Background and Objectives**

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed analgesics in veterinary medicine. NSAIDs are thought to influence the composition and function of the gut microbiota. As little is known about the recovery time of the gut microbiome post NSAID-treatment, we compared feces microbiome of sows treated with NSAIDs to that of control sows.

## Material and Methods

Twenty-two sows (parity range 1-5) on one farm were divided into three groups based on their NSAID medication history: never treated (control group, n=9), treated within a year (n=7), and over a year before sampling (n=6). Feces samples were cultured for Lactobacillaceae, which were identified with a colony PCR, and species were identified by comparing 16S PCR product sequences to the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST) database with 96% accuracy.

Kruskal-Wallis (SPSS) was used to find a difference in Lactobacillaceae species richness (observed) and total count (log10) between the groups.

#### Results

The Kruskal-Wallis test was found to be significant for culturable Lactobacillaceae species richness (H = 5.92, p = 0.05). The significant difference was seen between the control group and the sows treated within a year before sampling (p = 0.02 and adj. p = 0.05).

The total count of Lactobacillaceae tended to be different between the same two groups before adjusting the p-value (p=0.06).

### **Discussion and Conclusion**

These results shed light on the role of NSAIDs in the swine gut microbiome. In this study, the reduction of Lactobacillaceae seems to be linked to the use of NSAIDs. During the first year post treatment, richness of culturable species decreased significantly. A tendency for decrease was seen also in the total Lactobacillaceae count. While we acknowledge, that pain relief is vital to improve animal wellbeing, the sows might benefit from probiotic support during and after NSAID treatment to reinforce the recovery of the gut microbiota.

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## PELVIC ORGAN PROLAPSE IN LATE-GESTATION SOWS: FIELD EVALUATION OF A MEDICAL TREATMENT PROTOCOL

G. Wideman <sup>1</sup>, <u>G. Greaves</u> <sup>1</sup>, C. Werth <sup>1</sup>, K. Vilaca <sup>1</sup>, C. Lichty <sup>1</sup> <sup>1</sup>South West Ontario Veterinary Services, Stratford, Ontario, Canada

#### **Background and Objectives**

Pelvic organ prolapse (POP) is a common cause of sow removals across the swine industry. The welfare impact is severe and surgical treatment options are intensive and of limited value, leading to a high proportion of POP sows being euthanized. Euthanasia of a prolapsed sow leads to opportunity loss of a cull sow and nurse-off litter, negatively impacting management and the cost of production. The perineal scoring (PS) system specifically, offers a practical approach for identifying sows at risk for POP; as sows with a PS3 (severe swelling, redness and protrusion) have a higher risk compared to sows with a PS1. The study objective was to evaluate the efficacy of a medical POP treatment protocol in herds across Ontario, Canada.

#### **Material and Methods**

In June 2023, using a convenience sample of veterinary clinic clients, herds with a POP prevalence of  $\geq$ 2% were identified to implement the following protocol for sows with a PS3: wash tissue with cold water, replace prolapsed tissues (if possible), apply 10-20mL of Xylocaine 2% intravaginally once, administer 5mL of Salix<sup>®</sup> intramuscular (repeat every 12 hrs up to two more times), and administer Dexamethasone-5 [1mL/45kg] intramuscular once. If the treatment was successful (i.e., diminished swelling, redness, and protrusion), the sow was culled once the lactation period ended. If the treatment was unsuccessful, the sow was euthanized. Producers recorded treatment steps taken and outcome data, per sow.

#### Results

Herds varied in size (548-5370 sows), genetics, and management practices (e.g., stall and loose housing). To date, 78 sows were administered the protocol with 68% avoiding prolapse. Some herds were 100% successful at avoiding mortality due to POP in treated sows. Recovered sows lived longer and completed an average 21-day lactation period (p<0.001). Parity, body condition and time of treatment did not differ between sows that recovered and sows that did not.

## **Discussion and Conclusion**

This nonsurgical treatment for POP in sows with a PS3 may be a feasible option to improve welfare, complete a lactation period, and provide a cull sow opportunity compared to euthanasia. Investigation into the biological understanding of POP in sows remains important for informing treatment approaches.

#### HHM – Herd Health Management

## PORK MULTIPATH™ TECHNOLOGY: A NOVEL APPROACH FOR ASSESSING THE COMPREHENSIVE HEALTH STATUS OF PIG HERDS AND EFFICACY OF MEDICINE USAGE

<u>J. Gerszon</u><sup>1</sup>, B. Genz<sup>1</sup>, Y. Pollock<sup>2</sup>, B. Gleeson<sup>2</sup>, A. Morris<sup>3</sup>, R. Moser<sup>1</sup>, M. Sellars<sup>1</sup> <sup>1</sup>Genics Pty Ltd., 60 Research Road, St Lucia, QLD 4067, Australia <sup>2</sup>SunPork Group, Eagleview Place, Eagle Farm, QLD 4009, Australia <sup>3</sup>Riverbend Pork Group, 487-489 Ruthven St, Toowoomba City QLD 4350, Australia

## **Background and Objectives**

Numerous viral and bacterial pathogens are known to infect pigs, yet, little is known about the prevalence of co-infections, especially in commercial pig farming environments. To address this knowledge gap, this study aimed to evaluate the pathogen threshold of respiratory and enteric pathogens in pig herds using the Pork MultiPath<sup>™</sup> (PMP1 and PMP2) technology, which detects multiple pathogens simultaneously in a single reaction with sensitivity and precision.

## **Material and Methods**

Two longitudinal pathogen screening studies were conducted across three commercial piggeries with similar health status, comparable vaccination and medication programs, and similar production metrics but historically different levels of pleurisy and post-wean mortality. Aggregate samples (rope oral fluids and sock pooled faeces) were collected from pigs of different age groups (4 – 20 weeks of age). Extracted TNAs (RNA and DNA) were tested on Pork MultiPath<sup>™</sup> respiratory and enteric panels including internal control assays and GLP processing. PMP assays are run as a commercial service at Genics Pty Ltd (Brisbane, Australia). The presence of pathogen markers was determined as copies per reaction.

### Results

PMP1 detected M. hyrohinis, P. multocida, and H. parasuis as most prevalent respiratory pathogens. PMP2 identified E. coli genes for toxins - ST1, ST2, and fimbriae – F4 and F18 as well as Rotavirus B and C as frequently detected enteric pathogens. Strong associations of clinical symptoms and pathogen thresholds were discovered for H. parasuis and M. hyorhinis (respiratory pathogens) and B. pilosicoli (enteric pathogen), respectively. Additionally, the levels of several markers, namely E. coli F4, F5, F18, LT, ST1, and ST2, were associated with a higher likelihood of disease.

## **Discussion and Conclusion**

This study highlights the importance of monitoring the level of certain pathogens as useful indicators of disease in pig populations and guiding preventative measures. Both PMP panels are valuable robust surveillance tools capable of simultaneously monitoring multiple markers for respiratory (12) and enteric pathogens (19) in a single test.

HHM – Herd Health Management

## SUMMARY OF THE RESULTS OF 638 ATP BIOLUMINESCENCE TESTS TO MEASURE PIG FARMS CLEANLINESS

X. Mear <sup>1</sup>, T. Rousseau <sup>1</sup>, F. Larcher <sup>2</sup>, <u>E. Cantaloube <sup>3</sup></u> <sup>1</sup>SYNTHESE ELEVAGE, 11 Rue Marie Curie 35137 Pleumeleuc FRANCE <sup>2</sup>Chêne Vert, 4 rue Théodore Botrel, 22 600 Loudéac <sup>3</sup>Chêne Vert, 4 rue Théodore Botrel, 22600 Loudéac FRANCE

## **Background and Objectives**

ATP (Adénosine TriPhosphate) bioluminescence method is a pedagogical tool for on-farm rapid assessment of surface cleanliness. The aim of this study was to summarize the results of 638 ATP bioluminescence tests.

## Material and Methods

638 ATP bioluminescence tests were done during 76 disinfection controls in 41 farms. 56% of the samples were done in farrowing units, 19% in post weaning units and 25% in fattening units. Tests were done after disinfection in dry rooms by swabbing an area of 10 cm x 10 cm and were tested for microbial contamination level using an ATP bioluminescence meter (Relative Light Unit = RLU). Sampling sites were feeding and drinking areas, floor, wall over 1,5 meters and wall at animal height. For each sampling sites we had an "ATP index" (note 1 to 4, depending on the RLU value, according with a publication of the IFIP [ Corrégé, 2003]). An average of the indexes gave a score per farm. This score was then converted to a global evaluation index (Good – Average – Bad, adapted from Corrégé, 2003).

## Results

35% of the controls had a good global evaluation index, 34% average and 31% bad. The average ATP index (2,8) was higher in fattening units than in farrowing (2) and post weaning (2,1) units, p<0,001. This index was higher in drinking (3,1) and feeding (2,6) areas than on the floor (2,4) and on the walls (<2,2), p < 0,001. It was also higher for concrete and metal surfaces (2,4) than for plastic surfaces (1,8), p < 0,001. 94% of the farms used detergent in farrowing unit versus 69% in post weaning unit and 35% in fattening unit. ATP index was lower (2) with detergent than without (2,9), p<0,001.

#### **Discussion and Conclusion**

Some of our results agree with those obtained by Correge et al in 2003 by counting total aerobic bacteria. They also confirmed the interest of using a detergent to improve disinfection quality.

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## ASSESSMENT OF CLEANING AND DISINFECTION PRACTICES ON PIG FARMS ACROSS TEN EUROPEAN COUNTRIES

I. Makovska<sup>1</sup>, I. Chantziaras<sup>1</sup>, N. Caekebeke<sup>2</sup>, P. Dhaka<sup>3</sup>, J. Dewulf<sup>1</sup>

<sup>1</sup>Veterinary Epidemiology Unit, Department of Internal medicine, Reproduction and Population medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133,9820 Merelbeke, Belgium

<sup>2</sup>Biocheck.Gent BV, Belgium

<sup>3</sup>Centre for One Health, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India

## **Background and Objectives**

Biosecurity measures play a pivotal role in minimizing the risk of introducing and spreading infectious agents. Within internal biosecurity, cleaning and disinfection (C&D) procedures hold significant importance. The current study aims to assess the implementation of C&D procedures on pig farms in Europe and identify any potential gaps.

#### Material and Methods

Biocheck.UGent (https://biocheckgent.com) data from 14236 pig farms in 10 EU countries (Belgium, Finland, Ireland, Italy, Spain, Netherlands, Germany, Poland, Slovenia, and Serbia) collected between 2019-2022 were considered, and parameters from the questionnaire that are of interest to C&D measures were selected.

## Results

Out of the targeted 14236 farms, 74% (n=11866) of the respondents reported the presence of a hygiene lock and use of it by visitors. This use was especially high in the Netherlands (94%, n=107) and Germany (94%, n=58). In 55% (n=11511) the disinfection baths/boot washers were present at the entrance of the farm, with better implementation in Belgium (88%, n=8614), Finland (79%, n=2146), and Poland (75%, n=107). At the same time, a small percentage of farms (40%, n=5124) reported the presence of disinfection baths and/or boot washers or the practice of changing boots between compartments/units, and even fewer farms (19%, n=3090) had hand washing stations and/or hand disinfection equipment between compartments/units. It was observed that the protocol for C&D of equipment after use was present in almost half of the farms (51%, n=7420). The application of specific measures for the proper introduction of material was reported in only 22% (n=2846) of the farms. In terms of implementing C&D procedures after each production cycle, 79% of farms (n=9920) reported compliance, with Spanish (97%), Serbian (97%), Polish (96%), Italian (96%), and German (92%) farms showing the high rates of implementation. More than half of farms (65%, n=11516) reported following proper cleaning and disinfection procedures. However, the effectiveness of these practices (e.g. by using a hygienogram) was only validated by 1% of the farms (n=218).

## **Discussion and Conclusion**

The study underscores varied C&D practices on European pig farms. Fostering knowledge exchange improves industrywide biosecurity. Targeted interventions addressing these findings will strengthen internal biosecurity, ensuring animal health and reducing disease risk.

HHM – Herd Health Management

## ASSESSMENT OF THE RISK OF INFECTION WITH PRRS VIRUS FROM PASSING PIG TRANSPORTS

V.F. Jensen <sup>1</sup>, B. Lorenzen <sup>2</sup>, <u>L. Alban <sup>3</sup></u> <sup>1</sup>Danish Agriculture and Food Council, Axeltorv 3, Copenhagen, Denmark <sup>2</sup>Danish Agriculture & Food Council, Aarhus, Denmark <sup>3</sup>Danish Agriculture & Food Council: Animal Welfare and Disease Control, University of Copenhagen, Denmark

## **Background and Objectives**

Airborne transmission of PRRS virus (PRRSv) is an important risk factor to naïve pig herds, in particular within a few hundred meters from infected herds. Correspondingly, airborne spread of PRRSv could hypothetically occur from passing pig vehicles. The study aim was to investigate the probability of spread of PRRSv from passing pig vehicles to pig herds in Denmark.

## **Material and Methods**

The probability of airborne transmission from passing pig vehicles was estimated using a deterministic model. Due to lack of data on shedding and airborne transmission from pigs during transport, some justified assumptions were made. Only transport of growers around 30 kg was considered. The probability of transmission was set at 0.1% per year-accumulated-passage-time, based on infection dynamics in continuously positive weaner pig herds, with an estimated 5.6% of the pigs being viremic during transport. It was assumed that airborne transmission occurs only from vehicles passing within 500 meters distance; that all vehicles drive through areas of high pig density, each passing (exposing) 15 herds, all assumed to be virus-negative; that the vehicles pass close to the herds at 60 km/h giving a passage (exposure) time of 1 minute. In Denmark, there is around 160.000 grower transports annually; all transports were assumed to carry viremic pigs.

#### Results

The probability of transmission of PRRSv from vehicles transporting 30 kg growers to at least one pig herd in Denmark was estimated at 0.6% per year. This corresponds to a 95% chance that PRRSv would not be transmitted from passing pig vehicles to any Danish pig herds within a decade.

## **Discussion and Conclusion**

Young pigs are susceptible to infections and excrete more virus than adults. Shedding is high during the first 3-4 weeks after introduction of naïve pigs into a positive herd, and the probability of infection subsequently decreases rapidly. Therefore, the probability of transmission from slaughter pig transports is likely negligible compared to transport of 30 kg growers. The estimated probability of transmitting PRRS infection to naïve herds from passing vehicles was very low, mainly due to the assumed low excretion at the time of transport and the short duration of the passage close to the pig herd.

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## BREED PREDISPOSITION, ENVIRONMENTAL AND MANAGEMENT FACTORS FOR HAEMORRHAGIC BOWEL SYNDROME IN SWINE

F. Holenweger <sup>4</sup>, G. Schüpbach <sup>1</sup>, X. Sidler <sup>2</sup>, A. Hofer <sup>3</sup>, <u>A. Grahofer</u> <sup>4</sup> <sup>1</sup>Veterinary Public Health Institute, University of Bern, Liebefeld <sup>2</sup>Division of Swine Medicine, Department of Farm Animals, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland <sup>3</sup>SUISAG, Allmend 10, 6204, Sempach, Switzerland <sup>4</sup>Clinic for Swine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

## **Background and Objectives**

Haemorrhagic bowel syndrome (HBS) is a disorder characterized by sudden death in grower and fattening pigs. It causes economic losses and has an impact on animal welfare . The aetiology of HBS is not fully understood. Therefore, the aim of this study was to detect possible risk factors for the occurrence of HBS on herd level.

## **Material and Methods**

This case-control study included 50 case farms (mortality rate caused by HBS > 1.5%) and 50 control farms (mortality rate caused by HBS < 0.25%) with 600 or more slaughtered pigs per year. A questionnaire, covering general information about the herd, housing parameters, management and hygiene measurements of the pen and the feeding system was conducted. In addition, a herd examination of the pen was performed to evaluate the following parameters: pen size, number of pigs per pen, water flow rate, number of drinkers, height of drinkers, water pH, length of feeding trough, feed pH, air temperature and air draught. After the investigation, the pig density as well as the number of feeding spaces, feeding space width per pig and number of pigs per drinker were calculated.

#### Results

Having only PREMO® sired pig in the herdshowed to be a significant risk factor for HBS (Odds Ratio (OR) = 147) as compared to other breeds. Furthermore, pigs from two or more origins per batch were significantly more likely to develop HBS (OR = 52) in comparison with pigs from only one origin. Farms with 1 decimeter greater feeding place width per finisher pig had a lower HBS incidence (OR = 0.07). In addition, the frequency of cleaning of the distribution pipes (split up into categories) had a significant impact (p < 0.05) on the HBS incidence of a farm.

## **Discussion and Conclusion**

This study revealed four risk factors including sire breed PREMO®, number of origins of pigs per batch, feeding place width per animal and frequency of cleaning of the feed distribution system. These results corroborate that HBS has multiple risk factors that cannot solely be attributed to one management or housing category. Therefore, a thorough examination of every herd is necessary to identify possible risk factors.

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## CHANGES IN ANTIBIOTIC USAGE IN GROW-FINISHING FLOWS AFTER PRRSV-2 INTRODUCTION IN A NAÏVE FARROW-TO-FINISH SYSTEM

I.F. Machado <sup>1</sup>, T. Petznick <sup>1</sup>, A.P. Silva <sup>1</sup>, L. Karriker <sup>1</sup>, <u>D. Correia Lima Linhares <sup>1</sup></u>, G. De Souza E Silva <sup>1</sup> <sup>1</sup>Iowa State University, Veterinary Diagnostic and Production Animal Medicine

#### **Background and Objectives**

Responsible antibiotic usage (ABU) is important for both animal and human health, necessitating constant antimicrobial stewardship improvement. However, the presence of porcine reproductive and respiratory syndrome virus (PRRSV), a swine viral pathogen with immunosuppressive characteristics, potentially exacerbates bacterial co-infection clinical impacts, increasing ABU to treat affected animals. This study aimed to assess ABU changes in the grow-finish population after PRRSV-2 outbreak in a previously PRRSV-naïve sow farm of a farrow-to-finish genetic multiplier system.

## **Material and Methods**

Data from nursery and finishing phases were obtained, such as transferred animals, mortality, and ABU through oral (water) and injectable routes, and the ABU frequency across the system was calculated as the ratio of medicated to total animals. The population was categorized into PRRSV statuses, determined by PRRSV circulation within the sow farm: naïve, epidemic, and endemic. The "Pig treatment per animal days at risk" (PTDR) metric was calculated per route by dividing the medicated animals by animal days at risk for each lot. A negative binomial regression model was applied for overall (oral and injectable) and injectable PTDR to access incident rate ratios for each PRRSV status and phase (p-value<0.05). Water PDTR statistical difference between PRRSV-status was assessed using Kruskal-Wallis and Dunn tests (p-value<0.05).

## Results

135,000 animals were evaluated. The system followed the same antibiotic protocol across the study. Ampicillin was the most commonly administrated antibiotic (40% of total use), followed by lincomycin (39%) and enrofloxacin (21%) in the system. Regarding oral antibiotics, neomycin (78%), penicillin (16%), sulfamethoxazole (3%), and tylvalosin (3%). The nursery phase had a statistical difference between PRRSV statuses for overall PTDR, with an ABU increase of 3.8 and 2.5 times in the epidemic and endemic status, respectively. For water treatment, there was a statistical difference between the naïve and epidemic and endemic status. The finishing phase had a statistical difference in the injectable PTDR, with an ABU increase of 2.7 and 1.4 times in the epidemic and endemic status, respectively.

#### **Discussion and Conclusion**

There was an association between PRRSV status and ABU in the growing population, highlighting the importance of advocating for a system of care to prevent common diseases such as PRRSV.

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## DETECTION OF PORCINE CIRCOVIRUSES (PCV-2 AND PCV-3) AND MYCOPLASMA HYOPNEUMONIAE IN LUNG SAMPLES WITH PLEURISY LESIONS FROM SLAUGHTERED PIGS IN BRAZIL

E.R. Braga 1, A.K. Panneitz 1, F.M. Petri 1, M. Sibila 2, J. Segalés 3, L.G. De Oliveira 1

<sup>1</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil

<sup>2</sup>IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>3</sup>Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), and Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona,

## **Background and Objectives**

Porcine circoviruses (PCVs) and Mycoplasma hyopneumoniae (Mhyo) are etiological agents of the porcine respiratory disease complex (PRDC), which is of paramount importance to global swine husbandry. Pneumonia and pleurisy lesions frequently manifest in cases of PRDC. This study aimed to molecularly detect the presence of PCV-2, PCV-3 and Mhyo in lungs with different scores of pleurisy observed in swine slaughterhouses.

## **Material and Methods**

A total of 100 lungs were sampled by convenience to get 20 of each into the five categories defined by the slaughterhouse pleurisy evaluation system (SPES, scores 0 to 4), from a slaughterhouse located in the state of São Paulo, Brazil. Subsequently, the samples underwent qPCR for the detection and quantification of PCV-2, PCV-3, and Mhyo. The results were analyzed using GraphPad Prism 8.

## Results

PCV-2, PCV-3, and Mhyo were detected in lungs with all grades of pleurisy, with 65% (65/100), 32% (32/100), and 96% (96/100) of positive samples, respectively. Coinfection between PCV-2 and PCV-3 was identified in 22% (22/100) of the samples, while concomitant infection of all three pathogens occurred in 21% (21/100) of the lungs. Spearman's matrix correlation analysis revealed a significantly high correlation between Mhyo and PCV-2 quantification (0.73, (95% CI = 0.4240 - 0.8919); p-value = 0.0002) in lungs with pleurisy score 3.

## **Discussion and Conclusion**

There was a high detection of PCV-2 and Mhyo, while PCV-3 was identified less frequently in the studied lung samples. Such coinfections pose significant challenges for the prevention and control of PRDC, especially given the notable presence of PCV-2 and in lungs displaying pleurisy lesions. The strong correlation between the presence of PCV-2 and Mhyo suggests these pathogens in lung tissue. These results hold considerable epidemiological relevance for guiding future actions related to the control and treatment of the studied pathogens' infections. Acknowledgments grant#2023/01748-0, São Paulo Research Foundation (FAPESP).

#### HHM – Herd Health Management

## EFFICACY OF PRRS VACCINATION AND MANAGEMENT STRATEGIES TO CONTROL HIGH PATHOGENIC PRRS INFECTION IN NURSERY PIGS: A CASE REPORT

C. Laza <sup>1</sup>, M. Marcos-Cienfuegos <sup>2</sup>, R. Menjon <sup>3</sup>, <u>M. Jiménez</u> <sup>3</sup> <sup>1</sup>Caldesvet SA, Spain <sup>2</sup>MSD AH <sup>3</sup>MSD Animal Health

#### **Background and Objectives**

A new highly pathogenic (HP) PRRSV1 strain emerged in Spain in 2020 (Genbank ON571708). Up to date, there is limited information about the efficacy of commercial vaccines against this strain. The aim of the study was to determine if a combination of piglet PRRS vaccination and management measures could be an effective and profitable approach to control HP PRRSV1 in nurseries

## **Material and Methods**

The study took place in a PRRS positive but stable 1300-sow farm (site 1+2). In December-2021, a reproductive PRRS outbreak took place. PRRS was detected and ORF 5 sequencing confirmed strain ON571708. Emergency sow vaccination was performed, followed by implementation of herd-closure during 4 months, no adoptions policy and AI-AO management. Additionally, production was moved from 2- to 3-week batch system. By July 2022, all reproductive parameters were back to levels prior to the outbreak, and piglets' flow was PRRS negative However, nursery's mortality was still higher than expected. In August 2022 it was decided to vaccinate piglets at 2w of age (Porcilis® PRRS, intradermal, MSD Animal Health) for 1 year. Management strategies were maintained. Effect of vaccination on mortality and medication costs was determined by comparing pre- and post-vaccination batches. PRRS presence was tested by PCR on oral fluid samples at the end of the nursery phase. Statistical analysis (Chi-Square Pearson's test) was performed on monthly mortality and medication cost data taking the batch as the observational unit.

#### Results

Mortality in the nursery was significantly reduced from 7.39% in non-vaccinated to 3.73% in vaccinated batches (p<0.05), reaching similar mortality values as before the PRRS outbreak (3.4%). Medication costs related to antibiotic usage were also significantly reduced in 0.91€ (p<0.05), reaching lower values than before the PRRS outbreak. PRRSv was detected by PCR (ct 32) at the end of the nursery. Regarding profitability, calculated based on the reduction of mortality and medication costs, an extra benefit of 1,96€/piglet was obtained, including the cost of vaccination.

#### **Discussion and Conclusion**

In this trial, piglet vaccination and management measures were an effective and profitable combination to improve production parameters, as well as to reduce virus presence in nursery, after an outbreak of a HP PRRSV1 strain.

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## EVALUATION OF CLINICAL AND IMMUNIZATION SEQUENCES OF SAPOVIRUS AND ROTAVIRUS IN RELATED PIG FLOWS USING NEXT GENERATION SEQUENCING

<u>E. Russell, Ms</u><sup>1</sup>, B. Burton, Dvm<sup>2</sup>, A. Reever, Ms, Dvm<sup>1</sup> <sup>1</sup>Veterinary student <sup>2</sup>Veterinarian

## **Background and Objectives**

Sapovirus (SV) and rotavirus (RV) are prevalent causes of swine neonatal diarrhea. Cross-protection is limited within serogroups requiring immunization methods to be customized to individual flows. Custom RNA particle vaccines are developed based on sequences of viral proteins (VP), specifically VP7 and VP4 for RV and VP1 for SV. The objectives of this study were to investigate RV and SV diversity in related flows to help guide immunization strategies, pig flows, sanitation, and other control methods.

## **Material and Methods**

A multiplier flow and two commercial flows (sourced by the multiplier) were enrolled. These farms vaccinated pre-farrow with one type of SV vaccine for all farms and utilized master feedback for RV that is farm specific. Pooled diarrhea and feedback samples were collected from each sow farm and one recently weaned nursery over a two-month time period. All samples were tested by polymerase chain reaction (PCR) and next generation sequencing (NGS) for VP1, VP4, and VP7. Analysis was performed using Disease Bioportal.

#### Results

SV VP1 analysis revealed 5 distinct clades (2 or more sequences, 97+% homology). Two clades only included sequences from one flow, while all flows were represented in the remaining three clades. Most sequences were from nursery fecal samples. For RV analysis, both fecal and feedback samples were tested. VP7 sequences for RVC were present in two clinical diarrhea and one feedback sample. There were three clades where sequences found on the sow farm were not present in the feedback. Numerous RVAs were found in the nursery samples, but there was only a single RVA sequence found on one of the sow farms.

## **Discussion and Conclusion**

Limitations of this study include the duration of testing and difficulties interpreting partial sequences. This study found a wide diversity of RVs and SVs, as well as overlaps within related flows. In this system, pigs are single-sourced but can be placed in any available nursery so both viruses could be spread vertically or through contaminated trailers and nurseries. Readily available bioinformatic programs along with increasingly affordable and efficient sequencing techniques can be used by practitioners to combat these viruses.

HHM – Herd Health Management

## EVALUATION OF LUNG HEALTH AND PRODUCTION PARAMETERS BY STANDARDIZED METHODOLOGY (CLP) IN SLAUGHTER PIGS

F. Bago <sup>1</sup>, A. Palzer <sup>2</sup>, <u>C. Waehner</u> <sup>3</sup>, L. Kaebisch <sup>3</sup> <sup>1</sup>Tierarztpraxis Scheidegg, Germany <sup>2</sup>Veterinary pig practice Scheidegg, Bahnhofstraße 30, 88175 Scheidegg, Germany <sup>3</sup>Ceva Tieraesundheit GmbH Kanzlerstr. 4, 40472 Düsseldorf - Deutschland

## **Background and Objectives**

A fattening farm with 1300 pigs contacted their veterinarian due to increasing problems caused by coughing throughout the fattening period. In addition, an increase in lung lesions was noted during the slaughter process and lungs had to be discarded. Thanks to the local benefits – the pigs were fattened, slaughtered, and marketed all on the same property – intervention measures and their effects could be measured immediately.

## **Material and Methods**

The Ceva Lung Program (CLP) was carried out during different time points. Initial measurements were taken to set the baseline. After each intervention, another CLP was carried out by the same person, to minimize inter-personal biases. The EP- and APP-Indices were used for the evaluation of lung health. Piglet weights were measured at the end of nursery and later as slaughter weights. The days until slaughter were also evaluated. To improve the pig's lung health, vaccinations against Mycoplasma hyopneumoniae (Hyogen, Ceva Santé Animale) and Actinobacillus pleuropneumoniae (Coglapix, Ceva Santé Animale) were implemented. The cleaning and disinfection (C&D) protocol was adjusted.

#### Results

The EP-Index was reduced from 2.1 to 0.86, the APP-Index could be reduced from 0.98 to 0.47. Both indices account for gross lesions observed during slaughter. The proportion of modified MADEC Scores for no and very few lesions of lungs could be increased. In addition, the proportion of lungs adhering to the pleural cavity (most severe adhesions) was reduced from 8% to 2%. After implementation of the vaccines and a new C&D protocol, pathological coughing in the pens was no longer audible. Production parameters were improved.

## **Discussion and Conclusion**

Porcine lung health is implemented as factor of welfare by the European Food Safety Authority (EFSA). To properly assess this parameter, harmonized methodology should be applied. The Ceva Lung Program is an established lung scoring methodology, easy to carry out during the regular slaughtering process (fast) and reproducible. To increase animal welfare, measures like vaccinations should be taken into account and controlled. One way of controlling the taken actions, is the assessment of carcasses during slaughter. The CLP is an easy tool, taken together with production parameters, to define an increase in animal welfare.

#### HHM – Herd Health Management

## FARMER PROFILING WITH REGARD TO THEIR OBJECTIONS OR WILLINGNESS TOWARDS PRRS ERADICATION BY THE ADKA(R) CHANGE MANAGEMENT MODEL

D. Hendrickx <sup>1</sup>, M. Postma <sup>2</sup>, D. Maes <sup>3</sup>

<sup>1</sup>DAC ZuidOost Varkens en Pluimvee, DAC ZuidOost Holding BV, Deurne , Netherlands <sup>2</sup>Ghent University, faculty of Veterinary Medicine, Veterinary Epidemiology unit, Merelbeke, Belgium <sup>3</sup>Ghent University, faculty of Veterinary Medicine, Unit Porcine Health Management, Merelbeke, Belgium

## **Background and Objectives**

It is hard for veterinarians to convince farmers to eradicate the PRRS virus. In Hiatt's ADKA(R)®-model, people's awareness about a problem, their desire to do something about it, their knowledge, and their ability to make the change are scored. Hiatt's model indicates that one has to score 4 or 5 on all elements to make a change successful. A score <4 implies that there is an objection to that element.

The present study investigated the objections or willingness of pig farmers towards PRRSV eradication based on the ADKA(R)® model.

## Material and Methods

We designed a specific PRRS ADKA(R) scoring table, providing clear definitions for each element. Two veterinarians were trained to do an interview as a coach and to score each farmer in the ADKA(R) model. Twenty sow farms were randomly selected from the customers of one veterinary practice. The selected farms were PRRSV positive and vaccinated against PRRSV; the average number of sows was 1029 (min: 320, max 4112). Coaches visited the farms and did an interview, afterwards, they scored each farm.

### Results

For awareness, 7 farms scored ≥4 (range 2-5), for desire 5 (1-5), for knowledge 4 (1-5) and for ability 7 (1-5).

Only two farms had a score  $\geq$ 4 on each element, 2 farms on 3 elements  $\geq$ 4, 3 farms on two elements  $\geq$ 4, 3 farms on one element  $\geq$ 4.

Farms that scored  $\ge 4$  for awareness scored on average higher (3.66, range 2.66 - 4.66) on the other elements than farms that scored <4 (2.34, 1.66 - 3.5, p=0.004).

## **Discussion and Conclusion**

Major differences were observed between farms for the parameters of awareness, desire, knowledge, and farmers ability for PRRSV eradication. Results indicated that very few farmers were mentally prepared to eradicate PRRSV, with only two farms demonstrating a proper mindset. Recognizing PRRS as a problem enhances comprehension of other elements. The ADKA(R)-model aids veterinarians in being more targeted in their advice, allowing them to focus on those element(s) that are critical in a specific farm. The present research is ongoing and will hopefully show that this model helps veterinarians convince farmers to eradicate PRRSV.

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## IMPACT OF A SUDDEN ROOM TEMPERATURE DROP ON BODY TEMPERATURE IN WEANED PIGS

<u>J. Jourquin</u><sup>1</sup>, E. Vandoorn <sup>2</sup>, W. Verbeure <sup>2</sup> <sup>7</sup>Vetquin <sup>2</sup>Poulpharm, Izegem, Belgium

## **Background and Objectives**

Climate control is of major importance for the wellbeing, health and development of young pigs. Detrimental effects of temperature fluctuations on the pig's health during nursery, such as Streptococcus suis outbreaks, are described. However, little is documented how temperature fluctuations affect the body temperature (BT) of these pigs. In this abstract, two methods of temperature recording were used to investigate this.

## **Material and Methods**

Thirty-five weaned pigs were housed in a room with a target temperature set at 28°C and 50% relative humidity. Temperature chips (Thermochip mini) were injected in the semitendinosus muscle at the start of the trial. A daily read out was done at 8am when also the rectal temperature (RT) was measured. After 8 days, the room temperature was dropped gradually starting at 2am to reach a minimum of 18°C at 8am and brought back at target temperature by 2pm.

## Results

The average room temperature was  $27.64\pm0.65$  °C during the entire period excluding the 12 hours of fluctuation. The temperature dropped to 18.03 °C on day 8 (D8). The mean BT over the entire period was  $38.79\pm0.76$  °C when measured by temperature chip and  $39.35\pm0.38$  °C when measured rectally. The mean BT measured by temperature chip changed significantly from  $38.88\pm0.72$  °C on D7 to  $37.54\pm0.98$  °C on D8 and returning to  $38.86\pm0.70$  °C on D9 (p<0.0001), while the RT changed significantly from  $39.33\pm0.33$  °C to  $39.04\pm0.39$  °C returning to  $39.43\pm0.33$  °C for the same timepoints (p<0.0001).

## **Discussion and Conclusion**

The BT measured by temperature chip correlated with (p=0.0004;  $R^2=0.116$ ), but was systematically lower compared to RT measurement and had a higher standard deviation. For the registration of the core temperature rectal measurement may be a more sensitive method. The temperature chip recordings clearly showed a severe impact of the temperature fluctuation on the pig's thermoregulation, while the impact on the core temperature measured rectally, although also highly significant, was more subtle and may pass unnoticed. This trial shows that even a one-time, moderate and temporary drop of the environmental temperature has a significant impact on pigs.

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## OPTIMIZATION OF PIG AND PEOPLE FLOW MANAGEMENT BY CONTINUOUS ORAL FLUID (OF) SAMPLING FOR PRRS-SURVEILLANCE IN A NURSERY UNDERGOING ELIMINATION

<u>P.H. Rathkjen</u><sup>1</sup>, L.M. Jensen<sup>2</sup>, L.K. Tolstrup<sup>3</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Denmark <sup>2</sup>PORCUS Oerbaekvej 276, DK-5220 Odense <sup>3</sup>Boehringer Ingelheim

## **Background and Objectives**

The objective of this study was to understand PRRSV infection dynamics by continuous OF sampling in a nursery undergoing PRRSV elimination to improve pig and people flow management to achieve PRRSV negative status.

## Material and Methods

A Danish nursery site sourced with pigs from a PRRSV positive sow site undergoing elimination was selected. Samples from all age groups (1 - 8 week post placement (WPP)) in the nursery were performed monthly from 5 month before to 15 months after sow herd mass vaccination (MV) except for a 14 week period post mass vaccination. Four ropes per room for 30 minutes were placed per double-pens. OF were pooled by room and tested by qPCR. Pig flow, people movement and management procedures, especially during delivery of pigs, were adjusted according to apparent PRRSV circulation in the different age groups.

## Results

During the 5 months before the sow mass vaccination PRRSV was detected at different frequencies in all age groups, from 1 WPP (4 of 6 months, Ct's 27 – 31,6) until 7 WPP just before sale (4 of 6 months, Ct's 25,9 – 32,6). After the MV, no samples were positive from 1 WPP. From 6 months after mass vaccination all age groups from 1 - 4 WPP were negative. Delivery and picking procedures from the oldest pigs were changed resulting in eliminated PRRSV from 5 and 6 WPP. 7 WPP remained positive until 1 year after MV (average Ct 27,3). Only when procedures for delivery of pigs (especially people movements) were reevaluated, PRRSV were finally eliminated from the nursery.

## **Discussion and Conclusion**

It can be tricky to understand the exact PRRSV infection dynamics in a nursery especially when picking pigs from different age groups at delivery. Both people and pigs often move in risky manners leading to unintended spreading of virus. Based on this study, all age groups present on the site needs to be continuously monitored, (preferably monthly sampling), to understand infection dynamics, and correct inappropriate management and flow procedures. This is especially when pigs are picked for delivery in different rooms and ages, aiming for lower weight variation among sold pigs.

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## RELATIONSHIP BETWEEN ADG AND FCR ON DUTCH FINISHING PIG FARMS

<u>R. Jansen</u><sup>1</sup>, M. Steenaert<sup>2</sup>, C.O. Gomez-Duran<sup>3</sup> <sup>1</sup>Boehringer Ingelheim Vetmedica GmbH <sup>2</sup>Boehringer Ingelheim Animal Health Netherlands bv <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH, 55216 Ingelheim, Germany

## **Background and Objectives**

Feed conversion ratio (FCR) is the highest variable cost in the production of one kilogram of pork. Despite the economic impact in differences in FCR, not all production sites monitor FCR, leave alone to monitor it on a barn, room, or individual level. Average daily gain (ADG), however, is a parameter that can be relatively easy monitored on a farm level. In general can be said, that if pigs get healthier, that the ADG is higher, but that also FCR will be lower. The objective off this study is to establish a relationship between ADG and FCR, that could be used to estimate the impact of FCR if only data on ADG is available.

#### **Material and Methods**

Data obtained in a large-scale monitoring program was used to analyze correlation between ADG and FCR. Production data of in total 206 independent farms were analyzed, containing data over a period of at least 6 months of pig production and was adjusted to the standard live weight gain from 25 start weight until 117 slaughter weight. Data was tested for normality. Farms with data point outside the normal distribution were removed (n=8). The relation between ADG and FCR was tested using linear regression.

### Results

ADG and FCR have a high significant correlation (p<0,001). With every 10-gram increase of ADG, FCR is lowered with 0.012 units. This equals 1,1 kg of feed saved per pig. For full intact boars & gilts this effect was a lowering of 0.011 units (p<.0001; n = 175). For castrates and gilts, the effect was stronger but not significant due to a limited amount of data points (0.020 units, p=0.12, n=14). Aside to a lower FCR, daily feed intake increased as well with 16 grams for every 10 grams increase in ADG.

## **Discussion and Conclusion**

This data set gives indications to estimate an improvement in FCR based on ADG improvements. This can help to monitor the outcome off interventions (like for example vaccination) for a better understanding of the total economic value this is providing. When pigs grow faster, this is due to a combination of a better feed efficiency and a higher daily feed intake.

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## **RISK PERIODS FOR SOW MORTALITY IN DANISH SOW HERDS**

<u>T.B. Jensen</u><sup>1</sup>, M. Willkan <sup>1</sup>, H. Bak <sup>1</sup>, L.U. Hansen <sup>1</sup> <sup>1</sup>SEGES Innovation, Axeltorv 3, Copenhagen, Denmark

#### **Background and Objectives**

During the last years sow mortality has increased in Danish herds, and efforts are made to break the curve nationally. To implement effective strategies that will increase the survival rate, there is a need to gain more knowledge on when sows are at high risk of dying. This study investigates changes in sow mortality in relation to parity and stage of pregnancy.

## **Material and Methods**

Data originated from 108 herds delivering data to SEGES Innovation from 2021 to 2023. Data from 268.248 individual sows was included in the study and contained information about sow reproduction and causes of culling recorded by the farmer. Descriptive statistics and graphical illustrations were used to identify patterns in the data.

## Results

The overall sow mortality was 15.1 % (q25 = 11.6 %; q75 = 17.4 %) with 7.3 % being euthanized and 7.8 % experiencing sudden death. Mortality increased with parity ranging from 5.5 % in parity 1 sows to 7.2 % in parity 6 sows. Across parities more sows experienced sudden death compared to being euthanized. The ratio between sudden death and euthanasia was highest among sows in parity 1, 5 and 6 sows. According to farmers recordings the main reason for euthanizing sows was leg problems. For sows experiencing sudden death the main reason recorded by the farmers was "unknown".

We found an increase in mortality around day 28 after insemination and around the time of farrowing. The high mortality rate day 28 can be due to stress and fighting in relation to mixing of sows, when they are transferred from the insemination unit to the loose-house system in the gestation unit. For sows experiencing sudden death an increase in mortality was also seen from day 85 in gestation. Late gestation is a critical time where high producing sows are vulnerable with an increased risk of dying.

## **Discussion and Conclusion**

To reduce sow mortality attention must be given to sows in all parities and extra efforts is needed at the time of mixing of sows in the gestation unit and around farrowing. These results will be used to revise recommendations on sow management to farmers.

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## ULTRASONOGRAPHIC EVALUATIONS OF STOMACH AND COLOSTRUM INTAKE IN NEWBORN PIGLETS

S. Boulot <sup>1</sup>, G. Herve <sup>1</sup>

<sup>1</sup>IFIP-Institut du porc, Domaine de la Motte au Vicomte, BP 35104, 35651 Le Rheu

#### **Background and Objectives**

In large litters, poor colostrum intake and hypothermia are major causes of pre-weaning mortality, with high rates of empty stomachs (40 to 70%) in necropsied piglets. Early detection of colostrum deprivation would support better care and survival. However, subjective evaluations on liveborn are imprecise. The aim of this study is to evaluate the suitability of ultrasonographic stomach examination for future non-invasive real time detection of poor colostrum intake.

## Material and Methods

Study took place in IFIP experimental farm on piglets born in large litters ( $17.7 \pm 4.7$  total born) and found dead at birth (N = 20) or within 2 days (N = 20). Ultrasonographic examinations of lungs and abdomen were performed (5 MHz linear probe, Exapad®) in supine and lateral decubitus positions. Morphological analysis included birth weight, Intra Uterine Growth Retardation score (IUGR), hoof-tip status, wounds, or signs of crushing. All piglets were necropsied for confirmation of status (stillborn/liveborn) and analysis of stomach content. Reference pictures were collected after echo guided injections of milk (fresh, clotted) and air, in empty stomachs of stillborn piglets.

#### Results

Large size of the liver limits visualization, with supine position providing the best pictures. In stillborn piglets, stomach is easily located. It is full of amniotic fluid and hypoechogenic; pictures are dark and round, without artefacts. Replacement of amniotic fluid by milk shows echogenic round picture of stomach content, with clotting and air associated with perturbations or artefacts. In many liveborn piglets found dead, stomach is full of gas, with none or small colostrum particles. In these cases, abdomen and stomach contents are masked by aeration shadowing artefacts.

## **Discussion and Conclusion**

These preliminary results on piglets suggest that ultrasonographic pictures may vary according to milk intake, stage of digestion and cause of death. Despite suckling and swallowing may increase stomach air content and detrimental artefacts, this tool proved to be accurate for detection of empty stomachs in human beings. More data are required to evaluate accuracy on live animals.

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## 3D CAMERA SYSTEMS AND ARTIFICIAL INTELLIGENCE FOR DETECTING TAIL POSITION OF UNDOCKED PIGS

M. Schmid <sup>1</sup>, M. Terranova <sup>2</sup>, D. Kümmerlen <sup>3</sup>, <u>T. Echtermann <sup>3</sup></u> <sup>1</sup>Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland <sup>2</sup>ETH Zurich, AgroVet-Strickhof, Lindau, Switzerland. <sup>3</sup>Division of Swine Medicine, Department of Farm Animals, Vetsuisse-Faculty, University of Zurich, Zurich, Switzerland

## **Background and Objectives**

Since the number of pigs per farm in most countries is increasing, a reliable and time efficient monitoring system for pig health is of great concern. Therefor, monitoring systems based on automatic video analysis by artificial intelligence are being used both scientifically and commercially. If such a monitoring system can analyze the tail position in undocked pigs should be investigated by this study.

## **Material and Methods**

A total of 6 commercial available 3D camera systems were installed above the activity and feeding area in a farrow-tofinish research farm, 3 in the weaning unit and 3 in the fattening unit. Housed pigs per pen were 22 for the weaners and 16 for the fatteners. Only pigs with entire tails were used in the study. We followed one batch per age category. During the observation period, an existing algorithm for docked tails daily calculated the relative number of pigs with a low (0°), a medium (0°-45°) and a high (>45°) tail position based on the back line of the pig.

## Results

Overall a low position was measured in 47.9%, a medium position in 16.8%, and a high position in 35.3% on average of the complete observation period. In weaners there was a decrease of low tail position from 61.2% directly after entering the pens to 40.8% on the day before moving to the fattening unit. In fatteners we observed an increase in the relative number of pigs with low tail positions from 48.5% to 58.9% just before slaughter.

## **Discussion and Conclusion**

This study for the first time looked at the analysis of tail position in undocked pigs by artificial intelligence. The used algorithm showed comparable results to previous studies with docked tails. The decreasing number of low tails in weaners might be linked to the decreasing number of fights over time after mixing, and the increasing number of low tails in fatteners might be linked to less space per pig at the time before slaughter. If the algorithm can be used as a reliable tool for monitoring events of tail biting in undocked pigs should be investigated in future studies.

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## ASSESSING THE PRESENCE OF CLOSTRIDIOIDES DIFFICILE AND CLOSTRIDIUM PERFRINGENS TYPE A ON RUSSIAN FARMS SUFFERING FROM RECURRENT NEONATAL DIARRHOEA

J. Miguel <sup>1</sup>, D. Polutov <sup>2</sup>, A. Denisova <sup>2</sup>, A. Kolesnikov <sup>2</sup>, <u>D. Llopard <sup>3</sup></u> <sup>1</sup>*HIPRA, Amer (Girona), Spain* <sup>2</sup>*HIPRA Russia, Moscow* <sup>3</sup>*HIPRA, Amer, Spain* 

## **Background and Objectives**

Neonatal diarrhoea (ND) is a common problem in piglets. The cause of neonatal diarrhoea in piglets is often multifactorial and can involve bacterial pathogens such as Escherichia coli (E. coli), Clostridium perfringens type C and A (CpC & CpA), Clostrodioides difficile (C. difficile), or viruses such as Rotavirus or Porcine Epidemic Diarrhoea. As the prevalence of C. difficile and CpA on Russian farms has not been recently investigated, the objective of this study was to evaluate the incidence of both agents on farms with recurrent neonatal diarrhoea even though they were vaccinating against E. coli and CpC.

## **Material and Methods**

A total of 28 farms (sow size ranging from 1,500-12,500 - a total of 114,500 sows) distributed around Russia were included in the study. All the farms were vaccinated with different E. coli and CpC combo vaccines. A total of 166 litters were sampled with rectal swabs and placed on FTA Elute cards (Enterocheck kits) for polymerise chain reaction (PCR) testing. Cycle threshold (Ct) values were determined.  $\alpha$ -toxin of CpA was considered relevant within the Ct-value <26.

## Results

The average age of piglets at sampling was 7.7 days. 75% of the farms were positive for C. difficile, and 56.63% of litters. In relation to CpA, 100% of the farms were positive, with 30.1% of samples being positive in a high bacterial load. 22% of farms were positive for both pathogens when considering high CpA load.

## **Discussion and Conclusion**

This study demonstrates that C. difficile and CpA are both highly prevalent on Russian farms and need to be considered as potential pathogens of ND. Furthermore, the use of new diagnostic techniques can help with the detection of the pathogens involved. Preventive tools like vaccination are key to complementing the multifactorial control of ND.

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## CASE STUDY: KETOSIS IN HYPER-PROLIFIC FIRST PARITY SOWS DURING THE PERIPARTUM PERIOD

<u>M. Ros Fuertes</u><sup>1</sup>, P. Blanco<sup>1</sup>, I. Huerta<sup>1</sup>, J.A. Calderon Diaz<sup>2</sup> <sup>1</sup>Pig Improvement Company (PIC) <sup>2</sup>Pig Improvement Company

### **Background and Objectives**

Increased litter sizes has also translated into increased energy and nutrient requirement for sows during the gestation period. Higher energy demands could mean that sows enter in a negative energy balance and start to convert fatty acids to ketones. A high risk of pre-partum ketosis was reported in ewes associated to larger litters. In sows, indication of prepartum ketosis has also been reported. Therefore, the objective of this case study was to identify the incidence of subclinical ketosis in hyperprolific first parity sows during the peripartum period and to describe (re)productive parameters in sows with subclinical ketosis.

## **Material and Methods**

Hyper prolific Landrace x Large White primiparous sows (n=126) were used for this case study. Sows were fed a standard barley based gestation diet (2.15 Mcal NE/kg, 5 g/kg of SID Lys and 1.2 SID Leu:SID Lys ratio). Concentration of  $\beta$ -hydroxybutyrate (BHBA, mmol/L) was measured using a blood glucose and ketone bodies handheld monitor at approximately D111 of gestation. Concentration of BHBA  $\ge$ 0.1 mmol/L were considered indicative of subclinical ketosis. Individual sow body weight and information on total born and born alive piglets, litter weight, and sow average daily feed intake (ADFI) were collected.

### Results

Subclinical ketosis was observed in 25.4% of sows. In total, 20 and 12 sows had BHBA concentrations of 0.1 and  $\ge 0.2$  mmol/L, respectively. On average, sows with subclinical ketosis were 9.5 kg heavier at D111 of gestation than healthy sows. Total born (17.6±2.86 vs. 17.7±3.17), piglets born alive (16.5±2.37 vs. 16.4±3.11) and ADFI (5.1±1.22 and 5.6±0.95) were similar between both groups of sows.

## **Discussion and Conclusion**

A high incidence of subclinical ketosis was observed in this case study. However, BHBA concentrations were relatively low and thus, it is likely that large litter sizes did not impose enough of a metabolic stress on the sows to raise BHBA. A more controlled approach including a higher number of sows, more sampling points, access to individual feeding event records and varying litter sizes is needed to continue to elucidate the impact, if any, of ketosis on sow productivity

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## CORRELATION BETWEEN PRRSV AND SIV INFECTIONS AND FARM CHARACTERISTICS ON DUTCH SWINE FARMS

<u>J. Beek</u><sup>1</sup>, T. Nunes<sup>4</sup>, A. Schuttert<sup>2</sup>, E. Van Esch<sup>3</sup>, D. Giralt<sup>4</sup>, L. Nodar<sup>4</sup>, J. Miranda<sup>4</sup> <sup>1</sup>HIPRA Benelux <sup>2</sup>De Oosthof, The Netherlands <sup>3</sup>Technical Services Manager Swine, HIPRA Benelux <sup>4</sup>HIPRA, Amer (Girona), Spain

## **Background and Objectives**

The Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Swine Influenza Virus (SIV) are the primary agents of the Porcine Respiratory Disease Complex (PRDC) and represent a significant cost for swine producers. The infection in early stages of production is particularly costly and can be associated with different parameters, namely farm characteristics and the biosecurity strategy applied. Oral fluids are a reliable tool to check for PRRSV/SIV infection in the nursery, allowing a large number of individuals to be sampled. The goal of this study was to assess positivity for PRRSV and SIV in nurseries of Dutch farms and its association with the farms' characteristics and production system.

## **Material and Methods**

PRRSV and SIV presence was assessed by RT-PCR on oral fluid samples from 8-week-old piglets in 110 nurseries. The oral fluids were collected with cotton ropes into sterile plastic flasks and sent refrigerated to the laboratory within 24 hours. Data from the farms were collected through a written survey and analyzed to measure the association between PRRSV/SIV positivity and farm size (small <450 sows; medium 451-649 sows; large >650 sows), production system (on-site/off-site nursery) and gilt origin (external/internal).

#### Results

70 farms (63.6%) were positive for at least one of the viruses, including 24 farms (21.8%) that were simultaneously positive for both. The farm size was significantly associated with positivity, with large farms having higher positivity for PRRSV (59.3% versus 30.6%; p=0.04) and SIV (51.9% versus 30.6%; p=0.03) than small farms. Off-site nurseries had lower negativity for PRRSV than nurseries inside farrow-to-finish farms, but without statistical significance (42.6% versus 12.5%; p=0.07). The farms with external replacement gilts were also had higher positivity for both PRRSV (51.1% versus 32.7%; p>0.05) and SIV (53.3% versus 38.8%; p>0.05), although not significantly higher.

## **Discussion and Conclusion**

The results suggest a relevant presence of PRRSV and SIV in the nurseries, with almost 1/4 of farms having simultaneous circulation of both viruses. Farm characteristics, such as herd size, origin of the gilts, and segregated production (off-site nurseries) may also play a role in the positivity of the nurseries and should be taken into account in disease control programmes.

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## EFFICACY OF LATE INTERVENTION PRACTICES TO ELIMINATE PEDV FROM TWO MEXICAN SOW HERDS

A. Henao-Diaz 1, M. Ruy-Sanchez 2, D. Christy 2, H. Garcia 3, D. Case 3

<sup>1</sup>Bachoco <sup>2</sup>BACHOCO, MEXICO <sup>3</sup>ZOETIS, MEXICO

## **Background and Objectives**

After ~10 years, suckling piglet mortality caused by PEDv continues impacting Mexican pork production. Gilts immunization by PEDv exposure and herd closure protocols are recommended to control de disease. However, interruptions in control programs and surveillance allow for re-outbreaks. Since re-outbreaks cause less dramatic losses, late interventions are often improvised. This study describes the use of a commercial PED vaccine as a late intervention practice to control and eliminate PEDv from two Mexican herds after weeks of re-outbreaking.

## **Material and Methods**

After ~2 months of re-outbreak, the intervention program to eliminate PEDv from two ~2500 sows' herds was initiated by establishing the sows' PED-immunity status by an in-house PED- ELISA from 5 groups of sows at 5W-2W pre-farrowing. Then all sows were vaccinated according to manufacturer protocol. Traditional protocols including McRebel and facilities cleaning-disinfection continued. Pre-weaning mortality and other productive parameters were followed. The sows and piglets' PEDv-negative status by PED-PCR was followed according to the Sonora State Health Committee protocols. Pits and facility surfaces were also sampled to confirm the absence of environmental virus.

### Results

Quantitative (S/P) and qualitative (pos/neg) different PED-ELISA results were observed on sows regarding parity: sows of <3 parity showed lower or absence of PED-Abs. After immunization, all sampled sows resulted in PED-ELISA positives, so pre-weaning mortality reduced from 32% to 13%, and litters of <10 piglets recovered from 20.7% to 7% in the coming weeks. After fulfilling the authorities' requirements of four systematic negative PED tests, the herds were declared provisionally negative. The consistent recovery of productive parameters and the introduction of ~700 naïve gilts confirmed the negative herd status. Economical analyses for both farms on a yearly basis are pending.

## **Discussion and Conclusion**

Since re-outbreak is a risk, sows' immunity is fundamental for PEDv control over months or years post-outbreak. Commercial vaccine and antibody surveillance complement the traditional elimination programs by holding sows immunity and reducing susceptible subgroups.

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# EVALUATING THE EFFICACY OF LITTERGUARD LT-C VACCINATION FOR THE CONTROL OF ENTEROTOXIGENIC ESCHERICHIA COLI AND CLOSTRIDIUM PERFRINGENS INFECTIONS IN ENDEMIC PIG FARMS: A STEP TOWARDS ANTIBIOTIC ALTERNATIVES

N. Supimon 1, N.O. Khine 1, W. Niyomtham 1, J. Yindee 1, N. Prapasarakul 2

<sup>1</sup>Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. <sup>2</sup>Center of Excellence in Diagnostic and Monitoring of Animal Pathogens and Faculty of Veterinary Science, Chulalongkorn University. Thailand

## Background and Objectives

Neonatal diarrhea is a major problem in swine farms. The LitterGuardLT-C vaccination contains C.perfringens type C and inactivated Enterotoxigenic Escherichia coli (ETEC). The vaccination is to prevent neonatal diarrhea by passively transferring protective maternal antibodies to their piglets. The purpose of the study was to assess LitterGuard LT-C efficacy to prevent the cause of neonatal diarrhea in piglets raised in endemic farms comprising ETEC and C. perfringens.

## Material and Methods

From 9 farms, a total of 142 fecal swabs were individually collected from sows (Po and P1+), and piglets at 7 day of age. Fecal swabs collected were subdivided into 1.1) 72 samples from unvaccinated pigs and 1.2) 70 samples from vaccinated pigs from the same farm. The pregnant sows and gilts were vaccinated intramuscularly with 2 ml of LitterGuard LT-C at 11 weeks pre farrow and a booster at 3 weeks apart. After selective culture, the MALDI Biotyper was used to identify the bacterial colony. Samples would be tested by multiplex PCR and virulent factors associated with ETEC [2]. Multiplex PCR was also used to determine the types of clostridial toxin in all positive isolates [3].

#### Results

In vaccination trial, ETEC was detected at a rate of 5% in offspring from unvaccinated sows compared to 1.4% in offspring from vaccinated sows. The non-detection of C. perfringens type C restricts a comprehensive evaluation of the vaccine's efficacy. %PWM in vaccinated group is lower than non-vaccinated (2.7 and 4.9) especially in P0(5 vs 9.2).

## **Discussion and Conclusion**

This vaccination study revealed a significant decrease in the prevalence of ETEC among vaccinated pigs, indicating the efficacy of the LitterGuard LT-C immunization in preventing ETEC infections. This study demonstrated that the LitterGuard LT-C vaccination is effective in protecting piglets from E. coli scours on endemic farms.

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## FIRST EXPERIENCES OF THE DANISH PRRS REDUCTION STRATEGY

<u>N. Weber</u><sup>1</sup>, B. Lorenzen <sup>1</sup>, K. Møller <sup>1</sup> <sup>7</sup>Danish Agriculture & Food Council

#### **Background and Objectives**

A joint strategy, to reduce PRRSV in the Danish pig production, developed in a collaboration between veterinary authorities, slaughter companies, veterinarians, and pig producers, was published in May 2022. The objective of the national strategy is that 75% of all finishers will be PRRSV seronegative at slaughter and 85% of all sow herds will be PRRSV seronegative. To achieve the overall objective of the national reduction strategy, it is necessary to establish regional eradication programs to ensure that a systematic approach is applied to eradication in specific regional areas with high density of PRRSV seronegative herds.

## **Material and Methods**

A range of measures have been applied with the purpose of paving the road for a national reduction of PRRS. The most important measures are mandatory declaration of serological PRRSV status and a deduction system resulting in a reduced slaughter price on pigs from PRRS virus-positive herds. To support the reduction program, following projects are initiated: a) improvement of diagnostics during the regional eradication, b) monitoring program after regional eradication, c) follow-up on reinfections by data mining and questionnaire.

#### Results

As a result of the new PRRS measures a total of 16 regional eradication programs have, by November 2023, been established by voluntary initiatives of veterinarians, consultants, and pig producers. The regional programs include more than half of all Danish pig farms and are led by local program coordinators.

By November 2023 the proportion of PRRSV seronegative finishers delivered for slaughter has improved since the reduction strategy was published from 25% to 60%. The proportion of PRRSV seronegative sow herds have improved from 58% to 66%.

## **Discussion and Conclusion**

The Danish reduction program is off to a good start mainly due to many years of experience with handling PRRSV, via the Danish SPF system, and accumulated knowledge in close collaboration with veterinarians, universities, and other research institutions. Next step is to develop a follow-up monitoring system in regions which has been declared free of PRRSV.

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## LAMENESS ALTERS C-REACTIVE PROTEIN AND PIG MAJOR ACUTE PHASE PROTEIN IN GESTATING SOWS

N. Jakobsen <sup>3</sup>, I. Larsen <sup>3</sup>, N.R. Weber <sup>1</sup>, P. M. H. Heegaard <sup>2</sup>, K.S. Pedersen <sup>3</sup>

<sup>1</sup>Veterinary and Quality Services, Danish Agriculture and Food Council, F.m.b.A., Denmark

<sup>2</sup>Department of Health Technology, Technical University of Denmark, Kemitorvet 202, 2800 Kgs. Lyngby, Denmark <sup>3</sup>Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 2, DK-1870 Frederiksberg C, Denmark

### **Background and Objectives**

Lameness in sows is reported as one of the main reasons for early culling and is associated with reduced locomotion, pain, and a high antibiotic usage. The prevalence differs between housing systems and countries but is reported to be between 4.1% and 24.3%. The diagnostic tools to determine if the lameness is infectious or non-infectious are limited, but acute phase proteins could potentially fulfil this role. Hence the objective of this study was to investigate if and which acute phase proteins are altered in gestating lame sows compared to clinically healthy sows.

## **Material and Methods**

A case-control study was carried out in 12 conventional sow-herds in Denmark from May 2023 to July 2023. In total 50 pairs of lame and healthy sows were included, and matching was performed based on parity, expected farrowing date and pen. A blood sample was collected from the jugular vein and an ELISA performed to determine C-reactive protein (CRP), pig major acute phase protein (Pig-MAP), serum amyloid A (SAA) and haptoglobin (Hp) levels. A linear regression with Group (Lame vs. Healthy) and Herd as fixed effects was used to determine differences between means.

#### Results

The mean CRP level was statistically higher in lame sows compared to healthy sows (p<0.05) with lame sows having a mean CRP level of 24.69 µg/ml and healthy sows a level of 16.19 µg/ml. Pig-MAP levels were also significantly higher in lame sows compared to healthy sows (p<0.05) with lame sows having a mean Pig-Map level of 0.92 µg/ml and healthy sows a mean Pig-Map level of 0.81 µg/ml. For SAA and Hp no differences were found. Having one or more signs of inflammation on the affected limbs for the lame sows did not alter any of the measured acute phase protein levels.

## **Discussion and Conclusion**

Lameness in gestating sows resulted in elevated CRP and Pig-Map levels compared to clinically healthy sows, but evidence connecting the CRP or Pig-MAP level with underlying aetiology or prognosis is still needed before acute phase proteins can become a useful diagnostic tool to help determine whether antibiotic treatment should be initiated.

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## PIGMARKSAL SHOWED THE ASSOCIATION BETWEEN BODY WEIGHT AND SALIVARY INNATE IMMUNE MARKERS.

M. Matas-Quintanilla <sup>1</sup>, C. Piñeiro <sup>2</sup>, A. Miralles-Chorro <sup>3</sup>, J. Sánchez <sup>3</sup>, P. Fuentes <sup>3</sup>, <u>A.M. Gutiérrez</u> <sup>1</sup> <sup>1</sup>BioVetMed Research Group, Department of Animal Medicine and Surgery, Veterinary School, University of Murcia, 30100, Espinardo, Murcia, Spain <sup>2</sup>Animal Data Analytics, 30006, Segovia, Spain <sup>3</sup>CefuSA,30840, Alhama de Murcia, Murcia, Spain.

## **Background and Objectives**

Inflammation and acute phase reaction belong to the innate immune reaction of the body. It has been recently established the reference range values of several salivary innate immune markers including inflammatory markers, adenosine deaminase and S100A12, and acute phase proteins. The usefulness of these salivary determinations in field conditions has been also stated for several pathological conditions in growing pigs. The PigMarkSaL team has explored the usefulness of salivary biomarkers for animal performance prediction in clinically healthy weaning piglets in the present study.

## **Material and Methods**

The post-weaning phase of a commercial growing farm was monitored from week 0 to week 7. A total of 24 clinically healthy piglets at week 0 and 10 clinically healthy and 10 pigs with signs of disease at weeks 3, 5 and 7 were used in the study. Saliva samples were collected for all piglets before they were individually weighted. The concentration of C-reactive protein, haptoglobin, adenosine deaminase and S100A12 were quantified using previously optimized and validated assays. To analyze the association between body weight and salivary parameters, the Spearman correlation test was used. A linear regression analysis was performed to test for the salivary parameters that better explain the variations in body weight.

#### Results

The concentrations of all immune markers appeared inversely associated with the body weight of piglets. Haptoglobin levels showed the highest association with body weight, with a correlation coefficient of r = -0.70. The linear regression analysis showed a model that included haptoglobin, adenosine deaminase and S100A12 determinations with a correlation coefficient of r = 0.73 and a coefficient of determination of r = 0.53.

## **Discussion and Conclusion**

According to the PigMarkSaL results, an increase in the levels of salivary Hp could be interpreted as a sign of low animal performance due to its association with reduced body weight. Moreover, the model for body weight estimation using salivary parameters, specifically haptoglobin, adenosine deaminase and S100A12 quantifications, should be further explored to optimize the porcine production system monitoring. (Granted by PID2020-116310RB-I00; pigmarksal.com).

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## REDUCTION OF PRESSURE OF INFECTION AND MORTALITY AFTER INTRADERMAL PIGLET VACCINATION AGAINST PRRSV, MYCOPLASMA HYOPNEUMONIAE AND PCV2

<u>C. Ardies</u><sup>1</sup>, T. Nunes<sup>2</sup>, R. Jorda<sup>2</sup>, P. Hamon<sup>3</sup>, L. Manche<sup>3</sup>, K. Autret<sup>3</sup>, D. Giralt<sup>2</sup> <sup>1</sup>*HIPRA, 7 rue Roland Garros, Bat H, 44700 ORVAULT, FRANCE* <sup>2</sup>*Hipra, Amer (Girona), Spain* <sup>3</sup>*SELARL Vétérinaire des Aniocs, Pleyben, France* 

### **Background and Objectives**

Vaccination against the Porcine Respiratory and Reproductive Syndrome Virus (PRRSV), the Porcine Circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhyo) reduces the pressure of infection and the clinical manifestations on the infected animals. This case-report describes the changes of pressure of infection and mortality after the implementation of an intradermal piglet vaccination program.

## **Material and Methods**

On a French farrow-to-finish farm, a coinfection of PRRSV and PCV2 was diagnosed in nursery piglets and fatteners (RT-PCR and ELISA testing of blood samples), coinciding with increased mortality and higher demand for antimicrobial treatments, namely against arthritis. The piglets were only vaccinated against Mhyo. An intradermal vaccination programme was then implemented at weaning with Unistrain® PRRS and Mhyosphere® PCV. Blood samples were collected before and after the implementation (NV and V groups respectively) at 10 weeks of age, in the middle of fattening (16-19 weeks of age) and at 25 weeks of age. The antibody levels and viremia (PRRSV/PCV2) were assessed, respectively, by ELISA and RT-PCR at each blood sampling timepoint (ORF-5 sequencing was performed on PRRS-positive samples with Ct-value <30). The number of death pigs, individual antimicrobial treatments and animals diagnosed with arthritis were recorded weekly. The statistical analisys was performed using R software v4.3.

#### Results

At 10 weeks of age, the NV piglets were PCR-positive to PRRSV (wild-type virus) whilst all V samples remain negative to the virus. All samples were PCR-negative to PCV2, and PCV2 antibody titres were similar at 10 weeks (2429.5 versus 2293.2, p=0.527), but significantly higher in group NV at the middle of fattening (5108.8 versus 1490.7, p<0.001) and at 25 weeks (5143.1 versus 2056.7, p<0.001). Average weekly mortality was lower in group V (0.77 dead pigs versus 1.73), as were antimicrobial treatments (0.23 versus 4.94) and the number of animals diagnosed with arthritis (0.14 versus 3.37).

## **Discussion and Conclusion**

The vaccinated batches showed a milder seroconversion to PCV2, suggesting a significant reduction of viral circulation on the vaccinated population. Similarly, the results suggest a reduction of PRRSV circulation, with no virus being found on the samples from vaccinated animals. These variations were accompanied by a reduction of mortality, antimicrobial treatments, and arthritis incidence.

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## **RESPIRATORY TRACT INFECTIONS - AN INDICATION FOR EUTHANASIA IN PIGS?**

M. Miller <sup>3</sup>, J. Reinmold <sup>1</sup>, K. Gerdes <sup>1</sup>, E. Grosse Beilage <sup>1</sup>, M. Wendt <sup>2</sup>, I. Hennig-Pauka <sup>2</sup>, <u>N. Kemper <sup>3</sup></u> <sup>1</sup>Field Station for Epidemiology, Bakum, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

<sup>2</sup>Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

<sup>3</sup>Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour (ITTN), University of Veterinary Medicine Hannover, Foundation, Germany

### **Background and Objectives**

Infections of the respiratory tract are a well-known problem in today's pig husbandry at all stages of production. Besides the negative impact on animal health and welfare, decreased daily weight gains due to reduced feed intake, higher mortality rates and increased costs of medication can cause significant economic losses. This study, as part of the CARE-PIG-project, investigated the role of respiratory diseases in cases of fatal disease progression with final euthanasia.

## **Material and Methods**

Over 10 months, three trained veterinarians examined pigs on five different pig farms clinically, three days per week (Mo, We, Fr). Animals showing signs of respiratory tract infections were included in the study and individually treated with medication according to their symptoms. On all examination days, the clinical findings and additional photos and videos were taken to document course, severeness and development of the infections. In case the pig recovered, this was documented and the pig was dismissed cured. If recovery seemed unlikely or impossible, the pig was euthanized and examined pathologically. All findings were documented and analysed statistically with SAS (SAS Institute Inc., Cary, USA).

#### Results

Until November 2023, in general 507 pigs had been included in this study, with 80 ending in euthanasia and pathological examination. Respiratory tract infections that result in the need of individual treatment were not a common cause to include pigs in this study. In some cases, pigs showing signs of respiratory disease died an unexpected sudden death. In euthanized animals, only in very few cases pleurisy and pneumonia were diagnosed pathologically.

## **Discussion and Conclusion**

While respiratory tract infections diseases are one of the major problems in pig husbandry, in this study these diseases were not one of the main reasons for euthanasia. Nevertheless, infections of the respiratory tract should always be treated timely to provide the best possible prognosis and to minimize pain and suffering in the affected pigs. This work is financially funded by the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE; grant number 28N-2-008-01 "CARE-PIG").

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## THE ROLE OF OPPORTUNITY COST IN HEALTH MANAGEMENT AND COST CONTROL

L. Mulberry <sup>1</sup>, L. Lecznieski <sup>2</sup>, S. Chunqing <sup>3</sup> <sup>1</sup>Knowledge Ventures, LLC <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH, Germany <sup>3</sup>Boehringer Ingelheim Animal Health (Shangai) Co., Ltd

## **Background and Objectives**

Producers face the challenge of managing herd health while managing costs, however, effective cost control must consider both opportunity costs and accounting costs. Accounting costs are those tangible costs such as feed, labor, etc. Opportunity costs are losses from missed production potential, put simply, the additional profit that could have been achieved but was not. In swine production opportunity cost is commonly found in losses from reduced performance metrics. When managing costs, opportunity costs must be considered to ensure expected profits are achieved.

## **Material and Methods**

Five studies were conducted on Chinese finishingers to evaluate vaccine performance in two categories, PCV2 (Vaccines A, B, & C) and 2-dose RTU PCV2 & M.hyo (Vaccine D, E, & F), in a natural challenge environment. Individual pig records were kept on weight and mortality. Using this data, a bio-economic model simulated the growth and feed consumption of individual animals. Pigs were marketed in groups and optimized to maximize annual return over feed cost. A Chinese packer matrix was used to adjust the base price of each animal to measure the economic impact of variation. Mortality costs were calculated as the animal's market value plus feed consumed. Annual net returns were compared between the vaccine groups to evaluate economic performance.

### Results

Total vaccine cost was calculated as the purchase price of the vaccine plus the opportunity costs of impaired growth and mortality on a per head basis. Results are shown in \$US as total vaccine cost and (opportunity cost) and are as follows, Vaccine A=\$4.30(\$0), B=\$14.14(\$11.02), C=\$16.87(\$14.49), D=\$5.49(\$0), E=\$22.05(\$19.24), F=\$14.36(\$14.49). Results indicate that in most cases opportunity cost from impaired growth and mortality is significantly higher than accounting cost alone.

### **Discussion and Conclusion**

Opportunity cost can have a significant impact on economic performance and deserves careful consideration. While this study shows opportunity cost in vaccination, it also holds true in other areas of health management. When managing costs, it is important to calculate the economic impact of any changes in production performance to better understand the full cost.

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## ASSESSMENT OF EP- AND A.P.-LIKE LUNG LESIONS ON DUTCH AND BELGIAN FARMS USING THE CEVA LUNG PROGRAM SCORING SYSTEM, BETWEEN JANUARY 2022 AND OCTOBER 2023

<u>S. Van Colen</u><sup>1</sup>, E. De Jonghe<sup>1</sup>, P. De Backer<sup>1</sup> <sup>1</sup>Ceva Santé Animale, Brussels, Belgium

## **Background and Objectives**

Lung lesion scoring at the slaughter line is a useful tool to evaluate the respiratory health status of a pig farm and to evaluate the success of respiratory disease control programs, especially for enzootic pneumonia (EP) and swine pleuropneumonia. An overview of the lung screening results performed on slaughter pigs from Dutch and Belgian farms between January 2022 and October 2023 is presented in this report.

#### **Material and Methods**

Over a 22 months period, from January 2022 to October 2023, lungs of 203.171 slaughter pigs from 1403 batches of Dutch and Belgian farms were evaluated. To assess the lung lesions in a standardized manner, the Ceva Lung Program scoring methodology was used. Presence of bronchopneumonia which is suggestive for enzootic pneumonia (EP) caused by Mycoplasma hyopneumoniae (M.hyo), including percentage of affected surface of the bronchopneumonic lungs, was quantified. Based on these data, the EP-index was calculated. Presence of scarring and cranial pleurisy was evaluated. Presence and severity of dorsocaudal pleurisy, suggestive for Actinobacillus pleuropneumoniae (A.p.) infections, was scored and the APP-index was calculated.

#### Results

The median % of bronchopneumonic lungs was 6,67%, with Q1=1,21% and Q3=21,08%. The median % of affected surface of the bronchopneumonic lungs was 5,60%, with Q1=2,66% and Q3=7,54%. The median EP-index was 0,21 with Q1=0,03 and Q3=0,83. The median % of scarring was 3,92%, with Q1=1,75% and Q3=7,55%. The median % of cranial pleurisy was 1,06%, with Q1=0,00% and Q3=2,82%. The median % of lungs with dorsocaudal pleurisy was 10,26%, with Q1=3,39% and Q3=27,58%. The median APP-index was 0,32, with Q1=0,11 and Q3=0,82.

## **Discussion and Conclusion**

Earlier published data, summarizing the lung screening results in most European swine producing countries in 2021 showed a median % of bronchopneumonic lungs of 24% and a median % of lungs with dorsocaudal pleurisy of 8%. Comparing these data, a much lower prevalence of EP-like lesions and a slightly higher prevalence of A.p.-like lesions was observed in Belgium and the Netherlands, compared to the overall European situation.

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## COMPARISON OF MELOXICAM DOSING PROTOCOLS IN PERIPARTURIENT SOWS AND THE EFFECT ON FEVER REDUCTION AND PERFORMANCE

<u>E. Larsen</u><sup>1</sup>, A. Anderson Reever<sup>2</sup>, B. Burton<sup>2</sup>, B. Payne<sup>3</sup> <sup>1</sup>Lincoln Memorial University College of Veterinary Medicine, Harrogate, TN, USA <sup>2</sup>Suidae Health and Production, Algona, USA <sup>3</sup>Veterinary Pharmaceutical Solutions, St. Peter, MN, USA

## **Background and Objectives**

Periparturient inflammation and can result in negative implications for both the dam and litter. Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) that has been shown to have analgesic, anti-inflammatory, anti-endotoxic and antipyretic effects. In previous studies, a single dose reduced rectal temperatures at 12- and 24-hours post-farrowing, increased litter wean weights, and improved sow recovery. The objective of this study was to determine the effects of one versus two post-farrowing doses of meloxicam on maternal fever, piglet performance, and subsequent breeding metrics.

#### **Material and Methods**

In a 5,000-head sow farm, 403 sows within parity groups (gilts, P2-P3, P4+) were assigned to one of three treatment groups. One group received a single oral dose of compounded meloxicam (90 mg, 6 mL suspension), in the feeder post-farrowing, while a second group received an additional dose 48 hours later. The control group was not treated. Sow rectal temperatures were recorded upon farrowing completion and every 24 hours for 3 days post-farrowing. Fever was defined as a rectal temperature ≥103°F.

### Results

The percentage of sows with fever prior to treatment was similar (16-20%) between treatment groups. Only subtle numeric trends were noted between meloxicam treatment groups compared to controls for fever reduction (5-7%). A higher fever rate and greater response to meloxicam treatment were observed in first-litter gilts (14-23% above all parity averages) and at 72 hours post-farrowing, fever rate was lowest in the two-dose group, intermediate in the one-dose group, and highest in the control group (37.1%, 42.5%, 51.4%, respectively). Meloxicam treatment resulted in 7-9% more sows bred within 7 days post-weaning compared to the controls and the controls had the lowest retention rate to date. No differences in piglet mortality were observed due to unaccounted for piglets.

### **Discussion and Conclusion**

These findings were comparable to previously reported research. This study used a novel method of meloxicam administration (in the feeder vs. oral drench) which required minimal labor, was easy to administer and was consumed readily by most sows. The numeric trends identified in the study indicate it is worth exploring further benefits of a two-dose meloxicam protocol.

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## EFFECT OF THE ANTEROOM TO PREVENT PATHOGENS ENTERING AND SPREADING WITHIN THE PIG FARM

Y. Oh<sup>1</sup>, J. Cho<sup>2</sup>, D. Tark<sup>3</sup>, D. Yoo<sup>4</sup>, H. Cho<sup>2</sup>

<sup>1</sup>College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon, Republic of Korea

<sup>2</sup>College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan, Republic of Korea <sup>3</sup>Laboratory for Infectious Disease Prevention, Korea Zoonosis Research Institute, Jeonbuk National University, Iksan, Republic of Korea

<sup>4</sup>College of Veterinary Medicine, Chonnam National University, Gwangju, Republic of Korea

## **Background and Objectives**

Biosecurity plays a critical role in preventing and controlling the introduction and spread of infectious diseases. This is particularly evident in the case of pig farming as shown by the recent episodes of African swine fever (ASF) in Korea. The purpose of this study was to confirm the effect of pathogens entering and spreading within the pig farm on various types of anterooms, which has recently been controversial in Korea

## Material and Methods

A real-time qPCR for pathogens was performed by applying pathogens (porcine reproductive and respiratory syndrome virus, classical swine fever virus, porcine epidemic diarrhea virus, Erysipelothrix rhusiopathiae) to the anteroom floor for three pigpens and swabbing the floor from 0 to 30 meters along the hallway for 15 days.

#### Results

## **Discussion and Conclusion**

The anteroom is considered a core biosecurity facility, acting as a gateway to prevent visitors from entering pigpens where pigs are located. Therefore, this study proved that the anteroom as an internal biosecurity facility plays a very important role as a prevent of pathogens from entering and spreading within the pig farm.

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## EVALUATING ECONOMIC ASPECTS OF ENDEMIC RESPIRATORY DISEASES IN PIGS AND INTERVENING STRATEGIES

M. Boeters <sup>1</sup>, B. Garcia-Morante <sup>2</sup>, G. Van Schaik <sup>1</sup>, J. Segalés <sup>2</sup>, J. Rushton <sup>3</sup>, W. Steeneveld <sup>1</sup>

<sup>1</sup>Department of Population Health Sciences, section Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands.

<sup>2</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain.

<sup>3</sup>Institute of Infection, Veterinary and Ecological Sciences, School of Health and Life Sciences, University of Liverpool, Liverpool, UK.

## **Background and Objectives**

Understanding the losses in production and costs caused by individual and concurrent pathogens within the porcine respiratory disease complex (PRDC) provides opportunities to enhance farm profitability through improved disease management and prevention. However, while economic research in this area has been carried out, an overview on the economic impact of the PRDC and intervening strategies is lacking. Therefore, a systematic review was implemented to identify published information on the economic impact of the most significant PRDC pathogens and the costs and benefits of interventions.

#### Material and Methods

The systematic literature review was conducted following the PRISMA method. The primary pathogens included in the search were: the porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus 2, swine influenza virus, Mycoplasma hyopneumoniae, and Actinobacillus pleuropneumoniae. When applicable, the reported economic impacts were adjusted for inflation and the original currencies were converted to Euros.

#### Results

Fifty-eight (58) studies were deemed eligible for the purpose of this systematic review. Main findings were: (1) Studies mainly considered endemic scenarios in commercial fattening farms; (2) PRRSV was by far the most studied pathogen; (3) Most studies calculated the economic impact using primary production data, whereas twelve studies modelled the impact using secondary data only; (4) Seven different economic methods were applied across studies; (5) A large variation exists in the cost and revenue components considered in calculations, with feed costs and reduced carcass value included the most often; (6) The reported median economic impact of one or several co-existing respiratory pathogen(s) ranged from  $\in 1.70$  to  $\in 8.90$  per nursery pig,  $\in 2.30$  to  $\in 15.35$  per fattening pig, and  $\in 100$  to  $\in 323$  per sow per year; and (7) Vaccination was the most studied intervention.

## **Discussion and Conclusion**

This study provides an understanding of the current state of economic research on the PRDC and their control, by offering insight in the variation in studies, their methods, their advantages and limitations, and the reported impacts from the PRDC for pig production systems worldwide. To enhance future research, it is imperative to promote systematic approaches to economic evaluations that allow comparisons between different time periods and production systems.
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# EVOLUTION OF THE HB CONCENTRATION AFTER INTRODUCTION OF AN INJECTABLE GLEPTOFERRON/TOLTRAZURIL COMBINATION PRODUCT ON DUTCH SWINE FARMS.

<u>B. Cornelis</u><sup>1</sup>, P. De Backer <sup>2</sup>, J. Schumans <sup>3</sup>, S. Van Colen <sup>3</sup> <sup>1</sup>DVM Ceva Santé Animale <sup>2</sup>Ceva Santé Animale Benelux <sup>3</sup>Ceva Santé Animale. Naaldwiik. the Netherlands

#### Background and Objectives

Iron Deficiency Anemia (IDA) causes immune suppression and growth retardation in suckling piglets. Iron supplementation during the first days of life is a routine treatment to prevent IDA. One of the main parameters to identify IDA is measurement of the hemoglobin (Hb) level in the blood. In 10 Dutch farms, the veterinarian and farmer wanted to check if the Hb-levels could be improved by using Forceris (product F), an injectable combination product of iron (gleptoferron) and toltrazuril.

# **Material and Methods**

In 10 Dutch farms, blood samples of 708 piglets with an average age of 19.5 days were collected by puncture of an ear vein and Hb-levels were measured using the Hemocue® Hb 201+ analyzer. Piglets with an Hb-level < 90 g/l,  $\ge$  90 g/l and < 110 g/l and  $\ge$  110 g/l have respectively clinical anemia, subclinical anemia and optimal Hb-levels. In each farm, Hb-measurements were performed on piglets treated with the conventional treatment (n=387) and compared with Hb-levels of piglets treated with product F (n=321). 7 of the 10 farms were originally using an iron-dextran product, the others were using a gleptoferron product.

### Results

Hb-levels were significantly higher (p<0.05) and more homogeneous in piglets treated with product F, with average Hb-levels in farm A to J of 107.5 vs. 111.0, 103.3 vs. 109.6, 104.6 vs. 114.9, 97.7 vs. 106.6, 99.5 vs. 105.7, 97.7 vs. 104.2, 95.7 vs. 105.1, 98.9 vs. 105.9, 103.8 vs. 110.1 and 97.3 vs. 107.1 for piglets treated with the conventional product vs. product F respectively. Overall, in the 10 farms, the percentage of piglets with clinical anemia, subclinical anemia and optimal Hb-levels were respectively 28% vs. 46%, 56% vs. 47% and 16% vs. 6% in the piglets treated with the conventional product vs. product F.

# **Discussion and Conclusion**

In 10 different commercial Dutch farms, the use of an injectable combination of gleptoferron and toltrazuril (product F) resulted in significant higher and more homogeneous Hb-levels in suckling piglets, compared with alternative treatments.

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# FACTORS CONTRIBUTING TO THE RELATIVE PROFITABILITY OF PRODUCERS IN MAJOR PORK-PRODUCING COUNTRIES

<u>D. Holtkamp</u><sup>1</sup>, L.L. Schulz<sup>2</sup>, D. Hayes<sup>2</sup> <sup>1</sup>Iowa State University College of Veterinary Medicine, United States <sup>2</sup>Iowa State University College of Liberal Arts and Sciences

### **Background and Objectives**

The Global Swine Benchmarking (GSB) project was initiated in 2019 to benchmark the historical productivity and financial performance of producers in the major swine-producing countries. Each year, the relative cost of production and profitability of producers in each country are benchmarked. This study aims to use the data collected for the GSB project to assess the factors that contribute to the relative profitability of producers in different countries.

# **Material and Methods**

A breed-to-market production and economic model was built to conduct the analysis. Data for countries in Europe and the Americas comes from an international benchmarking network known as InterPIG, and data for countries in Asia comes from industry experts and published sources. The factors evaluated for their contribution to the relative profitability of producers in each country included market pig prices, feed prices, productivity, fixed costs, labor usage and wage rates. The analysis was conducted by setting the values for the factors and all other parameters in the model to the average for all countries. Each factor was then evaluated individually by setting the values for that factor to the reported values for each country, while the values for all other parameters in the model remained set at the average for all countries.

#### Results

The results reported are for 2021. The factors contributing to the highest relative advantage or disadvantage were market pig prices, feed prices, and productivity. Very high market pig prices in China that year gave producers a US\$2.19 per kg of carcass weight advantage over a country with average market pig prices. China's advantage over the USA was US\$2.20 since the USA had a US\$0.01 disadvantage relative to the average. Low feed prices in the USA and Canada gave producers a US\$0.24 and US\$0.16 per kg of carcass weight advantage. Producers in Europe benefited from relatively high productivity. Producers in Denmark had a US\$0.31 per kg of carcass weight advantage over a country with average productivity.

# **Discussion and Conclusion**

As global trade policies, animal disease, domestic regulatory environments and consumer demand evolve it is increasingly important to understand how the relative competitiveness of producers in various countries is changing over time.

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# FIELD STUDY COMPARING TWO COMMERCIAL VACCINES AGAINST BACTERIAL NEONATAL DIARRHOEA IN CHINA

T. Yang <sup>1</sup>, J. Miguel <sup>2</sup>, <u>W. Fei</u> <sup>1</sup>, M. Wu <sup>3</sup>, M. Solé <sup>3</sup> <sup>1</sup>HIPRA China, Beijing <sup>2</sup>HIPRA HQ, Amer (Girona), Spain <sup>3</sup>HIPRA, Amer (Girona), Spain

# **Background and Objectives**

Neonatal diarrhoea (ND) is a disease with a significant productive and economic impact. Mortality or weight at weaning are some of the production parameters affected. Although vaccines containing viral or bacterial antigens are commonly used, recurrent ND problems are still frequently observed. This study aimed to compare the efficacy of two different vaccines for the control of Escherichia coli and Clostridium perfringens type C (CpC) on farms suffering from ND in China.

# **Material and Methods**

Company 1 (C1) was using a local PED vaccine and an inactivated bacterin vaccine (V1) containing E. coli F4-F5-F6-F41, and LT-Toxin,  $\beta$  toxoid for CpC and an aluminum hydroxide-based adjuvant. Company 2 (C2) was using only V1.

Both companies switched V1 to SUISENG® (V2), a vaccine adjuvanted with HIPRAMUNE® G and subunit technology, with the same antigen composition.

Production parameters were recorded pre- and post-switch to V2 in both companies. In C1, data recorded from 5,122 and 3,323 litters; In C2, 1,202 and 998.

The % of litters and piglets with diarrhoea, mortality, average daily weight gain (ADWG), weaning weight, and piglets weaned per sow were compared. The statistical analysis was done using the R software. P-value < 0.05 was the limit for statistical significance. To compare the two groups, a T-test was performed.

#### Results

In C1, the percentage of litters and piglets with diarrhoea, mortality, ADWG, and weaning weight statistically improved after starting to use V2. In C2, significant differences were observed in the percentage of piglets with diarrhoea, the mortality rate, and the increase in the number of weaned piglets. Regarding the ADWG and weaning weight, no statistical differences were observed, but a tendency was. In both companies, the piglets born alive per sow remained similar during both periods.

# **Discussion and Conclusion**

Based on the results observed in both companies, SUISENG<sup>®</sup> provided greater protection against bacterial ND compared to V1. This improvement could be explained based on the greater immune response in the sows due to adjuvant and the inclusion of subunit antigens for E. coli adhesion factors. So, greater levels of immunoglobulins are transferred via colostrum providing better protection for the control of ND.

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# RELATION BETWEEN EP-LIKE LESIONS AND PLEURISY WITH PLUCK AND GUT LESIONS IN SLAUGHTERED PIGS IN NORTHERN PORTUGAL

<u>M.A. Rodrigues</u><sup>1</sup>, P. Teiga-Teixeira<sup>2</sup>, E. Teiga-Teixeira<sup>3</sup>, A. Esteves<sup>4</sup>, A.C. Coelho<sup>4</sup>, M.D.C. Fontes<sup>4</sup> <sup>1</sup>School of Agricultural and Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal <sup>2</sup>Food and Veterinary Division of Vila Real and Douro Sul, Directorate of Food and Veterinary Services of the North Region, General Directorate of Food and Veterinary Affairs, Portugal Department of Veterinary Sciences <sup>3</sup>Faculty of Sciences of the University of Porto, Porto

<sup>4</sup>Animal and Veterinary Research Center (CECAV), Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

### **Background and Objectives**

Respiratory diseases have a significant impact on the swine production industry. The macroscopic lung lesions most commonly detected during the post-mortem inspection routine in the slaughterhouse are enzootic pneumonia-like lesions (EP-like lesions), primarily related to Mycoplasma hyopneumoniae infection, and pleuritic lesions, usually associated with previous infections of Actinobacillus pleuropneumoniae.

This study aimed to determine the occurrence of EP-like lesions and pleurisy in 2142 finishing pigs from 18 different farms (central and southern Portugal, and northern Spain), the association between EP-like lesions and pleurisies, and the relationship with pluck and gut lesions (lobe scars, emphysema, lung congestion, pericarditis, liver milk spots lesions, steatosis, splenitis, pancreatitis, and enteritis).

# Material and Methods

For this purpose, EP-like lesions were scored per lobe. Pleurisy lesions were classified under the "Slaughterhouse Pleurisy Evaluation System" (SPES) score method. The presence of other lesions such as pericarditis, liver milk spots, splenitis, pancreatitis, lymphadenitis, and enteritis was also evaluated. Descriptive and multivariate logistic regression analyses were carried out. A variable was considered statistically significant when its P-value  $\leq 0.05$ .

#### Results

Among the sample, 41.1% of pigs presented EP-like lesions. Around 12% of the sample presented pleurisy. Following the EP-like lesions and pleurisy, liver milk spots and pericarditis were the most frequent lesions (4.8% and 3.6%, respectively). The higher the EP-like lesion score, the lower the incidence of a SPES score of 4 (OR=0.38, P<0.001). The higher the SPES scores, the lower the incidence of an EP-like lesion score of category 1 (OR=0.86, P=0.031) or category 2 (OR=0.71, P<0.0001). Pericarditis was associated with a higher incidence of SPES scores 1 (OR=8.57, P<0.001), 2 (OR=7.29, P<0.001), or 4 (OR=26.55, P<0.001).

# **Discussion and Conclusion**

Consolidated pneumonia lesions were the most common cause of partial rejection for human consumption, followed by pleuritic lesions, milk spot lesions, and pericarditis. Lung lesions are closely associated with other findings in tissues and organs with economic value, which can be subclinical or difficult to diagnose in live animals.

This study reinforces the importance of monitoring respiratory findings of pig carcasses at the slaughterhouse to assess the animal's production performance and welfare.

HHM – Herd Health Management

# TRACHEAL SAMPLE POOLING APPLIED TO MYCOPLASMA HYOPNEUMONIAE SURVEILLANCE

A.P. Serafini Poeta Silva<sup>1</sup>, R. Mugabi<sup>1</sup>, M. Rotolo<sup>2</sup>, S. Krantz<sup>3</sup>, R. Robbins<sup>2</sup>, D. Hemker<sup>2</sup>, A. Diaz<sup>4</sup>, A. Tucker<sup>5</sup>, R. Main<sup>1</sup>, J.P. Cano<sup>6</sup>, P. Hams<sup>2</sup>, C. Wang<sup>1</sup>, M.J. Clavijo<sup>7</sup>

<sup>1</sup>Iowa State University <sup>2</sup>PIC North America, Hendersonville, TN 37075 <sup>3</sup>Tosh Farms, Henry, TN USA <sup>4</sup>Pig Improvement Company, Hendersonville Tennessee, USA <sup>5</sup>University of Cambridge, Cambridge, UK <sup>6</sup>Pipestone Veterinary Services <sup>7</sup>Veterinary Diagnostic and Population Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, Pig Improvement Company, PIC®, Hendersonville, TN, USA

# **Background and Objectives**

The cost linked to M. hyopneumoniae (MHP) infection, and the availability of negative breeding stock has increased the adoption of elimination programs. Thus, there is a pressing need for precise, practical, and cost-effective surveillance programs. Tracheal sampling provides the highest sensitivity during early infection. However, the slow transmission of MHP requires large sample sizes for timely detection. The objectives of this study were to estimate the sensitivity of pooled testing of tracheal samples and to develop probability of detection estimates for tracheal samples pooled by 3, 5, and 10.

#### **Material and Methods**

Fifty MHP PCR-positive field samples were pooled by 3, 5, and 10 times using homogenized field MHP DNA-negative samples and tested in triplicate. Bayesian and spatial-spread models were used to estimate pool sensitivity and probability of detection estimates in tracheal samples using simulated field data based on a conservative scenario, 1,250 growing pigs (GDU site) with 0.08% MHP prevalence.

#### Results

The sensitivity was estimated at 0.96 (95% CI: 0.93,0.98) for pools of 3, 0.95 (95% CI: 0.92, 0.98) for pools of 5, and 0.93 (95% CI: 0.89,0.96) for pools of 10. All pool sizes resulted in PCR-positive if the individual tracheal sample Ct value was < 33. Regardless of pool size, sample size, or days post-infection, the probability of detection estimates remained similar (i.e., within 5%) compared to the individual estimates previously published by our group. Considering a 50-day quarantine, testing 30 individual tracheal swabs at 42 days post-introduction provides a 76% probability of detection. If adopting a pooling approach (5 or 10), the probability of detection only marginally decreases (72-74%) and testing cost is reduced.

# **Discussion and Conclusion**

This study showed that surveillance programs can incorporate tracheal pooling as a cost-effective strategy to maximize the detection of M. hyopneumoniae in negative populations. As a result, sampling guidelines with probability of detection estimates were proposed for various field-testing scenarios.

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# UNDERSTANDING THE TRANSEPITHELIAL ION TRANSPORT IN THE PIG STOMACH DURING GASTRIC ULCERS

<u>A. Kpogo</u><sup>1</sup>, J. Panisson<sup>2</sup>, M. Subramaniam<sup>3</sup>, R. Newkirk<sup>1</sup>, M. Loewen<sup>1</sup> <sup>1</sup>University of Saskatchewan <sup>2</sup>Prairie Swine Centre <sup>3</sup>University of Idaho

# **Background and Objectives**

Gastric ulcers increase significantly when pigs are fed fine particle size diets and exposed to stressful events. However, the exact causes of these gastric ulcerations remain unclear. Gastric acid production is known to be influenced by anionic secretion. The study aimed to evaluate the agonist-induced electrogenic secretory responses in the different sections of the stomach of pigs exposed to gastric ulcers. The pars oesophagea, cardia, fundus and pylorus were assessed in Ussing chambers.

# **Material and Methods**

Thirty-two pigs (initial BW of  $90.6\pm2.2$  kg), were fed for 14d and divided into 2 treatment groups (Control & Feedout). For Control, pigs were fed a coarse-ground wheat-pea based diets and for Feedout, pigs were fed diets with 40% air-classified pea starch (particle size  $10-20 \ \mu$ m) and exposed to an out-of-feed event, both recognized as potential factors contributing to gastric ulcer formation. For Feedout, feed was removed on day13 at 7am and reintroduced on day14 at 7am for another 24h before euthanasia. The Control group had feed throughout this period. After euthanasia, stomach was harvested and transported to the lab within 30 minutes and the different sections of the stomach mounted on Ussing chambers.

#### Results

The initial potential difference were significantly higher in the pars and cardia. The TEER was also high in the Control group compared to the feedout group in the pars oesophagea. Changes in short-circuit current ( $I_{sc}$ ) in the feedout group significantly increased following the activation of secretion via the adrenergic agonist (isoproterenol, forskolin, IBMX) and the cholinergic agonist (carbachol) in the pars oesophagea and cardia. Bumetanide (an inhibitor of the basolateral cotransporter 1, NKCC1), failed to significantly decrease  $I_{sc}$ . There were no significant secretory responses in the fundus and pylorus.

# **Discussion and Conclusion**

We report for the first time that the pars oesophagea becomes an actively secreting tissue during gastric ulcer conditions. Secretory responses were higher during ulcers as the control group of pigs showed no ulcers. Since bumetanide failed to inhibit NKCC1, we attribute the changes in the stimulatory I<sub>sc</sub> to bicarbonate (HCO<sub>3</sub>) secretion. In conclusion, HCO<sub>3</sub><sup>-</sup> may play a pivotal role in gastric ulcer conditions which could be as a result of a feedback mechanism.

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# VACCINATION OF PIGLETS WITH PRRSV-MLV PROMOTES NURSERY STABILITY AND REDUCTION OF MORTALITY

<u>V.D. Azeitona Martins</u><sup>1</sup>, T. Nunes<sup>3</sup>, R. Cordeiro<sup>2</sup>, D. Giralt<sup>3</sup>, L. Nodar<sup>3</sup>, G. Sena<sup>4</sup> <sup>1</sup>HIPRA Portugal <sup>2</sup>DVM Portugal <sup>3</sup>HIPRA, Amer (Girona), Spain <sup>4</sup>HIPRA Portugal, Malveira (Lisbon), Portugal

#### **Background and Objectives**

The Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) remains a challenge to swine production worldwide. Vaccination is a cornerstone of disease control and can contribute effectively to reducing the severity of the disease and associated mortality. The endemic circulation of PRRSV on a farm is more dependent on biosecurity measures and management strategies than on seasonal variations and so the need for an effective control is constant. Nonetheless, due to the variations in temperature, humidity and coinfecting pathogens during the year, the disease severity can vary and lead to the adoption of different strategies in different seasons. The aim of this report is to assess the effect of piglet vaccination on nursery mortality on a farm with an intermittent vaccination schedule.

# **Material and Methods**

On a PRRSV-positive swine farm with 750 sows (site 1 + site 2), a piglet vaccination programme with a MLV PRRS-vaccine (strain VP-046 BIS) had been in place since 2018. All the batches weaned between August and February of each year were vaccinated after weaning. Between March and July of each year, no piglet vaccination was performed. In July 2022, after a biosecurity breach, an increase in nursery mortality was observed in the non-vaccinated batches which was associated with the circulation of a wild-type PRRSV. Consequently, piglet vaccination was resumed. Between August 2020 and February 2023 the monthly mortality of the nursery animals was recorded, as well as the PRRSV-vaccination schedule of each month. A logistic regression with group as a factor and batch as a random factor was performed to assess mortality using R software v.4.3.

# Results

Vaccination of the piglets corresponded to lower levels of nursery mortality (3.1% versus 6.1%, p<0.001). The average mortality rate was above 3% in only 8/20 months in which the piglets were vaccinated, whilst nursery mortality was above 3% in 9/11 of the months in which the piglets were not vaccinated.

# **Discussion and Conclusion**

The results suggest a relevant contribution of piglet vaccination (with a MLV vaccine) to the maintenance of lower levels of nursery mortality during the year. A continuous vaccination programme can provide long-term stability that intermittent vaccination programmes are unable to.

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# ASSOCIATION BETWEEN CROSS-FOSTERING AND PIGLET BODY WEIGHT AT BIRTH WITH AVERAGE DAILY GAIN DURING THE LACTATION PERIOD

J. Camp Montoro <sup>1</sup>, J.A. Calderon Diaz <sup>2</sup>, <u>M. Ros Fuertes</u> <sup>1</sup>, J. Garcia <sup>3</sup>, S. Badia <sup>3</sup>, J.M. Herrero <sup>1</sup>, P. Magallon <sup>1</sup>, P. Blanco <sup>1</sup>, A. Vela <sup>1</sup>, I. Barbosa <sup>1</sup>, R. Sala Echave <sup>1</sup>, A. Arasil <sup>1</sup>, S. Shevchenko <sup>1</sup>, J. Lupi <sup>1</sup>, L. Zaragoza <sup>4</sup>, I. Huerta <sup>1</sup> <sup>1</sup>*Pig Improvement Company (PIC)* <sup>2</sup>*Pig Improvement Company* <sup>3</sup>*Piensos Costa S.A, Fraga, Huesca, Spain* <sup>4</sup>*Pig Improvement Company, Hendersonville Tennessee, USA* 

# **Background and Objectives**

Cross-fostering (CF) is a widely used management technique to increase piglet survival and to create more uniform litters both on terms of size and/or piglet weight. However, CF might be stressful for sows and piglets and stress can impact animal performance. We aimed to investigate the association between CF status and piglet birth weight with average daily gain (ADG) during the lactation period.

# Material and Methods

In total, of 3,602 piglets [Pietrain × (Landrace × Yorkshire)] originating from 322 hyper-prolific sows were weighed individually identified and their body weight was recorded within 24 hours after birth. Cross-fostering was practiced on the farm within 48h post-farrowing to standardise litter size as per normal farm management. Cross-fostering status was recorded (CF = 1,806 piglets and non-CF = 1,796 piglets) for each piglet. Birth weight was classified into different categories based on 100 g intervals ( $\leq 0.8$  kg up to  $\geq 2.0$  kg birth weight). Piglets were followed through the lactation period and weighed individually at weaning at 18.5±1.69 days of age. Piglet was considered the experimental unit. Data were analysed using a linear mixed model in R, including birth weight category, CF status and their interaction as fixed effects, and sow as random effect.

#### Results

Birth weight ranged from 0.54 to 2.06 kg and it was similar between CF and non-CF piglets  $(1.39\pm0.01 \text{ vs. } 1.41\pm0.01 \text{ kg}, \text{respectively}; P > 0.05)$ . Cross-fostered pigs had a lower ADG ( $152.5\pm2.98 \text{ g/d}$ ; P<0.05) compared to the pigs that were non-CF ( $171.7\pm2.90 \text{ g/d}$ ). Additionally, piglets with heavier birth weight had higher ADG during the lactation ( $150.4 \pm 4.51$  to  $177.6\pm4.31 \text{ g/d}$ ; Linear P<0.05) compared with piglets born at lighter weight. Finally, an interaction between CF and piglet birth weight category was observed (P<0.05) with piglets that were non-CF and heavier at birth having an improved ADG during the lactation period

# **Discussion and Conclusion**

Our results suggest that while light birth weight piglets (i.e. < 0.9 kg) may benefit from cross-fostering, this practice may negatively impact those piglets born at heavier weights.

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# **BIOSECURITY IN PIG FARMS OVER TIME IN IRELAND**

C. Correia-Gomes<sup>1</sup> <sup>1</sup>Animal Health Ireland, 2–5 The Archways, Carrick on Shannon, Co. Leitrim, Ireland N41 WN27

#### **Background and Objectives**

Biosecurity is paramount to the production of healthy animals. At farm it includes all measures taken to minimise the risk of introduction and spread of pathogens. The aim of this study was to quantify the biosecurity levels for the pig industry in Ireland over time and per production system.

# **Material and Methods**

The Biocheck.UGent scoring system was used. Since 2018 a scheme run by Animal Health Ireland funds one free biosecurity assessment, done by private veterinary practitioners, for commercial pig farms per calendar year. Production type in this study was aggregated into farrow-to-finish (FTF), farrow-to-weaner (FTW), weaner to finisher (WTF) and finisher (F) farms. All analyses were done using Rstudio.

# Results

From 2018 until end of June 2023, 921 assessments were done, and 378 pig units have been assessed. Accounting for 92.7% and 92.5% of the breeding and non-breeding animals in Ireland respectively. Considering the last assessment for all farms, external biosecurity scored higher than internal biosecurity. However, there is a wide variation of scores between farms. The areas with the lowest scores are the management of feed, water and equipment coming into the farms; the measures implemented between compartments, measures implemented at the farrowing and suckling period, and cleaning and disinfection procedures.WTF farms scored lower for overall and external biosecurity than the other farm types. FTF and FTW farms scored higher for external biosecurity than the other two farm types, while F farms scored higher for internal biosecurity than the other farm types.Around 38% of the farms had been assessed three or more times from 2018 to 2023. For this cohort, mean scores for external and internal biosecurity increased over time. This was mainly due to improvements in measures related to feed, water and equipment supply, personnel and visitors, vermin and bird control, measures between compartments and the use of equipment, and cleaning and disinfection.

#### **Discussion and Conclusion**

The Irish pig industry has made considerable efforts to improve and maintain a good biosecurity status. Further improvements in internal biosecurity will prevent disease spread within the unit and reduce disease prevalence for endemic diseases such as PRRS and colibacillosis, bringing additional economic benefits.

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# DECISION PATHS IN EUTHANASIA: HOW TO DECIDE THAT THE RIGHT TIME-POINT HAS COME?

#### J.D. Stoffregen 1, L. Kreienbrock 1, E. Grosse Beilage 2

<sup>1</sup>Department of Biometry, Epidemiology and Information Processing, WHO Collaborating Centre for Research and Training for Health at the Human-Animal-Environment Interface, University of Veterinary Medicine Hannover, Germany <sup>2</sup>Field Station for Epidemiology in Bakum, University of Veterinary Medicine Hannover, Germany

### **Background and Objectives**

The decision whether or not to euthanize a diseased or injured pig has to be re-evaluated on a daily basis. Given that pigs tend to mask their symptoms (among other reasons), the decision is often difficult to make. Besides barriers to decision making, recent publications have outlined a knowledge gap concerning the questions 'how the decision is actually made' and 'what role clinical signs have for observers over time'. Answers to these questions are crucial to develop on-farm euthanasia protocols and educate people in caretaking of pigs. Due to this, the goal of this study was to enhance understanding and generate an initial set of decision trees from the perspective of veterinarians.

#### **Material and Methods**

The study relates to the project CARE-PIG which aims at elaborating on the critical time-point for euthanasia of pigs by enhancing knowledge about crucial clinical signs and decision processes. Following a systematic literature review, a set of in-depth interviews with veterinarians was conducted and qualitatively analysed. Subsequently, results were discussed with experts to generate a shared account of generated decision trees.

#### Results

Preliminary results show that a general set of decision trees can be identified that vary depending on the disease or injury of concern. The more experience an interviewee has in euthanasia of pigs, the decision to euthanize results from analysis of the course of clinical signs rather than on signs identified at day one.

# **Discussion and Conclusion**

On a daily basis, pig veterinarians and farmers need to make a decision whether or not to euthanize diseased or injured animals. The decision shall avoid unnecessary pain and suffering of the animal and not at last, has to comply with jurisdiction. For veterinarians who consult their farmers in the development of on-farm protocols, the results provide initial insight, how to structure and adapt protocols for euthanasia over time as well as how to train caretakers.

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# DETECTION OF INTRAUTERINE PRODUCED ANTIBODIES AND PCR TESTING IN STILLBORN PIGLETS

<u>S. Cooremans</u><sup>1</sup>, H. Beulen <sup>2</sup>, R. Jansen <sup>3</sup> <sup>1</sup>Lintjeshof, Nederweert, The Netherlands <sup>2</sup>Merefelt Livestock Diagnostics, Nederweert, The Netherlands <sup>3</sup>Boerhinger Ingelheim AH, Alkmaar, The Netherlands

# **Background and Objectives**

Detection of antibodies in stillborn piglets can prove intrauterine infection. The **objective** of this study is to analyze whether the immunocrit-assay can detect antibodies in stillborn piglets.

### Material and Methods

Over a half year period, piglets submitted for routine diagnosis for stillbirth (SB) (farms with >8%SB) were included in this study. In total, 17 farms submitted fresh stillborn piglets. Stillbirth was confirmed by necropsy. From the heart serum blood was obtained and serum was separated and freezer stored (-80 °C) upon analysis. Serum was analyzed for antibodies, using the Radial Immuno Diffusion (RID; Triple J Farms swine IgG). The samples were alternatively tested with the immunocrit-assay (1), with an increase of centrifugation time of 10 minutes. Per farm, lung, liver and spleen were collected and pooled per 5 for PCR testing of PPV, PRRS, PCV2 and PCV3.

#### Results

In total, 157 still born piglets obtained from 15 farms were analyzed with both IC as RID. From these 157 samples, **13** samples, obtained from 3 farms, were positive for swine IgG using the RID assay (8.3%; 0.4-13.5 mg/ml; farm 1, 3 & 11). When using the immunocrit assay, **31** samples were positive (mean IC ratio 0.03; 0.01- 0.08). When a cut off value of IC 0.02 was used (<IC 2.0 neg; >=IC 2.0 pos), this resulted in a sensitivity of 92.3% and a specificity of 86,8%.Pcr testing: in 2 /15 farms SB piglets were positive for PRRS, 1/15 farms were positive for PPV, 9/12 farms were positive for PCV2.

#### **Discussion and Conclusion**

Prevalence of infections in SB is hardly reported. In total at 9/15 farms, positive PCR tests proved the presence of intrauterine infections with either PRRS, PPV, PCV2 and/or PCV3. The relevance of the presence of PCV3 is still unclear (2). The immunocrit assay proved to be a useful alternative for predicting the presence of intrauterine produced antibodies, which could be an indication of intrauterine infections related to SB. The test is fast and cheap and can be used before more expensive ELISA testing for specific infections is performed. With negative AB and PCR results, noninfectious causes of SB are more likely (6/15).

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#### DURATION OF MATERNALLY DERIVED ANTIBODIES OF PORCINE PARVOVIRUS IN GROWING PIGS

R. Renzhammer 1, B. Buchebner 1, U. Truyen 2, R.M. Kobialka 2, A. Abd El Wahed 2, M. Koch 1, A. Ladinig 1, C. Unterweger

<sup>1</sup>University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

<sup>2</sup>Institute for Animal Hygiene and Veterinary Public Health, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

### **Background and Objectives**

Since gilts and sows are regularly vaccinated against the porcine parvovirus 1 (PPV1), sometimes questions arise for the appropriate time point of basic vaccination of gilts due to different reports on the duration of maternally derived antibodies (MDA). Therefore, we aimed to investigate the presence of MDA in twelve, 18 and 24 weeks-old rearing pigs in nine different farrow-to-finish farms in Austria.

#### **Material and Methods**

In nine different farms, 3x10 serum samples were collected from twelve, 18 and 24 weeks-old-pigs respectively. All 270 samples were tested for the presence of PPV antibodies (Abs) by ELISA (INgezim® PPV, Ingenasa) and haemagglutination inhibition (HI) test. In addition, pooled faecal samples were collected from twelve weeks-old pigs in each farm for direct detection of PPV by PCR and virus isolation.

#### Results

While 14% of sampled growing pigs were Ab-positive by ELISA, PPV Abs were detected by HI test (titre: > 1:10) in 93% of tested samples. In total, 30% of sampled twelve-weeks-old pigs were positive by ELISA, while only 8% were positive by ELISA at the age of 18 weeks. An overall similar decline in Ab titres by HI test was observed from 12 to 24 weeks-old pigs in 5/9 farms. In the remaining four farms, high Ab titres (≥ 1:1280) were measured in samples from 24 weeks-old pigs, indicating a previous infection with PPV. Noteworthy, PPV was neither detected by PCR nor virus isolation in investigated faecal samples.

# **Discussion and Conclusion**

Our results emphasize that statements on the duration of MDA depend on the applied serological method and selected thresholds. In general, we could demonstrate a decline of MDA from twelve to 24 weeks-old pigs. While we could not detect PPV-DNA by PCR in faecal samples from twelve-weeks-old pigs, high Ab tires were measured in 24 weeks-old pigs in 4/9 farms indicating that infections with PPV field virus may frequently happen in six months-old pigs prior to first vaccination of gilts.

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# PREVENTIVE MEASURES AGAINST UMBILICAL OUTPOUCHINGS (HERNIAS): AN INTERNATIONAL SURVEY

M. Hansen <sup>1</sup>, I. Larsen <sup>2</sup>, T.B. Jensen <sup>3</sup>, C.S. Kristensen <sup>4</sup>, K.S. Pedersen <sup>2</sup>

<sup>1</sup>Copenhagen University <sup>2</sup>University of Copenhagen <sup>3</sup>SEGES Innovation, Axeltorv 3 Denmark <sup>4</sup>SEGES Danish Pig Research Centre, Aarhus, Denmark

# **Background and Objectives**

Umbilical outpouchings (UOs) in pigs are a multifactorial condition, and little is known about effective preventive strategies. Metaphylactic antibiotics for new-born piglets are used on some farms but are under scrutiny because of the growing concerns about antibiotic consumption, and therefore there is a need to focus on other strategies. This study presents an overview of the preventive measures against UO which are given as veterinary advice.

# **Material and Methods**

An online questionnaire addressing management and preventive aspects of UOs was made using SurveyXact and distributed by e-mail to veterinarians working within Porcine Health Management. The survey was distributed in the summer of 2021 and received 93 complete responses, mainly from 18 countries in Europe and a few from the USA, Canada, and Australia.

#### Results

Preventive measures related to hygiene included: disinfection and drying of the farrowing pen both pre- and post-farrowing; use of straw, sawdust, or powder-like materials in the pen; quick drying of piglets at birth; floor type and materials (plastic or rubber); and disinfection of the navel cord.Preventive measures related to the management of sows and piglets included gentle lifting and handling of piglets, adequate colostrum uptake, and shortening of the umbilical cord (must be dry when the navel cord is cut and the length should be 2–5 cm long), band-aid or clamps of UOs, avoid traction during birth help, oxytocin, moving piglets between sows, or diarrhea and cough in the piglets. Metaphylactic antibiotics were very often used within the first 96 hours for different causes, but prevention of umbilical infections was the most prevalent. More than nine different active compounds were used, with amoxicillin and tulatromycin being the most common.

# **Discussion and Conclusion**

Focus on hygiene, management, and antibiotics were all considered very important for the prevention of UOs in pigs. This study collected veterinarians' opinions on the prevention of UOs and can be used to inspire solutions in clinical practice and generate hypotheses for new research on this topic.

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# RELATIONSHIP BETWEEN BIOSECURITY SCORE AND AMOUNT OF ANTIBIOTIC USED AND PRODUCTION PARAMETERS IN WEAN-TO-FINISH PIG FARMS IN VIETNAM

T. Dang T. X. <sup>1</sup>, <u>H. Le Thanh</u><sup>2</sup> <sup>1</sup>Nong Lam university, HCMC <sup>2</sup>Nong Lam University, Ho Chi Minh City, Vietnam

### **Background and Objectives**

With the situation of disease outbreaks in pig production in southeast Asia, particularly in Vietnam, pig producers have tried to implement farm biosecurity (1). However, the awareness of farm biosecurity has still been low, even in large scale farms who feel reluctant to apply in the due to the lack of practical evidence of good biosecurity on production and antibiotic use, especially in the regular situation without big outbreaks. This study is aimed to show the relationship between biosecurity and production and health in which the amount of antibiotic usage become the global issue.

# **Material and Methods**

A study including 35 intensive grow-finisher pig farms (800-3000 heads) in the south of Vietnam was performed. Production parameters (i.e. FCR, mortality...) were collected from the database of those farms. Especially amount of each antibiotic used during production were recorded and then a value representative for the total amount of all antibiotics used of each farm was calculated. This value is called the treatment incidence (TI) values meaning that the number of animals out of a theoretical group of 1000 animals receiving a daily treatment with antimicrobials (2). In addition, all these farms were evaluated their biosecurity level using scoring system (Biocheck.Ugent<sup>™</sup>)(3). General linear models in which biosecurity scores were explanatory variables and production parameters (FCR, mortality), TI value were dependent variables.

#### Results

The result showed that there were significant relationships between biosecurity score and mortality and antibiotic use. Particularly, an increase of 1% unit of biosecurity score would reduce 0.1% of mortality and reduce 14 animals with daily antibiotic treatment in 1000 pigs. However, the relationship between biosecurity score and FCR was not clear.

# **Discussion and Conclusion**

These findings revealed the benefit of biosecurity improvement in the production and especially in antibiotic reduction. More farms included and other type of pig production should be studied to have a clearer picture of advantage from biosecurity.

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# RISK FACTORS ASSOCIATED WITH SPREAD OF AFRICAN SWINE FEVER VIRUS IN SMALL- AND MEDIUM-SCALE FARMS IN THE WESTERN REGION OF THAILAND

N. Am-In<sup>1</sup>, P. Photiphat<sup>2</sup>, S. Malicharn<sup>2</sup>, T. Bangkeaw<sup>2</sup>, B. Sorrasittsuksakul<sup>2</sup>, S. Nuanualsuwan<sup>3</sup>, D. Nilubol<sup>4</sup>

<sup>1</sup>Center of Excellence in Swine Reproduction, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Thailand

<sup>2</sup>Ratchaburi Provincial Livestock Office, Ratchaburi, Thailand

<sup>3</sup>Center of Excellence for Food and Water Risk Analysis (FAWRA), Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>4</sup>Swine Viral Evolution and Vaccine Development Research Unit, Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Thailand

### Background and Objectives

African swine fever (ASF) has developed into an endemic stage in Asia countries including Thailand. A pilot area-based control program designated as "Ratchaburi Sandbox" has been conducted. Keys in disease control are the understanding of risk factors associated with the spread of ASF virus (ASFV) and the introduction of ASFV. The objectives of the study were to gather and analyze crucial data related to risk factors that were associated with the spread of ASFV in the designated region

#### Material and Methods

Surveys were done during ASF outbreaks to identify and quantify risk factors for ASF transmission. Specifying and setting conditions in Microsoft Excel turned questionnaire data into risk factors. R's step AIC technique eliminates non-significant variables and lowers the model's Akaike Information Criterion. Risk variables' significance is verified by Student's t-test. SAS 9.3 (USA) performs risk factor regression logistic analysis

#### Results

The results of the study demonstrated that new pigs introduced without disease-quarantine, and/or exposure to contaminated environmental elements (OR=234.76) causes direct interaction between farm pigs and those outside the farm (OR=321.02). The risk of ASFV transmission by both in- and out-farms pig transport trucks and human were significantly high (p < 0.05). Failure to proper water-treatment, contaminated water created a twice non-significant statistical risk. ASF is 124 times more likely on farms without biosecurity. Farms with poor preventative measures had over 100-fold higher likelihood of ASF transmission (p<0.05). Large farms, corpse disposal, and on-farm pig sales relatively increased risk. ASF transmission is 45-fold higher on farms lacking disinfectants and knowledge. Non-targeted disinfectants increased ASF risk 12-fold on farms.

### **Discussion and Conclusion**

This risk assessment study might identify OIE Compartment-compliant farms. A compartment is physically separated from vulnerable animals to prevent infection. It uses spatial biosecurity systems and has an infection-related animal health status. The compartment's safety and control are verified by inspections, safety control protocols, and controls for international trade or disease prevention and control during animal movement. According to this report, fences should separate farms from the outside. Biosecurity and disinfectants are also stressed in the study to eradicate the ASF virus.

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# A SUMMARY OF THREE STUDIES TO ASSESS THE EFFECT OF AN ALGAE $\beta\text{-}1,3\text{-}GLUCAN$ ON COLOSTRUM QUALITY AND PIGLET IMMUNITY AND HEALTH

R. Neto 1, P. Bukowska 2, E. N´guetta 3, S. Ravichandran 2, A. Wealleans 2, S. Kirwan 4, M. Costa 5, V. Van Hamme 2 TKemin Europe NV

<sup>2</sup>KEMIN EUROPA NV <sup>3</sup>Kemin Europe N.V. <sup>4</sup>Kemin Industries, USA <sup>5</sup>Kemin South America, Brazil

#### **Background and Objectives**

With hyperprolific sows, colostrum management is paramount, piglets that ingest less colostrum and have lower levels of IgG are more likely to die early. It is key to increase colostrum quality and quantity. Three studies were designed to evaluate the effect of an algal  $\beta$ -1,3-glucan (B1,3G) (Aleta<sup>TM</sup>, Kemin) on colostrum quality, piglet immunity and health.

# **Material and Methods**

In the 3 studies, sows from commercial herds were randomly allotted to either a control (C) or treatment (BG) group:

1: 400 sows, C group, n=31, BG group, n=37, 1 g of B1,3G/sow/day from 85 days of pregnancy-weaning (P-W).

2: 2500 sows, C group, n=20, BG group, n=20, 200 g/t of feed of B1,3G from day 85 of P-W (21 days).

3: 190 sows, C group, n=18, BG group, n=19, 1 g of B1,3G /sow/day, entry to farrowing room to weaning (28 days).

Born alive (BA), born dead, birth weights (PBW), 18h (study 2), 21 days (study 3) and weaning weights were measured, mortality and incidence of diarrhoea (Study 3). Colostrum was harvested in study 2 (n=20) and 3 (all sows). Piglets blood sampled between 4-7 days to measure IgA (Study 1, n=30), IgG, IgA and IgM (Study 2, n= 20) (ELISA).Data were analysed in the Fit Model function of JMP 16. Differences considered a trend at P>0.05 and <0.1.

#### Results

Increase in Immunoglobulins in study 1, IgA (31.4 and 14.3mg/mL), IgG (40.9 and 27.0mg/mL), IgM (22.4 and 9.1mg/mL) and IgG for study 3 87.7 and 62.8mg/mL for BG and C respectively (P<0.05). Improved PBW in study 1 1.38 (C) to 1.5kg (BG) (P<0.05). In study 2, improved growth (18h), from 68 g to 102g (P<0.05) due to an increase in colostrum intake, 235 and 294g respectively, for C and BG (P<0.05). Mortality improved in study 3 from 10.1 to 6.0% for BG and C respectively (P=0.078).

# **Discussion and Conclusion**

Results of these studies support that algal  $\beta$ -1,3-glucan improves the immunological quality of colostrum and therefore the serological levels of immunoglobulins, having a very positive impact in the health of piglets, especially from hyperprolific sows.

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# ASSESSMENT OF A POSSIBLE RELATION BETWEEN HYGIENE PROTOCOLS MEASURED BY BIOLUMINESCENCE AND THE VIRAL LOAD: AN ORIENTATIONALLY STUDY

<u>V. Geurts</u><sup>1</sup>, K. Valentine <sup>1</sup>, S. Hänsel <sup>2</sup> <sup>1</sup>BioChek BV, Smart Veterinary Diagnostics <sup>2</sup>Hygiena Diagnostics GmbH

### **Background and Objectives**

Adenosine-triphosphate is the energy component molecule of all plant and animal cells which can be directly measured via bioluminescence expressed as RLU (relative light units). ATP-tests evaluate hygiene-and sanitation protocols on the spot within 10 seconds. ATP-bioluminescence detects, microbial cells, food residues, bacteria, fungi and yeast, which might persist after inadequate cleaning. Viruses do not contain ATP. The aim of the orientational study is to assess a possible relation between the ATP levels measured and viral load.

# **Material and Methods**

To investigate a possible relation between the amount of ATP- measured and the viral load of PRRSv, VetAssure<sup>™</sup>Swabs were used swabbing a defined area of 100 cm<sup>2</sup>. A sorting panel was divided into 3 compartments which were physically separated by glued slats to avoid cross contamination. Each compartment/group was inoculated with a fixed amount of a PRRS-MLV vaccine. Compartment1 served as a control meaning no cleaning and disinfection. Compartment2 was cleaned. Compartment3 was cleaned and disinfected. Compartments were sampled with VetAssure- and viral swabs on T0:at Inoculation, T1:after the described treatments, T2:45 min after T1.The RLU and the detected viral load are measured via the EnSURE®Touch-luminometer and a quantitative PRRSv-PCR(copies/500µl) to assess a possible relationship

#### Results

RLU-measurements and PRRSv loads per 100cm<sup>2</sup>:

 $Compartment1. \ Control: \ TO:2697_{RLU}-14155_{load/100cm2}T1:523_{RLU}-8375_{load/100cm2}T2:332_{RLU}-<600_{load/100cm2}Compartment2. \ Cleaned: \ TO:337_{RLU}-33428_{load/100cm2}T1:319_{RLU}-<600_{load/100cm2}T2:282_{RLU}-<600_{load/100cm2}Compartment3: \ Cleaned+ \ disinfection:T0:353_{RLU}-56850_{load/100cm2}T1:193_{RLU}-0_{load/100cm2}T2:198_{RLU}-0_{load/$ 

#### **Discussion and Conclusion**

Although the results are indicative, all groups showed a reduction in RLU and viral load from T0 onwards. The RLU reduction in the control group is likely caused by using a dry clean paper-tissue to ensure that the surface is dry and comparable to the other groups since they were also dried with a clean paper-tissue at T1. Swabbing alone may also cause a reduction in biocontamination. Several trends are seen in this trial. Cleaning and disinfection showed the lowest biocontamination followed by cleaning only. The lower the biocontamination the lower the PRRSv-load indicating a possible relation which justifies an extended study to confirm a statistically significant relation.

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### EFFECT OF AN ALGAE β-1,3-GLUCAN ON COLOSTRUM AND PIGLET PRE WEANING HEALTH

J. Silva <sup>1</sup>, <u>I. Silva</u> <sup>4</sup>, R. Neto <sup>2</sup>, M. Soto <sup>3</sup>, P. Folque <sup>4</sup>, D. Monteiro <sup>5</sup> <sup>1</sup>Universidade-de Trás-os-Montes-e-Alto Douro (UTAD), Department of Zootecnia, Vila Real, Portugal. <sup>2</sup>Kemin Europe NV <sup>3</sup>KEMIN EUROPA NV <sup>4</sup>Eurocereal, Malveira, Portugal <sup>5</sup>Universidade-de Trás-os-Montes-e-Alto Douro (UTAD), Department of Zootecnia, Vila Real, Portugal

#### **Background and Objectives**

Hyperprolific sows may be challenging as lower colostrum intakes lead to poorer survivability and performance. Algal  $\beta$ -1,3-glucan has been shown to improve colostrum quality, improving piglet health. The objective of this study was to assess the impact of a  $\beta$ -1,3-glucan on colostrum quality, piglet performance and health.

# **Material and Methods**

In a multiplication farm with 190 hyperprolific sows, 18 sows were randomly allotted to either a control group (C), regular basal diet or to the  $\beta$ -1,3-glucan group (BG), 19 sows (1g / sow / day of algal  $\beta$ -1,3-glucan (Aleta<sup>TM</sup>, Kemin)) from entry to farrowing room to weaning (28 days). Litters were weighed at birth (LWB), 24 h (time of fostering within treatment) and days 21 and 28. Deaths and diarrhoea were recorded. Farrowing length (FL) and pre-weaning mortality (PWM) were calculated, colostrum samples were tested for IgG and IgA and faecal samples from the piglets at 21 days of age (tested for calprotectin). Data were analysed via ANOVA with treatment as fixed effect in the Fit Model function of JMP 16. Differences considered a trend at P>0.05 and <0.1.

#### Results

No significant differences were observed for average parity, born alive, born dead, FL, LWB, weight and number of piglets at fostering, at 21 and 28 days.PWM was lower in the BG group than in the C group, 6.0 and 10.1% respectively (P=0.078). Cause of death was not assessed, however, fitter piglets who have consumed more, or better quality, colostrum are also less likely to die of non-infectious causes (Muns et al., 2016), no significant differences for the incidence of diarrhoea, 15.8 and 22.2 for the BG and C groups, respectively. Levels of IgG in colostrum were higher in the BG group, 87.7 mg/mL, than in the C group, 62.8 mg/mL (P= 0.043), levels of IgA and calprotectin were similar between the BG and C group.

#### **Discussion and Conclusion**

In the conditions of this study, supplementation of sows with an algal  $\beta$ -1,3-glucan resulted in lower pre-weaning mortality. This may have been a result of improved intestinal health, through improved passage of passive immunity, resulting in better survivability.

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# EFFECT OF TREATMENT PROTOCOL AND TYPE OF THE IRON ON IRON DEFICIENCY ANEMIA (IDA) AND HEMOGLOBIN (HB) STATUS AT WEANING PIGLETS IN INDUSTRIAL FARMS IN VIETNAM: AN OBSERVATIONAL STUDY

B. Nguyen <sup>1</sup>, N. Nguyen <sup>1</sup>, G. Morizur <sup>1</sup>, H. Karembe <sup>2</sup>, L. Meppiel <sup>3</sup>, <u>D. Sperling</u> <sup>4</sup> <sup>1</sup>Ceva Animal Health, Vietnam <sup>2</sup>Ceva Santé Animale, 10 Avenue de la Ballastière, 33500 Libourne - France <sup>3</sup>Ceva Santé Animale SA, 10 Avenue de la ballastière, CS 30126 – 33501 Libourne Cedex, France <sup>4</sup>Ceva Sante Animale, Libourne, France

### **Background and Objectives**

It is well established that insufficient iron intake in suckling pigs results in iron deficiency or development of Iron Deficiency Anemia (IDA). The objective of the current study was to evaluate the frequency of IDA on selected industrial farms in Vietnam and assess the effectiveness of different type and doses of iron products.

### **Material and Methods**

A survey was conducted on 10 large scale farms in different regions in Vietnam. In total, 1968 piglets were included and the hemoglobin (Hb) level at weaning (24 days of age, DOA) was assessed using HemoCue Hb 201+. A minimum of ten randomly selected litters (50- 726 piglets per farm) from different parity sows were selected and 3 piglets (small, medium, large) per litter were blood sampled. The effect of treatment protocol: dose of iron (200-300-400 mg/dose) and type of the iron (gleptoferron (GLF) vs. dextran) on Hb status was evaluated. Actual ranking criteria for Hb level were used: anemia Hb < 90 g/L, subclinical status  $90 \le Hb \le 110 g/L$  and optimal Hb level Hb> 110 g/L.

#### Results

Eight farms used dextran-based products, two used GLF. IDA was more frequently detected in piglets treated with dextran vs. gleptoferron, 17.7% (313) vs. 6.4% (13) piglets. Seven farms used increased dose of iron (dextran) (300- 400 mg/piglet). The highest dose of iron dextran (400 mg/piglet) significantly decreased the frequency of IDA and changed the distribution of the piglets in the remaining categories of Hb levels (p < 0.0001). When predicting Hb level per dose (only for dextran) with mixed linear regression, a dose of 400mg significantly increases the level of Hb comparing to a dose of 200mg (p= 0.0086) and 300mg (p< 0.0001). When predicting Hb by farm, type of treatment (all doses confounded) and its interaction, farms and treatment type appeared to be significant. The Hb level was significantly higher for GLF.

#### **Discussion and Conclusion**

IDA is frequently observed in industrial farms in Vietnam. GLF type of iron was superior to standard iron dextran type of iron. High % of farms were using increased dose of iron dextran compared to standard one to improve the Hb level of piglets.

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# IMPACT OF INTERNAL BIOSECURITY MEASURES ON PRRSV DISSEMINATION IN A HYPERPROLIFIC SOW HERD.

F. Gonzalvo <sup>1</sup>, I. Tardío Solans <sup>2</sup>, J. Casanovas Granell <sup>2</sup>, G. Abella Falcó <sup>1</sup>, A. Escoda <sup>2</sup>, J. Baliellas <sup>3</sup>, <u>L. Fraile</u> <sup>4</sup> <sup>1</sup>Boehringer Ingelheim Animal Health España <sup>2</sup>Cincaporc, Spain <sup>3</sup>Grup de Sanejament Porci, Lleida, Spain <sup>4</sup>University of Lleida, Lleida, Spain

#### Background and Objectives

Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) continues to be a major economic issue for the swine industry. The transmission of pathogens at farrowing can be reduced by the implementation of internal biosecurity measures, such as restricting piglet movements and improving hygiene between litters (McRebel protocol). The objective of this study was to evaluate the impact of McRebel protocol to reduce the PRRSv dissemination and their effect on the survival rate and weaning weight in a hyperprolific sow herd.

#### **Material and Methods**

This study was conducted in a low prevalence PRRSv unstable sow farm. Two different groups of 136 litters were included. In the McRebel group, piglet movements were only allowed during the first 24 hours of life and strict hygienic handling measures were adopted within the litter, including the use of needle-free devices. In the control group there were no limitation of movements throughout lactation and no special hygienic measures were adopted. All piglets were individually tagged at birth, to check movements between litters and weighed at 24 hours of life and at weaning. Five piglets per litter were bled at weaning. Samples were analyzed by RT-PCR for PRRSv detection in one pool per litter.

#### Results

Percentage of piglets transferred at 24 hours after birth was 8% and 41% in the McRebel and control group, respectively. These differences increased significantly at weaning. Thus, 16% of the piglets were swapped in the McRebel group vs 80% in the control group. Regarding the PRRSv transmission, significant differences were observed between groups (p<0.005). Thus, three per cent of the litters were positive in the control group whereas all litters were negative in the McRebel group at weaning. The average piglet weight of the McRebel group was 0.53Kg higher (p=0.004) than in the control group at weaning. However, a lower survival rate was observed in the McRebel group versus the control one (p=0.004).

### **Discussion and Conclusion**

Under the conditions of this study, McRebel protocol was able to decrease significantly PRRSv transmission in the maternity unit and significantly improved piglet weaning weight. However, the survival rate decreased when piglet movements were prohibited.

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# IMPLEMENTATION OF SANDBOX CONCEPT AND COMPARTMENTALIZATION TO REPOPULATE AND MAINTAIN FREE STATUS IN AN AFRICAN SWINE FEVER ENDEMIC AREA

D. Venkateswaran <sup>1</sup>, S. Muhammad <sup>1</sup>, N. Am-In <sup>2</sup>, S. Nuanualsuwan <sup>3</sup>, A. Tantituvanont <sup>4</sup>, P. Photiphat <sup>5</sup>, S. Malicharn <sup>5</sup>, T. Bangkeaw <sup>5</sup>, B. Sorrasittsuksakul <sup>5</sup>, <u>D. Nilubol</u> <sup>1</sup>

<sup>1</sup>Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>Center of Excellence in Swine Reproduction, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>3</sup>Center of Excellence for Food and Water Risk Analysis (FAWRA), Department of Veterinary Public Health, Faculty of Veterinary Science, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

<sup>4</sup>Department of Pharmaceutic and Industrial Pharmacies, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

<sup>5</sup>Ratchaburi Provincial Livestock Office, Ratchaburi, Thailand

# **Background and Objectives**

African swine fever (ASF) is a devastating disease. Following an outbreak, herds have been left empty or/with reduced productivity. Presently, ASF has developed into endemic stage. It is speculated that a disease-free zone can be implemented. The aim of this integrated research was to introduce a compartment system, with disease-free zone strategy, into medium and small sized farms in a pilot area called "Sandbox" in Ratchaburi province with a goal to repopulate pigs, restore productivity, control and maintain a disease-free status.

#### Material and Methods

The study utilized sandbox and compartmentalization strategies to manage ASF and FMD. A collaborative sandbox approach involved government officials, researchers, and the private sector in selecting a pilot area. Flexible ASF containment regulations were implemented, covering area selection, component identification, and relevant law assessment. Herd selection considered owner approval and a risk assessment for persistent ASFV presence. Prior to compartmentalization, a thorough risk analysis, including environmental factors and qPCR pig testing, was conducted. Measures to reduce ASFV included a repopulation protocol with cleaning and sentinel checks. Rigorous processes identified incoming gilt/pig sources using historical evaluation and PCR/ELISA testing. The compartmentalization phase involved step-by-step procedures for component identification, farm risk assessments, and potential disease pathways. Sub-compartments emphasized traffic flow separation, ensuring comprehensive containment through biosecurity, animal movement controls, and disease surveillance.

# Results

Two areas, each with a commercial pig farm (a 20-sow small herd and a 3,000-sow medium-scale operation), a feed mill, and a slaughterhouse, were selected based on predefined criteria. Contributing factors to ASF outbreaks included farm proximity, transportation, and worker-related issues. Challenges in controlling and repopulating small-holder farms included issues with feed quality and a lack of biosecurity knowledge. Risk assessment identified the potential contributors of recurrent ASF outbreaks including contaminated facilities, remaining pigs (carriers), and carcass disposal areas in selected herds. Diagnostic assays confirmed that small holding and medium-scale farms, following introduction of negative pigs, have maintained ASF free status for 18 and 12 months, respectively, despite nearby ASF outbreaks.

# **Discussion and Conclusion**

In conclusion, the implementation of sandbox and compartment, together with disinfection and introduction of naïve pigs, successfully maintaining ASF-free status in implemented herds.

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# ORAL VACCINATION AGAINST LAWSONIA INTRACELLULARIS IMPROVES THE QUALITY OF PORK MEAT AS ASSESSED BY RGB COLOR MODEL

<u>A. Scollo 1</u>, R. Bussi 1, A. Perrucci 1 <sup>1</sup>Department of Veterinary Science, University of Torino, Italy

#### **Background and Objectives**

Porcine proliferative enteropathy (PPE), also known as ileitis, is an infectious intestinal disease characterized by thickening of the distal small and proximal large intestinal mucosa because of enterocyte proliferation associated with the presence of an intracellular bacterium, Lawsonia intracellularis. This porcine intestinal pathogen has been identified as one of the main enteric pathogens of fattening pigs worldwide, causing economic losses in swine industry. This study was conducted to investigate the effect of an oral Lawsonia intracellularis vaccine against PPE on productive parameters and quality of pork meat as assessed by RGB (Red, Green, Blue) color model.

# **Material and Methods**

A total number of 2793 pigs (32,1±6,3 kg) were randomly allocated into vaccinated and control groups, housed in two identical barns in a commercial Italian growing site designated to heavy pigs production and historically experiencing PPE. Treated group was orally vaccinated at the arrival on site with a commercial attenuated L. intracellularis vaccine as following the manufacturer's instruction. Mortality and antibiotic consumption were observed during the whole trial. Images from 205 gracilis muscles were collected at slaughterhouse and processed with a software for color mapping using the RGB model.

#### Results

Mean slaughter weight was 164,9 kg. The vaccinated group showed significantly lower mortality (7,3 vs 9,7%; p=0,023) and antibiotic use related to enteric disease (2,89 vs 6,89 DDDvet; -58,1%) than those of the control group. Moreover, meat from vaccinated pigs had significantly lower values of color components Red (106,3 vs 109,9; p=0,024) and Green (66,1 vs 69,3; p=0,026).

#### **Discussion and Conclusion**

Findings confirm literature on efficacy of an oral vaccination in commercial pigs in reducing mortality and antibiotic use. The present study shows similar effects in the heavy pig production. Interestingly, meat from vaccinated pigs showed RGB colors values attributable to normal meat, whereas pigs belonging to the control groups showed higher red and green colors values closer to those at risk of PSE (pale, soft, exudative). A possible involvement of oxidative stress, glycogen imparity or pH variation in meat from infected pigs might be hypothesized. The possible impact of L. intracellularis infection on quality treats of pork meat should be investigated.

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# RISK FACTOR ANALYSIS OF FARMING PRACTICES FOR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRS) OF ALTERNATIVE PIG FARMS (APFS) IN MINNESOTA (MN)

<u>M. Medrano</u><sup>1</sup>, C. Corzo<sup>2</sup>, M. Culhane<sup>3</sup> <sup>1</sup>University of Minnesota <sup>2</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN <sup>3</sup>College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

#### **Background and Objectives**

As the US swine industry seeks to strengthen their defenses against the spread of Porcine Reproductive and Respiratory Syndrome (PRRS), understanding the role that farms of all types play in disease transmission is important. This includes those farms that are considered "alternative" in their production practices, namely, those that raise pigs with outdoor access. Therefore, this research seeks to identify any on-farm practices of alternative pig farms (APFs) in Minnesota (MN) that are risk factors for PRRS.

#### Material and Methods

A process was developed to identify and create an APF database that served as the study's target population. This population was then emailed an online survey using Qualtrics (Provo, UT) to gather information about their on-farm practices and invited to participate in a PRRS prevalence study with a sample size of 41 farms (n=200, 0.25/90/10). Interested producers were visited and oral fluid or serum samples were collected and tested via PRRS ELISA and RT-PCR. Farms were classified as PRRS positive if there was at least one positive ELISA positive sample (>0.4 S/P) and/or at least one positive RT-PCR positive sample (<40 Ct). To determine if there were any associations between different on-farm practices and PRRS status, univariable logistic regression was performed to calculate odds ratio from pre-selected variables based on t-test or fisher's exact tests.

# Results

198 APFs were identified, of which 54 (27%) responded to the survey and 24 were tested for PRRS. Five (21%) farms yielded at least one RT-PCR and ELISA positive sample, and three additional farms (33%) were positive only by ELISA. Hoop barns were associated (OR=16, CI95% 1.27-200.92) with ELISA positive status whereas using a farm specific vehicle had a sparing effect (OR=0.06, CI95% 0.005-0.760).

# **Discussion and Conclusion**

As expected, the PRRS virus is also present in APFs; however, the majority of the on-farm practices that were evaluated were not found to have an association with PRRS status. It is possible that other practices that were not evaluated, such as pig movement and farm location, may play a role in PRRS disease transmission within this population. Therefore, more information is needed.

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# **RISK FACTORS FOR TAIL BITING IN NURSERY-AGED PIGS**

<u>C. Munsterhjelm</u><sup>1</sup>, M. Tuominen-Brinkas<sup>1</sup>, H. Koskikallio<sup>1</sup>, K. Ahlqvist<sup>1</sup>, A. Valros<sup>1</sup> <sup>1</sup>Department of Production Animal Medicine & Research Centre for Animal Welfare, Faculty of Veterinary Medicine, Helsinki, 00014, Finland

#### **Background and Objectives**

Tail biting (TB) is a significant welfare problem in growing pigs. The behaviour often begins in the nursery.

#### Material and Methods

We performed one-day visits to ten Finnish farms rearing undocked pigs from weaning at about 4 weeks to 30 kg. On each farm tail health was assessed in 2-15 rooms (n=81) selected randomly within age strata, and risk factors for TB in 2-3 age-representative rooms with tail lesions (n=35). Tail health was assessed by scoring each pen (n=1217) as having visible tail lesions (wound, crust or reddening) in <10% or >10% of the pigs; or in the absence of lesions if there were hanging tails in no, <10% or >10% of animals. Risks were evaluated according to 54 multi-category environmental and animal-based questions on environment, hygiene, health, feeding and enrichment. Principal components of tail health were identified based on room level data including the percentage of pens in each of the five variables describing tail health. Three components explaining 84.5% of variance in the data were extracted: "High-prevalence TB" was loaded on by pens with >10% tail lesions and >10% hanging tails; "Low-prevalence TB" loaded on by <10% tail lesions and >10% hanging tails; and "Underlying TB" loaded on by <10% tail lesions and <10% hanging tails; factor scores were investigated by calculating non-parametric Spearman correlation coefficients in farm-averaged data.

#### Results

Tail health was unassociated with farm size or mortality. "Underlying TB" increased with resting in inappropriate areas in the pen, unrest (fighting or tail wagging), diarrhoea and perceived dusty air. "Low-prevalence TB" increased with a decreasing possibility for the group to eat simultaneously. "High-prevalence TB" increased with a larger proportion of animals with an empty belly.

### **Discussion and Conclusion**

Tail health was associated with only few of the 54 observed risk factors, indicating that the causes for TB were different on different farms. Feeding was the most important risk factor, emphasising it as a major cause of post-weaning stress. The lack of significance of enrichment-related factors was surprising given decent variability between farms.

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# SIMULTANEOUS PRRSV AND SIV INFECTIONS INCREASE ANTIMICROBIAL CONSUMPTION IN NURSERY

<u>J. Beek</u><sup>1</sup>, T. Nunes<sup>2</sup>, A. Schuttert<sup>3</sup>, E. Van Esch<sup>4</sup>, D. Giralt<sup>2</sup>, L. Nodar<sup>2</sup>, J. Miranda<sup>2</sup> <sup>1</sup>HIPRA Benelux <sup>2</sup>Hipra, Amer (Girona), Spain <sup>3</sup>De Oosthof, The Netherlands <sup>4</sup>Technical Services Manager Swine, HIPRA Benelux

#### **Background and Objectives**

Coinfections are the most frequent form of infection on pig farms, with the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Swine Influenza Virus (SIV) being the two major primary agents of respiratory coinfections. These viruses have the potential to interact and enhance the severity of symptoms and lesions, enabling the establishment of secondary bacterial infections and leading to an increased need for antimicrobial treatments. The aim of this study was to assess the correlation between positivity for PRRSV and SIV and the use of antimicrobial treatments in the nursery.

### **Material and Methods**

On 110 Dutch swine farms, oral fluids were collected from 8-week-old piglets with cotton ropes, conditioned in sterile plastic flasks, and sent refrigerated to the laboratory within 24 hours. Samples were tested by RT-PCR for PRRSV and SIV, and a written survey was performed on each farm to collect data on productive parameters and antimicrobial treatments during the nursery phase (measured in DDD – Defined Daily Dose).

#### Results

63.6% of the farms were positive for at least one of the viruses, with 21.8% being simultaneously positive for both. The double-positive farms had a higher rate of treatments against streptococcal infections (30.4% of animals versus 15% on double-negative farms, 12.5% on SIV-positive farms and 15% on the PRRS-positive farms). SIV-positive farms had higher DDD values (10.6 versus 6.4 on SIV-negative farms; p=0.01) and farms with lower SIV Ct values for the oral fluids showed higher levels of DDD to treat streptococcal infections (r=-0.25; p=0.09). No correlation was observed for the PRRSV Ct values and antimicrobial treatments, but lower Ct values were significantly correlated with higher mortality in the nursery phase (r=-0.32; p=0.04).

# **Discussion and Conclusion**

The results show that not only most of the farms were positive for at least one of the viruses, but that PRRSV and SIV infection during the nursery phase was associated with a higher incidence of streptococcal infections, increased antimicrobial treatments and mortality. These findings reinforce the need to establish proper control of PRRSV and SIV at farm level to reduce antimicrobial usage during the nursery phase, particularly on farms with a high incidence of streptococcal infections.

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# SUCCESSFUL ERADICATION PRRSV-1 FROM A DUTCH 3000 HEAD SOW FARM BY LOAD-CLOSE-HOMOGENIZE

<u>R. Janssen</u><sup>1</sup>, M. Steenaert<sup>2</sup> <sup>1</sup>De Varkenspraktijk, the Netherlands <sup>2</sup>Boehringer Ingelheim AH the Netherlands BV

### **Background and Objectives**

PRRS is endemic in most of the pig farms in the Netherlands. Despite the relative high success rate of Load-Close-Homogenize (LCH), the program has not been used in sow herds in the Netherlands very often. This case describes a Load-Close-Homogenize program to eradicate endemic circulating PRRSv-1 in a 3,000 head sow farm, farrow-to-wean, in the Netherlands.

### Material and Methods

The 3,000 head sow farm, farrow-to-wean, had an introduction of PRRS Wild Type Virus type 1 in October 2015 in combination with associated clinical reproductive disease. LCH started in November 2016. In week 1 and week 5 all pigs being 16 weeks of age and older were PRRS MLV mass vaccinated (ReproCyc® PRRS EU), and every 13 weeks after that. From week 1 onwards, the piglets were vaccinated PRRS MLV (Ingelvac PRRSFLEX® EU) at weaning (age 21 days) and removed from the sow farm. Monitoring from week 4 was done by PRRS PCR in at least 30 piglets prior to PRRS MLV vaccination, 1 piglet per litter, pooled 1:5, every 4 weeks. Week 25 the farm was opened again for introduction of PRRS WTV negative replacement gilts in the quarantine barn at the sow herd location.

#### Results

From 8 weeks in the LCH program, PRRS PCR was negative in serum from piglets prior to PRRS MLV vaccination. In week 24 in 1/15 pools was PRRS PCR positive. From week 28 onwards all PRRS PCR monitoring was negative and stayed PRRSv-1 negative for at least 6 years.

#### **Discussion and Conclusion**

Removing all weaned piglets from the farm was considered a major contribution to the success of the LCH program. It reduced the number of pigs in the farm by more than 50% and prevented in-farm pathogen shedding by weaned piglets. Continuation of PRRSv-1 MLV mass vaccinations every 13 weeks of all adult animals was based on the assumed risk of re-infection by PRRS WTV-1. This case demonstrates that a Load-Close-Homogenize program to eradicate PRRSv-1 from a farm can be successful in the Netherlands.

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# SURVEY ON RISK FACTORS AND THE IMPACT OF CONGENITAL TREMOR IN SWISS BREEDING HERDS

#### K. Moser<sup>2</sup>, M. Schweizer<sup>1</sup>, A. Grahofer<sup>2</sup>

<sup>1</sup>Institute of Virology and Immunology, Federal Food Safety and Veterinary Office (FSVO) and Vetsuisse Faculty University of Bern

<sup>2</sup>Clinic for Swine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

### **Background and Objectives**

In recent years, several cases of congenital tremor in piglets have been associated with the atypical porcine pestivirus (APPV). Only limited information is available about the prevalence at herd level and the impact of this disorder on pig farms. Therefore, the aim of this study was to gain further information about congenital tremor in piglets using an online questionnaire send to farmers of breeding herds in Switzerland and to analyse associations between this disorder and farm as well as management characteristics.

# **Material and Methods**

The online survey included a total of 17 questions covering general information about the herd, prevalence, and mortality data of congenital tremor, as well as implemented management procedures, and was submitted to 1337 farmers of pig breeding herds across Switzerland. The statistical processing of all data was performed in R, considering the level of statistical significance being p < 0.05.

#### Results

In total, 135 farms (response rate 10%) answered the survey. In the last two years, 40% of the farmers observed congenital tremor at least in one litter. This disorder caused 0.2% of the mortality rate of all piglets on the farm. However, the mortality rate of affected litters was on average 38 %. Compared to other breeds (Large White, Landrace, Pietrain), farms with Duroc (mean: 1.16 % vs. 0.5 %; p-value: 0.011) and Primera (Maternal line Large White x Landrace) (mean: 0.78 % vs. 0.44 %; p-value: 0.028) had a significantly higher prevalence of litters with congenital tremor. Furthermore, a parity effect could be detected. Litters of gilts were significantly more often affected by congenital tremor than older parity sows (mean: 2.2% vs. 0.9%; p-value: 0.007). One third of the farmers reported that congenital tremor has an impact on animal health and welfare, whereas two third didn't have an opinion or replied that the disorder didn't affect the general condition.

#### **Discussion and Conclusion**

This survey showed, that congenital tremor occurred frequently in Swiss breeding farms. Piglet losses within affected litters were high. Therefore, prophylactic measures as well as specific management procedures must be developed. Hence, further studies should focus on a possible breed effect and implementation of measurements to improve animal health and welfare.

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# UDDER THERMAL TEMPERATURE TO PREDICT LITTER PERFORMANCE: A PILOT STUDY

<u>R. Muns</u><sup>1</sup>, A. Jones <sup>1</sup>, C. Mulvenna <sup>1</sup> <sup>1</sup>Agri-Food and Biosciences Institute

#### **Background and Objectives**

Infrared thermography (IRT) measures surface temperature, detecting changes in heat transfer and blood flow. IRT has been used to identify stress, inflammation and disease. However, increased blood flow in the mammary gland detected as increased surface temperature could be associated with increased milk yield. Our objective was to explore whether IRT could be used to predict sow-litter performance.

### Material and Methods

Infrared images of 16 sows (parity 2-7) individually housed in conventional farrowing pens in a temperature controlled room were taken of each sows' udder (cranial end, including the first two mammary glands/teats) from a 60 cm distance (FLIR T650sc, FLIR Systems, UK) at before farrowing (day -1) and on day 28 of lactation. Images were evaluated using the FLIR Tools program. Emissivity was set to 0.96. Average temperatures were recorded for the udder (line immediately above the glands/teats) and for the teats. Sow and litter performance were recorded. Data was analysed using GenStat. Udder and teat temperature were analysed by ANOVA with day as main effect. Pearson's rank correlation analysis was conducted for all parameters.

#### Results

Udder and teat average temperatures were lower on day -1 than on day 28 (34.8 vs.  $37.2\pm0.29^{\circ}$ C, and 35.8 vs.  $36.8\pm0.18^{\circ}$ C; P<0.001, respectively). Udder temperature at day -1 was positively correlated with sow back fat levels (r=0.449, P<0.05) and sow body weight (r=0.537, P<0.05) before farrowing. Average teat temperature at day -1 was negatively correlated with litter weight gain (r=-0.581, P<0.01).

#### **Discussion and Conclusion**

Increased temperature from day -1 to 28 agrees with an increase in milk production potentially linked to increased blood flow into the udder. However, the lack of correlation between udder temperature and litter/piglet growth does not support our hypothesis. Interestingly, the negative correlation between teat temperature at day -1 and litter growth would support the link between IRT and health. Our work suggests that teats should be considered separately from the udder when assessing sow health/physiology using IRT. Further research is needed to better understand changes in udder temperature during lactation.

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# CASE STUDY: COMPARISON OF AN IRON-DEXTRAN INJECTION AND A TOLTRAZURIL/GLEPTOFERRON INJECTION FOR THE PREVENTION OF ANEMIA ON 3 BELGIAN FARMS

<u>E. De Jonghe</u><sup>1</sup>, P. De Backer<sup>1</sup> <sup>1</sup>Ceva Santé Animale, Brussels, Belgium

#### **Background and Objectives**

Iron Deficiency Anemia (IDA) negatively affects the immune response and growth in piglets. IDA is highly prevalent on Belgian farms. Three farmers wanted to improve the piglet health and on request of the veterinarian and farmer the anemia status of piglets at weaning was investigated. Beside other known haematological parameters, hemoglobine (Hb) concentration is the most frequently used for on-farm evaluation of the hematinic status of piglets and Hb evaluation was previously used for assessment of comparison of different types of iron supplementation on the health and productivity of piglets under field conditions. A comparison between their current iron supplementation and Forceris®, an injectable toltrazuril/gleptoferron combination product, was made.

# Material and Methods

Within each of the 3 Belgian farms (A, B, C), minimum ten randomly selected litters from different parity sows have been assessed. Half of the litters were treated with an iron-dextran injection (current treatment, group I) and half of the litters were treated with an injection of a toltrazuril/gleptoferron combination product (new protocol, group II). Within each litter, one large, one medium and one small piglet were sampled at weaning age. In total 120 piglets with an avg. age of 26 days were sampled. Blood was taken from the ear vein and Hb levels were measured using the Hemocue® Hb 201+ analyzer. Piglets were classified as follows: Hb levels < 90 g/l are considered to be anemic, Hb levels  $\ge$  90 g/l and < 110 g/l are suboptimal and Hb levels  $\ge$  110 g/l are optimal.

#### Results

The percentages of anemic piglets in group I compared to group II were higher on each farm (A: 33% vs. 0%, B: 27% vs. 7%, C: 23% vs. 7%). The percentages of piglets with optimal Hb levels in group I compared to group II were lower on every farm (A: 17% vs. 72%, B: 20% vs. 47%, C: 13% vs. 80%).

#### **Discussion and Conclusion**

There are less anemic piglets and more piglets with optimal Hb levels in group II on each farm. Based on these results, the 3 farmers decided to continue with the toltrazuril/gleptoferron combination product.

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# COMPARISON OF ON- AND OFF-SITE LUNG SCORING IN SLAUGHTER PIGS

V. Leppkes <sup>1</sup>, C. Waehner <sup>2</sup>, J. Kauffold <sup>3</sup>, H.L. Sigmarsson <sup>3</sup>

<sup>1</sup>Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany <sup>2</sup>Ceva Tiergesundheit GmbH Kanzlerstr. 4, 40472 Düsseldorf - Deutschland <sup>3</sup>Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

### **Background and Objectives**

Lung scoring at slaughter line (on-site) is common to assess lung health in pigs. Despite available and established scoring systems such as the Ceva-Lung-Program (CLP), scoring errors may occur on-site likely facilitated by the fast pace of the slaughter process. This study aimed to compare the results of on- and off-site (laboratory) lung scoring using CLP for reproducibility.

# **Material and Methods**

A total of 6.581 lungs from 51 farms were scored on-site, and a total of 5.053 from 137 farms off-site. All pigs originated from finisher farms all over Germany and were slaughtered between 3/2020 and 2/2022. Both scorings were done by the same person using CLP. Lesions recorded were EP-like lesions (EP), dorso-caudal pleurisy (DCP), cranial pleurisy (CP), interstitial pneumonia (IP), abscess (AS), acute pleuropneumonia (AP), scars and pericarditis (PC). Data was analysed by means of https://datatab.fr using the CHI<sup>2</sup> test also considering season as a factor.

#### Results

Significantly more EP (p=<0.001), DCP (p=0.02), CP (p=<0.001), IP (p=<0.001), AP (p=<0.001) and scars (p=<0.001) were detected by off-site scoring. Only AS was more frequently observed on-site (p=<0.001). Off-site scoring revealed significantly more EP (p=<0.001), DCP (p=<0.001) and CP (p=<0.001) in spring, while with on-site, EP and DCP (p=<0.001, p=<0.001) appeared more frequently in summer and autumn.

#### **Discussion and Conclusion**

Limits to the study were that lungs scored on- and off-site where not identical and from different farms. However, only one examiner scored always using CLP and during same season. The differences in prevalence of various types of lesions in different seasons indicate that the origin of lungs plays a more important role than the site of inspection. The off-site scoring allows more time available for scoring than on-site during the slaughter process, however the obtained results are not conclusive in terms of differences in accuracy. Both scoring methods are reliable even though differences in the results appeared.

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# DEVELOPMENT OF A STANDARDISED METHOD TO MEASURE THE INFECTIOUS LOAD IN A PIG PEN

L. Dahlin<sup>2</sup>, I. Hansson<sup>1</sup>, N. Fall<sup>2</sup>, M. Jacobson<sup>2</sup>, A. Sannö<sup>2</sup>

<sup>1</sup>Department of Biomedical Science and Veterinary Public Health, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

<sup>2</sup>Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

#### **Background and Objectives**

All-in, all-out with strict hygienic routines is necessary in modern pig production. Furthermore, a standardized, validated method is needed to quantitatively control the effect of these hygiene protocols. This study aimed to evaluate three different sampling methods to develop a standardised sampling method.

# **Material and Methods**

In a controlled environment, sterilized pig faeces were mixed with indicator bacteria (i.e. Enterococcus hirae, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus) and spread out in a pig pen. The retrieval rate of three different sampling methods were evaluated; swabbing by a (i) cloth and a (ii) sponge, which were analysed by standardised bacterial culture, counting of colony-forming units, and a (iii) cotton swab analysed by adenosine triphosphate (ATP) bioluminescence. Sampling was carried out before and after manual scraping of the surfaces. To determine sample-to-sample variability and critical control points, sampling by the cloth and the cotton swab was carried out directly after manual scraping and after cold-water high-pressure washing.

#### Results

In our preliminary results, sampling by the cloth or the sponge showed few differences in retrieval rate, whereas the swabs showed a very high retrieval rate. Sample-to-sample variability was low. No specific critical control points (CCPs) in the pen were identified.

#### **Discussion and Conclusion**

The cloth had slightly higher retrieval rate, usability and lower cost as compared to the sponge, which had lower variability but overall was harder to use. Since the ATP bioluminescence test measures ATP released from all cellular matter, sampling should be limited to visibly clean and dry surfaces. In the present study, surfaces were covered in faecal slurry, and thus, these results should be interpreted with care. No CCPs could be identified, however, in a clinical setting, corners and other areas potentially being more difficult to clean, are probably more critical and thus suitable to investigate.

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# EFFECT OF PARENTERALLY ADMINISTRATED TOLTRAZURIL ON LACTATION TO FINISHING PERFORMANCES

<u>G. Ramis</u><sup>1</sup>, F. Murciano<sup>2</sup>, B. Gonzalez-Guijarro<sup>1</sup>, L. Martínez-Alarcón<sup>3</sup>, A. Cuellar-Flores<sup>4</sup>, D. Espigares<sup>5</sup>, D. Sperling<sup>6</sup> <sup>1</sup>Departamento de Producción Animal. Universidad de Murcia <sup>2</sup>Facultad de Veterinaria, Universidad de Murcia, Spain <sup>3</sup>Instituto Murciano de Investigación en Biomedicina (IMIB), Murcia, Spain <sup>4</sup>Dpto. Producción Animal,. Facultad de Veterinaria., Universidad de Murcia, Spain <sup>5</sup>Ceva Salud Animal, Barcelona, Spain

<sup>e</sup>Ceva Animal Health France

### **Background and Objectives**

Cystoisosporosis remains the main parasitic disease affecting suckling piglets worldwide. Although the disease has been prevented over the last 30 years by management measures, hygiene and the routine administration of totrazuril to neonatal piglets, the parasites are still present in the populations at high prevalence. The administration of totrazuril has been done orally in the classical way until commercial preparations for parenteral administration have appeared on the market. The aim of this study was to evaluate the performance in suckling, nursery and fattening period of a batch of piglets treated with Forceris (Ceva Santé Animale, France)

# **Material and Methods**

The trial was carried out at the veterinary teaching farm of the University of Murcia. Ninety-two animals were left as control group without oral or parenteral toltrazuril administration (CON group) while 90 piglets were administered Forceris (F group) parenterally in the first 3 days of life. Piglets were weighed at birth (WAB), at weaning (WW), at entry to standardized fattening at 50 days of age (WN50d), and at the end of standardized fattening at 171 days of age (WN171d). Average daily gain was calculated for lactation (ADG\_S), nursery (ADG\_N), fattening (ADG\_F) and total (ADG\_T).

### Results

There was no difference in WAB (CON=1.52 vs F=1.49, p=0.490), nor in WW (CON=4.9 vs F=4.9, p=0.975). However, there was a significant difference in WN\_50d (CON=23.0 vs F=26.7, p<0.001) and WN\_171d (CON= 98.8 vs F=104.8, p=0.003). For growth, differences were observed in ADG\_N (CON=0.402 vs F=0.485, p=<0.001) and ADG\_T (CON=0.569 vs F=0.604, p=0.003), but not in ADG\_S (CON=0.135 vs F=0.137, p=0.712) or ADG\_F (CON=0.785 vs F=0.800, p=0.215).

#### **Discussion and Conclusion**

In this study the main difference observed was not during lactation, as has been observed in previous studies, but in nursery, suggesting late infestation by Cystoisospora suis, which was confirmed during the trialHowever, the difference produced during nursery is maintained in the fattening period and there is no compensatory growth by the CON animals, or at least it does not compensate the difference between groups. In conclusion, the administration of parenteral toltrazuril under the conditions of this study may result in the sale of up to five kilos more meat per treated animal to the slaughterhouse.

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# EFFECTIVENESS OF TWO STRATEGIES TO REDUCE PRE-WEANING ANEMIA AND COCCIDIOSIS IN TWO MEXICAN FARMS AND THEIR EFFECT ON POST-WEANING PERFORMANCE.

A. Henao-Diaz<sup>1</sup>, I. Garay-Guerra<sup>1</sup>, A. Cota-Valdez<sup>1</sup>, A. Ortega<sup>2</sup>, F. Chama<sup>2</sup>, .<sup>0</sup>, E. Aguilera<sup>2</sup>, J. Calveyra<sup>4</sup>

¹Bachoco ²CEVA, Mexico ⁴Ceva Animal Health, Brazil

#### **Background and Objectives**

Iron deficiency anemia (IDA) and neonatal coccidiosis challenge swine farms worldwide affecting the pigs' weight gain. This study compared two strategies to control IDA and coccidiosis in two Mexican farms.

# **Material and Methods**

A pre-study estimated the IDA and coccidiosis status including 30 piglets and 10 litters/farm. Then, two blinded studies including 40 litters (n=528) and 28 litters (n=351) were run at Sonora and Guanajuato states (Farms 1 and 2). Piglets were randomized into TT1 (IM Forceris®, [45mg toltrazuril+200mg Gleptoferron]) or TT2 (Dextran 200mgIM and toltrazuril 45mgOral) on day 2.5-3.5. Hemoglobin (onsite, HbHemoCue®) was estimated at weaning. Oocyst count (onlab, per-gram-feces OPG) was estimated at 10d and 17d. Body weight was measured pre-treatments, at weaning, and 80d. ANOVA analyzed results.

#### Results

Pre-study:

Farm 1 and 2: 70%(21/30) and 80%(24/30) of piglets showed IDA with Hb mean 10.04 g/dl and 9.3 g/dl, respectively. 20%(2/10) and 100%(10/10) of litters showed coccidiosis, with OPG mean of 3000 and 1868, respectively. Study:

Farm 1: at weaning, TT1 showed a lower IDA (28% vs 56%), with a higher Hb mean (11.38 vs 10.80 g/dL, P<0.05), lower coccidiosis peak (OPG 5331 vs 9337, P<0.05) and 0.6% less mortality. The mean weight was higher for TT1 at weaning (mean 5.73 vs 5.57, P<0.05) and at 80 days of age (mean 30.03 kg vs 28.78 kg, P<0.05).

Farm 2: at weaning age, the TT1 showed a lower IDA (46% vs 93%), with higher Hb mean levels (11.1 vs 8.7g/dL, P<0.05), similar coccidiosis peak (OPG 1944 vs 1939, P>0.05) and 5.4% less mortality; The mean weight was higher for TT1 at weaning (mean 6.98 vs 6.62, P>0.05) and at 80 days of age (mean 27.84 kg vs 26.25 kg, P<0.05).

#### **Discussion and Conclusion**

IDA and coccidiosis were different regarding the region: farm management, piglets management, and environment, may play a main role.

Regardless of the region, TT1 reduced ~45% IDA at weaning, and at 80d improved weight mean up to ~1.25kg and ~1.59kg in Farm 1 and 2; representing ~3.5 and ~4.45 \$USD/pig, respectively; likewise, a higher ROI would be expected closer to market age.

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# EFFECTS OF A PROBIOTIC DRINK IN SUCKLING PIGLETS - A PILOT STUDY

F. Zeeh <sup>3</sup>, A. Schnyder <sup>1</sup>, R. Graage <sup>2</sup>, D. Kümmerlen <sup>3</sup>

<sup>1</sup>Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

<sup>2</sup>Qualiporc Health Service, Qualiporc Cooperative, Appenzell, Switzerland

<sup>3</sup>Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland.

#### **Background and Objectives**

Diarrhoea in suckling piglets is a major issue in pig production. As bacterial pathogens are important in this context, antimicrobials are often used. However, antimicrobial usage is under debate and alternative strategies gain importance. Probiotics can be such an alternative, but studies in pigs are mainly focused on weaned piglets. Therefore, we tested the effect of a probiotic drink on the incidence of diarrhoea in suckling piglets in a pilot study.

# **Material and Methods**

In a breeding herd with a history of diarrhoea in suckling piglets, litters from one farrowing batch were randomly allocated in a treatment or control group. Piglets in the treatment group received 2 mL of a commercial probiotic drink 6 to 12 hours after birth. Control piglets remained untreated. Body weight was measured at day 0 (day of birth), day 16 (+/- 1 day) and before weaning (day 25 +/-1 day). Occurrence of diarrhoea, all antimicrobial treatments and losses were recorded.

#### Results

Five and four litters (62 and 46 piglets) were allocated to the treatment and the control group, respectively. Diarrhoea occurred in two and four piglets of the treatment and control group, respectively. Four piglets (2 per group) were treated by the farmer against diarrhoea with antibiotics. In the treatment and control group, 3/62 and 8/46 piglets died until D25 (p>0.05). The average daily gain (ADG) of the treated and control piglets from D0 to D16 and D25, in g/d were 201.1/ 221.8 (p>0.05) and 230.7/ 267.9 (p=0.005), respectively.

#### **Discussion and Conclusion**

Diarrhoea was nearly absent in this farrowing batch hampering a conclusion regarding potential reduction of diarrhoea. Unexpectedly, the ADG of the control group was significantly higher than that of the treatment group. Higher birth weights in the control group might have been a reason. When correcting for birth weight, no differences in the ADG were observed. Losses were numerically higher in the control group (4.8 versus 17.4%), potentially indicating a positive effect of the probiotic drink. Still, more research is needed to test the hypothesis of a positive effect of the probiotic drink.

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# PROTEXIN TM PROBIOTIC (ELANCO) TO IMPROVE THE PERFORMANCE OF PIGLETS FROM BIRTH TO PRE-FATTENING (69 DAYS)

<u>C. Alvarez</u><sup>1</sup>, O. Herran <sup>2</sup>, P. Sanchez <sup>1</sup>, A. Serna <sup>1</sup> <sup>1</sup>Elanco Animal Health <sup>2</sup>Nutrisolla Research Group

# **Background and Objectives**

The provision of probiotics in the first days of life of the piglet has been shown to modulate the intestinal microbiota, restore and improve the resistance of pigs to diseases and result in better productive performance. Evaluating the supply of alternatives other than antibiotics in piglets is important given the current pressures on the rational use of antibiotics in the livestock sector and the problem of antimicrobial resistance worldwide. The objective of this study was to determine the impact of administration to neonatal piglets (1 and 3 days of age) of a commercial probiotic Protexin (Elanco) on the percentage of mortality and feed conversion index from weaning to pre-fattening (69 days)

### **Material and Methods**

This study was carried out on a farm with 1000 sows with monthly batch management system, located at 2400 meters above sea level, in the department of Antioquia, Colombia. All litters corresponding to 4 birth groups were selected, to which 1 ml of probiotic was administered orally on day 1 and 3 of birth, follow-up was carried out until the end of the prefattening stage (69 days); The litters from 5 previous birth groups in which antibiotic treatment had been instituted on day 1 of life were taken as controls. Birth at weigh and after treatment was performed individually on day 21 and 69, In the statistical analysis, the relationship between the mortality and weight gain variables and their relationship with the use of probiotic was calculated using ANOVA test and Students t (JMP 15, SAS)

#### Results

Litters in treatment group obtained a lower feed conversion rate (weaning day 21 to pre-fattening day 69) (control value Vs treatment value) (0.62) than control group (P -value =0.0463); Likewise, the treatment litters had a lower mortality rate (1.8) than control litters and this is largely related to use of Probiotic (P-value=0.0240).

### **Discussion and Conclusion**

The results obtained demonstrate that there is a strong correlation between the use of probiotic in reducing mortality in pre-fattening, as well as an improvement in feed conversion; Unlike antibiotics, supplementation with probiotic had a positive impact on health of pigs and on some of the productive parameters.

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# ROTAVIRUS AND COMMON CO-INFECTING BACTERIAL PATHOGENS IN NEONATAL DIARRHOEA PROBLEMS IN EUROPE.

J. Miguel <sup>1</sup>, O. Boix <sup>1</sup>, L. Nodar <sup>1</sup>, D. Giralt <sup>1</sup>, I. Galé <sup>1</sup>, <u>J. Hernández-García</u> <sup>2</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA UK and Ireland

### **Background and Objectives**

Rotavirus is an important pathogen causing Neonatal Diarrhoea; type A was historically the most relevant but other types such as C and others are becoming more and more frequent in Europe.

Neonatal Diarrhoea problems involving Rotavirus frequently include other pathogens too, increasing the severity and economic impact of the problem at the same time they also complicate their treatment. This study evaluated probabilities of detecting key digestive bacterial pathogens overgrowth (high load) in Rotavirus-infected piglets.

#### **Material and Methods**

712 farms with neonatal diarrhoea problems throughout Europe were sampled in 2022 and 2023 following the Enterocheck PLUS protocol. Three litters per farm were sampled with rectal swabs -3 piglets/litter-.

Each litter provided one sample that was fixed in FTA cards to prevent quality decay, then tested by qPCR for key digestive pathogens such as Enterotoxigenic Escherichia coli virulence factors, Clostridium perfringens (A and C), Clostridioides difficile, Rotavirus (type A and C) and Porcine Epidemic Diarrhoea virus.

#### Results

56% litters tested positive to Rotavirus type C. C.difficile prevalence was 70%; coinfection with Rotavirus type C was present in 40%.

C.perfringens type A was present in 98% of samples and 53% with Ct values <26. High loads for both C.perfringens (Ct<26) and C.difficile (Ct<30) were found in 31% of litters, also combined with Rotavirus C in 19% of the litters sampled. The Odds Ratio for C.perfringens infected litters to be co-infected for C.difficile (with Ct<30) is 2.25 [2.00-2.54; 95% confidence].

E.coli virulence factors (fimbria F4-5-6 or LT toxin) were detected in 67% litters, 23% with Ct<30, and 14.8% when combining E.coli Ct<30 and Rotavirus type C presence. Other Rotavirus were found in 56% litters and multiple types were detected in 33% litters.

# **Discussion and Conclusion**

Rotavirus type C was found in lots of neonatal diarrhoea cases. It was frequently associated with bacteria such as E.coli, C.difficile or C.perfringens but also other rotavirus in at least 33% litters.

C.perfringens type A is not only virtually ubiquitous but also quite frequent at high load in 53% of the samples, and C.difficile is also associated in a high number of cases. Interventions should consider the polymicrobial nature of these processes.
#### HHM – Herd Health Management

# UNDERSTANDING THE SPATIO-TEMPORAL PATTERNS OF PELVIC ORGAN PROLAPSE (POP) IN THE SWINE INDUSTRY

J.P. Gómez-Vázquez <sup>1</sup>, <u>R. Robbins</u> <sup>3</sup>, M.J. Clavijo <sup>2</sup>, K. Aguirre <sup>4</sup>, P. Hams <sup>3</sup>, B. Martínez-López <sup>1</sup> <sup>1</sup>Center for Animal Disease Modeling and Surveillance (CADMS), School of Veterinary Medicine, University of California, Davis, CA, USA <sup>2</sup>Veterinary Diagnostic and Population Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, Pig Improvement Company, PIC®, Hendersonville, TN, USA <sup>3</sup>PIC North America, Hendersonville, TN 37075 <sup>4</sup>University of California, Davis

# **Background and Objectives**

**Porcine pelvic organ prolapse (POP)** is a condition that affects mainly peri-partum sows causing serious injuries to the animal reproductive tract. Given that no treatment exists, POP contributes significantly to removal of affected animals and overall farm mortality (Supakorn et al. 2017), which represent severe economic losses for the swine industry (Bhatia et al. 2023).Despite being one of the leading non-infectious causes of mortality in recent years, the underlying causes of POP remain poorly understood, and there is limited information regarding its baseline prevalence in the swine industry. The Midwest region in the United States represents a major hub for swine production, offering a unique setting to study the dynamics of POP. In this study, we investigated the spatio-temporal evolution of POP over recent years across 25 different states in the Midwest region of the US and parts of Canada.

### Material and Methods

The dataset comprised reports from over 300 breed to wean farms in multiple states, covering the period from 2018 to 2023 with a total number of 5,647 records. We used descriptive statistics to provide regional estimates of the POP prevalence, Moran's I to assess spatial autocorrelation, and space-time scan statistics (Kulldorff et al. 2005) to identify clusters of high POP incidence during the study period.

### Results

The average incidence per year has increased significantly from 1.03 in 2018 to 3.98 in 2023 with a median incidence of 3.33 for the whole study period. Moran's I revealed an increasing spatial dependence with cyclical patterns for the time period analyzed. We identified seven significant clusters during the study period, with relative risk estimates ranging from 2.09 to 12.67.

### **Discussion and Conclusion**

Our results provide supporting evidence that POP has had a significant increase in the swine industry in recent years. To the best of our knowledge, this analysis represents the first comprehensive evaluation of POP's temporal evolution and includes a spatial description of the at-risk population. Further analysis of the risk factors contributing to the disease would be beneficial to understand better the disease dynamics and provide recommendations for disease control.

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# EVOLUTION OF LUNG LESION SCORE AFTER IMPLEMENTATION OF A COMBINED MYCOPLASMA HYOPNEUMONIAE – PCV2 VACCINATION ON PIGLETS

C. Spindler <sup>1</sup>, A. Lefebvre <sup>2</sup>, J.C. Lorgere <sup>3</sup>, B. Maynard <sup>4</sup>, M. Charles <sup>4</sup>, <u>E. Lewandowski</u><sup>5</sup>, P. Leneveu <sup>4</sup> <sup>1</sup>Selas de Surfonds, 37 Rue Ettore Bugatti, 72650 La Chapelle Saint Aubin, France <sup>2</sup>HYOVET, 5 P.A. Carrefour du Penthièvre, 22640 Plestan, France <sup>3</sup>Farmapro, 6 P.A. carrefour du Penthièvre, 22640 Plestan <sup>4</sup>Ceva Santé Animale, 10 Avenue de la Ballastière, 33500 Libourne, France <sup>5</sup>Ceva Biovac, 4 rue 0 de Serres, 49 070 Beaucouzé, France

### **Background and Objectives**

Mycoplasma hyopneumoniae and PCV2 are 2 pathogens involved in Porcine Respiratory Disease Complex, a major issue for the pig industry. This study aims to describe the lung lesion evolution at slaughter after implementation of a combined Mycoplasma hyopneumoniae -PCV2 vaccine in replacement of another vaccine solution.

### **Material and Methods**

Farms, from a French cooperative, were retrospectively selected based on the following criteria: the implementation of Hyogen® - Circovac® vaccination on piglets and the use of lung scoring for monitoring pig respiratory health (Madec grid on 6 lobes; total score = 24) using Ceva Lung Program.

Lung scorings (30 lungs per batch as a minimum) one year "Before" and "After" implementation were compared. Analysed criteria were :

1/ the average Enzootic Pneumonia like Score (EPS). Its evolution is considered significant when over 0.5 2/ the percentage of Pneumonia Free Lungs (%FL).

The average of EPS and %FL per farm and per period were analysed.

PRRSv vaccination status of farm was also recorded (sows and piglets).

The study was extended for farms using the combine vaccine for at least 2 years in order to evaluate their situation in 2<sup>nd</sup> year After (After 2).

### Results

31 farms were included. Their lung scores improved significantly (Wilcoxon test):

- 0,8 of EPS (p = 0.009; 2.9 before vs 2.1 after)

+ 12 points of %FL (p = 0.016; 44% before vs 56% after).

19 farms (61%) significantly improved their EPS by more than 0.5.

PRRS vaccination status remained unchanged in most farms (22). For the other farms, change of PRRSv vaccination didn't induce the possible effect on EPS evolution : no improvement when vaccination implemented, no deterioration when vaccination stopped. PRRS vaccination status was therefore not involved in the scoring evolution described.

13 farms were included in the extended survey. Their EPS decreased by 1.3 when comparing Before (2.7) and After 2 (1.4) periods.

# **Discussion and Conclusion**

The evaluated vaccine solution significantly improved the respiratory health from first year of use and after. Results are consistent with a comparable study with Hyogen® (Spindler et al., 2018).

The improvement of scores is not biased by a change in PRRS vaccination status.

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# IMPROVING SOW UDDER HEALTH AT WEANING THROUGH A PLANT EXTRACT ORAL ADMINISTRATION

<u>B. Frederick</u><sup>1</sup>, P. De Cacqueray<sup>1</sup> <sup>1</sup>Newborn Animal Care SAS

### **Background and Objectives**

At weaning, emphasis is on piglets while dry-off management of sows often negleted. It' even more problematic that litter size and milk production continues to rise. More than ever, drying-off should require special attention to limit udder health issues and nonfunctional teats. Our study objective is to evaluate the nutritional complement effect on post weaning udder status.

### Material and Methods

Sixty-six Large White x Landrace sows were split into control and test groups. In the test group, sows received 15 mL of a gel (composed of echinacea, parsley, sage and B6 vitamin) on top of their last meal before weaning. Udder skin temperature (UST), udder engorgement (UE) and milk leakage (ML) scores were measured for three days following weaning (D+1, D+2 and D+3). UE was evaluated through a scoring from one (flabby and flat) to four (swollen and hard). ML scores rank from zero (no milk drops) to three (continuous leaking). Return-to-estrus was assessed with the heat standing behavior test.

### Results

At weaning, UE score repartition was different between groups (p<0.05, Khi<sup>2</sup> test). Twenty-nine percent of control sows have a UE score higher than three, versus 5% in the test group. Sow proportion with ML tended to be lower in the test group (56%) than in the control group (76 %, p<0.10, Khi<sup>2</sup> test). UST from D+1 to D+2 decreased in the control group from 33.05°C to 31.81°C versus 33.62°C to 30.80°C in the test group. The test group drop was significantly greater (p<0.01, Wilcox test). Simultaneously, sows proportion with a weaning-to-estrus interval greater than four days, tends to increase in the control group versus in the test group (56% vs. 35%, p<0.10, Khi<sup>2</sup> test).

### **Discussion and Conclusion**

UE and ML scores suggest that milk production decreased more rapidly in the test group. Furthermore, accelerated UST evolution, in the test group can be interpreted as a less inflammation based on several studies. Ours results show that a supplement may ease udder involution. Additional studies are underway to evaluate the product benefits on reproduction.

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# SOW MORTALITY IN DIFFERENT FEEDING PRINCIPLES FOR GROUP-HOUSED SOWS

L.U. Hansen<sup>1</sup>, T.B. Jensen<sup>1</sup>, M. Willkan<sup>1</sup> SEGES Innovation, Axeltorv 3, Copenhagen, Denmark

### **Background and Objectives**

Sow mortality in Danish herds has increased during the last years. The reasons may be due to management, but housing of gestation sows may also affect mortality. Gestation sows in Denmark are housed in groups with different feeding principles being either free assess stalls (FAS), electronic sow feeding (ESF) or floor feeding and vet feeding in long trough (FFT). This study investigated the effects of different feeding principles on sow mortality.

### Material and Methods

Complete data of all services, farrowings and deaths including death type was obtained from 108 Danish herds delivering data to SEGES Innovation from 2021 to 2023. Among the 108 herds, 29 % had FAS, 35 % had ESF, and 36 % had FFT. Data contained 268,248 sows and 766,229 complete reproduction cycles. Parity was grouped in 1-2, 3-4 and 5+. Mortality as frequency of sudden death and euthanasia during all ended reproduction cycles was modelled with a mixed effect logistic regression. Death time distributions were compared with a mixed effect cox regression.

### Results

From 2021 to 2023, 177,881 sows were replaced. Hereof 21,908 were euthanized, 24,019 experienced sudden death, and the rest were slaughtered. Herd size adjusted mean mortality was 12.9% (q25=10.3%/q75=14.4%) in FAS herds, 17.2% (q25=13.0%/ q75=20.6%) in ESF herds and 14.7% (q25=11.9%/ q75=17.3%) in FFT herds. Overall mortality was significantly better explained when including feeding principle (p < 0.00001) and its interaction with parity group (p<0.00001). Mortality was lowest for FAS across all parities, and parity group 1-2 had the lowest mortality in all feeding principles.

The results showed that the points in time in the reproduction cycle where sows most likely were euthanized or experienced sudden death did not change significantly between the three feeding principles.

### **Discussion and Conclusion**

This analysis confirms that the overall sow mortality differs across feeding principle, but also that the patterns of mortality rates related to parity are not related to the feeding principle. Furthermore, the analysis points out high-risk time intervals during the stage of pregnancy, which do not differ significantly between feeding principles. Thus, this study statistically confirms concepts of high-risk time intervals in pregnancy, where sow survival should be in extra focus.

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# THE DANISH SPF-SYSTEM IN 50 YEARS - WHAT'S NEXT?

<u>S. Dalsgaard</u><sup>1</sup>, K. Møller<sup>1</sup> <sup>1</sup>Danish Agriculture & Food Council

### **Background and Objectives**

The Danish SPF-system has been one of the world's best health systems for pigs for over 50 years in preventing disease and improving health, welfare and efficiency in the Danish pig production. But even successful systems need to be updated and keep in touch with modern structure and contemporary demands from the production. Therefore, the SPF-system is under evaluation and will be updated according to the needs from the pig producers and scientific recommendations.

### Material and Methods

In spring 2023 a pre-evaluation was conducted with semi structured interviews with a broad range of stakeholders. In the winter 2023/2024 an expert panel will review and analyze the system from a scientific and economic perspective with the purpose of providing specific recommendations for improvements. The recommendations from the expert panel will be combined with the results from the pre-evaluation and discussed with the stakeholders.

### Results

The pre-valuation identified 4 areas to investigate further: a) governance of the system, b) technologies used and relevant future technologies, c) veterinary issues, e.g., risk-based surveillance and d) customer service. All stakeholders emphasized the importance and the credibility of the system but also the need for developing the system to meet the demands from a modern pig production.

### **Discussion and Conclusion**

The Danish SPF system has a good foundation for developing and continue to bring value to the Danish pig production but is also challenged with the ever-increasing need for flow of animals between herds in Denmark and abroad. It is therefore important to combine the scientific recommendations from the expert panel with the economic and commercial needs in the pig production both from Danish farmers and foreign customers.

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# VALUES OF HAEMATOLOGICAL PARAMETERS DEPENDING ON THE SEASON AND THE HUSBANDRY SYSTEM IN ORGANIC PIG FARMS

<u>I. Golinar Oven</u><sup>2</sup>, J. Plut<sup>2</sup>, M. Hajdinjak<sup>1</sup>, T. Steferl<sup>2</sup>, E. Nadlucnik<sup>2</sup>, P. Njegovec<sup>3</sup>, M. Stukelj<sup>2</sup> <sup>1</sup>Laboratory of Applied Mathematics and Statistics, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia <sup>2</sup>Clinic for Reproduction and Large Animals, Clinic for Ruminants and Pigs, Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia

<sup>3</sup>VOA d.o.o, Domzale, Slovenia.

### **Background and Objectives**

There are many important reasons for determining haematological parameters in pigs: establishing a correct diagnosis, evaluating the health status of a pig and herd, early detection of diseases or poor growth performance. The ranges for most haematological parameters are quite wide and vary as they depend on many factors. The aim of the study was to determine whether the seasons and the type of husbandry influence the haematological parameters.

# Material and Methods

The study was conducted on two organic farms. The study comprised 3 groups of pigs. In each group there were 20 pigs of the indigenous Slovenian Krškopolski pig breed. The animals were kept under different conditions. The first group was kept indoors, the second in the same place but outdoor together with cattle, the third group was moved to another location and kept outdoor together with sheep. At the beginning of the study, all growers weighed approximately 30 kg. Individual blood samples were taken from anterior vena cava. The complete blood count was determined on 185 individual blood samples. The samples were analyzed with an automatic analyzer, the scil Vet abc PlusTM. Statistical analyses of the hematologic data were performed using one-way analysis of variance (ANOVA) and Tukey's HSD test or Welch's t-test, depending on the results of Bartlett's test for homoscedasticity.

#### Results

The complete blood count changed in all three groups depending on the season. In most cases, the seasonal pairs summer-autumn, spring-autumn and autumn-winter differed significantly. The seasons had the greatest influence on WBC (103/mm3), RBC (106/mm3) and MCH (pg) and the least influence on MCHC (g/dl), PLT (103/mm3) and on the haematological values of the animals kept in the barn. Depending on the type of housing, the complete blood count generally did not differ significantly in fall. The largest statistically significant differences were found comparing different pig groups in summer, especially between outdoor and indoor housing.

### **Discussion and Conclusion**

Seasons can influence hematologic parameters, as can housing type, especially comparing indoor and outdoor facilities. The differences between groups in complete blood counts were most pronounced in summer.

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# ASSOCIATIONS BETWEEN DISEASE STATUS AND PERFORMANCE, RESPIRATORY LESIONS AND ANTIBIOTIC USE IN IRISH PIG FARMS

<u>S. Mcauliffe</u><sup>1</sup>, E. Garcia Manzanilla<sup>2</sup>, M. Rodrigues De Costa<sup>2</sup>, S. Van Winden<sup>3</sup> <sup>1</sup>Royal Veterinary College & McAuliffe Pig Farms <sup>2</sup>Pig Development Department, Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermov, Co.

Cork, Ireland & School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland <sup>3</sup>Royal Veterinary College

### **Background and Objectives**

Serology is a useful tool to monitor disease status and vaccination efficacy in livestock. It can also be used to study the relationships between disease status and other variables like antimicrobial use. This study presents the first serology survey in Irish pig farms and uses different databases to study the associations between serology with biosecurity, antimicrobial use, productive performance and lesions found in the slaughterhouse.

### **Material and Methods**

A cross sectional serology was performed for 36 farms for the pathogens porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), Mycoplasma hyopneumoniae (MHyo), Actinobacillus pleuropneumoniae (APP), porcine parvovirus (PPV; only gilts and sows) and Erysipelothrix rhusiopathiae (Ery) at time points 0, 1-2, 3-4, 5-6 parities for sows, and 4, 7, 10, 13, 16, 19-21 and 21+ weeks of age for pigs. A total of 8 to 10 samples were collected for each time point. Those farms included in the serology survey that had data for productive performance, lung lesions, biosecurity, vaccination and antimicrobial use (n = 14) were included in a common database and a correlation study was performed.

#### Results

All farms were positive for APP, 72% were positive for SIV, 69% for MHyo and 47% for PRRSv. All farms were positive for Ery and PPV in gilts and sows and 6 farms were negative for Ery in growing pigs. APP was the main disease of concern and is the one with lower vaccination levels (14% of the farms). Vaccination for Ery, PPV and MHyo in positive farms was 100%. Vaccination for SIV and PRRS are mainly based on farmer's criteria with only 36% of the farms using it. The data from the serology was used to study associations with productive performance, lesions, AMU and biosecurity. Higher seroprevalence of SIV and PPV in sows were correlated to lower litters per sows per year (r < -0.76). PRRS and MHyo had associations with weaner and total mortality (r > 0.63). Lung lesions, AMU, and biosecurity did not show consistent correlations with serology

# **Discussion and Conclusion**

Sample size was too low for multivariable analysis but the results indicate the value of pursuing such an approach in some areas

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# CO-INFECTION OF VIRAL AND BACTERIAL PATHOGENS INVOLVED IN NEONATAL DIARRHOEA PROBLEMS IN EUROPE.

J. Miguel <sup>1</sup>, O. Boix <sup>1</sup>, L. Nodar <sup>1</sup>, D. Giralt <sup>1</sup>, I. Galé <sup>1</sup>, <u>J. Hernández-García</u> <sup>2</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA UK and Ireland

### **Background and Objectives**

Neonatal diarrhoea problems frequently are polymicrobial processes - initiated soon after birth by a low number of primary pathogens such as viruses and bacteria - that increase in severity once other pathogens commonly present in healthy animals get also involved causing production losses.

This study includes samples from farms with neonatal diarrhoea problems around Europe, and its objective was to provide information about the prevalence and co-infections of some key pathogens.

### **Material and Methods**

A total of 712 farms with neonatal diarrhoea problems were included in this analysis. Three litters per farm were sampled in 2022-2023. Samples with faecal material from the rectum were fixed in FTA-Elute cards and analysed by qPCR for key pathogens including Rotavirus type A and C, Clostridium perfringens type A and C, different types of Enterotoxigenic Escherichia coli, Porcine Epidemic Diarrhoea Coronavirus and Clostridioides difficile.

### Results

C.difficile was detected in 83% of farms (58% with Ct<30) and C.perfringens type A in 99% of farms (79% with Ct<26). Rotavirus was found in 89% farms, type C was detected in 66%, and type A+C co-infection occurred in 47% of the farms.

50% of the farms had co-infections of Rotavirus type C with high loads (Ct<26) of C.perfringens type A, 32% of farms had Rotavirus type C and Ct<30 for C.difficile, and 18% of the farms had Rotavirus C and Ct<30 for Enterotoxigenic E.coli.

### **Discussion and Conclusion**

Despite testing method sensitivity limitations, at least 66% of farms were infected with Rotavirus C and 47% co-infected with both Rotavirus type A and C. Co-infection with other pathogens at high load such as E.coli, C.perfringens or C.difficile occurred in more than 82% of farms.

These pathogens are known to increase severity and convalescent period together with higher mortality and need for antimicrobial use. Treatments and interventions must consider frequent pathogens such as these to be involved in polymicrobial processes; specific measures can reduce their pathologic effect and contribute to the control of the Neonatal Diarrhoea Disease Complex.

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# COMPARISON OF BATCH-TO-BATCH AND MASS VACCINATION APPROACHES IN A FARROW-TO-FINISH PIG HERD USING AN IMMUNITY MODEL FOR SWINE INFLUENZA

A. Niederberger <sup>1</sup>, <u>O. Mtaallah</u> <sup>1</sup>, A. Apolloni <sup>1</sup>, A. Jardin <sup>2</sup>, P. Creach <sup>2</sup>, G. Dauphin <sup>2</sup>, C. Hautefeuille <sup>1</sup> <sup>1</sup>CIRAD, UMR ASTRE, Univ Montpellier, INRAE, Montpellier, France <sup>2</sup>Ceva Sante Animale, Libourne, France

### **Background and Objectives**

Swine Influenza A Virus is one of the most important respiratory pathogens affecting pigs in farms. Vaccination plays a critical role in mitigating such challenges for better control; nevertheless, the impact of these control measures depends on several factors, among them the demographic dynamics, batch composition and the vaccination strategy choice. In this work, we present results from models developed to evaluate immunity rates achievable in a farrow-to-finish pig herd by comparing different vaccination strategies.

### **Material and Methods**

We developed a stochastic model to simulate post-vaccination coverage rates by age group in each stage in farrow-tofinish pig herds. Two years of herd activity were simulated and immunity rates determined along the duration. Two different vaccination schemes were compared: a "batch-to-batch" (BB) and "mass" (M) vaccination approaches. In mass vaccination, animals receive a single vaccine dose every 11 weeks. In batch-to-batch vaccination, animals receive doses at specific stages in each batch. In both scenarios the same number of doses were distributed. The vaccination was administrated at different stages, targeting both sows and rearing pigs. This contrasts with the conventional practice, which focuses on sows.

### Results

In seven batches systems, the median coverage rate in mass vaccination was 59%, with a 90% interval (48.53%, 86.36%) while, in batch-to-batch vaccination, the median rate was 56% with a 90% central interval (53.84%, 58.79%). In mass vaccination, the coverage rate peaked at over 80%, and the model was employed in various batch management production system. Mass vaccination is advantageous for pig herds managing with over five batches, while a batch-to-batch strategy is preferable for farms with fewer batches to ensure adequate coverage, especially in nursery. When maternal immunity is taken into account, batch-to-batch vaccination allowed a stable rate of maternal immunity, by strategically scheduling vaccinations just before farrowing, as compared to mass vaccination.

### **Discussion and Conclusion**

Our model allowed to compare two types of vaccination strategies at farm level and to provide inputs for farmers in the strategy to implement. This model will undergo extension to incorporate to different types of pig production, facilitating a comparative analysis of strategies involving distinct vaccine types and their implications at the production network level.

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# PREVALENCE AND SEVERITY OF ENZOOTIC PNEUMONIA AND PLEUROPNEUMONIA IN CZECH PIG FARMS BASED ON LUNG LESION SCORING IN 2023

<u>R. Krejci</u><sup>1</sup>, A. Dauvier <sup>2</sup>, J. Vanhara <sup>3</sup> <sup>1</sup>Ceva Animal Health, France <sup>2</sup>Ceva Sante Animale, France <sup>3</sup>Ceva Sante Animale Slovakia

### **Background and Objectives**

Monitoring of respiratory disease by lung scoring is beneficial to assess the farm health status. Clear correlation between lung lesions, the economic impact of the disease and the efficiency of vaccination has been reported. Ceva Lung Program (CLP) was confirmed as a valuable tool to establish the prevalence and severity of Enzootic Pneumonia (EP) and pleuropneumonia. The aim of this study is to evaluate the level of EP and A.p- like lesions on Czech pig farms in 2023 compared to the previous period.

### **Material and Methods**

The survey was conducted on conventional pig farms excluding those with the M.hyo and A.p. SPF status. A total of 4728 (3841 in previous year) lungs in 66 batches of slaughtered pigs were scored using the CLP method. Farms of origin were located evenly across the country, however they were not always the same as in 2022. Lungs from one farm were scored on average twice a year in 6 months interval approximately. EP-like lesions were recorded and scored. Dorso-caudal pleurisy (DP) suggestive for previous pleuropneumonia was scored to describe A.p-like lesions. Data were compared to the previous year.

### Results

The prevalence of 30% of BP was found, compared to 20% previously. The area of affected surface of lung parenchyma in pneumonic lungs reached 4.6% vs 2.5% in 2022. As for pleuropneumonia – 9,7% (vs 5.2% previously) of lungs were affected by dorso-caudal pleurisy lesions with the APPI index 0.2 compared to 0.12 in 2022. All values are expressed as median.

### **Discussion and Conclusion**

EP-like lesions both in terms of prevalence and severity have higher scores in lungs from Czech farms than in 2022. The values remain within the range of European averages. Lesions characteristic for A.p infections were also more prevalent with higher severity. Of course the farm factor plays an important role in those differences, neverthless this trend means a warning for the veterinarians and farm managers to improve the control measures for both diseases.

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# IMPACT OF EP- AND A.P.-LIKE LUNG LESIONS ON GROWTH OF FATTENING PIGS

<u>S. Van Colen</u><sup>1</sup>, P. De Backer<sup>1</sup>, G. De Reus<sup>2</sup>, O. Ninet<sup>3</sup>, H. Cotta<sup>3</sup> <sup>1</sup>Ceva Santé Animale, Brussels, Belgium <sup>2</sup>Ceva Santé Animale, Naaldwijk, the Netherlands <sup>3</sup>Ceva Sante Animale, Libourne, France

# **Background and Objectives**

Infections with Mycoplasma hyopneumoniae (M.hyo) and Actinobacillus pleuropneumoniae (A.p.) cause significant losses due to decreased performance. The aim of this study was to quantify the impact of bronchopneumonia (BP), suggestive for enzootic pneumonia (EP) caused by M.hyo, and dorsocaudal pleurisy (DCP), suggestive for A.p.-infections, on the growth of fattening pigs.

# **Material and Methods**

54.316 pigs, marked with an RFID ear tag, were slaughtered in a Dutch slaughterhouse between February 2022 and June 2023. At slaughter, the date of birth was read from the ear tag and linked to the slaughter date and carcass weight. The age at slaughter and carcass growth were calculated. These data were linked to the lung lesions, which were assessed using the Ceva Lung Program (CLP) scoring methodology. Each lung was assessed for the presence of BP and DCP and the extent of the lesions was evaluated.

### Results

Pigs without lesions (n=41.943) had a carcass weight of 98,29kg, an age at slaughter of 181,06 days and a carcass growth of 546 g/day. Pigs with BP (n=5.069), DCP (n=6.892) and BP+DCP (n=412) lesions had a carcass weight of 97,76 - 98,49 and 98,51 kg and were slaughtered at an age of 184,86 - 186,34 and 187,34 days respectively. This corresponds to a statistically significant lower carcass growth of 532 - 532 and 529 g/day respectively, compared to the pigs without lesions (p<0,05).

### **Discussion and Conclusion**

Both EP- and A.p.-like lesions have a significant impact on the pig's growth. A 14 g/day lower carcass growth was observed for both pigs with BP and DCP over the whole life of the pig. This corresponds to a loss of more than 2,5 kg carcass weight per pig in case pigs are slaughtered at 180 days of age or an increase of the time to slaughter by 4 to 5 days to obtain the same slaughter weight as healthy pigs. Taking preventive measures to minimize the effect of M.hyo and A.p. infections, will not only help to reduce the lung lesions, but will also improve the economic parameters of the farm.

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# IMPACT OF SOW PARITY AND BODY CONDITION MEASURED BY DIFFERENT TECHNIQUES ON COLOSTRUM AND MILK COMPOSITION

<u>L. Garza</u><sup>1</sup>, E. Udvarhelyi<sup>2</sup>, M.V. Falceto<sup>2</sup>, M.T. Tejedor<sup>3</sup>, A.M. Garrido<sup>2</sup>, O. Mitjana<sup>2</sup> <sup>1</sup>Department of Animal Pathology, University of Zaragoza, Spain; Ceva Salud Animal <sup>2</sup>Department of Animal Pathology, University of Zaragoza, Spain <sup>3</sup>Department of Anatomy, Embryology and Animal Genetics, Genetics Area, Faculty of Veterinary Medicine, University of Zaragoza

### Background and Objectives

An adequate quality and quantity of colostrum and milk intake are critical for piglet survivability. Previous studies determined sow parity can influence colostrum and milk composition. However, the impact of sow body condition (BC) on colostrum and milk composition is still limited. Different techniques can be used to evaluate BC in farms, such as Caliper, measurement of backfat (BFU) or loin muscle (LMU) thickness by ultrasounds, among others. Thus, the objective of this trial was to evaluate the effect of sow's parity and BC using different methods on colostrum and milk composition.

# Material and Methods

Forty-six sows with different parities were divided into three categories: Gilts (n=18),  $2^{nd}$ -4<sup>th</sup> (n=16) and  $\ge 5^{th}$  (n=12) parity. Sow's BC was measured by Caliper, BFU and LMU a week pre-farrowing and at weaning; and losses were calculated. Colostrum and milk samples of each sow were collected at farrowing and weaning, respectively. Parameters as percentage (%) of fat, protein, lactose and urea concentration (mg/L) were evaluated in colostrum and milk. Data normal distribution was evaluated using Shapiro-Wilk test. Comparison of colostrum and milk composition between sow parity's were performed using ANOVA test. Correlation between techniques for BC evaluation was done by Pearson coefficient (r). Pvalue was set <0.05.

#### Results

Gilts showed BC losses using Caliper and BFU compared to older sows that increased BC during lactation (p<0.05). The highest correlation between methods was Caliper-BFU (r=0.822), followed by Caliper-LMU (r=0.515) and BFU-LMU (r=0.495). Colostrum from gilts showed higher fat% than older sows (p<0.05). Urea concentration in milk from gilts was also significantly higher than older sows. Gilts showed lower lactose% in milk than other parities (p<0.05). A significant negative correlation (r=-0.352;p=0.021) in lactose% was found between colostrum and milk.

### **Discussion and Conclusion**

Parity had a significant effect on colostrum and milk composition, but no effect was detected regarding BC. Interestingly, BC losses observed in gilts could suggest fat mobilization, resulting in the highest fat% found in colostrum and milk in younger sows. Further research is needed to evaluate BC impact. Finally, correlation between methods for BC evaluation indicated that Caliper could be a practical alternative for sow's BC monitorization.

#### HHM – Herd Health Management

# INVESTIGATIONS ON ENVIRONMENTAL INFECTION PRESSURE OF ROTAVIRUS A IN FARROWING UNITS

A. Lebret <sup>3</sup>, T. Nicolazo <sup>3</sup>, C. Chevance <sup>3</sup>, <u>P. Berton</u><sup>1</sup>, E. Bousquet <sup>2</sup>, J. Jeusselin <sup>3</sup>, C. Teixeira-Costa <sup>3</sup>, V. Normand <sup>3</sup>, G. Boulbria <sup>3</sup>

<sup>1</sup>Virbac France, Espace Azur Mercantour, 3ème Rue, 06510 Carros, France <sup>2</sup>Virbac SA,13ème rue, 06510 Carros, France <sup>3</sup>Rezoolution Pig Consulting Services, Noyal-Pontivy, France

### **Background and Objectives**

Rotavirus (RV) is one of the main pathogenic agent involved in neonatal diarrhoea. This virus is highly infectious, massively excreted in feces, and quite persistent in environment and fomites. The objective of this study was to estimate RV presence in the farrowing environment in an endemic infected farm.

# **Material and Methods**

The study took place in a 600-sow-farrow-to-finish farm, weekly managed, with a good biosecurity level: respect of pig and human flows, all-in all-out management in farrowing units and strict cleaning and disinfection procedures. In this farm, RVA has been regularly evidenced by PCR since 2020 in 2 to 7 day-old piglets suffering from diarrhoea in association with evocative microscopic lesions. Starting February 2023, a RVA vaccine has been implemented in sows. Vaccination helped reducing drastically diarrhoea and antimicrobial treatments in piglets (more than 30% of affected litters before vaccination (period 1=P1) and less than 5% after vaccination (P2). Environmental samples (spots in farrowing rooms, clothes, hands, material) were collected using PBS-moistered wipes during 1st week of age of piglets, in the 2 periods P1 and P2 (on the 8th vaccinated batch). 6 samples were collected on P1 and 10 samples were collected on P2. All samples were analyzed individually for RVA genome by real-time RT-PCR.

#### Results

RVA genetic material has been found in building surfaces, material, and workers' hands and clothes, at both P1 (5/6 samples) and P2 (6/10 samples). Disinfected material remained negative before and after implementation of vaccination. Clean overalls and non-disinfected syringes were PCR positive at P1 and negative at P2. Ct ranged between 32 and 38.5.

### **Discussion and Conclusion**

RVA RNA could be evidenced in environment and fomites at both periods although in a lesser extent after vaccination. Persistence of the virus is facilitated in a context of high prevalence of RVA associated diarrhoea given the fact that RVs are highly excreted by feces. Even if further studies are needed to elucidate RVA infectiousness in contaminated surfaces, this study highlights the necessity of reinforcement of biosecurity measures to limit RVA circulation. RVA vaccination, by reducing clinical disease and consequently manipulations of piglets for treatments, could help reducing environmental contamination.

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# DOES A EUROPEAN SOW GUT MICROBIOTA EXIST? HARNESSING MICROBIOTAS FOR BETTER MANAGEMENT

<u>G. Vestergaard</u><sup>1</sup>, K. Filek<sup>2</sup>, S. Karlsen<sup>2</sup>, L.H.B. Hansen<sup>1</sup> <sup>1</sup>Chr. Hansen A/S, Animal and Plant Health & Nutrition, Bøge Alle 10–12, 2970 Hørsholm, Denmark. <sup>2</sup>Chr. Hansen A/S, Research and Development, Bioinformatics & Modeling, Bøge Allé 10-12, 2970 Hørsholm, Denmark

### **Background and Objectives**

The sow gut microbiota is crucial for performance and offers potential as a predictive management tool. Studies reveal various factors influencing microbial composition, including geographical location, suggesting each country may have its unique ideal microbiota. Furthermore, studies have shown changes in the sow gut microbiota during the perinatal period introducing an additional but controllable factor. Analyzing the composition at species level of sow gut microbiota from European farms at day 109 of gestation (d109) and day 21 of lactation (d21), we set out to identify if a European sow gut microbiota exists and to identify potential links between microbiota, individual bacterial species, and geographical location.

# **Material and Methods**

416 fecal samples from four Danish, four Spanish, four Dutch, two French and three UK sow herds (13 sows/herd) were analyzed using Oxford Nanopore sequencing of the V1-V8 region of the 16s rRNA. 10 out of 17 herds were administrating an in-feed probiotic to the sows. Sequencing data was processed and statistically analyzed combining sequencing data with data such as geographical location and sampling time.

### Results

4202 unique rRNA sequences were identified, many in which were present in all analyzed countries including known members of the sow gut microbiota such as Lactobacillus amylovorus and Streptococcus suis. Sampling time and country were both linked to the microbial composition (P=0.001 for both). However, time showed a higher effect-size than country (pseudo-f:46; pseudo-f:21, respectively). Interestingly, we observed lower alpha-diversity (P<0.001) and increased beta-diversity uniformity (P<0.001) at d21 compared with d109. Focusing on d21 samples, we found that parity was associated with the microbiome (P=0.002; pseudo-f:3) and probiotic use even more so (P=0.001; pseudo-f:7).

# **Discussion and Conclusion**

We show that timing of sampling matters more than country, indicating that there's a distinct European sow gut microbiota. Our ability to identify meaningful associations across samples at d21 such as parity and probiotic use underscores the practical application of viewing the European sow gut microbiota as a cohesive entity. We hope to show that the identification of a European sow gut microbiota could be linked to sow and litter productivity, and thus enable streamlined management practices across Europe.

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# RISKS FACTORS OF DIARRHEA ON PIGLETS IN FARROWING UNIT: A PRACTICAL TOOL FOR THEIR EVALUATION ON FARMS

<u>S. Brilland</u><sup>1</sup>, A. Jardin<sup>1</sup>, E. Lewandowski<sup>2</sup>, P. Leneveu<sup>1</sup> <sup>1</sup>Ceva Santé Animale SA, 10 Avenue de la ballastière, CS 30126 – 33501 Libourne Cedex, France <sup>2</sup>Ceva Biovac, 4 rue O de Serres, 49 070 Beaucouzé, France

### **Background and Objectives**

Neonatal diarrhea (ND) is one of the most common diseases described on pig farms today. It is responsible for major economic losses. The involvement of bacterial, viral or even parasitic infectious agents is frequently confirmed. However, non-infectious factors also play a role by helping the pathogens attachment or by weakenig the piglets for example. Therefore, preventing diarrhea is based on controlling risk factors by implementing therapeutic measures in addition to zootechnical, hygienic and dietary measures. Based on scientific publications and field experiences, Ceva technical team created a neonatal diarrhea risk factors audit. This publication presents the results of several audits carried out in 2023 in France.

### Material and Methods

The audit visits were carried out during the week of farrowing, the day after the supposed peak of farrowing. A checklist was filled up with the farm staff and was divided in 5 sections dedicated to management, feeding and watering, medical prophylaxis, comfort in farrowing unit (CIFU) and colostrum intake (CI). Concrete measurements of the nests comfort were registered with an infra-red thermometer. The CI quality was assessed by the measurement of piglets' body temperature at 24 hours of life with a rectal thermometer (Leneveu et al. 2022). A total of 47 criteria were evaluated; weighting is carried out to generate a final score out of 100 called the "overall prevention level".

### Results

The audit was carried out on 17 farms showing a chronic problem of diarrhea in maternity. The average score was 61/100 (min:34; max:75). The two sections with the lower level of prevention were "comfort" and "colostrum intake" (average score: 44/100 and 56/100 respectively). Concerning these sections, no farm for CIFU and only one farm reached a level of prevention above 2/3 of the maximum score for CI. It's reflecting a lack of comfort offered to piglets at birth and in the hours/days following birth and a lack of colostrum intake.

# **Discussion and Conclusion**

This study highlights that in farms facing recurrent diarrhea, the environmental comfort and the colostrum intake are often suboptimal. This audit enables to concretely identify the factors for a better digestive health of piglets.

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# SEASONAL VITAMIN D CONCENTRATION IN THE SERA OF KRSKOPOLJE PIGS IN ORGANIC FARMS IN SLOVENIA

<u>T. Steferl</u><sup>1</sup>, J. Plut<sup>1</sup>, M. Hajdinjak<sup>2</sup>, I. Golinar Oven<sup>1</sup>, E. Nadlucnik<sup>1</sup>, P. Njegovec<sup>3</sup>, M. Stukelj<sup>1</sup> <sup>1</sup>*Clinic for Reproduction and Large Animals, Clinic for Ruminants and Pigs, Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia* <sup>2</sup>*Laboratory of Applied Mathematics and Statistics, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia* <sup>3</sup>VOA d.o.o, Domzale, Slovenia.

# **Background and Objectives**

Vitamin D is an important micronutrient in pig production. However, as most pigs are reared indoors, there is little data on vitamin D in pigs raised in outdoor organic farms. The aim of our study was to determine serum concentrations of vitamin D in indigenous Krškopolje pigs in organic mixed outdoor farms in all seasons.

### **Material and Methods**

Grower pigs were divided into three groups: Low altitude outdoor pigs (n=19), high altitude outdoor pigs (n=19) and indoor pigs (n=19). Individual blood samples were taken and analysed using ELFA technique (Enzyme Linked Fluorescent Assay) once per season. Measured vitamin D levels of individual pigs were compared and statistically analysed using analysis of variance (ANOVA) and Tukey's HSD test or Welch's t-test, depending on the results of the Bartlett's test for homoscedasticity, to test for differences between the different groups of pigs and seasons.

### Results

Average serum concentrations of vitamin D in both groups of outdoor pigs peaked in summer (73,8 and 86,5 ng/ml) and then decreased in the following months, while serum concentrations of vitamin D in indoor pigs were lower during the same period (22,9 ng/ml). We observed that serum concentrations of vitamin D in the group of indoor pigs were highest in autumn (40,5 ng/ml). Statistical significance was found between different seasons as well as between different groups of pigs.

### **Discussion and Conclusion**

In our study, we found that vitamin D concentration in pig serum is highest in summer, when days are longest. There is lack of studies on serum vitamin D concentration in pigs, especially in organic farms. Our results showed that serum concentrations of vitamin D varied within the herd, but the average serum concentrations in both outdoor groups were higher in summer than reported in the study in outdoor herds in Denmark. Data from other study indicate that 30 ng/ml vitamin D in the blood is considered the minimum standard, but 50 to 80 ng/ml is required for optimal development. Minimum and optimum values for vitamin D serum concentrations are still under discussion.

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# SEROPREVALENCE OF LAWSONIA INTRACELLULARIS IN PREWEANED PIGLETS IN SPAIN

V. Rodríguez-Vega <sup>1</sup>, G. Abella <sup>1</sup>, S. Figueras <sup>1</sup>, R. Garcia <sup>1</sup>, F. Gonzalvo <sup>1</sup> <sup>1</sup>Boehringer Ingelheim Animal Health España, S.A.U.

### **Background and Objectives**

Porcine Proliferative Enteropathy (PPE) caused by Lawsonia intracellularis (Li), is one of the most prevalent enteric diseases worldwide (1). The vertical transmission of Li from sows to piglets during the lactation period has been proven (2) but the presence of maternal delivered antibodies (MDA) in the serum of piglets has not been assessed yet. Presence of MDA might interfere with successful immunization after parental vaccination by the IM route. A commercially available blocking ELISA for the detection of antibodies against L. intracellularis provided a tool to investigate the prevalence of antibodies. The objective of this study was to determine the prevalence of Li MDA in piglets in Spanish farms at the age of weaning.

# Material and Methods

The study was performed during 2023 in different regions of Spain. The participating veterinarians were asked to select farms supposed to have or not, PPE and showing or not, diarrhea signs. In total 15 farms were analyzed. Thirty due-to-wean piglets per farm were bled by jugular venipuncture at the age of weaning A total of 30 pigs per farm were sampled to detect a minimum prevalence of 10 % with 95% certainty. The samples were analyzed by using a blocking ELISA based on whole cell L. intracellularis antigen (SVANOVIR®) for the detection of IgG antibodies to L. intracellularis. A farm was considered positive when a single animal tested positive.

### Results

The percentage of positive farms was 73% while the percentage of positive piglets was 21% in total. The percentage of positive piglets per farm was between 3% and 80%

# **Discussion and Conclusion**

It is important to note that at the age of weaning 70% of farms have piglets with antibodies against Li. With an antibody seroconversion time of 3 weeks after natural infections with Li, these detected antibodies are most likely to maternally derived. The variation between farms was very high under the conditions of this study. The seroprevalence in positive farms was between 3% and 80%. The presence of MDA should be considered when implementing a parental vaccination program, as MDA can interfere with a successful immunization.

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# INFLUENCE OF TEMPERATURE ON SOW MORTALITY IN GESTATION BARNS

D. Yanguas <sup>1</sup>, J.A. Muñoz <sup>1</sup>, J.Q. Cabañes <sup>1</sup> <sup>1</sup>AGROCESA S.A.U.

### **Background and Objectives**

Climate change is already a reality for livestock production. Pigs cannot dissipate heat through the evaporation process, making them more susceptible to high temperatures than other animals. Several authors mark the thermoneutral zone of a sow between 15 and 21°C, so the aim of the study was to evaluate the temperatures that cause the death of sows in the gestation barns.

### Material and Methods

The study was performed in a Spanish 2200 sow farm, breed large white, non-hyperprolific. The death of sows in the gestation barns was recorded from May 2021 to September 2023, totaling 490 deaths. Temperature inside the barns was measured using four sensors at different locations. To evaluate the critical temperature influencing sow mortality, deaths were analyzed in relation to the number of hours that had exceeded 22°C, 23°C, 24°C, 25°C, 26°C and 28°C, as well as the maximum, minimum and average temperature, and the difference between the maximum and minimum temperature (VAR), all within the 1, 3, 7, and 14 days prior to death. Data were analyzed using SPSS software, were performed by U of Mann-Whitney test.

### Results

The correlation between sow mortality and the hours above certain temperatures begins when they exceed 8,57 hours above  $24^{\circ}$ C the day before death (p<0.05), increasing at higher temperatures, 3,1 hours above  $28^{\circ}$ C the day before death (p<0.001), but decreasing as the study periods extend, 38,52 hours above  $28^{\circ}$ C fourteen days before death (p<0.01). Factors like the maximum temperature (26,28°C), the average (24,08°C), and VAR (3,95°C) also influence sow mortality (p<0.005), not the minimum temperature.

### **Discussion and Conclusion**

The temperature to which sows are exposed can lead to their death. It seems that once the temperature exceeds 24°C, heat stress may become a lethal factor, and large temperature fluctuations can also induce irreversible stress. These findings are notably significant from the day prior to death, but the continued exposure to heat stress at high temperatures over extended periods is equally crucial.

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# STABILIZATION OF A SI AND PRRS POSITIVE BREEDING HERD IN THE NETHERLANDS USING HOMOLOGOUS VIRAL IMMUNIZATION

R. Van Gelderen <sup>1</sup>, I. Christiaens <sup>2</sup>, <u>R. Neto <sup>3</sup></u>, M. Williemsen <sup>3</sup> <sup>1</sup>Porc business bv <sup>2</sup>Poulpharm, Belgium <sup>3</sup>Kemin Biologics

### **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) and swine influenza virus (SIV) are economically important pathogens. These pathogens are difficult to control with commercial vaccines, which do not offer protection to new, heterologous challenge strains. The study assesses the efficacy of an autogenous PRRS and SI vaccine for the control of disease in a breeding herd.

# **Material and Methods**

A 4000-sow breeding herd was clinically affected by PRRS and SI (2022-2023). SIV circulation pre- and post-weaning was confirmed in nasal swab samples (30) and for PRRSV serum samples (20) were collected from each group followed by pooled PCR analysis (PoulPharm, Belgium). The circulating PRRSV was isolated from serum samples in porcine alveolar macrophage culture (PoulPharm, Belgium) and the ORF5 locus was sequenced (Pathosense, Ghent University). Circulating SIV was isolated and identified as H1N1 and H1N2 (PoulPharm, Belgium). Eight batches (800 each) of 3-day-old piglets were vaccinated intranasally with 2mL of the autogenous vaccine derived from the circulating isolates (KEMIN®AptiVax™Barricade™PRRS-SI). Mortality and weight gain data were collected at the end of the nursery phase and compared between periods with and without vaccine. Usage of water-soluble doxycycline was tracked for 6 months before and 3 months after the autogenous vaccination period. Results were analyzed with JMP fit model (P<0.05).

### Results

The average weight of autogenous PRRS and SI vaccinated pigs was 24.4kg and numerically greater than the weight of unvaccinated piglets (23.9kg). Use of the antimicrobial doxycycline decreased from 16kg to 12kg after vaccination. Compared to the unvaccinated groups, SIV circulation was significantly reduced in the 4<sup>th</sup> batch of pre-weaning piglets and undetectable from the 5<sup>th</sup> batch of vaccinates. Half of the vaccinated piglets were positive at weaning for PRRSV, likely MLV vaccine strain (one of the positive samples with a CT <24 had 99.39% homology (ORF5) with the MLV vaccine used in sows

### **Discussion and Conclusion**

The autogenous vaccine program provided protection during PRRSV and SIV challenge. Virus circulation and antibiotic usage was reduced after the implementation of the program, and weight gain improved at the end of the nursery period.

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# IMPACT OF PORCINE ENZOOTIC PNEUMONIA AND PLEUROPNEUMONIA ON CARCASS WEIGHT AND MEATINESS: QUANTITATIVE INSIGHTS FROM A MIXED-MODEL ANALYSIS

P. Przyborowska-Zhalniarovich <sup>1</sup>, P. Cybulski <sup>2</sup>, K. Janeczko <sup>4</sup>, R. Panek <sup>3</sup>, P. Iskrzak <sup>5</sup>, R. Lewko-Wojtowicz <sup>5</sup> <sup>1</sup>Department of Veterinary Public Health, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn <sup>2</sup>Goodvalley Agro S.A., Przechlewo, Poland <sup>3</sup>Ceva Animal Health, Poland

<sup>4</sup>Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Poland; Ceva Animal Health, Poland <sup>5</sup>Ceva Animal Health Poland

### **Background and Objectives**

Broncho-pneumonic and pleural lesions, symptomatic of Enzootic Pneumonia and Pleuropneumonia in swine, can affect carcass yield and quality. This study employs a statistical model to determine the effect of lesions, commonly found during abattoir inspection, on carcass weight and meatiness.

# Material and Methods

Data from abattoir inspections of seven pig batches totaling 679 pigs were subjected to a mixed-model analysis, incorporating the farm of origin as a fixed variable. Macroscopic pulmonary lesions were scored using CLP (Ceva Lung Program). CLP is a system based on a five-point Madec scale (0-4 points depending on the surface of the lobe affected), and modified SPES (Slaughterhouse Pleurisy Evaluation System) employing a 0-4 point system allowing evaluation of dorso-caudal pleurisy. The parts of the lungs evaluated included the left apical lobe, left cardiac lobe, left diaphragmatic lobe, intermediate lobe, right diaphragmatic lobe, right cardiac lobe, right apical lobe, as well as areas affected by cranial pleurisy, dorso-caudal pleurisy, and the presence of scars. These assessments facilitated a detailed analysis of the lesions' impact on carcass weight and meatiness.

#### Results

The model identified a significant decrease in carcass weight due to lesions in the right apical lobe and dorso-caudal pleurisy, with losses of 2.77 kg (p=0.01) and 2.29 kg (p<0.001), respectively. Moreover, the right apical lobe lesions were also found to significantly reduce carcass meat weight by 1.76 kg (p=0.02), while dorso-caudal pleurisy affected carcass meat weight with a reduction of 1.43 kg (p<0.001).

### **Discussion and Conclusion**

This study substantiates the negative impact of respiratory lesions on swine carcass weight and meatiness, highlighting a marked reduction associated with ventro-cranial consolidation due to enzootic pneumonia and dorso-caudal pleurisy afflictions. The findings indicate that enhanced disease management protocols are warranted, as the significant decrease in meatiness suggests current practices may be insufficient. Utilizing abattoir data could enable more precise herd health strategies by pinpointing the lung areas most susceptible to lesions.

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# MEDICATION SAFETY RISKS OF FINNISH SOWS - A QUALITATIVE OBSERVATION STUDY

K. Ahlqvist 1, M. Heinonen 1, A. Valros 1, A. Holmström 2

<sup>1</sup>Department of Production Animal Medicine & Research Centre for Animal Welfare, Faculty of Veterinary Medicine, Helsinki, 00790, Finland

<sup>2</sup>Faculty of Pharmacy, Division of Pharmacology and Pharmacotherapy, Faculty of Veterinary Medicine, Helsinki, 00790, Finland

### **Background and Objectives**

On-farm medication of sows is common. Complex medication management and use process of farms may contribute to medication safety risks, referring to safety risks associated with the use of medicines (e.g. using unclean equipment). These are largely unstudied. Our aim was to investigate the risks associated with the medication management and use process of sows during medications. This comprised implementation of medications, storage, equipment use, instructions and recording at farm level.

### Material and Methods

A convenience sample of 14 Finnish sow herds were recruited to an observation study in spring 2023. Herd size ranged from 150-1150 sows. Representatives of sow farms signed an informed consent and completed an electronic prequestionnaire. The lead author visited the herds once and collected the observation data together with the person responsible for sow medications using a semi-structured observation form. All data were analyzed using descriptive statistics.

### Results

The pre-questionnaire respondents were farm owners (86%) and workers (14%) responsible for sow medications. The workers were mainly the ones who started, administered, and monitored the treatments. According to the responses, 36% of the farms had non-Finnish speaking workers.

All farms (n=14) had only veterinary medicines in the refrigerator, the temperature of which was within the reference values on 10 farms (71%). Five farms (36%) monitored the refrigerator temperature daily and five farms (36%) weekly. Five farms (36%) marked the opening date to a medication package. Look-alike and sound-alike (LASA) medications were used in 14% of the farms.

Automatic medication syringes were used in 11 farms (79%) out of which nine (81%) had a functional syringe washing facility. Three farms (27%) said that they regularly disinfect their syringes. Eight farms (57%) changed the needle between sick sows. Written medication instructions were available in the piggery office for workers on eight farms (57%) and complete medication records on 11 farms (79%).

#### **Discussion and Conclusion**

Several medication safety risks were identified on the farms. Based on observations, there is room for improvement in several areas, including employee guidance, medication instructions, storage, equipment hygiene and record keeping. Larger-scale future studies are needed for risk identification and to develop systems-based strategies to proactively manage risks before harm occurs.

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# IMPROVEMENT ON REPRODUCTIVE PERFORMANCE IN AN ENDEMICALLY PRRS INFECTED BREEDING HERD AFTER IMPLEMENTING A VACCINATION PROGRAM WITH REPROCYC PRRS EU®

<u>Z. Tomic</u><sup>1</sup>, V. Miljkovic<sup>1</sup>, A. Veselinovic<sup>2</sup>, G. Lopez-Moreno<sup>3</sup> <sup>1</sup>Boehringer Ingelheim Serbia d.o.o. <sup>2</sup>Pig farm Dinamika, Serbia <sup>3</sup>Boehringer Ingelheim RCV GmbH & Co KG

### Background and Objectives

Porcine reproductive and respiratory syndrome virus (PRRSv) is one of the most important pathogens affecting swine health worldwide. Among the reproductive clinical signs are an increase of abortions, a decrease of farrow rate and of the number of piglets born alive. This study aimed to investigate reproductive performance before and after implementation of a vaccination program against PRRSv.

# **Material and Methods**

A 700 sows, Serbian farm. The farm had a history of increased number of abortions in the last stage of gestation. PCR testing of aborted fetuses confirmed PRRSv presence. Prior to the study, there was no PRRSv vaccination implemented in the farm. The PRRS vaccination program consisted of two mass vaccinations (4 weeks apart) of all sows and gilts with a commercially available PRRSv vaccine, followed by a routine mass vaccination every 3 months. Reproductive parameters were compared over a period of 12 months before and after implementation of the vaccination program. Statistical differences were investigated using Minitab statistical program. A return of investment (ROI) calculation was used with BECAL (Boehringer Ingelheim Economic Calculator).

### Results

Pregnancy rate increased by 3.53% (95% CI 0.72-6.34), farrowing rate increased by 8.08% (95% CI 4.10-12.06) and the number of piglets born alive per sow increased by 1.28 (95% CI 0.62-1.99). All these differences were statistically significant (P value < 0.05). The abortion rate decreased by 1.73% (95%CI 0.05-3.50), however this difference was not statistically significant (P value 0.056). The ratio of the benefits over the investments (ROI) was estimated to be 7.27 USD per each 1 USD.

# **Discussion and Conclusion**

PRRSv virus circulation at a low prevalence is limiting these farms to reach their full reproductive potential. The results obtained from this study show that adopting a vaccination program against PRRSv using ReproCyc PRRS EU® in farms with endemic PRRSv circulation will result in improvement of most reproductive parameters. The financial benefits obtained from the observed improvement clearly outweigh and justify the financial cost of a vaccination program.

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# INFLUENCE OF DIFFERENT VACCINATION STRATEGIES AGAINST PROLIFERATIVE ENTEROPATHY ON GUT MICROBIOME

<u>W. Van Herten</u><sup>1</sup>, H. Prüst<sup>2</sup>, R. Jansen<sup>3</sup> <sup>1</sup>DAC Zuid-Oost, Deurne, Netherlands <sup>2</sup>Boehringer Ingelheim Animal Health Netherlands bv <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany

### **Background and Objectives**

Oral vaccination against Lawsonia intracellularis is known to alter the gut micobiome which might be of influence on the incidence of tailbiting. This study was done to evaluate the actual change in microbiome after oral vaccination and to look for differences in the abundancy of specific bacterial species with an alternative vaccination strategy in a naturally infected swine herd. Infection was demonstrated by histology and PCR (ct 24) on intestinal tissue on a fattening pig after necropsy.

### **Material and Methods**

2 groups of pigs were used in this study, vaccinated simultaneously at entry of the same fattening barn im or orally respectively. In previous batches all pigs were vaccinated intramuscularly. im vaccinated group was vaccinated once, oral vaccinated group was revaccinated 8 weeks later. Faecal samples were collected on 8 (t1) and 12(t2) weeks after entering the barn, transported refridgerated to the vet practice and frozen at -18C before all samples being sent at once to the laboratory for 16s RNA gene sequencing on a GidION device.

#### Results

No significant differences on Alpha (Shannon)- or Beta diversity (Bray-Curtis dissimilarity index) were found at both timepoints between the two groups. At t1; 13 bacterial species were more abundant after im vaccination, whereas 9 species were more abundant after oral vaccination, both compared to the other method. At t2 only 5 species were more abundant in the im group, of which Streptococcus alactolyticus and Str. gallolyticus have been known to be related to a diseased gut, but 16 species in the orally vaccinated group (Dilaster scuccinatiphilus, Prevotella jejuni and Eubacterium pyruvativorans were most abundant, none of which are known to be related to disease or an unhealthy gut).

### **Discussion and Conclusion**

Use of oral vaccination has been shown to be beneficial in specific cases with tail biting (obsessive tailbiting), especially in cases of dysbioses of the gut. In the im vaccinate group Streptococcus alactolyticus is an opportunistic pathogen and Str. gallolyticus is barely found in the healthy gut, but known to be more abundant in humans in cases of (beginning of) cancer. Demonstrated changes in microbiome might give insights in methods to keep pigs with intact tails.

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# MONITOR SWINE RESPIRATORY DISEASE BY ELANCO LUNG LESION SCORING APPLICATION AND IMPLEMENT ANTIMICROBIAL PRUDENT USE IN INTEGRATED FARMS IN THAILAND.

W. Dee-Ampai<sup>1</sup>, P. Sandamuen<sup>2</sup>, W. Singthana<sup>1</sup>, A. Jiranaparat<sup>1</sup>, A. Ratanamongkolmas<sup>1</sup>, S. Sitthinan<sup>2</sup>, T. Panichpakdee<sup>2</sup>

<sup>1</sup>Elanco (Thailand) Ltd., Bangkok, Thailand. <sup>2</sup>Swine farm veterinarian, Thailand

### **Background and Objectives**

One of the pathogens that cause respiratory diseases in swine is Mycoplasma hyopneumoniae which can cause an economic impact and is difficult to eradicate from farms.Lung lesion scoring (LLS) is an evaluation method to quantify the amount of affected lung surface due to respiratory disease that may be caused by Mycoplasma hyopneumoniae (MHP) or Mycoplasma hyorhinis (MHR). which impacts farm production, and the producer can utilize this information to correctly make health and management decisions on the farm. This study objective is to use Elanco LLS application to monitor pneumonia and pleuritis and update antimicrobials susceptible as a guide for prudent use on the farm.

# Material and Methods

This study was conducted from April 2023 to May 2023, A total of 494 lungs from integrated pig farms in Thailand were evaluated for pneumonia and pleuritis with Straw's grid (score 0-10 : normal), score 11-40 : pneumonia and score >40 : severe pneumonia) and SPES method (classified lesion P0 : Absence of pleuritis lesions, P1: Ventro-cranial lesion: pleural adherence between lobes or at ventral border of lobes, P2: Dorso-caudal monoliteral focal lesion, P3: Bilateral type 2 lesion or extended monolateral lesion and P4: Severely extended bilateral lesion) by using Elanco LLS application.90 consolidated lung samples were collected to isolate and identify Mycoplasma spp. and MIC was conducted by the National Institute of Animal Health.

### Results

Of the 494 lungs evaluated, 83% were classified as normal (score 0-10), 15% were classified as pneumonia (Score 11-40) and 2% were classified as severe pneumonia (score >40). For pleuritis evaluation of the lungs, 77% were classified as P0, and 23% were classified as P1 to P4. Mycoplasma isolate and identified result was 9 MHP and 44 MHR isolation of 90 lung samplesFor the MIC result antimicrobial drugs susceptible to both MHR and MHP are Valnemulin, Tiamulin, Flofenicol, Enrofloxacin, Doxycycline, and Lincospectin.

### **Discussion and Conclusion**

Elanco LLS application can be used to monitor pneumonia and pleuritis on the farm and MIC against antimicrobial drugs guide to treat MHP and MHR properly on the farm.

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# LUNG SCORING SURVEY IN EUROPEAN COUNTRIES IN 2023

<u>R. Krejci</u>, A. Dauvier <sup>1</sup>, P. Mazerolles <sup>2</sup> <sup>1</sup>Ceva Sante Animale, France <sup>2</sup>Ceva Sante Animale, Libourne, France

### **Background and Objectives**

Scoring of lung lesions in the slaughter pigs provides an important information about the respiratory health in the pig population. Lesions suggestive for previous M.hyo or A.p. infections and their scoring were described before. Scoring of those lesions allows quantifying problems with enzootic pneumonia and pleuropneumonia. The aim of this survey was to collect the results of lung scoring performed in most of swine producing European countries in 2023.

### Material and Methods

Ceva Lung Program scoring methodology is implemented to score the lung lesions at many slaughterhouses and results are anonymously sent to central database with only a farm code and country denomination with no pre-selection of farms. The results were collected from all reports sent to CLP database from EU countries in the 12 months period from November 2022 till the end of October 2023. The median values were calculated for % of lungs with bronchopneumonia (%BP), % of affected lung parenchyma out of sick lungs (% parenchyma), % of dorso-caudal pleurisy (%DP) and APP index (APPI).

### Results

The total number of scored lungs was 593123 (vs 327588 in 2022) from 6192 reports (vs 3428 in 2022) from 2888 farms (one farm audited 2.1 times per year on average), which is 96 lungs per audit. There are differences among countries in number of farms, however most of audits (85%) come from Spain, France, Germany and Netherlands, countries slaughtering >55% of pigs in EU. The median value of BP was 26% compared to 30% in 2022. The median of affected parenchyma was 1,4 % compared to 2.7% in 2022. For DP the median was 3,5% vs 4.1% previous year and APPI index 0.09 vs 0.12 in 2022.

### **Discussion and Conclusion**

The extensive data set from EU countries in 2023 shows that there is a decrease in all parameters related to enzootic pneumonia and pleuropneumonia measured in slaughter pigs in comparison with the previous year 2022.

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# SAFETY MONITORING OF SOWS VACCINATED WITH A ROTAVIRUS VACCINE DURING GESTATION

M. Couteau <sup>1</sup>, P. Duval <sup>1</sup>, <u>P. Berton</u> <sup>2</sup>, S. Heliez <sup>1</sup> <sup>1</sup>Chêne Vert – Conseil Vétérinaire, 2 rue Pierre Harel, 35133 Lécousse, France <sup>2</sup>Virbac France, Espace Azur Mercantour, 3ème Rue, 06510 Carros, France

### **Background and Objectives**

Rotavirus (RV) is a pathogenic agent involved in neonatal diarrhoea. Since December 2022 in France, a commercial vaccine SUIGEN®ROTA COLI is available for prevention of RV clinical symptoms. Its adjuvant is an oil-in-water emulsion. As oil emulsions are susceptible to induce more adverse effects than aqueous adjuvants, this study aims to evaluate vaccine safety in 2 French commercial farms.

### **Material and Methods**

The tested vaccine was implemented in 2 farms where RVA circulation had been evidenced: 25 sows in farm 1, 22 gilts in farm 2, were vaccinated twice 3-4 weeks apart (V1 and V2). V1 was implemented by both veterinary practitioner and farmer. V2 was implemented by the farmer alone. At both V1 and V2, animals were monitored on the vaccination day (D0), 24h post-vaccination (D1), and one week after vaccination (D7). Following parameters were monitored: redness, swelling, pain, systemic signs. Rectal temperature was measured during V1D0, V1D1, V2D0 and V2D1 monitoring.

### Results

No local or systemic signs were noticed on V1D0 or V2D0 in farm 1 and 2. On V1D1, redness occurred in 20% of animals in farm 1; swelling: in 20 and 18% of animals in farm 1 and 2 respectively; pain was noticed in 16% of vaccinated animals in farm 1. At V1-D7, swelling was still noticeable in 4 and 9% of animals in farm 1 and 2 respectively, and pain in 4% of animals in farm 1.At V2D1, redness occurred in 40 and 5% of animals in farm 1 and 2 respectively; swelling: in 72 and 48% of animals respectively; pain was noticed in 16 and 5% of animals in farm 1 and 2 respectively. At V2D7, swelling was still noticeable in 16 and 5% of animals in farm 1 and 2 respectively. At V2D7, swelling was still noticeable in 16 and 5% of animals in farm 1 and 2 respectively. At V2D7, swelling was still noticeable in 14% of animals in farm 2.No rectal temperature raised above 39.3°C 24h after V1 or V2.

### **Discussion and Conclusion**

The vaccine induced frequently local reactions, more intense after V2. This is consistent with adverse effects mentioned in the SPC. Yet most of them had disappeared 7 days after vaccination. No rise of rectal temperature was noticed 24h after vaccination, suggesting that if present, this rise is transient. No systemic adverse effect was noticed.

# NUTRITION

# NUTR-PP-02

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# POTENTIAL ROLE OF MYCOTOXINS AND MYCOTOXIN DEACTIVATOR IN GASTRIC ULCER FORMATION

P. Cybulski<sup>1</sup>, M. Larska<sup>2</sup>, A. Wozniak<sup>3</sup>, A. Jablonski<sup>3</sup>, T. Stadejek<sup>4</sup>

<sup>1</sup>Goodvalley Agro S.A., Przechlewo, Poland

<sup>2</sup>Department of Virology, National Veterinary Research Institute, Pulawy, Poland

<sup>3</sup>Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences—SGGW, Warsaw, Poland

<sup>4</sup>Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Warsaw, Poland

### **Background and Objectives**

Ulcerative alterations are common findings in stomachs of slaughtered pigs. Although the disease was experimentally induced using different determinants, the importance of various on-farm triggering factors remains poorly understood. The aim of our study was to evaluate the influence of deoxynivalenol (DON), ochratoxin A (OTA), and supplementation of a mycotoxin deactivator on the development of the disease in finishing pigs.

### Material and Methods

The study was performed in 16 modern farms located in Poland. In order to assess the level of DON and OTA, every batch of feed (20 t) was analysed using Veratox ELISA tests (Neogen Corp., USA). The detection limit for DON and OTA was 0.1 ppb and 1 ppb, respectively. In selected farms the animals were supplied with Toxi-Tect A (LIKRA Tierernährung GmbH, Austria). Total number of 32264 animals was slaughtered in 360 batches between January 2013 and February 2017. Spearman rank correlation was used to identify relationships between the prevalence of gastric ulcers and the level of DON (0-0.74 mg/kg). Kruskal–Wallis equality-of-populations rank test was applied to nominal variables: detection of OTA (on the detection limit) and supplementation of mycotoxin deactivator (0-1 kg/t).

### Results

From 32264 stomachs examined, 54.9% (17703) had fully developed gastric ulcers. The range between 360 batches was evident (14-89.8%). A significant (p<0.05) association of the ulcer prevalence was found for supplementation of the mycotoxin deactivator (p=0.0008).

### **Discussion and Conclusion**

Even though the low concentration of DON and OTA had no impact on ulcer formation, the supplementation of the mycotoxin deactivator significantly correlated with the protection against the disease. The observation can indirectly indicate the negative impact of feed contamination with mycotoxins other than those analysed in our study. Despite the fact that many fungal metabolites have been described so far, their impact on pig health, including the influence on stomach integrity, requires further investigation.

NUTR - Nutrition

# AUTOLYZED BREWERS' YEAST IN SOW FEED RESULTS IN POSITIVE EFFECTS ON PERFORMANCE PARAMETERS OF HIGHLY PRODUCTIVE SOWS AND PIGLETS

P. Bisesti <sup>1</sup>, A. Müller <sup>2</sup>, <u>J. Schulze-Osthoff</u> <sup>3</sup> <sup>1</sup>Leiber GmbH, Bramsche, Germany <sup>2</sup>FutterAllianz Nordwest GmbH & Co. KG, Schneiderkrug, Germany <sup>3</sup>Leiber GmbH

### **Background and Objectives**

Success in commercial pig production systems depends on number of piglets reared. Health and fertility of sows and a continuous increase in body weight of piglets are the most important influence factors. A feeding trial was conducted to investigate the effect of autolyzed brewers' yeast on health and performance parameters of highly productive Danish sows.

# **Material and Methods**

The feeding experiment was conducted at a trial farm with 3000 sows in North-West Germany. 54 DANIC<sup>®</sup> sows were divided into two homogeneous groups according to parity. After birth, litters were equalized so that on average 17 piglets (DANIC<sup>®</sup> x PIC<sup>®</sup> 408) remained with each sow. Piglets that had fallen off severely were removed and placed collectively on foster mothers. Eight weeks before farrowing and during lactation period both groups received granulated feed either with autolyzed Saccharomyces cerevisiae yeast Leiber CeFi<sup>®pro</sup> (treatment) or without (control). Diets of both groups were isonitrogenous and isoenergetic. In the trial group soybean meal was partly replaced by 0.4% autolyzed brewers' yeast. In addition to sow milk all piglets received same prestarter diets from day 5. Health and performance parameters were recorded on individual animal basis during lactation period. A single-factor analysis of variance was used to calculate significant differences between the treatment groups. The statistical analyses were carried out using the SPSS software program (IBM SPSS, version 28). Differences were considered as significant at p<0.05.

### Results

Yeast autolysate treated sows consumed more lactation feed (p<0.05) and tended to have reduced weight loss compared to control group. 15.4 piglets were weaned per sow in trial group vs. 14.7 in control (p<0.05). Weight gain and growth rate of trial piglets were significantly increased (p<0.001). Corrected mean value of weaning weight showed an advantage for trial piglets of 532g.

# **Discussion and Conclusion**

This study shows that autolyzed brewers' yeast positively influences welfare and performance of highly productive sows and piglets. This may be a result of stimulated feed intake and relieved metabolism. Yeast autolysate contains bioactive compounds and is known for improving palatability and having prebiotic effects. Increased growth of piglets could be a consequence of better colostrum quality. Further research is needed.

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# THE EFFECTS OF DIETARY NET ENERGY LEVELS ON GROW-FINISH PERFORMANCE AND CARCASS CHARACTERISTICS OF MARKET GILTS IMMUNIZED AGAINST GNRF

L. Van De Weyer <sup>1</sup>, Y. Wang <sup>2</sup>, B. Hansen <sup>3</sup>, S. Pollmann <sup>4</sup>, J. Landero <sup>5</sup>, M. Young <sup>5</sup>, B. Bohrer <sup>6</sup>, A. Aldaz <sup>7</sup> <sup>1</sup>Zoetis Canada Inc, Kirkland, QC <sup>2</sup>Dept of Anim. Sci., The Ohio State University, Columbus, Ohio <sup>3</sup>BCH Consulting LLC, Atlantic, Iowa <sup>4</sup>DSP Consulting LLC, Alpine, Utah <sup>5</sup>Gowans Feed Consulting, Wainwright, AB <sup>6</sup>Dept of Animal Sci, The Ohio State University, Columbus, Ohio <sup>7</sup>Zoetis Inc, Parsippany, NJ, USA

# Background and Objectives

Study objectives were to determine the effects of three different dietary net energy (NE) levels (LOW–2.216, MID–2.341, HIGH–2.466 Mcal/kg) fed during the grow-finisher on live performance and carcass characteristics of market gilts managed with two doses of Improvest (IMP, also known as Improvac), an injectable gonadotropin-releasing-factor-analogue, or non-treated gilts (CON).

### Material and Methods

A total of 1,008 gilts (avg. start-wt 30.8 kg) were allocated by weight to 48 pens (21 pigs/pen) with treatments arranged as a  $2 \times 3$  factorial design with main effects of Improvest-treatment (**IMP** or **CON**) and NE-level (**LOW**, **MID**, or **HIGH**). An equal number of pigs were marketed from each of six treatment groups on study day-83 (21.9%), day-90 (21.9%), day-97 (21.9%), and day-104 (34.3%). Data were analyzed with PROC MIXED of SAS, with pen as experimental unit, fixed effects of IMP-treatment, dietary NE-level and their interactions, and a random-effect of pen location.

### Results

**IMP** gilts consumed more feed (6.8% greater ADFI; P < 0.01), grew faster (5.0% greater ADG; P < 0.01), were less efficient (1.5% greater F:G; P < 0.01), were heavier (3.5 kg HCW; P < 0.01), and were fatter (1.9 mm greater backfat-thickness, 0.9% less predicted-lean-yield; P < 0.01) than **CON** gilts. No difference in dressing-percentage between IMP and CON gilts was detected. There were significant effects of NE-level for ADFI, ADG, F:G, HCW, and dressing percentage. Gilts fed **LOW** NE-diets had the greatest ADFI, slightly lower ADG, the least efficient F:G ratio, the lightest HCW, and lower dressing percentage compared with **HIGH** (P < 0.01). There were no differences for backfat-thickness or predicted-lean-yield between NE-treatments. There were no interactions ( $P \ge 0.20$ ) for any performance or carcass variable.

# **Discussion and Conclusion**

**IMP** gilts achieved expected performance and carcass response levels at each NE level evaluated in this study (improved HCW of 2.6 kg for **LOW**, 3.4 kg for **MID**, and 4.5 kg for **HIGH**) vs. **CON** gilts. However, consideration should be given to the potential production impacts of feeding **LOW** NE diets when managing **IMP**-market gilts to ensure response can be consistently maintained.

NUTR - Nutrition

# PREMIX OF ESSENTIAL OILS BLEND AND EGG POWDER LOWERS THE FCR IN YOUNG PIGLETS- A PILOT STUDY.

<u>D. Peric</u><sup>1</sup>, M. Glisic <sup>2</sup>, M. Vidakovic <sup>3</sup>, J. Bošnjak-Neumüller <sup>3</sup>, M. Jovancevic <sup>3</sup>, J. Raj <sup>3</sup>, M. Vasiljevic <sup>3</sup> <sup>1</sup>Department of Animal Nutrition and Botany , Faculty of Veterinary Medicine, University in Belgrade, Belgrade, Serbia <sup>2</sup>Department of Animal Hygiene, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia <sup>3</sup>PATENT CO DOO., Serbia

### **Background and Objectives**

Weaning stress usually results in reduced feed intake (FI), an increased rate of diarrhoea, and developmental retardation. This stress often alters the structure and function of piglets' small intestines, impairing their capacity to digest and absorb nutrients effectively. Egg powder (EP)proteins, as well as essential oils (EO) use in post-weaning piglets are associated with positive effects on pig health and immune status. The aim of this study was to test the impact of EO and an egg powder mixture of additives on the production performance of post-weaned piglets.

### **Material and Methods**

The trial was conducted at the commercial pig farm where the piglets were allocated in floor pens and allowed to consume feed and water ad libitum. The study included 122 post-weaned piglets aged 25 days at the beginning of the trial. The trial lasted 21 days during thestarter phase of the dietary program. Piglets were divided into a control group (C) fed with a basal diet and a treatment group (T), where the piglets were fed the basal diet supplemented with 2 kg/ton of EO and EP premix (the formulation is the property of PATENT CO.). On the first and last day of the trial, the piglets' body weights (BW) were recorded individually, and the total FI was measured according to the discarded feed leftovers. At the end of the trial, the FCR was calculated for both groups.

### Results

All piglets remained in good health condition throughout the experiment, with no reported fatalities. On the first day of the trial, the average starting BW in the C group was  $6.66\pm0.96$  kg, while in the T group it was  $6.70\pm1.02$ . The difference in the BW of the Control and T group of piglets at the end of the trial was not found (P>0.05), however, the FCR was lower in the T group (1.476) in comparison to the C group (1.728), due to lower FI in the group of piglets from T group.

# **Discussion and Conclusion**

The addition of a premix of additives with active compounds originating from an EO blend and EP, led to a decrease in FCR value in post-weaning piglets under non-challenging conditions.

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# SUPPLEMENTATION OF PROBIOTIC CONTAINING BACILLUS SUBTILIS AND BACILLUS AMYLOLIQUQUEFACIENS IN SOWS DURING LACTATION ENHANCED FECAL SHORT-CHAIN FATTY ACIDS IN SUCKLING PIGLETS

<u>C. Srisang</u><sup>1</sup>, P. Taechamaeteekul<sup>1</sup>, S. Nuntawan Na Ayudhya<sup>2</sup>, L.H.B. Hansen<sup>2</sup>, P. Tummaruk<sup>1</sup> <sup>1</sup>Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand <sup>2</sup>Chr. Hansen A/S, Hoersholm, Denmark

# **Background and Objectives**

SCFAs play a crucial role in supplying energy to colonocytes and maintaining the metabolism and integrity of the intestinal mucosa. In the swine industry, probiotics have been used for decades as modulators of the gut microbiome. Alterations in the gut microbiome affect the production of metabolites, subsequently impacting the overall health of pigs. Therefore, this study aimed to investigate the effects of supplementation with a probiotic containing Bacillus subtilis and Bacillus amyloliquefaciens, administered during the transition and lactation periods, on the concentration of SCFAs in piglet feces at various time points during the suckling period.

# **Material and Methods**

The research was conducted at a commercial pig breeding facility in Central Thailand, involving 109 sows of the Canadian Landrace × Yorkshire breed, with parity numbers 1 to 5. These sows were split into two groups: CONTROL (61 sows) and TREATMENT (48 sows). The TREATMENT group received a lactational diet enhanced with the probiotic SolPreme® (consisting of B. subtilis – 541 and B. amyloliquefaciens – 516),, starting on the 109<sup>th</sup> day of gestation until the 21<sup>st</sup> day after farrowing. Fecal samples from 30 piglets, 15 from each group, were randomly collected on days 3, 7, and 21 for SCFA concentration analysis using gas chromatography with flame ionization detection. Statistical analysis was performed using SAS version 9.4, employing the general linear model procedure. The least-square means were compared between groups using the least significant differences test.

### Results

The SCFA concentration in piglet fecal samples was significantly higher in the TREATMENT group than in the CONTROL group ( $28.7 \pm 1.9 \text{ vs} 21.2 \pm 1.9 \text{ nM}$ , P = 0.011) on the 7<sup>th</sup> day of lactation. On days 3 and 21, however, the SCFA levels did not show significant differences between the two groups (P > 0.05).

### **Discussion and Conclusion**

Significant differences in SCFA levels were observed between the CONTROL and TREATMENT groups on day 7, unlike at other time points. This indicates that supplementing sows' diets with the Bacillus-based probiotic before and after farrowing may influence the gut microbiota of piglets, leading to alterations in microbial gut metabolic activity and its by-products.

NUTR - Nutrition

# THE EFFECT OF A COMBINATION OF ADDITIVE BLENDS IN FEED AND WATER WITH HYDROXYCHLORIDE CU ON INTESTINAL HEALTH AS ALTERNATIVE FOR AGP

<u>S. Van Kuijk</u><sup>1</sup>, G. Heim<sup>1</sup>, Y. Van Der Horst<sup>1</sup>, G. Boerboom<sup>1</sup> <sup>1</sup>Selko Feed Additives, Stationsstraat 77, 3811 MH Amersfoort, The Netherlands

### **Background and Objectives**

Presan FX (organic acid (OA), medium chain fatty acid and phenolic compound based feed additive (MCOAP)), Selko pH (OA based water additive), Fysal MP (OA based feed additive) and IntelliBond Cu were tested as antibiotic growth promoter (AGP) alternative on growth performance and intestinal health of weaned piglets.

### Material and Methods

120 piglets (Duroc\*Landrace\*Great White), (weaned at 21 days, weight ~6.0kg), were housed with 6 animals/pen and 5 pens/treatment. Four treatments fed starting 7 days post-weaning: (i) negative control (NC) without additives, (ii) NC with 30g/metric ton (MT) AGP and 2100g/MT ZnO on top (until day 14) (positive control, PC), (iii) NC with 2kg/MT MCOAP blend (Presan-FX, Selko, Tilburg, The Netherlands) and 215g/MT hydroxychloride Cu (IntelliBond C, Selko, Tilburg, The Netherlands) in feed, and 0.1% OA blend in water (Selko-pH, Selko, Tilburg, The Netherlands) (OACuW), (iv) NC with 2kg/MT MCOAP blend, 215g/MT hydroxychloride Cu and 3kg/MT OA (Fysal MP, Selko, Tilburg, The Netherlands) in feed (OACuF). Two feeding phases: (I) day 7-14, (II) day 15-42. Growth performance was measured at day 7, 14 and 42. On day 42, morphology of duodenum, jejunum and ileum, digestive enzymes in jejunum and colonic short chain fatty acids (SCFA) and microbiota (16s rRNA sequencing) were measured. Statistics were done in SPSS 20.0 using DUANKAN method (normal distribution), Mann-Whitney U test (microbiota diversity) and Kruskal-Wallis test (relative abundance microbiota).

### Results

OACuF increased daily gain compared to OACuW between day 15-42 (504.6 vs 427.1 g/pig/day, P=0.031). OACuF and OACuW positively influenced (P<0.05) villus height, crypt depth, villus:crypt ratio and colonic SCFA compared to NC and PC. Lipase activity was highest (P<0.001) in PC (752.32 U/L) and OACuW (854.25 U/L). Microbiota analysis showed most (P=0.005) observed species in PC (1475.0) and OACuW (1488.8). Microbial community deviated in OACuW and OACuF, resulting in less Bacteriodetes (NC=33.60%, PC=35.59%, OACuW=22.77%, OACuF=30.12%, P=0.025) and higher firmicutes/bacteriodetes ratio (NC=1.80, PC=1.65, OACuW=3.06, OACuF=2.22, P=0.017) compared to NC and PC.

### **Discussion and Conclusion**

Piglets fed combinations of OA, MCOAP and Cu had improved growth performance, gut morphology, enzyme activity, intestinal SCFA and a modified intestinal microbiota, compared to piglets fed NC or AGP (PC).

NUTR - Nutrition

# MORPHOLOGICAL ALTERATIONS ON THE SEMITENDINOSUS MUSCLE OF THE OFFSPRING OF SOWS SUPPLEMENTED WITH FUNCTIONAL AMINO ACIDS DURING GESTATION

<u>T.C. Caiafa De Alkmim</u><sup>1</sup>, J. Medeiros De Almeida<sup>1</sup>, A. Lacerda Shinkawa<sup>1</sup>, L. Pinheiro Moreira<sup>1</sup>, J.V. Lopes Ferreira <sup>1</sup>, D.K. Pires Oliveira<sup>1</sup>, I.M. Sátiro De Oliveira<sup>1</sup>, F.A. Pereira<sup>2</sup>, F.N. Alves Ferreira<sup>2</sup>, C. Speridião Silva Neta<sup>2</sup>, F. Radicchi Campos Lobato De Almeida<sup>1</sup>

<sup>1</sup>Laboratory of Structural Biology and Reproduction (Laber), Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte – MG

<sup>2</sup>Research Center "Professor José Maria Lamas da Silva", Agroceres Multimix, Patrocínio – MG

### **Background and Objectives**

Sows' nutritional management during gestation is a crucial factor influencing postnatal performance of the offspring. Alternative nutritional supplements, such as functional amino acids, may be offered to gestating sows to ensure optimal embryonic and fetal development. Thus, studying the effects of such supplementation on the performance of litters, particularly in commercially relevant systems organs such as the skeletal muscle, which will give rise to meat, is essential. The objective of this study was to investigate morphological alterations in the semitendinosus muscle (ST) of offspring from sows supplemented with an immunomodulatory functional amino acid concentrate during gestation.

### **Material and Methods**

Twenty-eight females (parities ranging from 1<sup>st</sup> to 7<sup>th</sup>) were randomly distributed among three treatments: T1 - sows receiving a control diet, with no supplementation, throughout gestation (n=9); T2 - sows receiving a control diet + supplementation (40g/sow) until the 60<sup>th</sup> gestational day (n=9); T3 - sows receiving a control diet + supplementation (40g/sow) throughout gestation (n=10). The supplementation was offered on top from the day of the first insemination until the 60<sup>th</sup> day of gestation (T2) or parturition (T3). Ten piglets from each treatment were euthanized at 26 and 145 days of age, when the ST was dissected and weighed. A fragment of ST was collected, fixed in paraformaldehyde 4% and histological slides obtained to determine the volumetric proportion of ST components. Data were analyzed using SAS software (2001) through analysis of variance, and means were compared using Tukey-Krammer post-test, with a significance of P<0.05.

### Results

At 26 days, no effects of supplementation were observed for ST weight. However, T3 animals showed higher proportion of myofiber nuclei and lower proportion of connective tissue than T1 (P<0.05), and both T2 and T3 exhibited higher proportion of blood vessels (P<0.05). At 145 days, T2 and T3 showed higher ST weight and sarcoplasmic proportion (P<0.05). Moreover, T3 presented higher proportion of blood vessels and T2, higher proportion of myofiber nuclei (P<0.01), and T1 exhibited higher proportion of adipocytes (P<0.01).

### **Discussion and Conclusion**

Taken together, the results demonstrate the benefits of supplementing gestating sows with functional amino acids on skeletal muscle structural parameters associated to meat quality.

NUTR - Nutrition

# THE EFFECT OF INTELLIBOND SUPPLEMENTATION ON THE IMMUNE SYSTEM OF WEANED PIGLETS UNDER LPS CHALLENGE

<u>S. Van Kuijk</u><sup>1</sup>, G. Boerboom<sup>1</sup>, E. Arevalo Sureda<sup>2</sup>, N. Everaert<sup>2</sup>, C. De Cuyper<sup>3</sup>, S. Millet<sup>3</sup> <sup>1</sup>Selko Feed Additives, Stationsstraat 77, 3811 MH Amersfoort, The Netherlands <sup>2</sup>KU Leuven, Department of Biosystems, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium <sup>3</sup>ILVO (Flanders Research Institute for Agriculture, Fisheries and Food), Scheldeweg 68, 9090 Melle, Belgium

### **Background and Objectives**

The essential trace mineral Zinc (Zn) plays an important role in immunity. This study assessed the effects of Zn supplementation from different Zn sources, in Escherichia coli lipopolysaccharide (LPS) challenged weaned piglets.

### Material and Methods

Weaned piglets (n=144, ~28 days of age) were allotted to 12 pen replicates per treatment. Pens were divided over two rounds, each housing 4 piglets (either gilts or barrows) and blocked on initial body weight. Three treatments: (1) negative control (NC) containing no added Zn, 15 ppm Cu from CuSO<sub>4</sub> and 32 ppm Mn from MnO, (2) inorganic treatment (INO) which is NC diet with 110 ppm Zn from ZnSO<sub>4</sub> added, (3) Hydroxychloride trace minerals treatment (HC) containing 110 ppm Zn, 15 ppm Cu and 32 ppm Mn from IntelliBond (Selko, Tilburg, The Netherlands). Diets were fed in two phases from day 0-14 and day 14-35. On day 9 pigs were injected with 160 µg LPS and on day 11 with 200 µg LPS. Growth performance was measured on day 0, 7, 14 and 35. Fecal consistency was scored daily between day 0-14 and weekly between day 14-35. Blood was sampled from 1 pig per pen on day 7 (96h before LPS), 11 (3h post LPS), 12 (24h post LPS), and 14 (96h post LPS), to measure immune parameters by ELISA.

### Results

Feed intake decreased on day 9, and body temperature increased on day 11, but animals recovered quickly. Zinc supplementation (both INO and HC) resulted in lower IL-1â levels compared to NC. The HC group showed lowest TNF-á levels, while TNF-á was higher in the INO group compared to the NC. IL-10 was numerically reduced in HC, compared to the NC and INO groups. Numerically, haptoglobin only increased in NC and INO during the first 24h post LPS. Differences in immune parameters were not reflected in growth performance. Fecal consistency was not affected, except on day 5 when zinc supplementation, regardless of source, improved fecal consistency.

# **Discussion and Conclusion**

Immune parameters, especially TNF-a and possibly IL-10, were affected by HC diets, indicating that piglets fed HC displayed a modestly enhanced resilience to an LPS challenge.

NUTR - Nutrition

# SUPPLEMENTATION OF THE PLANT SOLANUM GLAUCOPHYLLUM TO WEANER DIETS IMPROVES PERFORMANCE UNTIL SLAUGHTER

<u>K. Bühler</u><sup>1</sup>, A.E. Taylor <sup>2</sup>, S.C. Hazelden <sup>2</sup>, K. Mcdermott <sup>2</sup> <sup>1</sup>Herbonis Animal Health GmbH <sup>2</sup>University of Leeds, UK

### **Background and Objectives**

Weaning is a stressful period that can impact life performance. Lowering dietary calcium (Ca) in weaner diets reduces buffer capacity and antinutritive effects of high Ca levels. The plant Solanum glaucophyllum (SG) contains glycosylated 1,25(OH)<sub>2</sub>D, which supports Ca metabolism and could improve immune status.

### Material and Methods

240 weaned piglets (28 days of age,  $8.0 \pm 0.4$  kg) were distributed among 48 pens and fed one of four diets (starter: 0-15 days, grower: 16–42 days post-weaning): A control diet with 3100 ppm zinc oxide (ZnO) during the starter phase and no probiotics or organic acids at any time (PC), a control diet with probiotics and organic acids (NC), NC with 20 % reduced Ca (Ca<sub>low</sub>) or Ca<sub>low</sub> supplemented with SG (SG). SG was provided as 100 g Panbonis®/t of feed. At day 15, 10 piglets per treatment were euthanized for blood and intestinal samples to analyze tight junction proteins, intestinal morphology and cytokines. After 42 days, pigs were switched to a commercial diet. Final body weight was measured at slaughter.

### Results

Performance in the whole starter phase was comparable among treatments (p > 0.05). Nevertheless, a treatment effect on ADG and ADFI in the period 8 – 15 days could be observed (p < 0.05). Body weight on day 42 was the highest in treatment SG (33.64 ± 4.44 kg; p < 0.001). These pigs also tended to be the heaviest on day 113 (103.5 ± 7.16, p = 0.050). There was a treatment effect on DWG (p < 0.01) and FCR (p = 0.038) for the whole experimental period, with animals fed diet SG showing the best performance. The selected markers for intestinal integrity and immunity were comparable among treatments (p > 0.05).

### **Discussion and Conclusion**

The supplementation of SG to Ca reduced weaner diets improved performance. The effect was persistent until slaughter, although supplementation with SG ended 42 days post-weaning. As effects of SG were more pronounced in the grower phase, it was speculated that those piglets recovered faster from the weaning stress. To gain insight into the mode of action, trials with intestinal sampling throughout the weaner phase and trials with a higher challenge level need to be conducted.

NUTR - Nutrition

# EFFECT OF FEEDING CURVES FOR DUROC HYBRID PIGS ON CARCASS YIELD AND MEAT QUALITY

M.D. Lima 1, S.V. Ferreira 2, I.M.G. Lopes 3, N.C.S. Silveira 3, T.C. Figueiredo 4, F.R.C.L. Almeida 5, D.O. Fontes 3, . 0

<sup>1</sup>Department of Animal Science, Federal University of Minas Gerais, Brazil

<sup>2</sup>Danbred Brazil, Brazil

<sup>3</sup>Department of Animal Science, Federal University of Minas Gerais, Minas Gerais, Brazil

<sup>4</sup>Department of Technology and Inspection of Animal Products, Federal University of Minas Gerais, Brazil

<sup>5</sup>Departament of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, Minas Gerais, Brazil

### **Background and Objectives**

Duroc hybrids have stood out in swine farming due to their high marbling, resulting in better flavor and juiciness of the meat. The objective aimed to evaluate the effects of feeding curves on carcass yield and meat quality in Duroc hybrid pigs.

# **Material and Methods**

The research was carried out on an experimental farm in Minas Gerais, Brazil. A total of 216 Duroc-hybrid pigs (Duroc x Landrace x Large White - DanBred Brasil®) with an average initial weight of 24.80 kg and 63 days of age were distributed in a completely randomized design in a 2x3 factorial arrangement, with two sexual categories (SC) (108 immunocastrated males and 108 females) and three feeding curves: ad libitum (AL), feeding curve 1 (-15% in relation to AL consumption - CF1) and feeding curve 2 (-25% in relation to AL consumption - CF2). At the end of the experiment (125kg live weight), the animals were sent for slaughter in a slaughterhouse. After cooling period (16 to 20h), the entire carcasses, except for the head, feet, viscera and testicles, were sent to the boning room. Subsequently, the carcasses were deboned to obtain the weight and yield of the shoulder and loin meat cuts. Meat quality parameters such as cooking loss, marbling and color were measured through loin samples analysis.

#### Results

There was an interaction between feeding curves and SC for shoulder weight. The restriction increased loin weight and yield, however, it did not affect meat quality, which was influenced by SC, where females presented higher marbling scores.

# **Discussion and Conclusion**

The observed interaction is associated with the severity of the restriction (CF2) as the lower feed consumption reduced weight gain, carcass yield and shoulder weight. The greater loin weight and yield is associated with the effect of feeding curves, as CF1 animals were more efficient in converting dietary nutrients into muscle deposition. The higher marbling score for females is justified by the effective deposition of fat on the carcass compared to males, reflecting the deposition of intramuscular fat. It is concluded that use of feeding curves positively affects carcass yield in Duroc hybrids. However, it did not influence meat quality parameters.
NUTR - Nutrition

# NORWEGIAN BIOCHAR (OBIO) INCREASES DAILY GROWTH POST WEANING.

J. Haugegaard 1, K.S. Pedersen 2

<sup>1</sup>OEVET A/S, Naestved, Denmark

<sup>2</sup>Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark and OEVET, Naestved, Denmark

#### **Background and Objectives**

To find alternatives to the use of Zink oxide we investigated the efficacy of Norwegian biochar (+650 degrees centigrade pyrolysis of chipped pine tree and red spruce).

#### Material and Methods

In a large pigherd with E. coli weaning diarrhea 300 piglets from one weaning was spilt into 3 groups of 100 piglets each. Groups was 1) negative control, 2) given 1 % of biochar in the primary weaning diet for 8 days post weaning and 0,25 % of biochar in the second feed post weaning and 3) 2 % of biochar mixed in the primary weaning diet 8 days post weaning and 0,25 % in the second weaning diet. Weighing of piglets was done individually at weaning and again 35 days post weaning. Piglets was housed in 5 different pens for each group and were allocated randomly. Piglets was treated metaphylactic with antibiotics. Statistical analysis was done using a Linear mixed model.

#### Results

Average weight in the three groups was 6,5, 6,6 and 6,4 kg at weaning. 35 days post weaning the weight was 21,3, 22,9 and 20,9 kg respectively. The weight gain was 423, 466 and 414 g pr day respectively. The weight gain was significantly higher for group 2 compared with both group 1 (p = 0,033) and 3 (P=0,014). Group 1 and 3 did not differ. Mortality in the 3 groups was 4, 0 and 0 piglets.

### **Discussion and Conclusion**

The observed efficacy of biochar is seen under simultaneously use of antibiotics. The efficacy of 10 kg/ton is evident on the average daily gain. Future studies should be made, where the use of antibiotics is reduced using individual treatment with antibiotics. The 20 kg/ton inclusion rate did not show any difference to the negative control. This is probably due to a lower feed intake influencing the average daily gain. Mortality was evidently influenced however no significance can be calculated. Mixing the diet with biochar in the first post weaning feed by 10 kg/ton (8 days feeding) and in the second feed (2 weeks feeding) by 2,5 kg/ton increased the average daily gain significantly by 10 % in the first 5 weeks post weaning.

NUTR - Nutrition

# THE PROTECTIVE EFFECTS OF A BROAD SPECTRUM MYCOTOXIN ADSORBENT IN SOW DIETS

J. Van Soest <sup>1</sup>, <u>L. Vande Maele</u><sup>1</sup>, V. Vandendriessche <sup>1</sup>, A.J.L. Frio <sup>2</sup>, M.J. Serrano <sup>1</sup> <sup>1</sup>Orffa Additives BV, Breda, The Netherlands <sup>2</sup>First Ten Consulting Asia Pacific, Laguna, Philippines

#### **Background and Objectives**

Pigs are considered to be very sensitive to mycotoxins, especially sows and piglets. This highlights the need for solutions to reduce the negative effects of mycotoxins on pig performance. The aim of this trial was to study the effects of two broad spectrum mycotoxin adsorbents on sows and piglet performance, when fed to sows from late gestation until farrowing.

#### Material and Methods

A total of 100 primiparous and multiparous sows were divided over 3 treatments: control = basal gestation and lactation diet without mycotoxin binders; treatment 1 = control + mycotoxin adsorbent A at 0.15% inclusion; and treatment 2 = control + mycotoxin adsorbent B (Excential Toxin Plus, Orffa Additives B.V.) at 0.15% inclusion. Both adsorbents were broad spectrum products but contained different ingredients. The trial started at day 107 of gestation and lasted until weaning. Sow performance (daily feed intake, feed conversion ratio (FCR= lactation feed/litter weight gain), mortality) and piglet performance (average weaning weight, averaged daily gain (ADG), weaning litter size, mortality), were recorded. The diets were analyzed for mycotoxins. Differences between treatments were tested at 5% significance level (P < 0.05) and confidence interval of 95%.

#### Results

Aflatoxins (6.85 ppb), deoxynivalenol (103.65 ppb), beauvericin (8.5 ppb), moniliformin (5.53 ppb), enniatins (18.80 ppb), fusaric acid (35.55 ppb), fumonisins (217.10 ppb), mycophenolic acid (9.15 ppb) and zearalenone (27.10 ppb) were detected in the feed. Treatments 1 and 2 showed an improved FCR of 2.99 and 2.97 respectively, compared to the control (3.83) (P = 0.033).Piglets showed significantly higher average weaning weight for treatment 1 (7.25 kg/piglet) and 2 (7.14 kg/piglet) compared to control (6.17 kg/piglet) (P < 0.01). Furthermore, pre-weaning ADG was improved for treatment 1 and 2 (both 206 g/d) compared to control (170 g/d) (P < 0.01). Litter growth rate was significantly higher for treatment 2 (2.02 kg/d) compared to the control (1.68 kg/d) (P = 0.04).

# **Discussion and Conclusion**

Inclusion of a broad spectrum mycotoxin adsorbents in the diet of sows during gestation and lactation can improve sow performance (feed intake, FCR) as well as piglet performance (average weaning weight, ADG and litter growth rate).

NUTR - Nutrition

# A COMBINATION OF ADDITIVE BLENDS IN THE FEED AND WATER AND VARYING THE SOURCE OF CU AS ALTERNATIVE FOR ZNO

<u>S. Van Kuijk</u>, G. Heim<sup>1</sup>, Y. Van Der Horst<sup>1</sup>, G. Boerboom<sup>1</sup> <sup>1</sup>Selko Feed Additives, Stationsstraat 77, 3811 MH Amersfoort, The Netherlands

#### **Background and Objectives**

High levels ZnO are already banned in Europe and more regions are expected to follow. This study tested Presan FX (organic acid (OA), medium chain fatty acid and phenolic compound based feed additive (MCOAP)) and/or Selko pH (OA in water) and IntelliBond Cu in different combinations as ZnO replacer on growth performance and health status in weaned piglets.

#### Material and Methods

150 weaned piglets (Duroc\*Landrace\*Yorkshire) (age ~28 days, weight 8.78±0.18kg) were housed with 5 animals/pen, 6 pens/treatment. Five treatments were applied: (i) negative control (NC), (ii) positive control (PC) was NC with 1900 ppm ZnO on top during day 0-14, (iii) NC with 2 kg/MT MCOAP (Presan-FX, Selko, Tilburg, The Netherlands) added in feed (F), (iv) F plus 1 mL/L OA in water (FW) (Selko-pH, Selko, Tilburg, The Netherlands) and (v) FW with 125 ppm hydroxychloride Cu (IntelliBond C, Selko, Tilburg, The Netherlands) in feed (FWC). Diets NC, PC, F and FW contained 125 ppm Cu from CuSO4. Feeding Phase 1 was from day 0-14 and phase 2 from day 15-42. Growth performance was measured at days 0, 14, 28 and 42. Diarrhea incidence was measured daily on day 0-14. On day 14 feces was sampled to analyze microbiology (16srRNA) and volatile fatty acid (VFA) content. Statistical analysis was done in SAS 9.2 for growth performance (GLM procedure) and diarrhea incidence (Chi-square), and R for microbiota (Kruskal-Wallis test).

#### Results

Body weight, daily gain and feed intake were not different among treatments. Feed conversion ratio (FCR) was lowest in FW compared to PC between day 15-28 (1.321 vs 1.563, P=0.029). Overall study period, FCR tended to reduce in FW compared to PC (1.470 vs 1.589, P=0.060). The diarrhea incidence was lowest (P<0.001) in PC (3.94%) followed by FWC (11.32%), both lower than NC (18.21%). Although microbial communities tended to be separated between treatments, no treatment differences were observed on taxonomy level or fecal VFA.

#### **Discussion and Conclusion**

A combination of feed and water additives may provide an alternative for ZnO. Combining organic acids in both feed and water improved FCR compared to ZnO, while the diarrhea incidence was reduced with the combination of all additives compared to negative control.

NUTR - Nutrition

# COMPARISON OF 200 MG INJECTABLE IRON DEXTRAN VS. 400 MG IN EITHER A SINGLE TIMEPOINT OR SPLIT-DOSED INTO TWO TIMEPOINTS ON GROWTH AND HEMOGLOBIN IN PIGS FROM BIRTH TO FINISHING

<u>W. Lyons</u><sup>1</sup>, F. Gomez Cruz<sup>2</sup>, S. Krantz<sup>3</sup>, J. Pierce<sup>4</sup> <sup>1</sup>PHARMACOSMOS INC, WATCHUNG, NJ, USA <sup>2</sup>Western Veterinary College, University of Saskatoon <sup>3</sup>Tosh Farms, Henry, TN USA <sup>4</sup>JAMES L PIERCE CONSULTING

#### **Background and Objectives**

The industry standard for parenteral iron in piglets has been 200 mg given intramuscularly (IM). Recently published research has shown that an iron dosage higher than 200 mg has a better outcome on body weight and average daily gain (ADG). Previous studies investigated supplementing 200 mg iron dextran to piglets at two different timepoints prewean. This study's objective was to determine if dosing 400 mg of iron dextran split into two injections at a single timepoint is equivalent to dosing 200 mg at two different timepoints on the growth characteristics noted in previous studies.

# Material and Methods

590 piglets were enrolled at 24 hours of age. Enrollment consisted of three gilts and three barrows per litter. The piglets selected within litter were similar in weight. The treatment groups consisted of one 200 mg iron injection IM (control), two 200 mg IM injections at a single timepoint given in two injections (single), and two 200 mg IM injections given 5 days apart (split). Hemoglobin and weight were measured at enrollment, 6 days, weaning, end of nursery, and market. Wean age is the covariate for weaning weight and hemoglobin. Days on feed is the covariate for the wean to finish growth data. All reported results are P < 0.05.

#### Results

At weaning, Hemoglobin was increased in both treatment groups versus control. Weaning weight improved in the split group compared to the control. At end of nursery, pigs in the split and single groups were 1.04 and 0.86 kg heavier than the control. At marketing, both 400 mg iron dextran treatment groups were 6.35 kg heavier compared to control. ADG was improved 0.05 kg in both 400 mg iron dextran treatment groups compared to control pigs given 200 mg.

# **Discussion and Conclusion**

In production systems where pigs are picked up twice, implementing the second 200 mg dose of iron dextran adds little cost and labor. This study proves that 400mg of parenteral iron dextran supplemented to piglets at a single or split timepoint is cost-effective at improving ADG, market weights, and hemoglobin.

NUTR - Nutrition

# EFFECTS OF HIGH PROTEIN DIET SUPPLEMENTED WITH PROBIOTICS AND PROTEASE ON GROWTH PERFORMANCE, AND DIARRHEA INCIDENCE OF WEANED PIGLETS

M. Zhang <sup>1</sup>, L. Che <sup>2</sup>, C. Zhou <sup>1</sup>, L. Luo <sup>1</sup>, <u>S. Kirwan</u> <sup>3</sup> <sup>1</sup>Kemin (China) Technologies Animal Nutrition and Health <sup>2</sup>Sichuan Agriculture University, Chengdu, People's Republic of China <sup>3</sup>Kemin Industries, USA

#### **Background and Objectives**

Research has supported lower protein weaner diets to reduce the diarrhea risk and achieved good weaning, most farmers still prefer high protein diets for the sake of maximum growth performance and due to protein source limitations. For that reason, this study investigated the effects of different approaches to reduce diarrhea and enhance growth performance at a high dietary protein level.

# **Material and Methods**

160 weaned piglets (21 days, initial weight 6.12 kg) were randomly assigned to 5 treatments groups: LP, low-protein (18.27% crude protein); HP, high-protein (20.97% crude protein); HPPRO, high-protein supplemented with 300g/t probiotics (Bacillus sp. PB6 spores 2x10<sup>11</sup>/kg); HPPRS: high-protein diet with 300g/t protease (8000U/g); HPPRO-PRS: high-protein supplemented with 300g/t probiotics and 300g/t protease. Growth performance and diarrhea index were measured throughout; blood samples, cecum and colon content samples were collected on day 23. The chymus flora was detected by 16sRNA method. DNA was extracted by CTAB or SDS, and the purity and concentration of the extracted DNA were detected via gel electrophoresis. Major Acute-phase Protein and haptoglobin concentrations were determined in the blood plasma.

#### Results

Compared with LP group, the HP group had no significant effect on the growth performance of piglets, but HPPRO group and HPPRO-PRS group increased body weight on day 15 and day 22 (P<0.01). By day 22, HPPRO and HPPRO-PRS piglets had higher ADG and ADFI (P<0.01). Diarrhea in the HP group was increased for the whole experimental period (P<0.01). Diarrhea in the HPPRO, HPPRS and HPPRO-PRS groups was higher than LP group (P>0.05), but decreased compared to the HP group (P<0.01). The data of random block design was analyzed using the MIXED model of SAS 9.4, Except for growth performance, diarrhea and digestability which used pens as the statistical unit, other indicators were taken individual units.

# **Discussion and Conclusion**

In this experiment, feeding high instead of low protein diet, increased the diarrhea index of weaned piglets. The addition of probiotics and/or protease to high protein diet could reduce diarrhea and improve growth performance of piglets. Additionally, the addition of probiotics to high-protein diet reduced the abundance of harmful microorganisms in the colon.

NUTR - Nutrition

# EFFECTS OF RYE AS A FEED COMPONENT ON THE PREVALENCE OF SALMONELLA IN GILTS, SOWS AND REARING PIGLETS

<u>C. Homann</u><sup>1</sup>, V. Wilke<sup>1</sup>, I. Eckey<sup>1</sup>, C. Visscher<sup>1</sup> <sup>1</sup>University of Veterinary Medicine Hannover, Foundation, Institute for Animal Nutrition

#### **Background and Objectives**

Rye as a feed component offers nutritional benefits, especially due to its high content of non-starch polysaccharides (NSP). Microorganisms in the large intestine are able to convert them into short-chain fatty acids (SCFA), including butyrate. In experimental studies, butyrate has been shown to strengthen the epithelial barrier function in the colon by nourishing enterocytes and inhibiting the spread of Salmonella in the intestinal tract. Therefore, the objective of this study was to test under field conditions whether a diet containing rye as a component of the feed for gilts, sows and weaned piglets is associated with lower Salmonella prevalence.

# **Material and Methods**

Three piglet producers voluntarily participated in this study due to prevalent Salmonella problems. The farm-specific compound feed, containing wheat, barley and soybean meal as the main components, served as the control feed. In gilt integration and farrowing units, 30% rye was used in the experimental diet (R), while 25% rye was used in piglet rearing. To maintain the same energy and protein content, the experimental diet (without wheat) was adjusted to the respective control diet. To determine Salmonella prevalence, Salmonella antigen (KYLT® PCR) in boot swabs (n=1683) and fecal samples (n=267), and Salmonella antibodies (pigtype Salmonella Ab ELISA) in blood samples (n=1596) were analyzed according to a strict scheme. Statistical evaluation was conducted with SAS Enterprise Guide® (Wilcoxon rank sum test) and p < 0.05 was considered significant.

#### Results

In the evaluation of the positive boot swabs and fecal samples, Salmonella could only be detected intermittently. No difference was found between the feeding groups in any age group. The OD% values of the serum samples obtained were significantly lower when feeding rye in gilt integration (C 71.21±58.96, R 51.02±44.65) and in piglet rearing (C 27.30±33.62, R 12.33±23.67).

# **Discussion and Conclusion**

In conclusion, it can be assumed that rye has an effect on Salmonella, but that the amount used was not sufficient to achieve significantly lower detection rates in boot swabs and fecal samples. Further studies with higher proportions of rye in the compound feed are required to demonstrate the possible positive effects on salmonella prevalence.

NUTR - Nutrition

# EVALUATION OF THE NUTRITIONAL SUPPLEMENT VIUSID VET POWDER ON THE PRODUCTIVE BEHAVIOUR OF SOWS.

<u>J.C. Rodriguez-Fernandez</u>, I. Calero-Herrera <sup>1</sup>, V. Mendez-García <sup>1</sup>, K. Peña-Calzada <sup>1</sup>, B. Kukurtcu <sup>2</sup> <sup>1</sup>Department of Veterinary Medicine, Faculty of Agricultural Sciences, University of Sancti Spiritus <sup>2</sup>Scientific Department, Catalysis, S.L.

#### **Background and Objectives**

To improve the productivity of sows, different food additives are used to help satisfy their nutritional requirements, but they must be safe for people and the environment. The objective of the research was to evaluate the effect of providing the nutritional supplement on the productive behavior of the sows.

#### Material and Methods

Two homogeneous groups of pregnant sows were formed (35 control and 37 treated). Mixed with the concentrate, 10g/sow/day of the supplement was supplied. From 21 days before parturition and until weaning. Variables: live piglets per farrowing, stillborn, piglets weaned per litter (PWL), litter weight at birth (ILW), litter weight at weaning (FLW), weight gain (WG), weaning-fertilization interval, diarrhea in piglets, piglet deaths and body condition of the sow at weaning. The product contains malic acid, glucosamine, arginine, glycine, ascorbic acid, folic acid, monoammonium glycyrrhizinate, pyridoxine hydrochloride, cyanocobalamin, and calcium pantothenate and zinc sulphate. In addition, its components are subjected to a biocatalytic process of molecular activation to improve their biological activity and the biochemical reactivity of all its molecules, without affecting their molecular structure. For the analyses, Studen's T Test for homogeneous variances and the Hypothesis Test for Proportions were used.

#### Results

The treatment significantly affected (p<0.05) the variables: PWL (8.85 vs. 9.78); ILW (14.25 vs. 15.43 kg); FLW (74.13 vs. 86.72 kg); IW (59.88 vs. 71.29 kg) and significantly reduced diarrhea (p<0.05), 7.58% less in the treated group than the control. The other variables did not show significant differences.

# **Discussion and Conclusion**

Due to the variety of its composition, the mechanisms of action of the supplement are multiple; it has antioxidant, antiviral, antibacterial and immunomodulatory properties. Due to these properties, the treated sows achieved healthier and heavier litters than those in the control group. In the first weeks of life, the piglet fundamentally depends on the mother's health. Approximately one more piglet was obtained per birth and with a higher average weight (500 g more per piglet). It is concluded that VIUSID vet Powder significantly improves the productive behavior of sows and the piglets.

NUTR - Nutrition

# EVALUATION OF THE NUTRITIONAL SUPPLEMENT VIUSID VET POWDER ON THE PRODUCTIVE BEHAVIOUR OF YOUNG BOARS

<u>J.C. Rodriguez-Fernandez</u>, I. Calero-Herrera <sup>1</sup>, V. Mendez-García <sup>1</sup>, K. Peña-Calzada <sup>1</sup>, B. Kukurtcu <sup>2</sup> <sup>1</sup>Department of Veterinary Medicine, Faculty of Agricultural Sciences, University of Sancti Spiritus <sup>2</sup>Scientific Department, Catalysis, S.L.

#### **Background and Objectives**

The boar has a great impact on reproductive efficiency. Spermatogenesis requires a specific supply of energy and proteins, as well as macro and microelements, which is why the boar has a different nutritional demand than growing and fattening pigs. The objective of the research was to evaluate the effect of the nutritional supplement on the semen quality of young boars.

#### Material and Methods

Three groups were formed, with 10 boars/treatment (9 months old). Direct mounting was used, with 6 days of rest. Treatments: I-Control, II- 10g of supplement with the concentrate/boar, once a day for 6 weeks, starting from week 7 it was suspended for 45 days, the treatment was repeated for 6 more weeks. III-Same as II, but the dose was 15 grams. The evaluations were carried out at three times: At the beginning, two weeks after the end of the first treatment and two weeks after the end of the second. The product contains malic acid, glucosamine, arginine, glycine, ascorbic acid, folic acid, monoammonium glycyrrhizinate, pyridoxine hydrochloride, cyanocobalamin, and calcium pantothenate and zinc sulphate. In addition, its components are subjected to a biocatalytic process of molecular activation. For the analyses, Kolmogorov-Smirnov Test was used for goodness of fit, Levene's Test to evaluate homogeneity of variance, One-way ANOVA and Tukey's Multiple Range Test.

#### Results

In the initial evaluation, no significant differences were observed (p>0.05). At eight weeks, a significant improvement (p<0.05) was observed in the ejaculate volume of the group treated with 10g (211.7 mL vs 205.8 of the control); the other variables did not differ. In the last evaluation, a significant statistical difference (p<0.05) was only observed in ejaculate volume (216.3 vs. 209.3) and sperm concentration (185.5 vs. 177.5 x 106 spz/mL), in favor of those treated with 10g regarding control.

#### **Discussion and Conclusion**

Due to the variety of its composition, the mechanisms of action are multiple; it has antioxidant, antiviral, antibacterial and immunomodulatory properties. The antioxidant power of the product is high (11,587.95 imol TE/mL), which protects sperm cells from cellular damage. It is concluded that VIUSID vet Powder used as a nutritional supplement in young boars improves semen quality.

NUTR - Nutrition

# EVALUATION OF THE SUPPLY OF THE NUTRITIONAL SUPPLEMENT VIUSID DETOX VET. IN PIGLETS.

<u>J.C. Rodriguez-Fernandez</u><sup>1</sup>, I. Calero-Herrera<sup>1</sup>, V. Mendez-García<sup>1</sup>, K. Peña-Calzada<sup>1</sup>, B. Kukurtcu<sup>2</sup> <sup>1</sup>Department of Veterinary Medicine, Faculty of Agricultural Sciences, University of Sancti Spiritus <sup>2</sup>Scientific Department, Catalysis, S.L.

#### **Background and Objectives**

The use of antibiotics in animal feed has been questioned, fundamentally, due to the increase in bacterial resistance. Consequently, many alternatives began to be evaluated to establish other substances with similar beneficial effects, such as probiotics, prebiotics, acidifiers, etc. The objective of the research was to evaluate the effect of the nutritional supplement on the productive behavior of piglets.

#### **Material and Methods**

Three groups of 10 sows each were formed, with 10 piglets per litter. The treatments consisted of: I- Control; II- The piglets were given 3 mL of the supplement orally, on the day of birth and at 3 days of age, from 10 days onwards it was mixed once a day with the food, until weaning; III- Same as II, but 5 mL was supplied. The product contains malic acid, glucosamine, arginine, glycine, ascorbic acid, folic acid, monoammonium glycyrrhizinate, pyridoxine hydrochloride, cyanocobalamin, and calcium pantothenate and zinc sulphate. In addition, its components are subjected to a biocatalytic process of molecular activation. It also contains 12 species of probiotic bacteria. For the analyses, Kolmogorov-Smirnov Test was used for goodness of fit, Levene's Test to evaluate homogeneity of variance, One-way ANOVA, Tukey's Multiple Range Test and Hypothesis Test for Proportions.

### Results

Significant statistical differences (p<0.05) were observed between the 5 mL treatment and the control, in the variables: piglets weaned per sow (9.20 vs. 7.40 kg), final litter weight (54.28 vs. 34.94 kg), gain weight (41.07 vs. 21.73 kg) and food consumption (15.6 vs. 7.1 kg). Diarrhea and deaths were also significantly reduced (p<0.05) in those treated. The group treated with 5 mL showed the greatest differences with the control group. The 3 mL dose did not differ from the control.

# **Discussion and Conclusion**

VIUSID DETOX vet combines the benefits of probiotics with the antioxidant, antiviral and immunomodulatory effect of the rest of the components. This composition favors the best state of health of the piglets, reducing diarrhea and increasing the consumption and use of nutrients. It is concluded that the supplement significantly improves the productive behavior of piglets, mainly the 5 mL dose.

NUTR - Nutrition

# EVALUATION OF THE VIUSID VET (POWDER AND SOLUTION) NUTRITIONAL SUPPLEMENT IN GROWING-FINISHING PIGS.

<u>J.C. Rodriguez-Fernandez</u>, I. Calero-Herrera, V. Mendez-García, K. Peña-Calzada, B. Kukurtcu<sup>2</sup> <sup>1</sup>Department of Veterinary Medicine, Faculty of Agricultural Sciences, University of Sancti Spiritus <sup>2</sup>Scientific Department, Catalysis, S.L.

### **Background and Objectives**

The use of antimicrobials as additives has been limited worldwide. The European Union decided to ban antibiotics as food additives in January 2006. Consequently, many alternatives are being studied to find other substances with beneficial effects. The objective of the research was to evaluate the effect of the nutritional supplement on the productive performance of growing-fattening pigs.

# Material and Methods

Two experiments were carried out, with 210 pigs each, with 40 days of initial age. 5 pens/treatment were used with 14 pigs each. In each experiment, 3 treatments were evaluated: First experiment I-Control group, II-Treated group, for 90 days with 1.5g of supplement (Powder) per kilogram of concentrate and III-Treated group equal to II, but with 2.0g. Second experiment I-Control group, II-Group treated for 90 days, with 1.5g of supplement (Powder) per kilogram of concentrate and III-Treated group equal to II, but with 2.0g. Second experiment I-Control group, II-Group treated for 90 days, with 1.5g of supplement (Powder) per kilogram of concentrate and III-Group treated the same as II, but with 1.5 mL of the supplement in solution. The duration was 170 days. Both the powder and solution contain malic acid, glucosamine, arginine, glycine, ascorbic acid, folic acid, monoammonium glycyrrhizinate, pyridoxine hydrochloride, cyanocobalamin, and calcium pantothenate and zinc sulphate. In addition, its components are subjected to a biocatalytic process of molecular activation. For the analyses, Kolmogorov-Smirnov Test was used for goodness of fit, Levene's Test to evaluate homogeneity of variance, One-way ANOVA, Tukey's Multiple Range Test and Hypothesis Test for Proportions.

### Results

In the two experiments, the supplement (powder or solution) significantly improved (p<0.05) weight gain/pen (from 9.1 to 11.5% more) and feed conversion, with respect to pigs in the control groups, it also significantly reduced the deaths. Doses of 2.0g; 1.5g and 1.5 mL per kilogram of concentrate did not differ between them.

# **Discussion and Conclusion**

Due to the variety of its composition, the mechanisms of action are multiple; it has antioxidant, antiviral, antibacterial and immunomodulatory properties, which improve the health and well-being of the animals that consume it. The antioxidant power of the product is high (11,587.95 imol TE/mL). It was concluded that the supplement (powder or solution) improves the productive behavior of growing-fattening pigs.

NUTR - Nutrition

# FEED ADDITIVE PACKAGE WITH MICROENCAPSULATED ZINC ALLOWS PERFORMANCE MAINTENANCE IN PIGLETS FED WITH LOW ZNO CONTENT AND REDUCED PROTEIN

<u>G. Ramis</u><sup>1</sup>, F. Murciano<sup>1</sup>, J. Orengo<sup>1</sup>, L. Martínez-Alarcón<sup>2</sup>, B. González-Guijarro<sup>1</sup>, A. Cuellar-Flores<sup>1</sup>, E. Landeau<sup>3</sup>, S. Benaben<sup>3</sup>

<sup>1</sup>Dpto. Producción Animal,. Facultad de Veterinaria., Universidad de Murcia, Spain <sup>2</sup>Instituto Murciano de Investigación en Biomedicina (IMIB), Murcia, Spain <sup>3</sup>JEFO EUROPE, TECHNICAL DEPARTMENT, FRANCE

### **Background and Objectives**

The EU ban on the use of zinc oxide at therapeutic levels in 2022 has limited the amount that can be added to 150 ppm in piglets feeds and has led to a clear loss of gut health on farms. Therefore, it is imperative to look for new strategies that would comply with the allowed addition of ZnO. Within those new strategies, inclusion of microencapsulated zinc with higher bioavailability. The aim of this study was to determine the productive performance of piglets fed a low ZnO alternative way with the addition of a feed additive in protein reduced feed.

# Material and Methods

Three groups of 224 piglets each were set up wih base feed without zinc: one group with addition of 3000 ppm ZnO (CON+), another with ZnO at 150 ppm (CON-) and the third with addition of Jefo Package, providing 120 ppm of Zn, on top of a reduced protein feed (J). Piglets were weighed at weaning (WW), at the end of prestarter (W15d) and at the age of transfer to fattening (W49d). Growth was calculated at each phase (ADG1-15, ADG15-49, ADG1-49), as well as FCR per pen.

#### Results

There were no differences for WW (4.9 kg for all groups) and there was a difference for W15d between CON+ and J with CON- (7.52, 7.25 and 6.9 kg, respectively; p<0.001), whilst there was a difference between CON+ compared to J and CON- for W49d (23.6, 22.1 and 21.55 kg, respectively, p<0.001). The same differences were assessed for ADG1-15 (p<0.001), ADG15-49 (p<0.001) and ADG1-49 (p<0.001). There was no statistically significant difference in FCR among groups (CON+=1.79, J=1.88, CON-=1.94) despite a numerical improvement with J group in comparison to CON-

#### **Discussion and Conclusion**

Group J, was in all parameters between CON+ and CON-, with no differences with the former in the first 15 days postweaning. Even though there were no differences with CON- in some parameters, it must be considered that group J had a lower protein content in the feed, with a consequent reduction in the cost of production. Therefore, there was a better production efficacy in the J animals group compared to the CON- group.

# IMMUNOLOGY AND VACCINOLOGY

# IMM-PP-01

IMM - Immunology and Vaccinology

# CA-CAS-01-A IS A POTENTIAL CELL LINE FOR THE ISOLATION AND REPLICATION OF AFRICAN SWINE FEVER VIRUS

S. Lee <sup>1</sup>, Y. Kim <sup>2</sup>, J. Cha <sup>3</sup>, K. Chathuranga <sup>3</sup>, H. Kwon <sup>1</sup>, M.H. Kim <sup>1</sup>, W. Jheong <sup>2</sup>, J.Y. Lee <sup>1</sup>, I. Yoon <sup>1</sup>, S. Yoo <sup>4</sup>, <u>L. Jong-Soo</u> <sup>3</sup>

<sup>1</sup>ChoongAng Vaccine Laboratories, Daejeon, 34055, Republic of Korea

<sup>2</sup>Wildlife Disease Response Team, National Institute of Wildlife Disease Control and Prevention, Gwangju 62407, Republic of Korea

<sup>3</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea <sup>4</sup>Choong Ang Vaccine Laboratory Co., Ltd., Daejeon, 34055, Republic of Korea

# **Background and Objectives**

African swine fever virus (ASFV) is the causative agent of the highly lethal African swine fever disease that affects domestic pigs and wild boars. In spite of the rapid spread of the virus world-wide, there is no licensed vaccine available. The lack of a suitable cell line for ASFV propagation hinders the development of an effective vaccine. For ASFV propagation, primary macrophages and monocytes have been widely studied. Obtaining these cells can be time-consuming and expensive, making them unsuitable for mass vaccine production. The goal of this study was to validate the suitability of novel CA-CAS-01-A (CAS-01) cells, which was identified as a highly permissive cell clone for ASFV replication in the MA-104 parental cell line for live attenuated vaccine development.

# **Material and Methods**

MA-104 subpopulations were infected with ASFV for seven passages, and ASFV replication was evaluated by RT-PCR for ASFV DNA and TCID<sub>50</sub> assays. ASFV was passaged for 12 consecutive passages in CAS-01 cells, and infectivity, growth kinetics were evaluated. Moreover, cytopathic effect and hemadsorption properties were evaluated.

#### Results

Through a screening experiment, maximum ASFV replication was observed in a particular sub-clone compared to other sub-clones of MA-104 with 14.89 and log<sub>10</sub> 7.5±0.15 Ct value and TCID<sub>50</sub>/ml value, respectively. The selected sub-clone was denoted as CA-CAS-01-A (CAS-01) cell. CAS-01 cells were deposited in the Korean Collection for Type Cultures (KCTC) under accession no. KCTC 14568BP. Stable replication and adaptation of ASFV were observed over the serial passage. When CAS-01 cells are inoculated with ASFV, replication of ASFV was significantly enhanced after 4<sup>th</sup> passage, and it was confirmed via Ct value for ASFV DNA, HAD<sub>50</sub>/ml assay, TCID<sub>50</sub>/ml assay. Moreover, when infected with the adapted virus, cytopathic effects, and hemadsorption were observed similar to those in primary porcine alveolar macrophages.

# **Discussion and Conclusion**

Collectively, the CAS-01 cell line can be maintained in vaccine laboratories and exhibits numerous valuable properties for the replication and adaptation of ASFV. Therefore, CAS-01 cells will be invaluable for advancing our understanding of ASFV and developing technologies to combat it, such as live-attenuated vaccines. [National Research Foundation (2021R1A6A1A03045495) and Ministry of Environment (NIWDC-2021-SP-02)]

IMM – Immunology and Vaccinology

# ASSESSMENT OF THE SAFETY AND EFFICACY OF G2B TYPE LIVE ATTENUATED PED VACCINE IN THE KOREAN SWINE FARMS.

<u>G. Kim 1</u> 1DAESUNG MICROBIOLOGICAL LABS. CO., LTD.

#### **Background and Objectives**

Porcine epidemic diarrhea (hereafter referred to as PED) is a non-zoonotic viral disease characterized by watery diarrhea, dehydration and weight loss. More recently, the Genotype has been changed to G1a, G2a and G2b types, causing enormous economic damage in South Korean domestic pig industry. It is recommended that healthy pregnant sows get vaccinated at least twice to prevent PED in neonatal piglets. G2b type live attenuated vaccination is required to prevent PED caused by G2b type PEDV in a more efficient way.

# Material and Methods

The vaccine used in this study was manufactured in compliance with the KVGMP (Good Manufacturing Practice for Veterinary Pharmaceutical in Korea) standards. To assess the vaccine efficacy and safety, clinical trials were conducted at three pig farms in South Korea. Clinical trials were conducted by dividing pregnant sows into groups. The first dose was administered 4~5 weeks before farrowing and the second dose was administered 2 weeks after the first dose. 10 doses were administered to the Safety groups at once.

#### Results

Sows in Vac. and Safety group farrowed naturally with a successful lactation without any adverse effects of the vaccination. No clinical symptoms were observed in pregnant sows and neonatal piglets. In serological tests, there was no significant difference between the sows in Vac. and UnVac. groups before vaccination. When compared to sows in UnVac. group, SN titer, colostrum antibody titer of the sows in Vac. group were significantly higher at 2 weeks after vaccination and at farrowing time. In addition, when compared to the piglets in the UnVac. group, it was confirmed that the serum neutralizing antibody titer of the suckling piglets in the Vac. group were significantly higher until weaning.

# **Discussion and Conclusion**

The 'DS PED-Q Live PigVac.' was assessed as safe for pregnant sows. No clinical symptoms were observed and sows farrowed naturally. And, it has been proven to be effective by measuring the antibody titer. It was observed that antibodies were formed in pregnant sows, and maternal antibodies were successfully supplied to piglets. In conclusion, this G2b type live attenuated vaccination can prevent or reduce PED infection which causes economic damage not only in South Korean domestic pig industry, but also around the world.

IMM – Immunology and Vaccinology

# IMMUNE RESPONSE OF EXPERIMENTAL BACTERINS AGAINST STREPTOCOCCUS SUIS IN PIGS

<u>M. Gottschalk</u><sup>1</sup>, M. Segura<sup>1</sup> <sup>1</sup>University of Montreal, Canada

#### **Background and Objectives**

Streptococcus suis is one of the most important bacterial pathogen affecting nursery pigs and is also a zoonotic agent of critical concern, mainly in Asia. There is no commercial vaccine and the only alternative practitioners have is the use of autogenous vaccines (bacterins) based on the predominant strain(s) recovered from diseased pigs in the affected farm. The objectives of the current study were to evaluate the effect of following parameters on the immune response and protection induced by a S. suis bacterin: a) different adjuvants; b) the use of a concentrated bacterial supernatant added to the bacterin formulation; c) the use of a single strain vs the combination of several strains (belonging to different serotypes, a common practice in the field).

# **Material and Methods**

For all objectives the virulent S. suis serotype 2 P1/7 strain was used.

Objective 1: Similar six bacterins were produced. The only difference among them was the use of six different adjuvants. Objetive 2: The use of a concentrated bacterial supernatant added to the bacterin formulation was compared to the use of washed bacteria.

Objective 3: The use of a single strain vs the combination of several strains (belonging to different serotypes, a common practice in the field) was also compared.

For all experiments: piglets were vaccinated at 3 and 5 weeks of age and challenged at 7 weeks of age with the serotype 2 P1/7 strain by intraperitoneal route. Immune response and protection was evaluated.

#### Results

-Different immune response and protection was observed depending on the adjuvant used.

-Adding concentrated supernatant to the bacterin did not increase protection, even in the presence of higher levels of hemolysin (suilysin), previously described as being protective.

-A multivalent (five different serotypes) bacterin was as effective as a monovalent bacterin, suggesting that the use of multiple strains in a bacterin does not affect the protective capacity of the vaccine.

#### **Discussion and Conclusion**

In theory, producing S. suis autogenous vaccines (bacterins) is simple. However, there are so many ways to produce them that it is literally impossible to compare autogenous vaccines produced by different laboratories. A better standardization of bacterin production will benefit the swine industry.

IMM – Immunology and Vaccinology

# ANTIBIOTIC REDUCTION AND CONTROL OF GLäSSER'S DISEASE THROUGH VACCINATION

T.C. Gomes Dutra <sup>1</sup>, T. Gasparini <sup>1</sup>, <u>L. De Lucas</u> <sup>2</sup> <sup>1</sup>Hipra Saúde Animal, Ltda <sup>2</sup>HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

Glaesserella parasuis is known as the cause of Glässer's disease. Although serotypes 4 and 5 are the most frequently identified in Brazil, other serotypes have recently been increasingly associated with GD outbreaks possibly due to inappropriate antibiotic therapy which points towards immunoprophylaxis as the most assertive preventive measure against GD. The aim of this study was to evaluate the effect of vaccination with a vaccine containing serotype SV1 and SV-6 on the performance of nursery and finishing piglets on a farm with circulation of SV-5- SV-2 and SV-13 serotypes.

# **Material and Methods**

22,271 piglets were studied. Group 1 (n = 11,136) was not vaccinated. In group 2 (n = 11,135), piglets were vaccinated at 3 days of age and at weaning, and their mothers were vaccinated 6 weeks pre-farrowing and 21 days later. During the nursery and finishing periods, ADG, FCR, mortality and antibiotic treatments were recorded.

#### Results

The mortality rate in the nursery and fattening periods was significantly lower in G2 compared to G1 (0.65% vs 3.56%) and (0.15% vs 2.61%), respectively. In addition, no samples (0/16) from G2 piglets which died during this study were positive for G. parasuis and all samples (16/16) of G1 dead piglets were positive for G. parasuis. The amount of mg of antibiotic consumed per kg of pig in G2 was significantly lower than in G1 (317mg VS 428mg) and below the Brazilian average, which is 350 mg. Vaccinated animals showed better FCR and ADG in the nursery and fattening phases (449 g and 975 g, respectively) when compared to the G1 animals (399 g vs 970 g).

#### **Discussion and Conclusion**

These results demonstrate that the circulation of more than one serotype on the same farm may cause more clinical cases of GD and increase mortality and the use of antibiotics. In this study, the efficacy of vaccination against Glässer's disease to control the disease and to reduce antibiotic therapy in pig farming is also demonstrated.

#### IMM – Immunology and Vaccinology

# ASSESSMENT OF THE SAFETY AND EFFICACY OF THE 4 ML COMBINATION OF HIPRAMUNE® G ADJUVANTED VACCINES AGAINST SWINE ERYSIPELAS & PORCINE PARVOVIRUS AND BORDETELLA BRONCHISEPTICA & TOXIGENIC PASTEURELLA MULTOCIDA.

<u>A. Puigredon</u><sup>1</sup>, S. Cros<sup>1</sup>, J. Miguel<sup>1</sup>, I. Galé<sup>1</sup>, I. Ballarà-Rodriguez<sup>1</sup>, M. Simon-Grifé<sup>1</sup>, M. Fontseca<sup>1</sup>, J. Montané Giralt<sup>1</sup> <sup>1</sup>*HIPRA, Amer (Girona), Spain* 

#### **Background and Objectives**

The combined application of vaccines in swine provides many advantages, including an improvement in animal welfare and a reduction in labour time and costs. However, the in-use stability and effectiveness of the mixture needs to be considered. The aim of this study was to evaluate the safety and efficacy of the combined administration of two commercial vaccines.

#### **Material and Methods**

Thirty-seven gestating gilts were randomly distributed into three groups: Group A (n=13) was vaccinated with 4ml of the combination of ERYSENG®PARVO and RHINISENG®; Group B (n=12) was simultaneously vaccinated with 2ml of each vaccine, one on either side of the neck; Group C (n=12) was injected with 4ml of PBS. All the groups received two doses, at 6 and 3 weeks before farrowing. Rectal temperature and local reactions were checked at the time of vaccination, 6 hours post-vaccination, 24 hpv and 48 hpv after each dose. Reproductive data were collected and compared between groups. The serological response against Swine Erysipelas and Porcine Parvovirus was assessed at the administration of the first and second doses of the vaccines, and 1 week post-farrowing using two ELISA kits (CIVTEST® SUIS SE/MR and INGENASA PPV). Serum samples were also tested by a microagglutination assay used to quantify the antibody response against Bordetella Bronchiseptica.

#### Results

The administration of the vaccines, either alone or in combination, produced mild to moderate inflammation at the injection site. Moreover, they caused a transient increase in body temperature within the first 6 hpv, which spontaneously resolved within 24 hpv. No vaccine-associated systemic reactions were observed. Furthermore, no differences between groups were observed regarding reproductive performance. Regarding the humoral immune response, no differences were seen at any time point between GA and GB and both groups had a significantly increased antibody response (p<0.05) compared to GC.

#### **Discussion and Conclusion**

This study demonstrated that the combination of ERYSENG®PARVO and RHINISENG® was safe and produced an antibody response similar to their separate administration despite fewer injections being performed. This integrated approach represents a promising solution for optimizing vaccination protocols in terms of efficiency and resource management in the context of animal health.

IMM – Immunology and Vaccinology

# IMPACT ON PERFORMANCE OF IMMUNOLOGICAL BOOSTER VACCINATION WITH RESPISURE ONE® IN PIG FARMS IN BRAZIL

K.A. Franz<sup>2</sup>, <u>A. Aldaz<sup>1</sup></u>, D.C. Veit<sup>2</sup>, D.P. Tres<sup>2</sup>, V. Fernandes<sup>2</sup>, E.G. Franco<sup>2</sup> <sup>1</sup>Zoetis Inc, Parsippany, NJ, USA <sup>2</sup>Zoetis Inc.

#### **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is highly prevalent in pigs all over the world. It causes chronic respiratory disease that is often complicated with other bacterial and viral infections, resulting in suboptimal production performance and financial losses for pig producers. The objective of this study was to measure the impact of an additional booster vaccination against Mhyo on zootechnical parameters in the growing-finishing phase, in 3 different production systems.

# **Material and Methods**

The study was conducted in 3 large production systems (A, B and C) between 2020 and 2023. In addition to vaccination against Mhyo at 25 days of age with a single dose product, the treatment group received a second booster vaccination was carried out between 60 and 90 days of age. Zootechnical parameters and condemnation at slaughter were compared between the Treatment group (with booster) and Control group (only initial vaccination.

#### Results

In production system A, better results were observed in the Treatment group, with a reduction in feed conversion of 20 grams, 1.1 kg higher average slaughter weight, and a reduction of 11.5% in condemned carcasses for slaughter, being the control group favored by the higher mortality with a 10% reduction. In production system B, there was a 43% reduction in the incidence of Mhyo lung lesions at 150 days of life for the Treated group. In production system C, there was a 3% reduction in mortality, 50 grams of improvement in feed conversion, 22% savings in the cost of medicines, and 960-gram additional average slaughter weight, with a 59% reduction in carcass condemnations due to respiratory rate for the Treatment group compared to the control.

#### **Discussion and Conclusion**

Mhyo remain a challenge in the three production systems evaluated. Prevalence at weaning has a direct correlation with the economic impact during the finishing phase. It has been reported the effect of multiple vaccinations against Mhyo reduce the incidence and transmission. This study confirms that a booster vaccination between 60 and 90 days of age may reduce the negative impact of the agent in finishing pigs, helping to improve zootechnical performance, reducing the cost of medicines, and reducing the condemnation rates at slaughter.

IMM – Immunology and Vaccinology

# INTRADERMAL VACCINATION AGAINST LAWSONIA INTRACELLULARIS INCREASED PERFORMANCE AND REDUCED ENVIRONMENTAL IMPACT DUE TO ANTIBIOTIC USAGE AND EMISSIONS OF N, P AND CO2

<u>J. Kock</u><sup>1</sup>, C. Renken<sup>2</sup> <sup>1</sup>Practitioner, Tierarztpraxis OM GmbH, Vechta <sup>2</sup>Intervet Deutschland GmbH, MSD Animal Health, Unterschleißheim, Germany

#### **Background and Objectives**

Lawsonia intracellularis (Li) is present in most German pig farms and negatively influences health, performance, and resource-efficiency in pig production. In addition to economic threats, the demands from politics and society towards a more sustainable pork production are increasing. The goal of this study was to evaluate the effect of an intradermal Li vaccination on performance and economic parameters, as well as the environmental impact due to antibiotic usage, emission of N, P and CO<sub>2</sub>.

# **Material and Methods**

This case took place in a wean-to-finish pig farm in North-Western Germany with history of ileitis caused by Li. Piglets were vaccinated against PCV2, Mhyo and PRRSV (3 weeks of age, woa) and APP (7<sup>th</sup> and 11<sup>th</sup> woa). Performance data over two years (2020-2022) was recorded, i.e. feed conversion ratio (FCR), antibiotic usage. An historical control (3 fattening batches; oral vaccination against Li; n=9295) was compared to a subsequent period where pigs were vaccinated intradermally (ID) at 6 woa (3 fattening batches; intradermal vaccination; Porcilis®Lawsonia ID; n=9303). N and P excretion difference between groups was calculated with the official manual from the Lower Saxony Chamber of Agriculture using different standardized feed ratios by entering performance data the model. The carbon footprint (CO<sub>2</sub>-e) was calculated using the agricultural GHG Calculator "TEKLa" (based on a German-wide calculation standard) from the Lower Saxony Chamber of Agriculture.

#### Results

According to the vet's and farm records, lleitis-associated signs almost disappeared. Performance data showed an improved FCR in the intradermally vaccinated pigs (oral 2.65 vs ID 2.59) which means an economic benefit of 2.11  $\epsilon$ /pig produced (mean feed price mean 288  $\epsilon$ /t). Use of antibiotics due to clinical enteric disease was reduced (treatment days per pig: oral 4.4 vs. ID 1.1; -75%), likewise emission of N (oral -3.1% vs ID -3.4%), P (-3.2% to -3.7%) and CO<sub>2</sub> (-1.5%) were lowered.

#### **Discussion and Conclusion**

Under the conditions of this field observation, intradermal Li vaccination led besides the clinical and economic benefits to more resource-efficient results. This study suggests that Li vaccination may be a valuable and sustainable tool in modern pig production.

IMM – Immunology and Vaccinology

# AN ACTIVE SEROLOGICAL SURVEILLANCE OF CLASSICAL SWINE FEVER IN SWINE HERDS FROM MAY 2021 TO APRIL 2023 IN CHINA

<u>K. Wang</u><sup>1</sup>, Q. Hu<sup>1</sup>, J. Wu<sup>1</sup>, Y. Lu<sup>1</sup>, C. Mah<sup>2</sup> <sup>1</sup>Swine Business Unit, Zoetis China, Shanghai, P.R China, 200050 <sup>2</sup>Global Commercial Development Livestock, Zoetis Inc.

### **Background and Objectives**

Classical Swine Fever (CSF) is a highly contagious viral disease of worldwide important and one of the WOAH-listed diseases, CSF outbreaks in China has been decreasing over time in recent years. Under such situation, an active serological surveillance was conducted in CSF-vaccinated herds to understand better the immune response differences of the current various vaccination protocols in the sow herds and the wean-to-finish herds from May 2021 to April 2023.

# **Material and Methods**

A total of 16,913 serum samples were collected from 202 sow herds and 200 wean-to-finish pig herds in various production phases from 30 provinces in China. Among them, these sow herds were mass-vaccinated 2-4 timely annually, typically pre or after farrowing. A single and two shots of CSV vaccination protocols were used in 152 and 48 wean-to-finish pig herds respectively. All sera samples were tested for antibodies against CSF virus by commercial ELISA kits. The seroconverted results were analyzed by a box-and-whisker plot based on various vaccination protocols and production phases.

#### Results

Disregard of vaccination protocols, the CSF-seropositive rate of sow herds was already above 93% in the lower quartile and was above 95% in the median of box plot. For wean-to-finish herds with a single vaccine shot, the CSF-seropositive rate of herds in the lower quartile decreased over time from 80% at 2-week-old to the lowest at 20% at 8-week-old, then gradually increased to the peak level of 82% at 20-week-old; For herds using two shots of vaccine, the seropositive lower quartiles by production phases showed similar trends, but reached the lowest level at 20% earlier at 6-week-old and then gradually increased to the peak level of 68% at 20-week-old. Interestingly, the medians of box plot in the wean-to-finish herds with either one or two shots of vaccine were above 80% at 14 weeks of age and older.

#### **Discussion and Conclusion**

The results showed that the CSF vaccination for the sow herds in China were at high satisfactory levels. As for the weanto-finish herds, the single shot vaccination protocol can be successful if the vaccination timing is suitable. Further research should be conducted to understand the effect of CSF vaccines on immune dynamics in the field.

IMM – Immunology and Vaccinology

# CLINICAL EFFICACY OF AN AUTOGENOUS VACCINE FORMULATED WITH THREE CLINICAL STRAINS OF STREPTOCOCCUS SUIS SEROTYPE 9 SELECTED BY FLOW CYTOMETRY ANTIGENIC ASSAY

R. Oppitz Ferreira <sup>1</sup>, F. Zerbielli <sup>1</sup>, V. Maldaner <sup>1</sup>, D. Bordignon <sup>2</sup>, M. Gheller <sup>2</sup>, A. Spricigo <sup>2</sup>, A.P. Mori <sup>3</sup>, M. De Souza Costa Tamanini <sup>3</sup>, A. Holtz <sup>3</sup>, R. Petri <sup>3</sup>, D. Baldasso <sup>4</sup>, J.A. Guizzo <sup>4</sup>, L.C. Kreutz <sup>5</sup>, <u>R. Frandoloso <sup>5</sup></u>

<sup>1</sup>Laboratory of Microbiology and Advanced Immunology, University of Passo Fundo, Passo Fundo, Brazil <sup>2</sup>Cargill Alimentos, Campinas, Brazil

<sup>3</sup>Vaxxinova, Vargem Grande Paulista, Brazil

<sup>4</sup>AFK Imunotech, Passo Fundo, Brazil

<sup>5</sup>Laboratory of Microbiology and Advanced Immunology, University of Passo Fundo & AFK Imunotech, Passo Fundo, Brazil

### **Background and Objectives**

Streptococcus suis is a diverse pig pathogen comprising 29 different serotypes that are defined by the antigenicity of the polysaccharide capsule. In Brazil, the control of S. suis is carried out mainly by using autogenous vaccines which are only effective when the antigenic design is appropriate. The objective of this work was to demonstrate the effectiveness of an autogenous vaccine formulated with three clinical strains of S. suis serotype 9 selected using the flow cytometry antigenic assay.

#### **Material and Methods**

The study was conducted on a farm located in the west of Santa Catarina, Brazil, throughout 2023. The farm had a history of frequent outbreaks of arthritis and meningitis due to S. suis serotype 9 infection. From January to May, piglets were immunized with the Govaxx vaccine (Vaxxinova, Brazil) based on a single strain of S. suis serotype 9 (AFK-168/22 strain, VAC-A), and from June onwards, piglets began to receive the Govaxx vaccine based on three different strains of the S. suis serotype 9 (AFK-027/23, AFK-116/23 and AFK-141/23, VAC-B). Piglets were immunized at day (D) 21 and D35. Blood samples were collected at D21, D35 and D49 to assess the anti-S. suis serotype 9 IgG titers by indirect ELISA. The mortality rate was assessed over the first 10 months of the year. The experiment was approved by CEUA-AFK n<sup>o</sup> 022/2023.

#### Results

The VAC-A and VAC-B vaccines were immunogenic and 14 days after revaccination piglets showed 6.8-fold seroconversion (p=0.0012) in comparison to D35. The mortality observed in piglets immunized with VAC-A during the first 5 months of the year was not statistically different (p=0.1421) from the rates observed at the end of 2022 (pre-vaccination). In contrast, after one month of readjusting the antigenic composition of the vaccine (VAC-B), mortality rates due to S. suis were reduced by 43.28% in July, 57.97% in August, 42.13 % in September, and 50.31% in October.

## **Discussion and Conclusion**

We recently demonstrated that clinical strains of S. suis serotype 9 can display different antigenic profiles; here we proved that the success of an autogenous vaccine based on S. suis serotype 9 is dependent on the inclusion of all antigenic variants isolated from systemic sites of diseased animals.

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# AN INACTIVATED PCV2A/PCV2B/MYCOPLASMA HYOPNEUMONIAE VACCINE IS SAFE IN PREGNANT SOWS AND GILTS IN ALL STAGES OF GESTATION

C. Venegas-Vargas <sup>1</sup>, Y. Diamondidis <sup>1</sup>, L. Taylor <sup>1</sup>, M. Smutzer <sup>1</sup>, A. Heinz <sup>1</sup>, T. Hansen <sup>1</sup>, <u>M. Balasch</u><sup>2</sup>, M. Bandrick <sup>1</sup> <sup>1</sup>Zoetis Inc, Kalamazoo, MI. USA.

<sup>2</sup>Zoetis Manufacturing&Research Spain S.L.

#### **Background and Objectives**

Considering the benefits that gilt/sow porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhyo) vaccination can bring to the breeding herd in terms of reduction of PCV2 circulation and for homogenizing the herd immunity, it is key to demonstrate the safe use of PCV2/Mhyo vaccines when used in different stages of gestation. The goal of this study was to test field safety of two serials of CircoMax Myco®-like vaccine (containing inactivated PCV2a, PCV2b, and Mhyo) when administered to pregnant gilts and sows.

# **Material and Methods**

A total of 360 pregnant swine in one of the three gestational stages, were enrolled and administered a dose of CircoMax Myco<sup>®</sup>-like vaccine (IVP) or Control (CP) in two USA commercial farms. Within each site and gestation period, treatments were randomized to pigs using a generalized randomized block design. All sows/gilts were observed on day 0 (within 4 hours post-vaccination) and day 1 for local and systemic reactions. From day 2 until one-week post-farrowing all Adverse Health Events (AE) were recorded and after day 14 AE were recorded only if they required veterinary intervention and/or treatment. Piglets born to the enrolled sows/gilts were observed for one-week post-farrowing. Outcomes evaluated included: injection site reaction scores, if a sow ever had an AE, pregnancy and farrowing outcomes, and litter outcomes. Frequency summaries were generated and evaluated by treatment and by site and treatment.

#### Results

No IVP-related abnormalities were observed within 4 hours of treatment and (0) injection site reactions were never observed. None of the AE were attributed to the vaccine as determined by the study investigators. Four AE were determined as unknown, and unlikely related to IVP. Two of the four events occurred in control animals, therefore unrelated to the IVP. Greater than 90% of piglets per litter per group were normal each day observed. Reproductive outcomes and litter performance were not biologically different among IVP and CP treated animals and were within site historic and average industry benchmarks for USA pork production.

#### **Discussion and Conclusion**

These data confirm the safety of CircoMax Myco<sup>®</sup> when administered as a single 2 mL dose IM to pregnant swine across all gestation stages, under field conditions.

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# CHARACTERIZATION OF THE HOMOLOGOUS AND HETEROLOGOUS PCV2 ANTIBODY NEUTRALIZING ACTIVITY IN VACCINATED ANIMALS IN A PCV2D/PRRSV CHALLENGE MODEL

M. Kroeger 1, E. Fano 2, A. Sponheim 3, P. Pineyro 4

<sup>1</sup>Iowa State University

<sup>2</sup>Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA; Pipestone Veterinary Services, Pipestone, MN, USA <sup>3</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA

<sup>4</sup>Department of Veterinary Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

# **Background and Objectives**

PCV2 is an economically significant, highly prevalent pathogen and is the etiological agent of porcine circovirus-associated disease (PCVAD). Vaccination does not prevent viral replication and infection, potentially leading to gaps in immunity caused by heterologous infection and coinfection. While cross-protection amongst different PCV2 subtypes has been demonstrated, questions remain regarding the full immunological efficacy of commercial vaccines against homologous and heterologous challenges. Therefore, this study aims to assess the serum neutralizing activity in PCV2 vaccinated animals followed by homologous and heterologous infectious challenges.

#### **Material and Methods**

On study day 0 (D0), 60 three-week-old commercial pigs were vaccinated with Ingelvac PRRSV MLV. Additionally, pigs received a PCV2a vaccine (n=20), PCV2d vaccine (n=20), or no PCV2 vaccine (n=20). On D28, all 60 pigs were inoculated with 1 mL IM and 1 mL IN of PCV2d (5 log10/2ml dose) and 2 mL IM of 1-7-4 PRRSV (4.0 TCID<sub>50</sub>/mL). Mortality was recorded throughout the study and all remaining pigs were euthanized on D56. Serum samples were collected on D0, 28, 35, 42, 49, and D56 and were analyzed for viremia, total IgG (ELISA), and neutralizing antibodies (NA) were evaluated in virus neutralizing assays (VNA) using eight PCV1-2 chimeric viruses representing a, b, and d subtypes.

#### Results

The mortality rate was significantly higher in the nonvaccinates (60%) compared to the vaccinates (10%). PCV2d vaccinates had significantly lower viremia from D35-56 compared to the PCV2a vaccinates. Both vaccinated groups had similar total antibody levels throughout the study duration and the nonvaccinates had a delayed response following challenge. On D56, all groups had similar NA titers against the PCV1-2d chimeric challenge strain by VNA. On D56, PCV2d vaccinates had significantly higher NA titers than PCV2a vaccinates against three PCV1-2d chimeric constructs by VNA. Furthermore, the PCV2a vaccinates had significantly higher NA titers against two PCV1-2a and two PCV1-2b chimeric constructs by VNA.

#### **Discussion and Conclusion**

Differential subtype-dependent NA activity was observed following vaccination and homologous/heterologous challenge. Virus neutralization assays may be a stronger indicator of vaccine protection as compared to total antibody methods. Further studies should assess the cross-neutralizing antibody dynamics following vaccination in the absence of challenge and coinfection.

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# COMPARISON OF TWO VACCINATION PROTOCOLS AGAINST AUJESZKY'S DISEASE IN CHINA

<u>W. Fei</u><sup>1</sup>, T. Yang <sup>1</sup>, D. Angelats <sup>2</sup>, S. Romero <sup>2</sup>, M. Hsun Wu <sup>2</sup>, W. Pan <sup>3</sup> <sup>1</sup>HIPRA China, Beijing <sup>2</sup>HIPRA, Amer (Girona), Spain <sup>3</sup>Guangxi Xionggui, Nanning, Guangxi, China

# **Background and Objectives**

Aujeszky's disease (AD), caused by the pseudorabies virus (PRV), has posed a significant threat to the Chinese swine industry. AD is characterized by severe neurological symptoms and high mortality rates in affected pig populations. Vaccination against AD has become a crucial control measure. Most of the vaccines against AD have deleted glycoprotein E (gE) to differentiate between vaccinated and naturally infected animals (gE+). By suppressing gE, the vaccines enable serological detection of infected animals, facilitating epidemiological monitoring and disease control. The aim of this study was to assess the comparison between 2 different protocols to control virus circulation.

#### **Material and Methods**

Trial was done on a Chinese farm with 8623 sows and 889 gilts divided into 4 different production lines (PL). This farm had a vaccination protocol consisting of 4 mass vaccinations of sows/year with AUSKIPRA®GN (AD live vaccine with deleted gE, HIPRA). 166 blood samples (60, 11, 40 and 55 from PL 1, 2, 3 and 4 respectively) were collected at the end of February2023 from sows with different parities to check the prevalence of gE with ELISA (CIVTEST® SUIS ADVgE, HIPRA). In March2023, the ADV vaccination protocol was changed (not the vaccine) to 3 mass vaccinations/year and an additional cycle vaccination 40 days before farrowing. 6 months later, in September2023, 224 blood samples (100, 40, 40 and 44 from PL 1, 2, 3 and 4 respectively) from sows with different parities were collected to perform the ELISA gE test.

#### Results

At the end of February2023, the gE-positivity rate was 20.5% (34+/166) and in September2023 the gE-positivity rate was 12.5% (28+/224). A logistic regression was performed, considering parity and line as random effects. The results demonstrated a statistically significant reduction (p-value=0.01) in the prevalence of gE-positive animals following 3 mass vaccinations/year and an additional administration of AD vaccine 40 days before farrowing.

# **Discussion and Conclusion**

Choosing a vaccine with proven efficacy is essential, a well-designed vaccination protocol is equally critical. In this trial, higher vaccination pressure helps to reduce the circulation of the field virus, which in this study has been evaluated through gE seropositivity.

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# EFFICACY OF THE ERYSIPELAS VACCINE SYVAC ERY UNDER FIELD CONDITIONS

<u>M.L. De Arriba</u><sup>1</sup>, C. Fernandez<sup>1</sup>, C. Casal<sup>2</sup>, A. Aguaron<sup>2</sup>, E. Roca<sup>1</sup>, L. Delgado<sup>2</sup> <sup>1</sup>Laboratorios Syva <sup>2</sup>Laboratorios Svya

#### **Background and Objectives**

Swine erysipelas, when uncontrolled, has a significant economical impact in porcine production. Laboratorios Syva is the Marketing Authorisation Holder of the vaccine Syvac Ery, an erysipelas vaccine that includes inactivated Erysipelothrix rhusiopathiae as active substance and mineral oil as adjuvant. The efficacy of the vaccine was assessed under field conditions and compared to a positive control vaccine based on aluminium and ginseng adjuvants.

# Material and Methods

The study was designed as a positive controlled multicentric study to investigate the efficacy of the vaccine in 12-week-old pigs under commercial conditions. It was carried out in three farms located in Spain, with a history of erysipelas infection and involved 555 healthy pigs monitored from vaccination to finishing. Animals were distributed in two vaccinated groups (n=240) and a sentinel group (n=75, non-vaccinated). Assessment of efficacy was based on the proportion of animals showing typical skin lesions caused by E. rhusiopathiae and as secondary parameters the serology and the growth performance.

#### Results

No rhomboidal erythema skin lesions were detected throughout the study. The only clinical signs compatible with erysipelas infection were cases of lameness, similar in all groups, and for which E. rhusiopathiae was excluded as etiological agent. There were no differences between vaccinated groups neither on the weights nor on the growth performance. However, a significant higher antibody response (p<0.05) was detected in the group vaccinated with Syva's vaccine when compared to the positive control group. The number of seropositive animals was significantly higher in this group on days post vaccination 27, 69, 78 and 91, being day 49 the only point in which differences were not significant. Antibody titres were also significantly higher during all timepoints.

#### **Discussion and Conclusion**

The use of erysipelas vaccines, administered to 86% of the piglets in the study, prevented the appearance of the disease. In absence of the infection, the vaccine developed by Syva induced a better humoral response across (i.e. higher antibody response and more seropositive animals) than the positive control used.

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# ILEITIS VACCINATION: ASSESSMENT OF IMMUNE RESPONSE AND CLINICAL SIGNS IN PIGS CO-INFECTED WITH LAWSONIA INTRACELLULARIS AND BRACHYSPIRA HYODYSENTERIAE

S. Chagas 1, P. Jensen 2, E. Paladino 3, L. Mendonça Pascoal 4, S. Von Berg 5, C. Gebhart 6, F. Vannucci 7 <sup>1</sup>PhD student at Universidade Federal de Goiás and University of Minnesota

<sup>2</sup>University of Minnesota - College of Veterinary Medicine

<sup>3</sup>University of Minnesota

<sup>4</sup>Escola de Veterinária e Zootecnia, Universidade Federal de Goiás

<sup>5</sup>MSD Animal Health, Munich, Germany

<sup>e</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

7Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA

# Background and Objectives

Lawsonia intracellularis (LI) and Brachyspira hyodysenteriae (Bhyo) co-infections are reported in the field, but with a lack of information about their interaction. This study was conducted to assess the level of infection and immune response (local and systemic) in pigs vaccinated for LI and co-infected with LI and Bhyo.

# Material and Methods

Pigs were distributed into 5 groups: vaccinated and co-infected with LI and Bhyo (V-CO, n=21), non-vaccinated and co-infected with LI and Bhyo (NV-CO, n=18), vaccinated and infected with LI (V-L n=21), non-vaccinated and infected with LI (NV-L, n=12) and non-vaccinated and non-challenged (NC, n=8). The vaccine used was Porcillis® lleitis (MSD Animal Health). Animals were challenged with LI 22 days after vaccination (0dpi) and with Bhyo 29 days after vaccination. Parameters analyzed: fecal score (1=normal feces to 5=watery diarrhea), LI immunohistochemistry (IHC) of ileal fragments (0=negative to 4= >75 to 100%) of crypts infected and, serum IgG and ileal lavage IgA (Immunoperoxidase Monolayer Assay).

#### Results

Overall fecal score averages were V-CO=2.3, NV-CO=2.5, V-L=1.6, NV-L=1.9, NC=1.1. There was an increasing trend on the fecal scores of NV-CO and NV-L over time. V-L had an increasing trend from 7dpi to 11 dpi, followed by a decreasing trend from 16dpi to 21dpi. Regarding IHC, both vaccinated groups had lower IHQ scores on 14 (V-CO=0.5; V-L=0.67) and 21 dpi (V-CO=0.83; V-L=0.83) compared to non-vaccinated groups regardless of the infection type (14dpi NV-CO=2, NV-L=1.67; 21dpi NV-CO=2.5, NV-L=2.67). Serum and ileal lavage samples results are being analyzed and will be presented at IPVS 2024.

# **Discussion and Conclusion**

This represents the first study assessing the impact of LI vaccination in pigs experimentally infected with Bhyo. Co-infected animals showed higher fecal scores when compared to animals infected with LI only. Vaccinated groups had lower fecal score values than the corresponding non-vaccinated groups. Bhyo seems to worsen LI clinical signs, but the LI vaccination still was able to reduce clinical signs. IHC showed that the vaccine reduces LI gut infection in both co-infected and LI-infecteded groups. Overall, Bhyo and LI have a synergistic effect in terms of clinical signs and LI vaccine reduces LI gut colonization even in co-infected animals.

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# IMPACT OF VACCINATING SOWS FOR PORCINE CIRCOVIRUS TYPE 2 (PCV2) ON THE IMMUNITY AND REPRODUCTIVE PERFORMANCE OF THE HERD

A. De Paula Teixeira <sup>1</sup>, L. Fiorin Hernig <sup>1</sup>, E. De Conte <sup>1</sup>, <u>R. T. Lippke <sup>1</sup></u> <sup>1</sup>Boehringer Ingelheim Animal Health Brazil

#### **Background and Objectives**

Sow's vaccination for porcine circovirus type 2 (PCV2) can improve reproductive performance throughout the reproductive cycles, reducing losses. This study aimed to evaluate the effect of sow vaccination for PCV2 on their immunity and reproductive performance.

### Material and Methods

The longitudinal study was carried out on a commercial farm with 4,000 sows to evaluate the serologic status of PCV2 in the sows and the reproductive impact of PCV2 vaccination on them. On day zero (D0), blood was collected from 125 sows, one week postpartum. After collection, the vaccinated group (V) (n=65) received a single dose of commercial vaccine for PCV2 (Ingelvac Circoflex®), and the control group (n=61) was not vaccinated (NV). The parity age distribution was the same between treatments. The identification and quantification of immunoglobulin G (IgG) titers in the serum extracted from these samples were made using the ELISA test (BioChek) on D0 and on days D42, D91, D126, and D182 after vaccination. In addition, reproductive data from the sows were collected in the farrowing following vaccination. The means comparison was performed using the T-test.

#### Results

Vaccination of sows increased (p<0.01) anti-PCV2 IgG levels to levels higher than those of the unvaccinated group over the six months of evaluation. On D0, antibody levels were similar (p=0.48) between groups (V=1.58 and NV=1.65 S/P). At 42 days after vaccination, the highest antibody titers (V=2.87 and NV=2.33 S/P) were observed in this group, which remained higher than the unvaccinated group until D182 (V=2.19 and NV=1.94 S/P). Sows in the vaccinated group had an average of 1.02 piglets born (V=15.1 and NV=14) and 0.84 piglets born alive (V=13.4 and NV=12.6) more than the unvaccinated group and a lower percentage of mummified piglets (V=1.9% and NV=2.3%). There was no statistical difference despite reproductive results between groups.

# **Discussion and Conclusion**

Sow's vaccination led to an improvement in the immunity against PCV2, keeping anti-PCV2 antibody levels high throughout the six months of evaluation. Vaccinated sows had an average of 1.02 and 0.84 total born and born live, respectively, more than the unvaccinated group. Mass vaccination, followed by one vaccination in each reproductive cycle, is recommended for a herd approach.

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# MAT-SEROCONVERSION OF SOWS AFTER BASIC IMMUNIZATION AGAINST DIFFERENT LEPTOSPIRA SEROVARS

<u>S. Mascher</u><sup>1</sup>, S. Mück<sup>1</sup>, P. Astrid<sup>2</sup>, A. Ullerich<sup>3</sup>, K. Strutzberg-Minder<sup>4</sup> <sup>1</sup>Veterinary practice Tierärzte Team Tiefenbach GmbH <sup>2</sup>MSD Animal Health <sup>3</sup>IVD GmbH, Innovative Veterinary Diagnostics, Seelze, Germany

<sup>4</sup> IVD GmbH, Innovative Veterinary Diagnostics, Seelze, Germany

# **Background and Objectives**

In case of fertility problems due to suspected Leptospiral infections, Microscopic Agglutination Test (MAT) is a standard diagnostic tool to determine infection in a sow herd but without the possibility to differentiate between infected and vaccinated animals<sup>1</sup>. Single, low Bratislava titers are not unusual in unvaccinated German sow herds and not necessarily connected to clinical problems, while other serovars can rise to remarkable heights<sup>2</sup> following an infection. After introduction of a commercial vaccine in 2018 the interpretation of MAT-results for different serovars is challenging especially after vaccination. This study aims to show courses of MAT-seroconversion after basic immunization in two commercial farms to support field vets in drawing the right conclusions from laboratory results.

# **Material and Methods**

Breeding sows of two commercial piglet producing farms in Southern Germany were immunized twice with Porcilis<sup>®</sup> Ery+Parvo+Lepto. During the following six months before the next booster vaccination, every four weeks blood samples (12 vaccinated; 6 non-vaccinated sows) were taken to determine MAT titers against various Leptospira serovars.

#### Results

Pace, extent, and duration of MAT titers differed between serovars, with the maximum consistently reached four weeks after the second vaccination. Courses of seroconversion induced by the serovar Bratislava occurred in all samples with the highest titers as opposed to Pomona with a few low titers. MAT-Titers of other investigated Serovars were between Bratislava and Pomona. Apart from the Australis serogroup (serovars Bratislava and Australis), all other MAT titers were almost back to the baseline level two months after the second vaccination.

#### **Discussion and Conclusion**

Data from this study indicate that MAT-results from blood samples taken more than 8 weeks after basic immunization can be interpreted like results from unvaccinated herds. As the Australis serogroup i.e. the serovar Bratislava is endemic in Germany and field contacts are present seroconversion after Bratislava-vaccination can in many farms be interpreted as a booster effect of a former field infection. In contrast, the serovar Pomona is rarely diagnosed in Germany and Pomona-MAT-seroconversion after vaccination stays low and short. Though seroconversion is not connected to protection neither in vaccinated nor in unvaccinated herds the results of this study can help field practitioners in interpreting MAT lab results.

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# PIGS VACCINATED WITH A PRRSV1 MLV DEVELOPED POTENT IMMUNITY AGAINST JAPANESE PRRSV2 STRAINS

S. Romero <sup>1</sup>, J. Miranda <sup>1</sup>, M. Fenech <sup>1</sup>, M. Shibata <sup>2</sup>, <u>I. Ballarà</u> <sup>1</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA Japan, Tokyo

### **Background and Objectives**

PRRS is one of the most economically significant diseases in the swine industry. PRRS Modified live vaccines (MLV) are a reasonable choice for pig immunization. Cell-mediated immunity (CMI) after MLV vaccination is responsible for limiting the duration of viraemia and the spread of the virus. For PRRS, humoral immunity provided by neutralizing antibodies (NA) is developed slowly and do not reach high titres. Evaluation of CMI and NA provided by MLV PRRS vaccines is important to assess protection against field strains. The objective of this trial was to determine the humoral (NA) and cellular (CMI) immunity conferred by a PRRSV1 MLV against Japanese PRRSV2.

#### **Material and Methods**

10 naïve pigs were vaccinated with UNISTRAIN® PRRS (PRRSV1 MLV, HIPRA) at 2 weeks of age. Peripheral blood mononuclear cells (PBMC) and serum were collected 4 weeks after vaccination. CMI and NA were evaluated against 7 Japanese PRRSV2 (Nagasaki11-14, P192-5, EDRD1, PRRSKU-27-156K, 338, 345, 348). CMI was evaluated by IFN-ã-SC using ELISPOT assay. Humoral immunity was measured by virus neutralization assay. VP046 (vaccine virus) was included in the panel of strains tested and its stimulation acts as the comparative response to the Japanese strains by means of Kruskal-Wallis test (Non-parametric).

#### Results

Animals vaccinated developed IFN-ā-SC response against the Japanese PRRSV2 analyzed. This response was comparable to the response against vaccine virus (except Nagasaki11-14) demonstrating an equal cellular activation. Moreover, between 70-100% of the vaccinated animals responded when stimulated with each virus strain. Vaccinated animals produced NA against 3 Japanese strains (Nagasaki11-14, EDRD1 and 345). However, although CMI provided by these strains was remarkable, results of NA of strains P192-5, PRRSKU-27-156K, 338 and 348 were not conclusive.

### **Discussion and Conclusion**

PRRS NA production after a single dose of any MLV is limited, developed slowly and doesn't reach high titres. Thus, the CMI generated by a PRRS vaccine plays an important role in protection against the challenge. These results confirms that UNISTRAIN® PRRS can confer NA against 40% of the strains tested. Regarding CMI, this vaccine induced potent CMI against almost all the strains tested. Consequently, this PRRSV1 MLV is a good choice to control Japanese PRRSV2.

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# VACCINATION WITH A PRRSV MLV IN SOWS INDUCES NEUTRALIZING ANTIBODIES AGAINST PANEL OF CONTEMPORARY FIELD STRAINS

L. Aguirre <sup>1</sup>, H. Clilverd <sup>1</sup>, J. Miranda <sup>2</sup>, <u>I. Ballarà <sup>2</sup></u>, M. Martín <sup>1</sup>, E. Mateu <sup>1</sup>

<sup>1</sup>Department de Sanitat i Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain <sup>2</sup>HIPRA. Amer (Girona). Spain

# **Background and Objectives**

The lack of cross-protection is one of the major obstacles when it comes to immunizing against PRRSV. The genetic variability of the virus, along with the complexity of the immune response and the variability of the response depending on the host, are factors that contribute to the difficulty of immunization against current field strains. The present study aimed to assess the capability of neutralizing diverse PRRSV-1 contemporary strains in sows vaccinated with a PRRSV-1 MLV.

### **Material and Methods**

For this purpose, 15 sows at 45 days of gestation were used. Sows were vaccinated with Unistrain® PRRS by mass administration every 4 months. Serum samples were obtained 3 months after the last vaccination, and then after 21, 54 and 75 days. Sera were analyzed by RT-qPCR, ELISA (pigtype PRRSV Ab - INDICAL) and viral neutralization test against the homologous strain (Unistrain® PRRS) and a panel of 5 strains: one was isolated in 2006 from serum of a boar from a Portuguese farm (strain 3267), thus contemporary of the vaccine strain, and the other four were field strains isolated in 2022 (strain A), 2018 (strain B) and 2019 (strain C and D) from sera of piglets from Catalonian farms.

#### Results

All animals were negative by RT-qPCR at all examined timepoints. Regarding ELISA results, all sows were seropositive throughout the sampling period (range of S/P values: 0.94 - 3.74). At the first sampling, all sows had neutralizing antibodies against the vaccine strain (mean log<sub>2</sub> of  $6.2\pm1.9$ ) that did not significantly increase (mean log<sub>2</sub> titer of  $6.8\pm1.0$  at day 75). Titers of neutralizing antibodies against strain 3267 and contemporary field strain B were similar, with mean log<sub>2</sub> titers at first sampling of  $5.4\pm2.0$  against strain 3267 an of  $4.4\pm2.3$  against strain B, with no significant differences throughout the study. Regarding the neutralization of the other field strains, titers were lower (mean log<sub>2</sub> titers at first sampling of  $3.0\pm2.0$ ,  $3.0\pm2.1$  and  $2.3\pm2.1$  for strain A, C and D, respectively) and did not show a significant increase.

### **Discussion and Conclusion**

In this study, sows vaccinated with Unistrain® PRRS presented broad neutralizing activity in vitro, especially against the homologous strain and two of the field strains analyzed.

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# VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE ENCAPSULED IN SBA-15 NANOPARTICLES REDUCED LUNG LESIONS IN PIGLETS

F.M. Petri <sup>1</sup>, C.S. Malcher <sup>1</sup>, M.L. Mechler-Dreibi <sup>1</sup>, E.R. Braga <sup>1</sup>, A.K. Panneitz <sup>1</sup>, G.A. Aguiar <sup>1</sup>, T. Da Silva Martins <sup>2</sup>, M. Carvalho De Abreu Fantini <sup>3</sup>, O.A. Sant'Anna <sup>4</sup>, H.J. Montassier <sup>1</sup>, <u>L.G. De Oliveira <sup>1</sup></u> <sup>1</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil <sup>2</sup>Department of Chemistry, Federal University of São Paulo (UNIFESP), Diadema, SP, Brazil <sup>3</sup>University of São Paulo (USP), Physics Institute, São Paulo, Brazil. <sup>4</sup>Butantan Institute, São Paulo, Brazil.

# **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo), causes porcine enzootic pneumonia (PEP), leading to substantial losses in global swine farming. Experimental nanotechnology vaccines have shown very promising results. In this study, a single-dose intramuscular vaccine, encapsulating Mhyo proteins in SBA-15 silica nanoparticles, was developed to immunize piglets against PEP.

#### Material and Methods

At 21 days of age, 39 piglets were housed in the Swine Medicine Laboratory-UNESP. On day 24 (D0), they were immunized or not and divided into G1 (commercial vaccine), G2 (SBA-15 vaccine), and G3 (sterilized saline–control). On D21, challenged with 10<sup>6</sup> CCU of Mhyo. Serum, nasal, and laryngeal swab samples were collected from D7 to D56 for anti-Mhyo antibody quantification (ELISA) and bacterial detection (qPCR). Lung lesions were evaluated macroscopically and microscopically after necropsy on D56. Statistical analyses used GraphPad Prism 10.

#### Results

Median macroscopic lung lesion scores were: G1 (7.1%), G2 (10.08%), and G3 (23.37%). Group comparisons revealed significant differences in total lung lesion area related to Mhyo, notably G1 vs G3 (p=0.01) and G2 vs G3 (p=0.01). Histopathology revealed bronchus-associated lymphoid tissue (BALT) lesions (0-4), significant in G1 vs G3 (p=0.01) and G2 vs G3 (p=0.04). Immunized groups showed anti-Mhyo IgG by D14 and IgA from D28; control group turned positive on D49. Mucosal response, IgA, was detected from D21 in G1 and G2. qPCR results showed varied shedding among vaccinated groups, G1 on D28 and D35, and G2 on D28, with lower bacterial loads and shorter shedding periods compared to the control group.

#### **Discussion and Conclusion**

The results reveal a significant reduction in lung lesions in vaccinated groups, indicating the vaccine's potential against Mhyo-induced lesions. Histopathological analysis reveals specific BALT lesions, with notable differences between the vaccinated and control group. The serological response shows anti-M. hyo IgG and IgA responses, emphasizing the humoral response. Nasal swab IgA detection suggests a role in preventing Mhyo adherence. qPCR results show varied shedding among vaccinated groups, hinting at the vaccine's potential influence on elimination dynamics. In summary, the SBA-15 vaccine holds promise in reducing lesions, modulating the immune response, and offering effective strategies against PEP. Grant#2021/11914-0 and Grant#2021/14515-9, São Paulo Research Foundation (FAPESP).

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#### CAN IMMUNOCRIT BE USED AS A MONITORING TECHNIQUE FOR VACCINATION AND INFECTION STUDIES?

M. Sagrera 1, M. Sibila 2, A. Llorens 3, J. Pastor 4, L. Garza 5, D. Espigares 5, J. Segalés 6

<sup>1</sup>IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), 08193 Bellaterra, Spain / Ceva Salud Animal, Barcelona, Spain

<sup>2</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain.

<sup>3</sup>IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>4</sup>Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona (UAB), Barcelona 08193, Spain

<sup>5</sup>Ceva Salud Animal, Barcelona, Spain

<sup>e</sup>Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), and Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona,

# **Background and Objectives**

The immunocrit is a cost-effective and straightforward technique traditionally used to assess passive immunity transfer in newborn piglets. However, it has not been previously used for monitoring the effect of vaccination and/or infections. Therefore, this study aimed to assess the immunocrit values of pigs at weaning and evaluate their evolution post-vaccination and subsequent viral challenge under experimental settings, using porcine circovirus 2 (PCV-2) as pathogen model.

#### **Material and Methods**

Serum samples (n=204) were obtained from a PCV-2 experimental study, with six groups of 12 animals each (H-V, M-V, L-V, H-NV, M-NV, and L-NV), classified by their PCV-2 IgG ELISA S/P ratio (high [H], medium [M] or low [L]) preimmunization and vaccination status (V or NV). Piglets were vaccinated at study day 0 (SD0; 3 weeks of age [woa]), challenged with PCV2b at SD21 (6 woa) and necropsied at SD42 (9 woa). Sera from all pigs at each time-point were tested for PCV-2 antibodies, immunocrit ratio (determined using 40% ammonium sulfate in microhaematocrit capillary tubes), and total protein (TP, measured by a refractometer). In addition, a proteinogram (including TP, albumin, and á-1, á-2, â, and ã-globulins concentrations) was performed on a subsample (n=42).

#### Results

PCV-2 antibodies showed (although with different S/P values between groups at the different time points) a descending dynamic from SD0 to SD21 and an ascending one from SD21 to SD42 in all groups except H-NV (which decreased from SD0 to SD42). Similar dynamics were observed with immunocrit and ā-globulin detection, but with no differences among groups. Refractometer readings were fairly constant, but followed comparable dynamics in H-V, L-V and M-NV groups, and a mild ascending pattern from SD0 to SD42 for the rest. Linear correlations between techniques were low to moderate, being the most robust ones found between immunocrit and refractometer (r=0.41) and immunocrit vs ā-globulins (r=0.39).

#### **Discussion and Conclusion**

Our results suggest that immunocrit allows monitoring pigs under vaccination and/or infection scenarios. Although variation in postweaning pigs was subtle, it decreased slightly after SD0 and increased after challenge (SD21), in a similar dynamic to that of PCV-2 ELISA S/P values.

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# CASE REPORT: EFFECT OF EARLY PRRSV VACCINATION IN PIGLETS

<u>G. Abella Falcó</u><sup>1</sup> <sup>1</sup>Boehringer Ingelheim

#### **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most damaging diseases in the swine industry, having negative effects typically affecting breeding herd reproductive parameters as well as pig productivity parameters. The implementation of sows and piglet vaccination has had a significant positive impact on reproductive and productive parameters. This study analyzes the evolution of productive parameters after a change in the control program through the application of early piglet vaccination after a PRRSV outbreak.

### **Material and Methods**

Located in the northeast region of Spain, the 1150 lberian sow farm produces 18kg piglets. The farm was PRRSv positive, confirmed by PCR in tongue tips, the sows were vaccinated on a quarterly basis. In May 2022 there was a PRRSv outbreak, affecting reproductive and productive parameters. The detected strain was a high pathogenic PRRSv strain. In November 2022, the farm started a double sow mass vaccination (Reprocyc PRRS EU<sup>®</sup>). In January 2023 early vaccination of piglets was implemented by administration of 1ml IM of Ingelvac PRRSFLEX EU<sup>®</sup> during the first week of life. Production data was recorded in a weekly basis: Total born alive piglets/farrow (NBA), number weaned piglet per sow (WP/sow), number losses per week in farrowing unit (Loss/S1) and nursery (Loss/S2). Two different periods were analyzed 2022 (control) vs. 2023 (vaccine). For the statistical analysis Software R was used.

#### Results

Results of the main productive parameters before and after the early piglet vaccination implementation are the following: 0,41more piglets/sow were weaned after the piglet vaccination was implemented. And reduction of 10,62 piglets lost/week was also observed in this period. These results show a general improvement in the production data analyzed. Weaned piglets/sow increased and mortality was also reduced in nursery.

### **Discussion and Conclusion**

The setup of early prrs vaccination in piglets, together with sow vaccination, helped not only to increase the number of weaned piglets but also to reduce the number of losses per week in the nursery after a severe PRRS outbreak. When the farm recovers the Positive stable status, the recommendation would be to vaccinate the piglets at weaning, following the producer indications

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# CASE STUDY: LOWER PRRSV CIRCULATION IN A SIDE BY SIDE EVALUATION USING PRRS MLV VACCINATION GIVEN AT AN AGE DIFFERENT TO THE LABEL

<u>K. Koenders - Van Gog</u><sup>1</sup>, K. Vandamme<sup>2</sup>, B. Garcia-Morante<sup>3</sup>, M. Steenaert<sup>4</sup> <sup>1</sup>Lintjeshof Veterinary Practice and Merefelt Livestock Diagnostics

<sup>2</sup>Lintieshof Vet Group. The Netherlands

<sup>3</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain. <sup>4</sup>Boehringer Ingelheim Animal Health Netherlands bv

#### **Background and Objectives**

On a 3000 head sow farm (farrow to grow), 8 weekly batches piglets were vaccinated PRRS MLV and compared to non-vaccinated pigs. The objective was to measure PRRS MLV vaccination efficacy to decide to vaccinate all piglets for a prolonged period.

## Material and Methods

All pigs were housed in the same building, production phases separated by hygiene locks.Per weekly batch a subgroup was PRRS MLV vaccinated (Ingelvac PRRSFLEX®) (Vx) at processing 3-5 days of age, versus non-vaccinated (Con).Every weekly batch was followed up taking serum samples (n=800), piglets from gilts (PG) and piglets from sows (PS). Due To Weaning (DTW) 30 Vx + 30 Con (10 PG and 20 PS per group). End Of Nursery (EON) 20 Vx + 20 Con (10 PG and 10 PS per group). PRRS rt-PCR, pooled 1:5.We identified groups CT-low (CT<23; 0.25 percentile value) and CT-high (CT>28; 0.75 percentile value). Additionally, we identified weekly batches that contained at least one CT-low result.

#### Results

DTW 14/96 pools PRRS-PCR positive. DTW pools CT-high Vx 5/48, Con 2/48.

EON 40/64 pools PRRS-PCR positive. EON pools Vx CT-high 3/32, Con 1/32. EON PG pools Vx 5/16 CT-low positive, Con 10/16 (p<0.10).EON Vx 2/8 batches had at least one CT-low pool, versus Con 5/8.Post weaning mortality in both groups was low and not significantly different. No differences in clinical evaluation between both groups were observed.

### **Discussion and Conclusion**

After weaning infectious pressure increased in both groups. 1/8 batches tested PRRS PCR negative from birth to end of nursery suggesting PRRS containment within batches is possible. PRRS CT-value indicates viral load: higher CT value, lower viral load. And vice-versa. EON PG there was a statistical trend of viral load being lower in Vx versus Con. EON the viral load in Vx was lower versus Con. EON Vx less **batches** contained high viral load pools, versus Con. The findings suggest PRRS MLV vaccine efficacy as described in the SPC, even when vaccinated in the first week of life.We conclude: 1) Strict biosecurity is important to contain virus within subpopulations. 2) PRRSFLEX vaccination at processing was able to reduce the viral load in the vaccinated animals, which adds to the control of PRRS infections.

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# COMPARISON OF THE PROTECTION AGAINST PCV2 EXPERIMENTAL INFECTION WITH INTRADERMAL OR INTRAMUSCULAR PCV2/M.HYO VACCINES IN THE PRESENCE OF MATERNALLY-DERIVED ANTIBODIES.

C. Pedernera Romano <sup>1</sup>, M. Simon-Grifé <sup>1</sup>, L. Acal <sup>1</sup>, <u>M. Roca</u> <sup>1</sup>, J. Soy <sup>1</sup>, A. Mayola <sup>1</sup>, A. Morral <sup>1</sup>, R. Serradesanferm <sup>1</sup>, R. March <sup>1</sup>, M. Sitjà-Arnau <sup>1</sup>, J. Montané Giralt <sup>1</sup> <sup>1</sup>*HIPRA. Amer (Girona). Spain* 

#### **Background and Objectives**

Porcine Circovirus type 2 (PCV2) is a worldwide, potentially severe loss-causing pathogen. Due to the ubiquitous nature of PCV2 in the pig population, most dams have been exposed to the field virus or vaccinated, resulting in piglets with varying levels of passively acquired PCV2 maternal antibodies. The aim of this study was to evaluate the efficacy of two vaccination protocols against PCV2 in the presence of high levels of maternally-derived antibodies (MDA).

# **Material and Methods**

Three-week old piglets with high levels of MDA (mean S/P ratio = 1.9 [BioCheck PCV2 Antibody Test kit]) against PCV2 were randomly distributed into three groups (15 piglets/group). Group A (GA) was vaccinated with MHYOSPHERE® PCV ID (0.2 ml / dose) using Hipradermic®, Group B (GB) was vaccinated with an intramuscular PCV2/M.hyo vaccine (2 ml / dose). A group of non-vaccinated piglets served as controls (GC). All the piglets were challenged at 13 weeks of age with a PCV2b strain. Serum samples and nasal swabs were collected weekly for determination by qPCR (Vetmax<sup>TM</sup>) of viraemia and nasal shedding respectively. At 28 days post-infection all the pigs were euthanized, and mesenteric and inguinal lymph nodes, tonsils and lungs were collected for PCV2 quantification. Viraemia and nasal shedding were analysed using the area under the curve (AUC), as an indicator of the viral load over the study period.

#### Results

The mean AUC in serum was significantly lower (p<0.05) in GA (19.9) and GB (15.5) compared to GC (55.7). Vaccination also resulted in a significantly lower AUC (p<0.05) for nasal shedding in GA (18) and GB (24) than in GC (63.7). The mean PCV2 tissue load ( $log_{10}$  genomic copies/ml) was significantly lower (p<0.05) in the vaccinated groups than in the control group (GA; GB vs GC) in mesenteric lymph nodes (3.4; 3.1 vs 5.2), inguinal lymph nodes (3.2; 2.5 vs 5.2), tonsils (3.4; 3.0 vs 5.5) and lungs (4.5; 4.1 vs 5.7).

#### **Discussion and Conclusion**

The results of the present study demonstrated that MHYOSPHERE® PCV ID provides PCV2 protection such as the intramuscular vaccine tested, even when used in pigs with high MDA at the time of vaccination.

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# EFFECT OF ENTERISOL ILEITIS® ON THE CONTROL OF SALMONELLA INFECTION IN FATTENING PIGS UNDER FIELD CONDITIONS.

<u>V. Rodriguez Vega</u><sup>1</sup>, F. Gonzalvo<sup>1</sup>, S. Figueras<sup>1</sup>, I. Piñas<sup>2</sup>, R.C. Mainar<sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health España, S.A.U. <sup>2</sup>Departamento de Patología Animal, Facultad de Veterinaria. Universidad de Zaragoza

#### **Background and Objectives**

Salmonellosis is a major problem in public health, concurrent infections of Salmonella and Lawsonia intracellularis are a risk factor for Salmonella shedding. Oral vaccination against L. intracellularis reduces both the shedding and the prevalence of Salmonella at slaughter in L. intracellularis co-infected herds The objective of this study was to assess if the vaccination with Enterisol lleitis® reduces Salmonella seropositivity in pigs during the fattening period under field conditions.

# **Material and Methods**

The study was conducted on three batches of pigs from two wean-to-finish farms positive for Salmonella and L. intracellularis. Each batch was split into two groups (1.000 pigs each), vaccinated group (VG) and control group (CG). Before vaccination 40 pigs from each group were bled to confirm that no serological differences were found. Vaccine was administrated through drinking water to 8-weeks-old pigs. Per group 20 pigs were individually ear-tagged and bled at 1, 2 and 3,5 months after vaccination (sampling 1,2 and 3 respectively). Serum samples were analyzed by an indirect ELISA..ELISA OD% values were compared between groups by Mann-Whitney test, and seroprevalence by Chi-squared test. The Kaplan-Meier curve was used to compare the average time to seroconversion in both groups.

#### Results

Significant differences in median OD% values and seroprevalence were observed between groups for the last sampling and when all sampling were taken together, both were lower in the VG. For sampling 3 the odds of being seropositive in the VG was 0.49 times that of the CG. In total 30% of the pigs from the CG had seroconverted one month after vaccination, 38% two months after vaccination and 50% at the end of the study. From the VG group only 10% of the pigs seroconverted two months after the vaccination, and 26% at the end of the study. The relative risk of seroconverting was about three times higher in the CG (2,92; IC95%=1,36-6,32).

# **Discussion and Conclusion**

There was a significant lower number of Salmonella seropositive pigs and lower rates of seroconversion in the VG. Seroconversion in the VG occurred mostly at the end of the fattening period, so vaccination may have a positive impact in the control of Salmonella.

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# EFFECT OF SOW VACCINATION AGAINST INFLUENZA A ON PASSIVE IMMUNITY

<u>A. Puigredon 1</u>, S. Cros 1, J. Miranda 1, L. De Lucas 1, I. Ballarà-Rodriguez 1, M. Simon-Grifé 1 1HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

Vaccination of breeding females against the Influenza A virus (IAV) not only provides immunity to the breeding herd, but also passive immunity to the offspring. Passive immunity plays a key role in reducing IAV transmission. The aim of this study was to provide information on the effect of sow vaccination on passive immunity.

#### **Material and Methods**

Eighteen gestating sows were randomised into three groups taking into account their antibody levels: Group A (n=6) was vaccinated with GRIPORK® at 6 and 3 weeks before farrowing; Group B (n=6) was vaccinated with an inactivated trivalent vaccine against influenza at 5 and 2 weeks before farrowing; Group C (n=6) received two doses of PBS following the same vaccination scheme as group B. The humoral immunity response was evaluated in the sows at 3 weeks after the first dose and 1 and 3 weeks post farrowing. Maternally-derived antibodies (MDAs) against influenza were evaluated in 30 piglets per group at different timepoints (1, 3, 5, 7 and 9 weeks of life). Sera were examined using a commercial ELISA kit designed to detect antibodies against type A influenza nucleocapsid (CIVTEST-Suis, Laboratorios Hipra SA, Amer, Spain). The piglets' serology was analysed using a mixed linear model for repeated measures, considering the sows and piglets as a random effect. The sows' serology was analysed using a mixed linear model for repeated measures considering the sow as a random effect.

#### Results

In the sows, significantly higher levels of antibodies were observed in Group A compared to Groups B and C at 3 weeks after the first vaccine dose and at 1 and 3 weeks post-farrowing (GB p=0.028; GC p=0.003). The level of MDAs against influenza in piglets from Group A vaccinated sows was higher at all the timepoints studied compared with the piglets from GB (p=0.009) and GC (p=0.005). No significant differences between piglets in GB and GC were observed (p=0.805).

# **Discussion and Conclusion**

GRIPORK® showed a greater increase in antibody titres in sows compared to the trivalent inactivated vaccine. In addition, the acquired passive immunity conferred to the piglets was higher and lasted until 9 weeks of age.
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# EVALUATION OF THE POTENCY OF A COMMERCIAL VACCINE TO PREVENT CLOSTRIDIOIDES (CLOSTRIDIUM) DIFFICILE INFECTION IN SWINE

V.S. Amarante <sup>1</sup>, T.G.V. Souza <sup>1</sup>, C.P. Ramos <sup>1</sup>, <u>R.O.S. Silva</u> <sup>1</sup> <sup>1</sup>Universidade Federal de Minas Gerais

#### **Background and Objectives**

Clostridioides difficile infection (CDI) is the main cause of antibiotic-associated diarrhea in humans and is a major cause of neonatal colitis in piglets. Research suggests potential transmission of strains between animals and humans, emphasizing the public health importance of preventing CDI in swine. Recently, the first commercial vaccine for pigs reached the market; however, this product has not been evaluated yet. The objective of this study was to test the potency of a commercial vaccine (SUISENG® Diff/A, Hipra, Spain) against C. difficile in swine.

# **Material and Methods**

Eighteen sows from a commercial farm were divided into two groups: group I (n = 12), which received two doses of the vaccine 6 and 3 weeks before parturition, while sows from group II (control), consisting of non-vaccinated sows.

## Results

Titers of neutralizing antibodies were evaluated using a serum neutralization test in Vero cells, with a detection level of 0.06 IU/mL. Before vaccination, all animals from both groups were negative for neutralizing antibodies against A/B toxins. Three weeks after the first vaccine dose, sows from group I showed a mean titer of  $(1.06\pm2.46 \text{ IU/mL})$ , while those from the control group were negative. After the second dose, the mean titers of group I increased to  $1.60\pm2.29$ . Piglets from group I showed high levels of neutralizing antibodies  $(1.74\pm2.22 \text{ IU/mL})$ , similar to the titers observed in their mothers.

### **Discussion and Conclusion**

This study conclusively demonstrated that the tested vaccine induces neutralizing antibodies against A/B toxins in pregnant sows and successfully transfers them to piglets through colostrum. The subsequent phase of this investigation will focus on confirming whether this vaccine can effectively diminish the prevalence or mitigate clinical signs of CDI in these animals.

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# IMPROVEMENTS IN COLOSTRUM, PIGLET HEALTH AND PERFORMANCE THROUGH SUPPLEMENTATION OF AN ALGAE a-1,3-GLUCAN ON SOWS DIETS

<u>J. Hložková</u><sup>1</sup>, P. Bukowska<sup>2</sup>, A. Wealleans<sup>2</sup>, R. Neto<sup>2</sup>, V. Van Hamme<sup>2</sup> <sup>1</sup>Afeed a.s, Opava, Czech Rep <sup>2</sup>KEMIN EUROPA NV

### **Background and Objectives**

With hyperprolific sows, colostrum management is ever more important as piglets' health pre and post weaning is decided by colostrum intake. The objective of this study is to assess the impact of an algal â-1,3-glucan (Aleta<sup>™</sup>, Kemin) on colostrum, viability and performance of the piglets pre- and post-weaning.

### **Material and Methods**

A 2000 sow breeding herd in CZ was chosen. The study was split in 2 phases:

- 1: twenty five sows were included in a Control group (C) receiving a basal diet and 22 sows to a BG group
- 2: Four batches, one control, C (n=87 sows) followed by three batches BG (1 to 3) of 87 sows each.

BG groups were supplemented with 1 g / sow /day of a-1,3-glucan from 85 days of gestation to weaning.

Colostrum was collected (all sows, Phase 1) and assessed (IgG and IgA). Piglets born alive (BA) and dead (BD), weak piglets (WP) (<700g) at birth, weaning weight (WW) and number of weaned per sow (WPS) were recorded and analyzed in both phases. For phase 2, pre and post-weaning diarrhoea, mortality and treatments were also assessed. Data were analysed in the Fit Model function of JMP 16. Differences considered significant at p<0.05

### Results

In Phase 1, numerical differences were observed for BA, BD, WP, WW (6.9 and 6.6kg for BG and C, respectively). Significant increases (P<0.05) were observed on IgA 25.7 and 15.3 (SED=15.3) and IgG 114.9 and 74.3 mg / ml (SED=74.3) for BG and C, respectively.In Phase 2, the 2nd batch, BG 2 had a significantly lower WPS than Control and BG 1 and BG3, 12.6, 13.0, 13.2 and 13.2 for BG2, C, BG1 and BG3 respectively (P<0.05). The WW was higher in the BG groups than in C and increased from batch to batch, 6.7, 7.2, 7.5 and 7.8 kg for C, BG1, BG2 and BG3 respectively (P<0.05). Pre-wean mortality was 9.03 and 12.1 for BG and C respectively (P>0.05).

#### **Discussion and Conclusion**

Results of these studies support that algal â-1,3-glucan improves the immunological quality of colostrum, leading to higher weaning weights and other health parameters that determine piglet performance.

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# INTERPRETATION OF PRRS PCR RESULTS IN PIGLETS DEPENDS ON CHARACTERISTICS OF THE PRRS MLV VACCINES USED

<u>K. Koenders - Van Gog</u><sup>1</sup>, T. Wijnands<sup>2</sup>, B. Garcia-Morante<sup>3</sup>, M. Steenaert<sup>4</sup> <sup>1</sup>Lintjeshof Veterinary Practice and Merefelt Livestock Diagnostics <sup>2</sup>Lintjeshof Vet Group, The Netherlands <sup>3</sup>Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain. <sup>4</sup>Boehringer Ingelheim Animal Health Netherlands bv

## **Background and Objectives**

In farms that are not yet successful in delaying the main timepoint of piglet PRRSv infection to at least 5-6 weeks of age it may be indicated to vaccinate in the first week of life. In the absence of a registered PRRS MLV product an alternative PRRS MLV vaccine was used. The objective of this study was to evaluate changes in PRRSv PCR status before and after the change of vaccine.

# Material and Methods

In 16 farrow-to-grower herds, the registered product (Suvaxyn PRRS MLV; 'Before'), vaccinated at 3 to 5 days of age, was replaced one on one by an alternative product (Ingelvac PRRSFLEX®; 'After'). Diagnostic evaluation was done by blood sampling, per monthly farm visit 30 piglets due to weaning (DTW) (n=2070) and 20 piglets End Of Nursery (EON) (n=1290). Piglets were vaccinated in the 'Before' and 'After' situation. Samples were pooled 1:5 and tested by PRRS PCR (Bio-T Kit PRRS DIVA).

## Results

PRRS PCR results of serum samples pooled 1:5 before and after changing the PRRS MLV product:Before DTW 57% PRRS positive (n=126), EON 83% PRRS positive (n=153)After DTW 22% PRRS positive (n=283), EON 78% PRRS positive (n=104)

## **Discussion and Conclusion**

DTW After showed less PRRS PCR positive results versus Before. These findings could be explained by differences in the shedding time of the vaccine strains following vaccination; shedding of product 'After' is less than product 'Before'.EON the PRRS PCR positive results were comparable. This could be explained by PRRS field virus infections in the nursery that was suggested by PRRS ORF5 sequencing results in the 'After' period in which PRRS wild type virus was found in the nursery in 6 out of 8 farms. We conclude that at interpretation of PRRS PCR results in piglets following PRRS MLV vaccination, one should consider the specific characteristics of the vaccines used, like persistency on a population level. (The use of the alternative, not for this age registered product is allowed by EU law in particular to avoid causing unacceptable suffering and is under responsibility of the responsible veterinarian).

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# MONITORING THE IMPACT OF MYCOPLASMA HYOPNEUMONIAE VACCINATION ON PRODUCTIVE PARAMETERS UNDER FIELD CONDITIONS

P. Sánchez <sup>1</sup>, M. Sagrera <sup>2</sup>, D. Espigares <sup>2</sup>, <u>L. Garza <sup>2</sup></u> <sup>1</sup>Ars Alendi S.A., Spain <sup>2</sup>Ceva Salud Animal, Barcelona, Spain

### **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is the causative agent of enzootic pneumonia, an important respiratory disease that affects mainly growing and finishing pigs. This disease causes significant economic losses associated with decreased feed efficiency, reduced average daily gain, and increased medication cost. Commercial vaccines against Mhyo are extensively used for controlling this pathogen and improving such performance parameters. Therefore, the objective of this project was to compare the effect of vaccination against Mhyo on productive parameters in wean to finish farms.

# **Material and Methods**

Twenty-eight consecutive piglet batches (n=2,000) were selected from a Mhyo positive one-site farm with 2,500 sows. Three-weeks-old piglets were vaccinated against Mhyo with vaccine A (A1-A24 batches) or B (Hyogen®, B25-B28), following the manufacturer's instructions. Piglets were located in different wean to finish farms, except for the last six batches that were located in three farms (2 batches/farm), under equal housing conditions. During the growing-fattening period, average daily weight gain (ADWG), feed conversion rate (FCR) and medication cost were recorded and compared using t-test (Welch's t-test when including all A batches). Mortality rate was also calculated and compared with Chi-square test. Total production cost per batch ( $\epsilon$ /kg) at the end of fattening period was also calculated.

#### Results

When comparing all batches, piglets vaccinated with vaccine B showed a higher ADWG (608 g) than those vaccinated with vaccine A (588 g) (p<0.1), except when compared with only the 3 A batches from the same farm (618 g). B batches showed a lower FCR (2.38) than A (2.48 for all and 2.47 for three batches) (p<0.1). Mortality rate was also significantly lower in B (9.1%) than in both analyses for A (13.0% and 14.1%, respectively) (p<0.05). Medication cost was also lower in B (5.82 $\in$ ) than in three A batches (6.23 $\in$ ), but higher when all A batches (5.54 $\in$ ) were considered (p<0.1).

# **Discussion and Conclusion**

Piglet vaccination with vaccine B significantly improved mostly all measured performance parameters compared to vaccine A. Therefore, this study confirmed that piglet vaccination with Hyogen® is a valuable tool to improve the productive parameters and reduce the economic losses associated to Mhyo in affected farms.

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# ALTERNATIVE IMMUNIZATION STRATEGIES TO CONTROL PRRS USING INACTIVATED VACCINES IN NURSERY PIGLETS

<u>B. Iriarte</u><sup>1</sup> Piensos Costa, Spain

#### **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) is one the most economically important pathogens affecting swine industry worldwide. Piglet vaccination using attenuated live (MLV) vaccines against PRRSV has been used for controlling the impact of the disease in nursery period. Nevertheless, no information regarding the effect of piglet vaccination using inactivated vaccines is available. Therefore, the aim of this exploratory study was to evaluate the effect of piglet vaccination against PRRSV with an inactivated vaccine on productive parameters as mortality rate during lactation and nursery period.

# **Material and Methods**

This trial was conducted in an unstable PRRSV positive farrow-to-nursery farm with 1,700 sows, during seven consecutive weekly batches of piglets. The batches were confirmed as PRRSV positive at birth by RT-PCR. Four non-vaccinated (NV) batches (n=5,495) were compared with three vaccinated (V) batches (n=4,124) during nursery period. Piglets included in V batches were vaccinated with 1mL of Progressis® (Ceva, France) at 3 days of age and revaccinated with 1mL at 3 weeks of age. Piglets from all batches were under equal housing conditions. Mortality rate was recorded at lactation and nursery period and were compared between V and NV batches with Chi-square test. P-value was set at 0.05.

#### Results

Seven consecutive batches of piglets (n=9,619) were included in this trial. Mortality rate during lactation was lower in group V (20.43%) compared to group NV (21.03%), with a 0.60% difference between the two groups. Similarly, mortality rate in piglets during nursery period significantly decreased (p<0.05) in group V (8.66%) compared to NV (11.02%), which is a difference of 2.36% between groups.

## **Discussion and Conclusion**

Piglet vaccination against PRRSV with an inactivated vaccine at three days and three weeks of age reduced the mortality rate in lactation and nursery period, compared to non-vaccinated batches, especially in the nursery period, where the difference was statistically significant. Obtained results suggest piglet vaccination with inactivated PRRSV vaccines could be a potential alternative for reducing the PRRS impact during lactation by 6/1000 suckling piglets and nursery period by 23/1000 weaners.

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## COMMUNICATION BETWEEN INFLAMMATORY CYTOKINE GENE EXPRESSION AND MUCOSAL ANTIBODY IMMUNE RESPONSE IN PIGLETS IMMUNIZED WITH SILICA SBA-15 VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE

F.M. Petri <sup>1</sup>, M.L. Mechler-Dreibi <sup>1</sup>, A.K. Panneitz <sup>1</sup>, E.R. Braga <sup>1</sup>, M. Carvalho De Abreu Fantini <sup>2</sup>, T. Da Silva Martins <sup>3</sup>, O.A. Sant'Anna <sup>4</sup>, H.J. Montassier <sup>1</sup>, <u>L.G. De Oliveira</u> <sup>1</sup> <sup>1</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil

<sup>2</sup>University of São Paulo (USP), Physics Institute, Šão Paulo, Brazil.

<sup>3</sup>Department of Chemistry, Federal University of São Paulo (UNIFESP), Diadema, SP, Brazil <sup>4</sup>Butantan Institute, São Paulo, Brazil.

# **Background and Objectives**

The effective protection against Mycoplasma hyopneumoniae involves cytokines and immunoglobulin A (IgA). Vaccination commonly uses adjuvants to boost immune responses. This study explores silica SBA-15 nanoencapsulation in piglet immunization against M. hyopneumoniae, assessing the immune response and its impact on lung tissue colonization while examining the intricate communication between inflammatory cytokine gene expression and mucosal antibody responses.

## Material and Methods

At 24 days of age (D0), 48 piglets were immunized or not and grouped into G1 (commercial vaccine), G2 (SBA-15 vaccine), and G3 (SBA-15 without antigen) and G4 (sterilized saline). On D21, they were challenged with 10<sup>6</sup> CCU of M. hyopneumoniae. On D56, euthanasia was performed, and lung samples were collected for DNA and RNA extraction. The qPCR targeted M. hyopneumoniae p102 gene. The cDNA from RNA extraction was analyzed for gene expression (IL-4,IL-6,IL-8,IL-17,IFN-å and TGF-å genes). IgA was detected in BALF samples by ELISA. Statistical analyses were performed using GraphPad Prism 10.

## Results

Lung tissue quantification revealed varying values: G1  $(3.3 \times 10^3 \pm 1.5 \times 10^5)$ , G2  $(2.6 \times 10^4 \pm 7.1 \times 10^5)$ , G3  $(3.65 \times 10^4 \pm 8.5 \times 10^4)$  and G4  $(1.59 \times 10^5 \pm 3.89 \times 10^5)$ . Significant differences were observed between G1 and G4 (p=0.01) and G2 and G4 (p=0.04). IgA OD medians in BALF were: G1: 0.73, G2: 0.6, G3: 0.39, and G4: 0.47. Cytokine gene expression varied, with correlations like in G1: IL-17xIL-8 (rho=0.63,p=0.04), IFN-axIgA BALF (rho=0,76,p=0,003) and IgA BALFxqPCR lung (rho=0.63,p=0.02). In G2: TGF-axIL-6 (rho=0.65,p=0,02). In G3: IL-17xIL-8 (rho=0.84,p=0.004) and IL-17xIgA BALF (rho=0.75,p=0.006) and in G4: IL-6xTGF-a (rho=0.75,p=0.004).

## **Discussion and Conclusion**

Variability in lung tissue quantification highlighted significant differences among groups, suggesting distinct immunization responses. G1 and G2 demonstrated notable reductions, indicating potential efficacy, while G4 showed higher values, suggesting increased tissue colonization. IgA levels in BALF exhibited a clear trend, emphasizing diverse mucosal responses to vaccination. Correlations between cytokine gene expression and IgA levels revealed intricate interactions, with positive associations in G1, regulatory patterns in G2, and robust responses in G3. The findings highlight the promising potential of silica SBA-15 as an adjuvant in piglet immunization against M. hyopneumoniae. FAPESP #2021/11914-0 and #2021/14515-9.

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# COMPARATIVE ANALYSIS BETWEEN NEEDLE FREE INTRAMUSCULAR VACCINATION PROCESSES VERSUS INTRADERMAL

<u>A. Uribe 1</u>, J. Reynaga 1, P. Peimbert 1, F. Pinal 1 1BOEHRINGER INGELHEIM ANIMAL HEALTH MEXICO

#### **Background and Objectives**

Intramuscular vaccination has been one of the most frequently used methods in porcine production systems, however, there are risks associated with needle use (Madapong et al. 2021). Currently, there are new device technologies for needleless vaccination by Intramuscular (IM) and Intradermal (ID) routes (Dalmau et al., 2021), however, it is necessary to evaluate the vaccination type involved with the devices considering vaccine residue could be left on the skin (Otake et al. 2002; Chase et al. 2008; Temple et al. 2017). The purpose of this field study was to compare needle free intramuscular vaccination versus intra dermal vaccination by measuring the residue volume percentage left on the skin of the pig.

# **Material and Methods**

Sixty 28-day old animals were chosen randomly and two groups were created. Group 1 was vaccinated intramuscular and group 2 was vaccinated intradermal. Ingelvac FLEX Combo (2 mL) was used for the intramuscular vaccination and the vaccine against M. Hyopneumoniae and Porcine Type 2 Circovirus (0.2 mL was used for intradermal vaccination. Commercially available 75 mm long micro-capillary tubes were used for vaccine residue collection (microhematocrit tubes). The residual volume left on the skin was measured in millimeters immediately after each vaccination. Finally, a Mann-Whitney statistical analysis was performed to determine if a significant statistical difference exists.

#### Results

Vaccine residual measurements were made through the capillary microtube from groups 1 and 2. The results indicate that the group vaccinated intramuscularly resulted in an average of 0.013% vaccine residue, whereas for the group vaccinated intradermally the average residue was of 2.32% ( $p \le 0.05$ ).

### **Discussion and Conclusion**

The vaccine residual proportion on the skin for the intradermal vaccination was 17.8 times greater than compared to the intramuscular vaccination, which was statistically significant. Additionally, it was observed during this study that there was less bleeding of the pigs vaccinated intramuscularly. This study shows that intramuscular administration resulted in less vaccine residues on the skin compared to intradermal vaccination. Intramuscular vaccination ensured a full dose of vaccine whereas intradermal vaccination resulted in administration of less than a full dose, which might have an impact on vaccination compliance.

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# COMPARISON OF THREE DIFFERENT PCV2 AND M.HYO COMBINATION VACCINES IN REGARDS TO PIG PERFORMANCE IN TWO FARMS IN CHINA.

C. Sun <sup>1</sup>, Y. Wang <sup>1</sup>, C. Yin <sup>1</sup>, <u>L. Lecznieski</u><sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health (Shanghai) Co.,Ltd.,Shanghai <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH, Germany

## **Background and Objectives**

In the past years, several PCV2 and M.hyo ready-to-use (RTU) vaccines have been launched in China. As Porcine Circovirus Disease (PCVD) and M.hyo may cause significant performance losses, it is crucial to continuously investigate the outcome of the available vaccines under Chinese field conditions. Two studies were performed in two pig farms separately located in the Gansu and Shannxi provinces in China in 2023. Both farms had respiratory problems and farmers were not satisfied with the pig performance parameters of current RTU vaccines. The objective of the studies was to evaluate the effect on performance of different PCV2/Mhyo vaccination protocols, using RTU vaccines (T2 and T3) compared to Ingelvac CircoFLEX® and Ingelvac MycoFLEX® freshly mixed - FLEXcombo® (T1).

# Material and Methods

The first study included a total of 340 animals (T1: n=170, T2: MNC RTU n=170) and the second study included 240 animals (T1: n=120, T3: Local RTU n=120). Pigs were tagged with RFID and individually weighted. Treatments were administered IM at weaning. The primary parameter was ADWG, the secondary parameter was mortality, both calculated from wean-to-finish period. Data were analyzed by ANOVA using Minitab.

#### Results

Pigs vaccinated with T1 had significantly higher ADWG compared to T2 and T3 (Study 1: 728g vs 655g; Study 2: 661g vs 618g), ( $p \le 0.05$ ). Pigs in T1 also showed better market weight uniformity with lower standard deviation (Study 1: 9.96 vs 15.55; Study 2: 12.90 vs 15.60) and lower mortality rate (Study 1: 8.2% vs 14.7%; Study 2: 5.0% vs 11.7%).

### **Discussion and Conclusion**

In face of difficult market situations, such as low pig price and/or high feed costs, it becomes even more important to reduce costs and improve farm efficiency. The economic benefit obtained through the studies, by the improvement on ADWG and reduction on mortality rate was calculated considering the current pig and vaccines prices. When market pig price is 14RMB/kg, farms vaccinated with T1 could earn 216 RMB/pig and 171 RMB/pig more than T2 and T3, respectively. The outcomes observed in this field studies shown the influence of PCV-2 and M.hyo vaccine selection on farm efficiency and profits.

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# COMPARISON OF TWO DIFFERENT PCV2 VACCINES WITH REGARD TO ADG IN CHINA

<u>G. Marika</u><sup>1</sup>, O. Duran<sup>2</sup>, C. Sun<sup>3</sup>, L. Huang<sup>4</sup> <sup>1</sup>Boehringer Ingelheim Vetmedica GmbH <sup>2</sup> Boehringer Ingelheim Vetmedica GmbH, Germany <sup>3</sup>Boehringer Ingelheim Animal Health (Shanghai) Co.,Ltd.,Shanghai <sup>4</sup>Boehringer Ingelheim Animal Health (Shanghai) Co. Ltd., Shanghai, China:

#### **Background and Objectives**

Since the launch of the first commercial PCV2 vaccine in China (Ingelvac CircoFLEX®) several other vaccines have been licensed in China.Due to the economic impact of PCVD it is crucial to continuously investigate the effect of the available vaccines under Chinese field conditions.

## **Material and Methods**

Two studies were performed in two finishing sites of two groups in China. Both studies were conducted in endemically infected farms with PCVD.Both studies included 3 different vaccine groups and an unvaccinated control group each. The first study a total of 853 animals (CF n= 211, vaccine B n= 211, vaccine c n=212, unvaccinated control n=219) and the second study included 1563 animals (CF n=400, vaccine A n=385, vaccine B n=390, unvaccinated controls n=388). The primary parameter was ADG, which was calculated based on the starting weight (at weaning), the finishing weight and the duration of fattening. Data were analyzed by ANOVA using SAS JMP. The null hypothesis was that vaccination did not improve ADG.

# Results

Vaccination with CF resulted in both trials in the significantly highest ADG (780 and 719 g) ( $p \le 0.05$ ) as shown by the results of the ANOVA, whereas the local vaccines had ADGs that were numerically higher, but not significantly different from the unvaccinated controls (>0.05). Both unvaccinated groups had the lowest ADG (751 and 673 g) confirming the economic impact of PCVD. It is concluded from this study that vaccination against PCVD is crucial for improved economical performance.

## **Discussion and Conclusion**

PCVD is considered as being one of the most costly swine diseases – both from a herd health and an economic perspective. All PCV2 vaccines used in both trials improved numerically ADG, but vaccination with Ingelvac CircoFLEX® resulted in the highest ADG ( $p \le 0.05$ ).

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# EFFICACY OF AN INTRADERMAL HOMOLOGOUS AUTOGENOUS PRRS VIRUS VACCINE ADMINISTERED AT 3 DAYS OF AGE AGAINST A HOMOLOGOUS PRRS CHALLENGE

<u>A. Ladinig</u><sup>1</sup>, S. Dürlinger<sup>1</sup>, H. Kreutzmann<sup>1</sup>, T. Rümenapf<sup>2</sup>, I. Christiaens<sup>3</sup>, R. Neto<sup>4</sup>, M. Williemsen<sup>4</sup> <sup>1</sup>University Clinic for Swine, University of Veterinary Medicine Vienna, Austria <sup>2</sup>Institute of Virology, Department for Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria <sup>3</sup>Poulpharm, Belgium <sup>4</sup>Kemin Biologics

## **Background and Objectives**

PRRSV is one of the most significant pathogens in pigs. With autogenous vaccines being used in Europe, the objective of this study was to evaluate the protective effect of an autogenous intradermal PRRSV vaccine in piglets, against a homologous infection.

# **Material and Methods**

Forty-eight PRRS negative piglets were included. Piglets were randomly allotted to 1 of 3 groups, positive control (PC) (not vaccinated (NV), challenged), negative control (NC) (NV, non-challenged) and vaccinated challenged (VC) vaccinated intradermally at day -38 (KEMIN® AptiVaxTM Barricade PRRS AUT15-33). Piglets from the PC and VC groups were challenged intranasally at day 0 with PRRSV AUT15-33 1x10<sup>5</sup> TCID<sub>50</sub>. Clinical signs were assessed daily and body weight measured on days -38, -24, -15, 0, 7, 14, 21 and 27. Serum samples and nasal swabs were collected regularly. Necropsies were carried out at 10 and 27 days post challenge (DPC), data analysed by ANOVA and Bonferroni.

#### Results

No severe clinical signs were observed. At 27 DPI piglets in VC, NC and PC groups weighed 24.8 kg, 27.6 kg and 23.8 kg respectively (P>0.05). From 0 to 7 days, the ADG was 396.0, 501.3 and 375,9 g /day for VC, NC and PC groups, respectively (P>0.05). From 14 to 21 DPC, the ADG was 552.7<sup>a</sup>, 829.5<sup>b</sup> and 471.4<sup>a</sup> g/day for the VC, NC and PC groups, respectively. From 21 to 27 DPC, the NC and VC had similar ADG, 710.0 g/day, and the PC had an ADG for the same period of 643.1 g/day (p>0.05). ADG from 14 to 27 days was 625.5<sup>a,b</sup> g/day for VC, 774.5<sup>a</sup> for NC and 550.7<sup>b</sup> for PC (different superscripts indicate p<0.05). The AUC for PRRSV genomic copies in serum samples and nasal swabs for the first 10 DPC were lower in VC compared to PC pigs (P>0.05).

#### **Discussion and Conclusion**

In the conditions of this study, limited in the statistical analysis by the number of piglets included, use of a intradermal PRRS autogenous vaccine at 3 days of age against a homologous challenge 38 days later was effective in maintaining good performance levels in piglets challenged with a pathogenic PRRSV strain early in life.

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# EVALUATION OF A NOVEL PED VACCINE, PEDQ, IN PROTECTION OF NEWBORN PIGLETS AGAINST PEDV INFECTION

C. Chang <sup>1</sup>, T. Luo <sup>1</sup>, <u>P. Wu <sup>1</sup></u> <sup>1</sup>*Reber Genetics Co., Ltd.* 

#### **Background and Objectives**

Passive immunity has been proven important for protection of newborn piglets against porcine epidemic diarrhea virus (PEDV) infection. In this study, the potential of an injectable PED vaccine in inducing PEDV-specific antibodies in pregnant sows, which would pass on to the newborn piglets for protection against PEDV infection, was evaluated.

## Material and Methods

Pregnant sows were intramuscularly vaccinated with one dose of PED vaccine (vaccination; n = 10) or adjuvant only (control; n = 3) at 8, 5, and 3 weeks before farrowing. Blood samples and oral swab samples were collected from each sow (n = 13) at each vaccination and parturition for antibody analysis. Newborn piglets from both vaccination (n = 83) and control (n = 32) groups were challenged with a Taiwan locally isolated genotype 2b PEDV (strain Pingtung 52) at 3 days old. Clinical signs and fecal scores were recorded daily and fecal swab were collected daily for virus shedding analysis from all challenged piglets. Blood samples were collected from 5 piglets per sow at 0 and 10 days post challenge (dpc) for antibody analysis. At the end of study, survival rate of piglets in both groups were calculated. Statistical analysis was performed using One-way ANOVA (antibody level and virus shedding), Mann-Whitney U test (fecal score), and Chi-square test (survival rate). A p-value < 0.05 was considered statistically different between two groups.

## Results

Pregnant sows in vaccination group had higher PEDV-specific antibody titers at parturition, including serum IgG and oral IgA antibodies. Piglets born from vaccinated sows had higher serum PEDV-specific IgG antibody titer and survival rate (80% vs 41%) than piglets born from control sows. During PEDV challenge, piglets born from vaccinated sows also exhibited less clinical signs and had lower virus shedding.

## **Discussion and Conclusion**

Vaccination of pregnant sows with an injectable PEDV vaccine, PEDQ, can enhance PEDV-specific antibody responses and provide passive immunity to newborn piglets against PEDV infection and improve survival rate. This demonstrates that induction of PEDV-specific antibody in pregnant sows via vaccination is an efficient way to protect newborn piglets from PEDV infection and lower the mortality rate as a result of PEDV infection among newborn piglets.

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# EVALUATION OF THE IMMUNOGENICITY OF A NOVEL ORAL MUCOSAL VACCINE FOR THE PROTECTION OF PIGLETS AGAINST GLÄSSER'S DISEASE

A. González Fernández <sup>2</sup>, ó. Mencía Ares <sup>2</sup>, M. Petrocchi Rilo <sup>2</sup>, R. Miguélez Pérez <sup>2</sup>, V. Acebes Fernández <sup>2</sup>, A. Perelló Jiménez <sup>1</sup>, C.B. Gutiérrez Martín <sup>2</sup>, <u>S. Martínez Martínez <sup>2</sup></u>

<sup>1</sup>Hunting Resources Research Institute

<sup>2</sup>Unit of Microbiology, Department of Animal Health, Veterinary Faculty, University of León, León, Spain

#### **Background and Objectives**

Glässer's disease, caused by Glaesserella parasuis, is a prevalent post-weaning condition responsible for significant economic losses in the swine industry. Due to the interference of maternal antibodies, sow vaccination is the most common approach, which may have a limited protection. To overcome this limitation, this study evaluates a novel piglet vaccination in the oral mucosa to enhance immunity.

## **Material and Methods**

The vaccine comprised the recombinant antigen TbpB<sup>Y167A</sup>, deficient in porcine transferrin binding protein, generated from the Nagasaki strain of Glaesserella parasuis. Eight colostrum-deprived piglets received 0.2 ml of the vaccine via oral mucosal administration at 15 and 30 days of age. Immunization was performed using the Comfort-In® device (Gamastech, Italy). Five non-vaccinated colostrum-deprived piglets were included as control. Serum and mucosa samples from all piglets were collected on day 15 (pre-first dose), day 30 (pre-second dose), and day 45 (post-second dose). IgG (on plates coated with inactivated bacteria and recombinant protein) and IgM concentrations from serum, along with IgA from mucosa, were quantified using ELISA plates. Data analysis was performed using Wilcoxon rank-sum test.

#### Results

A significant increase (p < 0.05) in all evaluated immunoglobulins was observed after the first (day 30) and second dose (day 45) of the vaccine. IgM and IgA levels post-vaccination were observed to be higher than IgG levels (p < 0.05). Remarkably, vaccination in the oral mucosa demonstrated a noteworthy increase in IgA concentrations in mucosa in vaccinated animals compared to control pigs. (0,2234  $\pm$  0,0511 vs. 0,7273  $\pm$  0,0998 on day 30; 1,8134  $\pm$  0,0358 vs. 2,1961  $\pm$  0,0790 on day 45).

# **Discussion and Conclusion**

The results underscore that vaccination in the oral mucosa induces a humoral protective immune response in colostrumdeprived pigs, extending beyond serum immunoglobulins to mucosal ones, which constitute the first barrier for bacterial colonization. Notably, the recombinant antigen TbpB<sup>Y167A</sup>has been shown to be suitable for a subunit vaccine targeting Glaesserella parasuis, in contrast to previous studies. In conclusion, this innovative vaccination method demonstrates immunogenicity as it elicits a humoral immune response against Glässer's disease in piglets, presenting itself as a promising tool for future disease control.

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# FIELD ASSESSMENT OF IMMUNOGENICITY OF TWO DIFFERENT AUTOGENOUS STREPTOCOCCUS SUIS BACTERINS

M. Schibalski <sup>1</sup>, C. Waehner <sup>2</sup>, A. Gaudreau <sup>3</sup>, E. Thibault <sup>4</sup>, M. Segura <sup>3</sup>, M. Gottschalk <sup>5</sup>, <u>H. Gantelet</u> <sup>6</sup> <sup>1</sup>Tierärztliche Gemeinschaftspraxis Artland, Quakenbrück, Germany <sup>2</sup>Ceva Tiergesundheit GmbH Kanzlerstr. 4, 40472 Düsseldorf - Deutschland <sup>3</sup>Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada <sup>4</sup>Ceva Biovac, Beaucouzé, France <sup>5</sup>Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Québec, Canada <sup>6</sup>Ceva Biovac, 6 Rue Olivier de Serres, 49070 BeaucouzE, France

## **Background and Objectives**

Streptococcus suis (S. suis) is a major porcine pathogen causing important morbidity and mortality worldwide. The use of autogenous bacterins is widespread; however, their composition is variable and depends on the production processes of the companies preparing the autovaccines. Our study was performed to evaluate the humoral response induced by two autogenous bacterins from two different compagnies.

# **Material and Methods**

Two formulations of S. suis serotype 2 plus S. suis serotype 7 bacterins were studied. Eleven pre-parturient sows (never vaccinated against S. suis before) were vaccinated twice with a 2 mL dose at a 21 day-interval for groups A and B in a sow farm. The group C was included as an unvaccinated control. To evaluate transfer of maternal immunity, only one piglet per litter was randomly selected. The magnitudes (total Igs) of the vaccine-induced antibody response were evaluated by ELISA test against serotype 2 and 7 as coating antigens. An opsonophagocytosis assay (OPA) was performed to assess the capacity of serum antibodies to induce a killing of bacteria.

#### Results

In sows, the two autovaccines (A and B) induced a significant increase of anti-S. suis antibodies only against serotype 7 compared to the basal level already present in the unvaccinated animals,

and no difference against serotype 2 was observed. In piglets (by maternal immunity transfer), only one autovaccine induced a significantly stronger level of total antibodies against serotype 7 (group A).In addition, the induction of potentially protective antibodies against serotype 2 (OPA results) in piglets was only successfully demonstrated for group A.

#### **Discussion and Conclusion**

Our results showed that the protocols used to produce autogenous bacterins are a major element to induce an effective immune response against S. suis.

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# GENERATION AND EVALUATION OF CELL-ADAPTED VACCINE CANDIDATE AGAINST AFRICAN SWINE FEVER VIRUS

H. Kwon <sup>1</sup>, M. Kim <sup>1</sup>, J. Cha <sup>2</sup>, Y. Kim <sup>3</sup>, J.Y. Lee <sup>1</sup>, I. Yoon <sup>1</sup>, W. Jeong <sup>3</sup>, I. Mo <sup>4</sup>, S. Yoo <sup>1</sup>, <u>L. Jong-Soo</u> <sup>2</sup> <sup>1</sup>ChoongAng Vaccine Laboratories Co., Ltd, Daejeon, 34055, Republic of Korea <sup>2</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea <sup>3</sup>National Wildlife Disease Control Institute, Gwangju, 58131, Republic of Korea <sup>4</sup>Avinext, Cheongju, 28382, Republic of Korea

### **Background and Objectives**

African Swine Fever (ASF) remains one of the most devastating viral diseases affecting domestic pigs and wild boars, posing substantial threats to the global food safety. Recently, ASFV has extended its geographical area from Central Europe to East Asia, causing tremendous economic losses to the local swine industry. The absence of a commercial vaccine heightens the vulnerability of swine populations to widespread outbreaks. The aim of this study was to develop a safe and efficient live attenuated ASFV vaccine that offers protection against ASFV in domestic pigs.

## Material and Methods

To develop a cell-adapted live attenuated ASFV strain that replicate effectively in a established cell line, South Korea/Wild boar/Hwacheon/2020 virus was serially passaged into the CA-CAS-01-A cell line. Replication of ASFV in CA-CAS-01-A cells increased with the increasing number of passages, and a specific single clone of ASFV (ASFV-KOR.INJE.MEC-01.2022) was isolated via plaque purification after 18 passages. To assessed the genetic changes acquired during the adaptation, next-generation sequencing (NGS) was performed. In order to define the safety and efficacy of this attenuated vaccine candidate ASFV-KOR.INJE.MEC-01.2022 was administrated intramuscularly to swine. ASFV-specific antibody production, disease severity and survival in lethal parental ASFV strain challenge was determined.

#### Results

NGS analysis of ASFV-KOR.INJE.MEC-01.2022 virus indicated that it lacked 12 genes in the MGF region of the genome compared to the parental strain. ASFV-11893 was completely attenuated in pig as pigs inoculated with 2 doses of 10<sup>5</sup> HAD<sub>50</sub> remained healthy without signs of the disease. Importantly, all immunized pigs conferred 100% protection against lethal parental ASFV challenge, which correlated with high ASFV-specific antibody titers. Importantly, deleted genes were antagonized the host's innate antiviral immune response, confirming their virulence.

### **Discussion and Conclusion**

Overall, these results suggest that ASFV-KOR.INJE.MEC-01.2022 could be a safe and potential attenuated candidate against the current ASFV outbreak. Additionally, Our findings suggest that the attenuated virulence of ASFV-KOR.INJE.MEC-01.2022 might be mediated by increased innate antiviral immune response. [National Research Foundation (2021R1A6A1A03045495) and Ministry of Environment (NIWDC-2021-SP-02)]

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# HISTOLOGICAL EVALUATION IN ILEUM FROM ORAL VACCINATION WITH ENTERISOL ILEITIS

S. Jardon <sup>1</sup>, V. Quintero <sup>2</sup>, F. Pinal <sup>3</sup>, <u>L. Cruz <sup>3</sup></u> <sup>1</sup>FESC UNAM <sup>2</sup>Independent advisor <sup>3</sup>Boehringer Ingelheim Animal Health Mexico

## **Background and Objectives**

Ileitis is a globally important enteric disease. This disease is characterized by thickening of the intestinal mucosa due to the proliferation of epithelial cells in the intestinal crypt (McOrist et al 1995). Lesions are mainly found in the ileum, but lesions can be found in the jejunum and colon (Jacobson et al 2003). The aim of this study is to compare the efficacy of vaccination against ileitis by decreasing the degree of villus injury (Enterisol Ileitis, Boehringer Ingelheim AH).

## **Material and Methods**

Two groups of pigs were formed on the production line, each of 69 pigs. The vaccinated group (G1) received a 2 mL dose of Enterisol lleitis vaccine orally at 18 days of age, while the control group (G2) received 2 mL of saline orally at the same age. The intestines were checked at slaughter in a slaughterhouse. Sterile cotton swab samples were taken from 10 pigs in each group to perform the detection technique for Lawsonia intracelullaris by RT-PCR. The proliferation of crypt cells was qualified, considering one stratum as normal and two to three strata as proliferation. The stratification of the epithelium in the upper third of the villi was also considered, considering one to two strata normal and proliferating from 3 or more strata. Measurements of villus width and length were statistically analyzed with the student's t-test for independent samples ( $p \le 0.05$ ).

#### Results

Villi width has a statistically significant difference between vaccinated and control groups (p=0.0013) and villus length has a statistically significant difference between vaccinated and control groups (p=0.001). More stable crypts with 1 to 2 strata were observed in the vaccinated group establishing a mild proliferative enteropathy compared to the control group with a moderate enteropathy.

# **Discussion and Conclusion**

The use of the oral vaccine against Lawsonia intracellularis favors the reduction of the degree of villus injury, thus obtaining multiple benefits such as a better and efficient absorption of nutrients, improvement of productive parameters such as daily weight gain and the reduction of clinical problems that generate expensive treatments.

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# IMMUNOGENICITY AND SAFETY OF SBA-15-BASED VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE IN PIGLETS

G.A. Aguiar <sup>1</sup>, F.M. Petri <sup>1</sup>, D.G. Da Silva <sup>1</sup>, <u>L.G. De Oliveira</u> <sup>1</sup> <sup>1</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil

#### **Background and Objectives**

Mycoplasma hyopneumoniae, the pathogen responsible for porcine enzootic pneumonia (PEP), leads to economic losses in the pork industry. There is a need for new vaccine technologies, therefore, safety assessment and immune response are essential to evaluate performance. This study aimed to evaluate the immunogenicity of a vaccine incorporating nanostructured silica SBA-15 as an adjuvant, focusing on safety parameters and evaluation of immunoglobulins.

# Material and Methods

Twenty-four pigs at 21-day-old were housed in the Swine Medicine Laboratory (FCAV/Unesp Jaboticabal) with water and fed ad libitum (CEUA#003841/23). On day 24 (D0), they were divided into G1 (commercial vaccine), G2 (SBA-15 vaccine) and G3 (SBA-15 without antigen). Prior to vaccination on D0, baseline measurements for body weight (BW) and rectal temperature (RT) were established, and blood samples were collected for assessment of IgA and IgG by the SDS-PAGE technique. Thereafter, physical parameters and blood sampling occurred at D1 and D21. Statistical analyses were performed using Rstudio 4.3.0.

#### Results

Average daily weight gain (ADWG) was the same for the three groups, 0.08 kg/day. Significant differences in rectal temperature (RT) were observed on G3, showing lower temperatures after the vaccination (D0=38.9°C; D1=38.7°C; D21=38.2°C; p=0.027), as for G1 and G2, no significant differences for RT were found. Significant results extended to IgA concentrations at D21, with G3 with higher concentrations post-vaccination (2.80 mg/dL) in comparison to G1 and G2 (2.11 mg/dL and 2.40 mg/dL, respectively) (p=0.0003). Additionally, IgG concentrations had significance values on G1 at D21 (G1=16.80 mg/dL; G2=14.30 mg/dL; G3=14.05 mg/dL; p=0,007).

## **Discussion and Conclusion**

Understanding the safety and efficacy of the nanostructured SBA-15 vaccine is important. Notably, the vaccine had no interference with animals ADWG. Moreover, RT remained consistent post-vaccination, underscoring essential safety. The modulation of the immunoglobulins, particularly IgA on vaccinated animals and control group, was crucial for mucosal protection, alongside the stimulation of the dendritic cells to produce IgG. In conclusion, the SBA-15 silica vaccine demonstrated both safety and an immune response in piglets. Grant#2021/11914-0 and Grant#2023/04557-1, São Paulo Research Foundation (FAPESP).

IMM – Immunology and Vaccinology

# IMPROVED PIGLET PERFORMANCE AFTER SOW'S VACCINATION AGAINST C. DIFFICILE IN PORTUGAL.

V.D. Azeitona Martins <sup>1</sup>, E. Duque <sup>1</sup>, <u>O. Boix</u> <sup>2</sup> <sup>1</sup>HIPRA Portugal <sup>2</sup>HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

Clostridium difficile (C. difficile), is a bacterium that can lead to severe gastrointestinal infections, causing neonatal diarrhoea, weight loss, and reduced overall health. Beyond immediate health concerns, the impact of neonatal diarrhoea on young piglets can extend to their growth and productivity in later stages. The present study aims to quantify the improvement in piglet performance after immunization against C. difficile.

### **Material and Methods**

This study was conducted on a commercial Portuguese herd comprising 420 sows, farrow to finish, showing a recurrent neonatal diarrhoea problem. The farm was positive for E. coli F4 and F5, Clostridium perfrigens type A, Clostridium difficile and Rotavirus A and C. The experimental design compared 26 consecutive batches. G1 was vaccinated with Vaccine A (adjuvanted with á-tocoferol) and consisted of the average recorded data from 19 consecutive batches from April to November 2022. G2 was vaccinated with Suiseng® Coli/C mixed with Suiseng® Diff/A and included 9 batches from November 2022 until February 2023. All the vaccinations were performed according to the manufacturer instructions. Preweaning mortality and weight at weaning were monitored in both periods.

#### Results

The average pre-weaning mortality in G1 exhibited an overall mortality rate of  $9,91\% \pm 2,20$  during the lactation phase, which was significantly higher than the  $7,99\% \pm 0,82$  overall mortality recorded in G2 (p<0.05). These significant differences were translated to 0.93 more piglets/sow at weaning (13,35 ± 0,43 [G1] vs 14,28 ± 0,99 [G2]). Regarding the piglet weight at weaning significant improvements in body weight at weaning were monitored (5,71 ± 0,36 [G1] vs 6,35 ± 0,34 [G2]). These significant differences were translated to extra 14.5 kg/litter (76 ± 3,69 [G1] vs 90,5 ± 4,65 [G2]).

### **Discussion and Conclusion**

This study highlights the significant advantages of immunization against C. difficile A and B toxins, showcasing lower piglet mortality rates and improved performance at weaning. These findings emphasize the crucial role of C. difficile immunization in enhancing the health and productivity of commercial pig herds, offering valuable insights for effective herd management.

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# SAFETY OF A TRIVALENT PCV2A/PCV2B/MYCOPLASMA HYOPNEUMONIAE VACCINE ADMINISTERED BY INTRAMUSCULAR ROUTE WITH NEEDLE-SYRINGE OR WITH A NEEDLE-FREE INJECTION DEVICE (NFID)

<u>M. Balasch</u><sup>1</sup>, M. Cabana<sup>1</sup>, M. Buxeda<sup>1</sup>, A. Rojo<sup>1</sup>, J. Marx<sup>2</sup>, N. Mangarova<sup>3</sup>, A. Aldaz<sup>4</sup>, J. Angulo<sup>5</sup> <sup>1</sup>Zoetis Manufacturing & Research Spain, S.L. <sup>2</sup>Zoetis Inc, Kalamazoo, MI. USA. <sup>3</sup>ZOETIS Benelux, Mercuriusstraat 20, 1930 Zaventem, Belgium <sup>4</sup>Zoetis Inc, Parsippany, NJ, USA

<sup>5</sup>Zoetis Inc, 1040 Swabia Ct, Durham, NC 27703 – United States

## **Background and Objectives**

Needle-Free (NF) vaccine administration offers clear advantages compared to the use of conventional needles: improves animal wellbeing, eliminates the risk of broken needles and carcass defects in the meat, avoids needle stick injuries in caretakers, and reduces iatrogenic pathogen transmission. Even though vaccines are commonly delivered through needles, the use of Needle Free Injector Devices (NFID) offer an alternative to capture all benefits of NF. The studies reported here compare the safety of a PCV2a/PCV2b/Mycoplasma hyopneumoniae vaccine administered intramuscularly with either a NFID or a conventional syringe-needle.

## **Material and Methods**

Two studies were conducted; piglets were randomly allocated to two treatment groups, 10 to 12 pigs each, and received intramuscularly either the vaccine (CircoMax Myco®) or a saline/PBS by: 1 mL at 3 days of age and 2 mL 2 to 3 weeks later. In Study 1 pigs were dosed with a syringe-needle, while in Study 2 with a NFID (Pulse FX®). Rectal temperatures and injection site reactions were monitored during 4 and 14 days after each administration, respectively. Microscopic examination of the injection sites was conducted at the end of the studies.

#### Results

In both studies there was an increase in rectal temperature with a maximum of 1.35°C and 2.05°C, respectively. Swellings were also observed, with a diameter below 2 cm in study 1, lasting 10 days, and 2 to 5 cm in study 2, lasting 6 days. In study 2, following 1<sup>st</sup> vaccination, hematomas were observed in 66.7% of the vaccinated and in 55.6% of the control pigs. All resolved spontaneously after 4 days. After 2<sup>nd</sup> vaccination no hematomas were observed. Microscopically, both administration systems induced granulomatous inflammation at the injection site in 100% and 67% of the vaccine-treated pigs, respectively.

## **Discussion and Conclusion**

The safety profile of both administration methods was almost equivalent at three weeks of age. The high pressure needed to administer 1 mL with a NFID in a newborn piglet induced transitory hematomas. For this reason, it is important to calibrate and adjust the NFID before each administration to the optimal pressure for a successful vaccination.

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# TONSILLAR DETECTION OF MYCOPLASMA HYOSYNOVIAE IN GROWING PIGS BASED ON VACCINATION STATUS

H. Schwecke 1, E. Mcdowell 2, A. Sponheim 3, J. Nerem 2, E. Fano 4, R. Valeris-Chacin 5, M. Pieters 6

<sup>1</sup>University of Minnesota, College of Veterinary Medicine, St. Paul, MN

<sup>2</sup>Pipestone Veterinary Services

<sup>3</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA

<sup>4</sup>Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA; Current affiliation: Pipestone Veterinary Services, Pipestone, MN, USA

<sup>5</sup>Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX

<sup>e</sup>Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; Swine Disease Eradication Center,

## **Background and Objectives**

Mycoplasma hyosynoviae is a commensal bacterium that can induce lameness in growing pigs, causing significant animal welfare concerns, increased antibiotic usage, and pork production issues. Information regarding prevention and control measures for M. hyosynoviae is highly needed. The aim of this study was to evaluate the effect of dam and/or pig vaccination on the tonsillar colonization with M. hyosynoviae in growing pigs.

# **Material and Methods**

The study was performed at a sow farm and downstream sites. Dams were vaccinated with an autogenous bacterin at 5 and 3 weeks pre-farrow or remained unvaccinated. Piglets born to these dams were either unvaccinated or were vaccinated twice during lactation, lactation and nursery, or nursery (8 experimental groups). Tonsillar swabs were collected from a subset of growing pigs (n=200; ~10.5 to 14.0 weeks of age) including all treatment groups. Swabs were processed for detection of M. hyosynoviae by PCR. The proportion of pigs that were detected positive for M. hyosynoviae by PCR per treatment group were analyzed using a Pearson's Chi-square test using R, and results were considered significant at p < 0.05.

# Results

The percentage of pigs positive for M. hyosynoviae per treatment group, born to unvaccinated or vaccinated dams were: unvaccinated 72% and 84%, vaccinated during lactation 56.7% and 76.5%, vaccinated during lactation and nursery stage 58.6% and 50%, vaccinated during nursery 48.4% and 66.7%, respectively. The statistical analysis showed that the percentage of M. hyosynoviae positive pigs was similar among experimental groups (p >0.05).

#### **Discussion and Conclusion**

Under the conditions of this investigation the tonsillar colonization of growing pigs with M. hyosynoviae did not differ, regardless of the dam or piglet vaccination status. However, the commensal nature of this bacterium implies interpretation challenges and the significance of bacterial colonization on disease presentation remains uncertain. As this investigation is ongoing, samples from other production stages have been collected and will be assessed to further determine the effect of dam or piglet vaccination in the overall bacterial colonization with M. hyosynoviae. In addition, clinical and production data will be analyzed to assess the effect of the application of an autogenous bacterin on lameness development.

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# VACCINATION OF 3-DAY-OLD PIGS WITH A PRRSV-1 MODIFIED LIVE VACCINE INDUCES SIMILAR NEUTRALIZING TITERS THAN 3-WEEK-OLD VACCINATION

L. Aguirre <sup>1</sup>, F. De Mergelina <sup>2</sup>, J. Miranda <sup>2</sup>, I. Ballarà <sup>2</sup>, M. Martín <sup>1</sup>, E. Mateu <sup>1</sup>

<sup>1</sup>Department de Sanitat i Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain

<sup>2</sup>HIPRA, Amer (Girona), Spain

### **Background and Objectives**

Maternally derived antibodies (MDA) may interfere with the immune response to vaccination. The present study aimed to compare the development of neutralizing antibodies (NA) after vaccinating piglets with MDA either at 3 days or 3 weeks of age with two different MLV PRRSV-1 vaccines.

# **Material and Methods**

The study was performed in two farms (F1 and F2) that vaccinated sows with Unistrain® PRRS or Suvaxyn® PRRS. In F1, 29 piglets were vaccinated with Unistrain at 3 days of age (3d), half of them intradermally (ID; n=15) and the rest intramuscularly (IM; n=14). Another group of 29 piglets was vaccinated at 3 weeks of age (3w) ID (n=13) or IM (n=16). In F2, 26 piglets were vaccinated IM with Suvaxyn at 3d (n=13) or 3w (n=13). Sera were collected at 3, 21, 28 and 56 days of age to assess the homologous titers of NA. In both farms, 5 non-vaccinated pigs were kept as controls.

#### Results

In F1, all pigs were seropositive at 3 days of age (average neutralization titer against Unistrain® PRRS:  $4.1 \log_2 \pm 2.1$ ). The titers of NA decreased in all groups at 21 days of age, including the not vaccinated controls ( $0.3 \pm 0.9^{a}$  for ID3d,  $1.9 \pm 1.6^{ab}$  in IM3d,  $0.4 \pm 0.9^{a}$  in ID3w,  $2.2 \pm 1.9^{b}$  in IM3w and  $2.7 \pm 1.0^{ab}$  in the placebo group; p<0.1). Afterward, NA increased at day 56, with  $\log_2$  averages of  $3.3 \pm 1.9$  for ID3d,  $4.1 \pm 2.1$  for IM3d,  $2.7 \pm 1.6$  for ID3w, and  $2.8 \pm 2.1$  for IM3w (non-significant). There were no significant differences between ID and IM at any date. Regarding F2, animals also had NA at day 3 (average titer against Suvaxyn® PRRS:  $4.7 \pm 1.7$ ) that decreased at day 21 ( $3.3 \pm 1.7, 2.5 \pm 1.3$  and  $1.8 \pm 0.8$  for 3d, 3w and placebo respectively). In contrast to F1, NA did not increase at day 56 ( $1.4 \pm 1.5$  and  $0.8 \pm 1.3$   $3.0 \pm 3.6$  for 3d, 3w and placebo respectively).

#### **Discussion and Conclusion**

The results of the present study showed that the administration of Unistrain® PRRS at 3d or 3w by the IM or ID route produced similar results in terms of neutralizing antibodies. In the case of Suvaxyn® PRRS, titers did not increase after vaccination, which might be explained by interference of MDA with the development of the humoral response.

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# CLINICAL EFFICACY OF A SOW ROTAVIRUS VACCINE ON PIGLET NEONATAL DIARRHOEA

<u>E. Bousquet</u> <sup>1</sup>, A. Lebret <sup>2</sup>, T. Nicolazo <sup>2</sup>, C. Chevance <sup>2</sup>, J. Jeusselin <sup>2</sup>, C. Teixeira-Costa <sup>2</sup>, V. Normand <sup>2</sup>, P. Berton <sup>3</sup>, A. Morillo Alujas <sup>4</sup>, G. Boulbria <sup>2</sup>

<sup>1</sup>Virbac SA, 13ème rue, 06510 Carros, France
<sup>2</sup>Rezoolution Pig Consulting Services, Noyal-Pontivy, France
<sup>3</sup>Virbac France, Espace Azur Mercantour, 3ème Rue, 06510 Carros, France
<sup>4</sup>Get in Touch, 22400 Monzon, Huesca, Spain

# **Background and Objectives**

Rotavirus type A (RVA) is often isolated in piglet neonatal diarrhoea. This study was performed to assess efficacy of a sow vaccine (SUIGEN® ROTA COLI, combining E. coli F4/F5/F6/F41 and RVA) in the prevention of piglet neonatal diarrhoea.

# **Material and Methods**

Study took place in a farrow-to-finish farm, with a history of neonatal diarrhoea due to RVA. Twenty-three pregnant sows from the same batch were randomly allocated to T or C group according to parity. Tested vaccine was administered twice at 6 and 3 weeks before farrowing in all sows of the T group. In C group, farm vaccine (combining E. coli F4/F5/F6 and Clostridium perfringens type C toxoid) was administered once at 3 weeks before farrowing on sows and twice on gilts (6 and 3 weeks before farrowing). Issued piglets were individually examined daily during the first week of life then weekly till weaning (3 weeks). Fecal consistency and general health were scored. When required, diarrhoeic piglets were individually treated orally with an amoxicillin 10% powder until diarrhoea stopped (20 mg/kg/d). Amoxicillin use was calculated per litter by dividing the used amount of amoxicillin by total litter weight (mg amoxicillin per kg biomass). Groups were compared by GLM model.

#### Results

The estimated probability of diarrhoea was lower in the T than in the C group (respectively 0.669 and 0.808, p < 0.005). Most of diarrhoea cases occurred during first week of life. The estimated probability of mortality was not different between groups (respectively 0.098 and 0.089, p > 0.05). The mean oral amoxicillin mg per kg biomass was numerically lower in T than in C group (respectively 23.9 and 36.6 mg/kg), variance being lower in T group (p < 0.05).

# **Discussion and Conclusion**

The tested vaccine induced a significant decrease of diarrhoea incidence, though it remained high in both groups. As both groups were housed in the same farrowing rooms, pathogens may have circulated between crates, due to a high infection pressure. The antimicrobial usage decrease can be linked to reduction of diarrhoea incidence and severity. Monitoring of following batches in the same farm after use of the tested vaccine confirmed that decrease.

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# COMPARATIVE EFFICACY OF FOUR PCV-2 VACCINES IN CONVENTIONAL PIG FARMS IN THAILAND

S. Sonna <sup>1</sup>, P. Tippitak <sup>1</sup>, J. Korntipasasi <sup>1</sup>, <u>E. Bousquet</u> <sup>2</sup> <sup>1</sup>Virbac Thailand Co., Ltd, Chaengwattana Rd., Parkkred, Nonthaburi, Thailand <sup>2</sup>Virbac SA,13ème rue, 06510 Carros, France

#### **Background and Objectives**

PCV-2 is one of the most common pathogens in swine production. In Thailand, PCV-2d is the predominant genotype. Though cross protection has been proven in challenge tests, the differences of genotype between vaccine strains may play a role in PCV-2 infection prevention and subsequent zootechnical performances of vaccinated pigs in field conditions. Thus, objective of this study was to compare survival rate from wean to finish between different PCV-2 vaccines in Thai field conditions.

## **Material and Methods**

During two successive weeks, 3000 healthy weaned piglet per week from different breeding farms were allocated to four wean to finish units (750 pigs per unit) from the same geographical zone with the same sanitary status, biosecurity and management. PCV-2d infection had been confirmed in the breeding farms of origin by sequencing of strains isolated from blood or lymph nodes in one-day-old piglets (by Veterinary Diagnostic Center, Kasetsart University, Thailand). Each unit was allocated to a PCV-2 vaccine administered at the arrival of piglets: Suigen PCV2 (whole inactivated PCV-2d vaccine, 1 ml IM), another whole inactivated PCV-2 vaccine A (0.5 ml IM), a PCV-2 ORF-2 subunit vaccine B (2 ml IM) or another whole inactivated PCV-2b vaccine C (2 ml IM). The survival rate of pigs from weaning to finishing was recorded and compared between groups by the Chi-square test, then by the Fisher's exact test for pairwise comparisons.

#### Results

Over 2 weeks of inclusion, 1500 pigs were allocated to each vaccine group. The survival rate was significantly different between groups (p < 0.001), being the highest with the Suigen PCV-2 (95.6%) compared to the control vaccines (A: 85.8%, B: 92.8%, C: 91.5%).

#### **Discussion and Conclusion**

The four vaccines were compared during the same period, but in different wean to finish units. However, geographic proximity, management standardization and similar sanitary status for these units allow to evaluate survival rates between these vaccines. Higher survival rate with the PCV-2d vaccine may be linked to the presence of PCV-2d genotype in breeding farms from which piglets were issued. Thus monitoring of PCV-2 vaccines efficacy is still recommended.

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# EFFECT OF VACCINATING SOWS AND THEIR PIGLETS AGAINST GLÄSSER'S DISEASE ON A COLOMBIAN FARM SUFFERING FROM POLYSEROSITIS

L. De Lucas<sup>2</sup>, R.D.C. Hinestrosa<sup>1</sup>, Y.L. Parra<sup>1</sup>, D. Giralt<sup>2</sup> <sup>1</sup>Hipra Colombia S.A.S. <sup>2</sup>HIPRA, Amer (Girona), Spain

## **Background and Objectives**

Glaesserella parasuis is the aetiological agent of Glässer's disease, a common pathology in the pork industry with a high prevalence in the post-weaning period. Vaccination is one of the strategies to prevent the impact of the disease. In this study, we evaluated the efficacy of vaccinating sows and their offspring for proper control of the mortality associated with confirmed polyserositis in the post-weaning period on a Colombian farm.

# **Material and Methods**

On a 1,200 sow farm, a high presence of Glaeserella Parasuis was confirmed by PCR in the polyserositis lesions, which was affecting the performance of nursery pigs. As the antibiotic therapy was not properly controlling the disease, a vaccination protocol with Hiprasuis® Glässer was implemented in sows at 80 days of gestation and piglets at 25 and 35 days of life. A one-year period before and after implementing the vaccination protocol was compared in terms of mortality, FCR and ADWG in the nursery phase involving 30,000 piglets in total. A logistic regression analysis was performed using R software v4.03 to compare mortality in the two groups.

#### Results

One year after implementation of the vaccination protocol in sows and piglets, the mortality rate in the nursery phase had decreased by 2.47 points, significantly moving from 3.38% to 0.91% (p<0.001). With regard to the daily weight gain, although a statistical analysis was not performed, the parameter had improved by 16 grams per day (from 529g to 545g) after one year of vaccinating sows and their offspring.

#### **Discussion and Conclusion**

This case report shows the efficacy of Glässer disease control with a correct immunization protocol not only of the piglets but also of the sow herd, as sows are the source of Glaeserella Parasuis and piglet colonization is known to occur very early in life.

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# EVALUATION OF THE SEROLOGICAL PROFILE IN ANIMALS VACCINATED AT 1ST WEEK OF LIFE WITH A MLV AGAINST AUJESZKY DISEASE BY DIFFERENT ROUTES OF ADMINISTRATION.

<u>A. Puigredon</u><sup>1</sup>, M. Simon-Grifé<sup>1</sup>, S. Romero<sup>1</sup>, J. Miranda<sup>1</sup>, I. Ballarà-Rodriguez<sup>1</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain

#### **Background and Objectives**

The aim of piglet vaccination against Aujeszky's disease at early ages is to immunize them before they become infected naturally. It has been reported that maternal antibody interference may hamper the development of the immune response induced by early vaccination against Aujeszky's disease. To overcome this negative effect, alternative vaccination routes have been proposed. The aim of this study was to evaluate the serological profile of animals vaccinated with a commercial Aujeszky's disease vaccine at 1 week of age (woa) by intramuscular (IM), intradermal (ID) and intranasal (IN) administration.

## Material and Methods

Eigth gestating sows from a gE-negative farm that vaccinated against ADV every 4 months were distributed into 4 groups (2 sows/group): at 1 woa, 10 piglets/sow were randomly selected and vaccinated with Auskipra®GN by ID route with Hipradermic® device (group A), IM (group B), IN (group C), and group D remained unvaccinated. At 9 and 12 woa all groups were vaccinated by the IM route. The immune response was evaluated in all piglets at different time points (1, 3, 5, 7, 9, 12, 15, 20 woa). Sera were examined by a blocking ELISA test designed to detect the presence of antibodies against gB of ADV (CIVTEST® SUIS ADVgB, HIPRA) and by a blocking ELISA test designed to detect the presence of antibodies against gE of ADV (CIVTEST® SUIS ADVgE, HIPRA).

#### Results

All the animals were gB positive until 9 woa. At 12 woa the percentage of gB positive animals was significantly higher in groups A and C (100%) compared to groups B (79%) and D (78%) (p<0.05). At 15 and 20 weeks of age, the percentage positivity of gB titres was significantly higher in groups B and D compared to A (p<0.05). All tested animals remained gE negative throughout the study.

## **Discussion and Conclusion**

Vaccinating with one dose of Auskipra®GN ID and IN at 1 woa induced humoral immune responses against ADV, overcoming maternally derived antibodies. A delayed revaccination should be considered in ID groups to avoid vaccine neutralization at 9 and 12 weeks of age.

#### IMM – Immunology and Vaccinology

# IMMUNE PROFILE AND CLINICAL EFFICACY OF THE FIRST SINGLE-DOSE AND BROAD-SPECTRUM VACCINE AGAINST GLAESSERELLA PARASUIS

<u>R. Frandoloso</u><sup>3</sup>, H. Falcão Da Costa<sup>1</sup>, I.R. Honorato Gatto<sup>1</sup>, P. Consuelo Arancibia<sup>1</sup>, M. Antão Cavalcanti Pizani<sup>1</sup>, F. Hirose<sup>1</sup>, F. Nielsen Almeida<sup>1</sup>, J. Antônio Guizzo<sup>2</sup>, C. Cerutti Dazzi<sup>3</sup>, I. Giacobbo<sup>2</sup>, L.C. Kreutz<sup>3</sup> <sup>1</sup>Ourofino Saúde Animal, Cravinhos, Brazil <sup>2</sup>AFK Imunotech, Passo Fundo, Brazil <sup>3</sup>Laboratory of Microbiology and Advanced Immunology, University of Passo Fundo & AFK Imunotech, Passo Fundo, Brazil

#### **Background and Objectives**

The antigenic diversity found within and between serovars of G. parasuis (Gps) represents a major challenge in trying to control Glasser's disease by using classical vaccines. As well documented, cross-reactivity between the different serovars of this agent is low or even absent. Here, we present the immunological and clinical characterization of the first broad-spectrum vaccine against Gps.

#### Material and Methods

A total of 88 SPF piglets, 21 days old, were divided into 11 groups (G1 - G11) and used in this study. The piglets (G1 to G5) were immunized intramuscularly at day 21 (D21) with 2 mL of the Safesui Glässer ONE vaccine (Ourofino Saúde Animal, Brazil). Group G11 was immunized with a competitor vaccine based on Gps SV5. Groups G6 to G10 were inoculated with PBS. During the study at D21, D28, D35, D42 and D49, serum samples were collected to titrate IgG to SV1, SV4, SV5 and Non-Typeable (NT) by Indirect ELISA. On D42, groups G1 and G6 were challenged intranasally with lethal dose of Gps SV1; groups G2 and G7 with Gps SV4; groups G3, G8 and G11 with Gps SV5; groups G4 and G9 with Gps NT; groups G5 and G10 with Gps SV7. After the challenge, the animals were clinically evaluated for 14 days. The experiment was approved by CEUA-UPF n<sup>o</sup> 016/2022.

#### Results

All piglets immunized with Safesui Glässer ONE developed high titers of IgG anti-Gps (> 6,000), 21 days after a single vaccination, against all SVs tested here. After challenging, all control animals (groups G6 to G9) died and only 1 animal from G10 survived. In contrast, high survival rates were observed in immunized animals from groups G1 (71%), G2 (85%), G3 (100%), G4 (83%), and G5 (100%). In contrast, only 20% of the animals in the G11 group reached the end of the study.

## **Discussion and Conclusion**

In this study we demonstrated the effectiveness of Safesui Glässer ONE (SGO) against 5 different serovars of Gps, which, together, are responsible for causing more than 80% of GD cases in Brazil. The efficacy of SGO vaccine was higher than that achieved by the main vaccine available worldwide for GD.

#### IMM – Immunology and Vaccinology

# IMPACT ON PRODUCTIVE PARAMETERS IN SUCKLING PIGLETS AFTER SOW VACCINATION AGAINST NEONATAL DIARRHOEA INCLUDING C. PERFRINGENS TYPE A

S. Cárceles <sup>1</sup>, M. Guerra <sup>3</sup>, M. Toledo <sup>3</sup>, E. Goyena <sup>2</sup>, E. Ruiz <sup>3</sup>, S. García <sup>3</sup>, J. Díez <sup>3</sup>, <u>D. Espigares <sup>1</sup></u> <sup>1</sup>Ceva Salud Animal, Barcelona, Spain <sup>2</sup>Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain

<sup>3</sup>Agropecuaria Casas Nuevas, Murcia, Spain

### **Background and Objectives**

Neonatal diarrhoea (ND) remains a challenge for swine farms reducing productive parameters and increasing economic losses and antimicrobial use in piglets during suckling period. Many factors can influence the occurrence of ND, including bacteria and viruses, sometimes in coinfection. The aim of this study was to assess the efficacy of sow vaccination with a vaccine against E. coli and C. perfringens type C and type A in comparison to another vaccine against E. coli and C. perfringens type C.

# Material and Methods

The study was carried out on a 2700 sows farrow commercial farm located in the Southeast of Spain. Piglets in the first of days of life were affected with creamy feces lasting several days. Laboratorial diagnostic was performed and CPA detection containing beta2 toxin was confirmed. Two groups were compared: Control group '22 (CG) included sows vaccinated according to the usual farm protocol and vaccine against E. coli/C. perfringens C and Enteroporc group '23 (EG) which sows were vaccinated with Enteroporc Coli AC (Ceva Santé Animale) at 5 and 2 weeks before farrowing. Productive parameters of litters coming from CG and EG born between July 31<sup>st</sup> July to October 15<sup>th</sup> in the years 2022 and 2023, respectively, were recorded. To evaluate the efficacy of these vaccines, the following parameters were recorded during suckling period: born alive and weaned per litter (BA/L and W/L), mortality (%M) and antibiotic consumption (AC). Results were compared using Pearson Chi-square test or T-test. Significant differences were considered when p<0.05.

#### Results

A total of 1207 litters in CG and 1223 litters in EG were included. Despite there was significant difference for BA/L in control group vs EG [CG:14.43; EG: 13.40 (p<0.001)], W/L [CG:11.65; EG: 11.72] showed numerically differences comparing litters from vaccinated or not vaccinated sows. Statistical difference was found for mortality [CG:19.04; EG: 12.76 (p<0.001)] and for AC (mg/kg) [CG:86.95; EG: 54.84 (p<0.001)] between compared groups.

### **Discussion and Conclusion**

Under these field trial conditions, vaccination of sows against E. coli/C. perfringens (C and A) (Enteroporc Coli AC) improved significantly productive parameters in suckling piglets and reduced diarrhoea treatments, being an effective tool for controlling neonatal diarrhoea.

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# PERFORMANCE OF ANIMALS VACCINATED WITH DIFFERENT VACCINES FOR PCV-2 AND MYCOPLASMA HYOPNEUMONIAE

A. Fiuza 1, E. De Conti 1, P. Motta 3, R. Simões Santana 2, R. Gonçalves 1, R. Lippke 1, . 0

<sup>1</sup>Boehringer Ingelheim Animal Health Brazil <sup>2</sup>Agropecuária FETZ <sup>3</sup>Vetmarket Agronegócios

## **Background and Objectives**

Vaccination is an important tool to control Porcine Circovirus type 2 (PCV-2) and Mycoplasma hyopneumoniae (Mhyo). However, some vaccines that cause more severe adverse reactions can directly impact the pig's performance. Thus, this study aimed to measure average daily gain (ADG) and weight gain in pigs vaccinated with different vaccines for PCV-2 and Mhyo.

### **Material and Methods**

The study was carried out on a commercial farm with 2450 sows. This sow heard vaccinated only piglets at weaning and replacement gilts at ±150 days for PCV-2. A total of 1290 piglets, equally divided into males and females, were ear-tagged, randomized into two treatments (T1 and T2), and vaccinated at 22 days of age. They were also weighed individually at 22, 64, and 153 days of age. Both treatments received one dose vaccines. The T1 was vaccinated with Porcilis PCV® and M+Pac®, and the T2 was vaccinated with Ingelvac Circoflex® and Hyogen®. Statistical analysis was performed considering a 90% confidence interval.

### Results

The mean initial weight ( $\pm$ 5.8kg) was the same between treatments (P>0.1). In the nursery (64 days), no difference was observed between treatments in terms of weight (T1=20.09 kg vs T2=20.43 kg, P>0.1) and ADG (T1=0.340 Kg vs T2=0.348 Kg, P>0.1). At finishing (153 days), pigs in T2 obtained 1.57 kg more per animal (T1=104.48 kg vs T2=106.05 kg, P<0.1) and had higher ADG (T1= 0.948 kg vs T2= 0.962 kg, P<0.1).

## **Discussion and Conclusion**

This result is in line with other studies, in which it was shown that oily vaccines (T1) cause greater vaccine reactions and might impair pig weight gain performance. Pigs vaccinated with an aqueous polymer (T2) adjuvant vaccine showed better performance. Therefore, the performance of pigs vaccinated can be affected by the different types of vaccines used.

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# PREVALENCE OF PCV2 GENOTYPES AND EVALUATION OF T-CELL EPITOPE RELATEDNESS SCORES BETWEEN FIELD STRAINS AND VACCINES IN POLAND

A. Mańka 1 Zoetis Poland

#### **Background and Objectives**

PCV-2 has high evolutionary rate and constantly evolves. Commercial PCV-2a-vaccines have provided effective PCV-2-SD-control, but genetic gap between field strains and vaccines has widened<sup>2</sup>. The aim of this study was to generate information on the prevalence of PCV-2-genotypes in Polish pig farms and determine T-cell-epitope relatedness scores between Polish field strains and vaccines.

#### **Material and Methods**

Thirteen PCV-2-positive samples were collected from Polish pig farms (vaccinated and unvaccinated) from June-September 2020. Samples were genotyped using RT-PCR and sequenced. Each ORF2 field sequence were analyzed using PigMatrixEpitope prediction tool to quantify degree of antigenic relatedness based on T-cell-epitope content. Potential T-cell-epitopes were identified for SLA-class-I and SLA-class-II-alleles using EpiVaxToolkit and SLA-binding matrices generated from the PigMatrix<sup>3</sup>. By using the EpiCC-algorithm, the content of the T-cell-epitopes were compared to that of two-PCV-2a-vaccines (Vac1,Vac2) and PCV-2a+PCV-2b-vaccine (VacAB).

#### Results

Predominant genotype was PCV-2d-85% followed by PCV-2b-15%. PCV-2a was not isolated.

Thirteen PCV-2-field sequences were sent to EpiVax and analyzed using EpiCC. Results were visualized in a radar plot grouped by genotype. For each strain the EpiCC-score of VacAB is represented by orange dot and the two PCV-2a-based vaccines are represented by green (Vac1) and blue dots (Vac2) respectively.

The more common epitopes between field and vaccine strain, the higher the EpiCC-score and the closer the dot to the baseline is and to the circumference of the circle, and then the broader immunological coverage is expected provided by the vaccine Figure .

Comparing EpiCC-scores beetwen Polish PCV-2 field strains and vaccines it can be determined that VacAB provides: 49.3% improvement of coverage on PCV-2b and 32.67% on PCV-2d compared to Vac1,

38.89% improvement of coverage on PCV-2b and 26.31% on PCV-2d compared to Vac2.

### Discussion and Conclusion

In Polish pig farms PCV-2d was found as predominant genotype, followed by PCV-2b. PCV-2a was not detected. For the analyzed Polish PCV-2 field strains VacAB (CircoMaxMyco) provided broader immunological coverage based on T-cell-EpiCC score compared to Vac1 & Vac2 (two PCV-2a-based vaccines).

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# REDUCTION OF SOW SUDDEN DEATH AFTER IMMUNIZATION AGAINST CLOSTRIDIUM NOVYI IN ARGENTINA.

A. Cugno <sup>1</sup>, R. Amilcar <sup>1</sup>, R. Garcia <sup>1</sup>, M. Colitto <sup>2</sup>, E. Daffunchio <sup>2</sup>, S. Fernandez <sup>2</sup>, R. Manzano <sup>2</sup>, <u>O. Boix</u> <sup>3</sup>, L. Nodar <sup>3</sup> <sup>1</sup>Porcal <sup>2</sup>Hipra Argentina <sup>3</sup>HIPRA. Amer (Girona). Spain

## **Background and Objectives**

In recent years, one of the most significant challenges in swine production has been the increased death rate in sows, especially during the peripartum period. Clostridium novyi (C. novyi) is an anaerobic bacterium that causes sudden death with necrotic hepatitis. The aim of the present study was to evaluate the productive and economic impact after immunization against C. novyi.

## **Material and Methods**

The present study was conducted on a commercial farm with 1,600 sows in Córdoba, Argentina. The study timeline was divided into two different periods (T1-T2). T1 ran from June 2020 until March 2021, and T2 extended from July 2021 until August 2022. During the study periods, two experimental groups were compared: one group received Suiseng<sup>®</sup> containing C. novyi in T2, while the control period (T1) used another commercial vaccine without C. novyi. To compare sudden mortality between the groups, a logistic regression was performed. To calculate the economic impact of the vaccination, the prices of GP-sows ( $\epsilon$ 477) and GGP ( $\epsilon$ 660) provided by the company were considered.

#### Results

In T1, an average of 7.11 sows died per month, equivalent to a 5.33% annual mortality rate, while in T2, this number decreased to 3.36 sows per month, meaning a 2.52% annual mortality rate. During the 110-130 days post-breeding, sudden mortality in T1 occurred at a rate of 4.55 sows per month, equivalent to a 3.42% annual mortality rate. In the second period, it decreased by 36%, with a rate of 2.90 sows per month, equivalent to a 2.18% annual mortality rate. The mortality rate in young sows (between Parity 0 and Parity 3) decreased from 3.5% in T1 to 1.55% in T2. The losses recorded due to sudden deaths were  $\epsilon$ 9,680 in GGP and  $\epsilon$ 33,708 in GP-sows in T1, while they were  $\epsilon$ 3,960 and  $\epsilon$ 16,354, respectively, in T2. The economic reduction of losses due to sudden deaths amounted to  $\epsilon$ 23,074.

#### **Discussion and Conclusion**

This study demonstrated a 36% reduction in peri-partum sudden deaths and a 94% reduction in primiparous sows after immunization with Suiseng<sup>®</sup>. This decrease in mortality resulted in an annual additional gain of €23,704.

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# SEROLOGIC PROFILE FOR PCV2 IN HERDS WITHOUT SOW'S VACCINATION

A. De Paula Teixeira <sup>1</sup>, E. De Conti <sup>1</sup>, L. Fiorin Hernig <sup>1</sup>, <u>R. T. Lippke <sup>1</sup></u> <sup>1</sup>Boehringer Ingelheim Animal Health Brazil

### **Background and Objectives**

Gilts are typically vaccinated for porcine circovirus type 2 (PCV2) around weaning and receive a booster at 150 days of age. Suppose they are not vaccinated again, the immunity begins to be stimulated only by contact with the agent in the environment, which can occur in an irregular and varied way. Therefore, litters may have compromised passive immunity to PCV2, generating instability in the herd. Thus, this study aimed to evaluate the health status of unvaccinated sows against PCV2.

## Material and Methods

The serologic profile of three commercial breeding farms was evaluated, with 4500 sows each. It was done in tree equal periods (March 2021 (1), May 2022 (2) and September 2022 (3)), totaling 270 blood collections from sows in different reproductive cycles. Blood samples were collected from lactating. The serum was analyzed using the direct ELISA test (BioChek) for the detection and quantification of immunoglobulins G (IgG) for PCV2.

### Results

The results showed a variation in IgG titers for PCV2 among sampling timepoints of the same farm, as well as between different farms. Farm A had S/P of 2.38 ( $\pm$ 0.61), 2.99 ( $\pm$ 0.47) and 2.43 ( $\pm$ 0.62) at 1, 2 and 3, respectively; Farm B had S/P of 2.88 ( $\pm$ 0.58), 1.95 ( $\pm$ 0.89) and 3.06 ( $\pm$ 0.32) at 1, 2 and 3, respectively; and Farm C had S/P of 2.9 ( $\pm$ 0.50), 2.46 ( $\pm$ 0.68) and 1.77 ( $\pm$ 0.49) at 1, 2 and 3, respectively. There is also variation between sows in different reproductive cycles, with IgG titers being higher in gilts (3.03, mean from the three farms) and decreasing in sows of higher parity order (2.13).

# **Discussion and Conclusion**

Therefore, herd immunity is not stable for PCV2. These herds may, over time, even show a drop in titers in some sows to levels that make them susceptible targets for infection and clinical manifestation of circovirus, as well as their litters. One way to avoid this is through sow vaccination. This study concluded that there is a wide variation in immunity to PCV2 in sows on farms. This variation can directly influence the health status of the herd, as well as the immunity of their respective litters.

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# SHORT AND LONG-TERM EFFICACY EVALUATION OF A SINGLE-DOSE PCV2B AND MYCOPLASMA HYOPNEUMONIAE-BASED VACCINE IN PIGS

L.C. Kreutz <sup>1</sup>, H. Falcão Da Costa <sup>2</sup>, I.R. Honorato Gatto <sup>2</sup>, P. Consuelo Arancibia <sup>2</sup>, R. Caetano Monti Guedes <sup>2</sup>, F. Hirose <sup>2</sup>, F. Nielsen Almeida <sup>2</sup>, J.A. Guizzo <sup>3</sup>, C. Cerutti Dazzi <sup>1</sup>, I. Giacobbo <sup>3</sup>, R. Frandoloso <sup>1</sup>

<sup>1</sup>Laboratory of Microbiology and Advanced Immunology, University of Passo Fundo & AFK Imunotech, Passo Fundo, Brazil <sup>2</sup>Ourofino Saúde Animal, Cravinhos, Brazil

<sup>3</sup>AFK Imunotech, Passo Fundo, Brazil

## **Background and Objectives**

Porcine circovirus 2 (PCV2) and Mycoplasma hyopneumoniae (Mhyo) are ubiquitously found in pigs and coinfections cause severe respiratory disease that impairs productivity. Early vaccination of piglets has been effective in reducing morbidity and mortality. Here we evaluated the onset and duration of immunity and clinical efficacy of a bivalent vaccine formulated with PCV2b and Mhyo.

#### Material and Methods

Ninety-six SPF piglets (G1-G4; G9-G12) and 96 conventional piglets free of antibodies to Mhyo (G5-G8; G13-G16) were used to evaluate the efficacy of the vaccines applied as single 2ml dose IM on piglets at D21: experimental vaccine A (G1, G5, G9, G13); vaccine B (G2, G6, G10, G14); commercial vaccine (G3, G7, G11, G15), and placebo – PBS (G4, G8, G12, G16). Piglet were challenged at D42 (PCV2: G1-G4; Mhyo: G5-G8) or at D175 (PCV2: G9-G12; Mhyo: G13-G16) to evaluate the onset or duration of immunity (OOI/DOI) respectively. Relevant biological samples were collected prior to vaccination and afterwards to evaluate protection parameters.

#### Results

At challenging (D42/OOI) all piglets had neutralizing antibodies to PCV2; afterwards, the viral load on blood was lower on piglets from G1 which, at necropsy, had lower signs of PCV2 replication. After D175 PCV2 challenging, viremia was not detected in piglet from G11; on lymph nodes the viral load was similar between groups. Regarding Mhyo, after D42 challenge, higher levels of anti-Mhyo IgG antibodies were detected in G7 piglets; serum and mucosal IgA anti-Mhyo were also detected in most piglets, but at higher levels on G7 and G15 piglets. Mhyo load on tracheal mucous and lung lesions were similar in vaccinated piglets but lower compared to control piglets. After the late challenge (D175), Mhyo load on broncho-tracheal mucous was similar within vaccinated piglet but lower compared to control piglets.

#### **Discussion and Conclusion**

Here we demonstrated the short and long-lasting efficacy of a bivalent vaccine in reducing PCV2 and Mhyo load on selected organs and reducing tissue damage on vaccinated piglets. Vaccine A performed similarly to current available commercial vaccine and should be a valuable tool to control PCV2 and Mhyo infection in pigs.

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# A CLINICAL CASE OF EARLY PCV2 INFECTION

<u>A. Jardin 1</u>, S. Brilland 1, R. Krejci <sup>2</sup> <sup>1</sup>Ceva Santé Animale, 33 500 Libourne, France <sup>2</sup>Ceva Animal Health, France

#### **Background and Objectives**

Porcine circovirus type 2 (PCV2) is associated with several PCV2-associated diseases such as PCV2 systemic disease (PCV2-SD). PCV2 vaccines are massively used and are considered as effective products. Nevertheless, according to Segales and Sibila (2020), vaccine-induced protection can be compromised under certain circumstances such as an improper vaccination, an onset of the vaccinal immunity occurring after the natural infection or a vaccination impaired by interfering maternally derived antibodies (MDA) or concomitant infections.

Here we report a clinical case of PCV2-SD on vaccinated pigs and the investigations performed.

# **Material and Methods**

In late 2022, a 600-sow farrow to finish farm was facing cases of PCV2-SD like syndrome on fatteners especially on future gilts and their brothers (3/4 Large White \*1/4 Landrace). These events occurred even though a PCV2 vaccination was implemented at 3 weeks of age on all piglets. PCV2-SD was confirmed by the clinical and laboratory findings (including typical histological lesions combined with high amount of PCV2 in lymphoid tissues). Based on Segales and Sibila (2020), investigations were done to understand this PCV2 outbreak.

#### Results

As no PCV2 sows' vaccination was implemented, the hypothesis of a significative MDA interference was excluded. The implementation of the PCV2 vaccine on piglets was checked and was conformed to the vaccine label. No PRRSV infection at the time or close to PCV2 vaccination was detected. To investigate PCV2 early infections, 15 piglets of 3, 5, 7 and 9 weeks of age were blood-sampled and PCV2 RT qPCR were performed on pool of 3 serums. At 3 and 5 weeks of age, 2 pools out of 5 revealed to be PCV2 PCR positive (maximal load of 5,46\*10<sup>s</sup> copies/ml).

## **Discussion and Conclusion**

In this farm, the PCV2-SD outbreak despite PCV2 vaccination appeared to be explained by the occurrence of a PCV2 natural infection before the onset of the vaccinal immunity. This hypothesis was confirmed by the major clinical improvements observed after the implementation of several actions including a PCV2 sows' vaccination to stop the early PCV2 infections: the proportion of losses was reduced of 3 points in nursery and of 4 points in fattening unit.

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# A FIELD OBSERVATION OF SURVIVAL RATE AND GROWTH IMPROVEMENT AFTER SWITCHING PCV2 VACCINES IN A PIG BREEDING FARM IN HUNAN PROVINCE

S. Lin <sup>1</sup>, C. Sun <sup>1</sup>, S. Fang <sup>1</sup>, <u>L. Lecznieski <sup>2</sup></u> <sup>1</sup>Boehringer Ingelheim Animal Health (Shanghai) Co., Ltd., Shanghai <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH, Germany

## **Background and Objectives**

Porcine Circovirus Disease (PCVD) caused by PCV2 virus is an important syndrome that affects pigs, generating immune suppression and increasing the susceptibility to other pathogens. The clinical signs usually involve weight loss (wasting) and reduction in batch uniformity, impacting on the farm performance. In situations of high PCV2 infection pressure and/or co-infection with other pathogens, some PCV2 vaccines available in the Chinese market seems not to provide adequate protection. This field observation of a before-after study investigates the improvement in survival and growth rates of replacement gilts following the change of PCV2 vaccine.

#### Material and Methods

This case occurred in a 1200 breeding farm in Hunan province of China. Pigs selected as replacement gilts at 14 weeks of age were injected with 1ml of a local PCV2 vaccine from January to November 2022. Under this vaccination program, respiratory signs were observed at end of nursery and finishing stages. Several sick pigs were necropsied. Lung and kidney lesions were observed and PCV2 virus antigen was stained and detected by Immunohistochemistry in lymph nodes. PCV2 was detected in oral fluids by qPCR with very low ct value (ct=20) at nursery and finishing stages. In December 2022, the herd veterinarian decided to change the PCV2 vaccination program, and started using Ingelvac CircoFLEX®. Survival rate on nursery and finishing stages were recorded and analyzed (Before: Jan-22 to Nov-22, After: Jan-23 to Aug-23). Pig weight was tested and calculated the 100kg adjusted age. Data were analyzed by SPC chart in Minitab.

### Results

According to the before-after analysis, the survival rate on nursery stage did not change. However, survival rate on finishing stage was significantly improved by 2.4% (from 95.2% to 97.6%) ( $p \le 0.05$ ) after changing the PCV2 vaccine. The time to reach 100kg adjusted age was shortened from 190 days to 172.6 days.

# **Discussion and Conclusion**

PCV2 is a ubiquitous virus and might impact commercial farms and multiplication herds. The vaccine choice may impact on the performance outcomes. In this study, we observed that replacement gilts vaccinated with Ingelvac CircoFLEX® had better survival rate, faster growth and needed shorter time to achieve the ideal selection weight.

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# ALTERNATIVE SOLUTIONS FOR POST WEANING DIARRHOEA CAUSED BY ENTEROTOXIGENIC E.COLI EXPRESSING F4 AND LT TOXIN

A. Martínez <sup>1</sup>, J. Camarasa <sup>2</sup>, L. Nodar <sup>3</sup>, J. Miguel <sup>3</sup>, <u>O. Boix <sup>3</sup></u> <sup>1</sup>Vall Companys S.A. <sup>2</sup>Hipra España <sup>3</sup>HIPRA. Amer (Girona). Spain

#### **Background and Objectives**

Following the ban on zinc oxide and restrictions on antimicrobial usage, post-weaning diarrhoea in piglets has emerged as a primary challenge in the swine industry, resulting in significant economic losses. One of the main contributors to this issue is Enterotoxigenic Escherichia coli expressing virulence factors like F18, F4, and LT toxin. Despite the implementation of various preventive measures, finding effective solutions remains elusive. This abstract aims to assess the effectiveness of a neonatal diarrhoea E. coli sow vaccine in addressing this unresolved problem and enhancing piglet health and overall farm productivity.Principio del formulario

# Material and Methods

This study was conducted on a commercial Spanish herd comprising of 1,800 sows working on a weekly batch management. The farm exhibited a high positivity rate by PCR for F4 and LT toxin of E. coli. The trial compromised of 6 consecutive batches. Batches 1, 3 and 5 were vaccinated with Suiseng® Coli/C, receiving two 1 ml injections at 7 and 21 days of age (G1). G2 was not vaccinated and included batches number 2, 4 and 6. The post-weaning mortality rate was recorded throughout the nursery phase.

#### Results

The average post-weaning mortality rate in G1 was 2.2%, which was significantly lower than the 3.3% overall mortality rate recorded in G2 (p<0.05).

### **Discussion and Conclusion**

In this instance, the off-label use of Suiseng<sup>®</sup> Coli/C, which contains F4 and LT toxin, led to a significant reduction in mortality. Drawing upon these results, the use of parenteral vaccines containing the pathogenic factors responsible for post-weaning diarrhoea are solutions for its control.

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# COMPARISON OF IMMUNE RESPONSE AFTER INTRADERMAL AND INTRAMUSCULAR VACCINATION AGAINST PRRS, PCV2 AND M. HYOPNEUMONIAE (MYHO) IN A FINISHING HERD

N.H. Nguyen <sup>1</sup>, D.N. Tran <sup>2</sup>, H. Lin <sup>3</sup>, O. Romanov <sup>2</sup>, T. Thi Bich <sup>2</sup>, L.D.E. Ellerma <sup>4</sup>, <u>R. Del Pozo Sacristán <sup>4</sup></u> <sup>1</sup>Department of Infectious diseases and Public health, Faculty of Animal sciences and veterinary medicine, Nonglam University, Linhtrung ward, Thuduc city, Hochiminh city, Vietnam. <sup>2</sup>MSD Animal Health, Vietnam <sup>3</sup>MSD Animal Health Innovation Pte Ltd, 1 Perahu Road, Singapore <sup>4</sup>MSD Animal Health

## **Background and Objectives**

PRRSV, PCV2 and Mhyo are pathogens within porcine respiratory disease complex (PRDC). Vaccination is a key tool to control PRDC. Vaccination by intramuscular (IM) injection has been applied for many years. It has limitations such as: stressing the animal, affecting the quality of meat, risking iatrogenic disease transmission. Intradermal (ID) vaccination is an alternative way of application. This study compares the immune response and zootechnical performance after intradermal vaccination against PRRSV, PCV2, and Mhyo (ID Group) and intramuscular injection (IM Group), in finishers under standard farming conditions in Vietnam.

## Material and Methods

In total, 200 five-day old pigs were randomly assigned to two different groups: Group 1 (ID-G; n=100) and Group 2 (IM-G; n=100). At 14 days of age, piglets were vaccinated against PRRSV; ID-G intradermally with PRIME PAC® PRRS using IDAL 3G and IM-G using a PRRS MLV vaccine intramuscularly. PCV2 and Mhyo vaccination: at 21 days of age; ID-G was vaccinated intradermally using IDAL 3G TWIN with Porcilis® PCV ID and Porcilis® M HYO ID ONCE vaccine while IM-G was vaccinated intramuscularly with a combined PCV2 and Mhyo vaccine. Pigs were weighed individually at 24, 35 and 175 days. Zootechnical parameters such as ADWG and Mortality rate were also recorded separately. Blood samples were collected (20 pigs/group) at 10, 70, 105 and 54 days and evaluated for antibody response for PRRS, PCV2 and Mhyo.

#### Results

Performance of both groups showed no statistical difference (P>0.05) for both ADWG (ID-G: 0.637; IM-G: 0.636) and Mortality rate (ID-G: 4%; IM-G: 4%). Antibody titers for PRRSv revealed marked increase for both groups post vaccination but there was no statistical difference between two groups. Mhyo and PCV2 antibody titers for ID group were significantly higher compared with the IM group.

## **Discussion and Conclusion**

The study showed that intradermal route of vaccinating animals with PRRSv, PCV2 and M. hyopneumoniae was able to show similar response with that of intramuscular route. Administering vaccines via this route has additional benefits associated with needle-free vaccination such as reducing stress and improving farm biosecurity.

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# COMPARISON OF THE EFFECT OF 2 DIFFERENT VACCINES ON THE FREQUENCY OF CLINICAL SIGNS AND ASSOCIATED MORTALITY CAUSED BY NEONATAL DIARRHEA

C. Casanovas <sup>1</sup>, S. Cregenzan <sup>2</sup>, I. Tardío Solans <sup>2</sup>, M. Adsuar <sup>2</sup>, L. Cons <sup>2</sup>, A. Escoda <sup>2</sup>, J. Casanovas Granell <sup>2</sup>, <u>D. Espigares</u><sup>1</sup>

<sup>1</sup>Ceva Salud Animal, Barcelona, Spain <sup>2</sup>Cincaporc, Spain

## **Background and Objectives**

Neonatal diarrhea is a common multifactorial problem involving management, facilities, and different infectious agents that include Rotavirus type A, B and C, PEDV, TGE, Enterotoxigenic E.coli (ETEC), C.perfringens type A (CPA) and C (CPC), C.difficile, Enterococcus hirae and Cystoisospora suis. The objective of this study was to compare 2 different commercial vaccines in the control of neonatal diarrhea.

# **Material and Methods**

The trial was performed on a 3500 nulliparous sow unit, as it had started production recently. A high percentage of litters suffered diarrhea around 24-48 hours after birth, despite being vaccinated with an E.coli + CPC vaccine (vaccine A). Laboratory tests confirmed the presence of CPA producing Alfa and Beta-2 toxins, E. hirae, Rotavirus type C, C. difficile and ETEC F41. Results from 479 sows vaccinated with vaccine A were compared with 659 sows with an E. coli + CPC + CPA vaccine (Enteroporc Coli AC), named vaccine B in a cohort retrospective study. The vaccination scheme applied was the same for both products: 2 doses at 5 and 2 weeks before farrowing. Mortality related to diarrhea during the first week of life and the % of litters affected with each type of commercial vaccine were recorded.

#### Results

Diarrhea-related mortality was statistically significantly reduced (p<0,001) from 8.12% (Vaccine A) to 2.78% (Vaccine B) The prevalence of affected litters was statistically significantly reduced (p=0,006) from 83.5% (Vaccine A) affected litters to 73.3% (Vaccine B)

### **Discussion and Conclusion**

The vaccination induced protection against CPA alpha and beta 2 toxins provided by Enteroporc coli AC significantly reduced diarrhea-related mortality of piglets during the first week of life. The prevalence of litters with clinical diarrhea also decreased significantly, although it remained high, indicating possible multifactorial nature of this type of diarrhea in combination with impact of immaturity of sow population on newly established farm. The presence of other infectious agents and environmental, colostrum quality of first parity animals and hygiene deficiencies should be considered beside together with the vaccination in case of management of neonatal diarrhoea problems.
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# COMPARISON OF THE EFFICACY OF TEN MESOMYCOPLASMA HYOPNEUMONIAE VACCINES ON THE INCIDENCE OF LUNG LESIONS ASSESSED AT SLAUGHTER IN SPAIN.

M. Lasierra <sup>1</sup>, M. Carmona <sup>1</sup>, <u>D. Espigares</u> <sup>1</sup> <sup>1</sup>Ceva Salud Animal, Barcelona, Spain

### **Background and Objectives**

Despite all efforts performed to reduce economic losses caused by Mesomycoplasma hyppneumoniae (Mh), this continues to be an important concern for worldwide swine herds. Vaccination against Mh is a common tool to prevent and control Enzootic Pneumonia (EP). Evaluation of Ep-like lesions in slaughtered pigs is a good indicator to estimate the incidence of Ep on farms and assess the effectiveness of the prevention measures applied. The purpose of this study was to compare the incidence of Ep-like lesions at slaughter in batches non vaccinated or vaccinated with 10 different commercial vaccines.

## **Material and Methods**

The analysis included 451,900 lungs from 2,807 batches from different farms around Spain and slaughtered between January-2016 and October-2023 in different Spanish slaughterhouses. The animals were no vaccinated or vaccinated with 10 different commercial vaccines, monovalent one dose (Mh) (vaccine H, 1,2,3,4), monovalent two-dose (Mh) (vaccine 5, 6, 7) and bivalent one dose (Mh-PCV2) (vaccine 8,9). The scoring system used was the Ceva Lung Program which assists in evaluating the presence, incidence, and impact of EP using modified Madec grid.For each batch the following parameters were calculated: Enzootic pneumonia index (Ep\_index) Broncho-pneumonic lungs (BPL) (%) Affected surface out of all lungs (ASAL) (%) Affected surface of Bronco-pneumonic lungs (ASPL) (%) Results were compared through Kruskal-Wallis test with pairwise comparisons and p-value was set at 0.05.

#### Results

Batches vaccinated showed statistically (p<0,05) less Ep-like lesions (EP\_index, BPL, ASAL, ASPL) than no vaccinated. Batches vaccinated with Vaccine H showed statistically lower Ep\_index than vaccines 1, 2,3,4,5,6,7,8,9 (p<0.05). Considering BPL, Vaccine H is statistically lower than vaccines 1,3,4,5,6,7,8,9 (p<0.05). In relation to ASAL, Vaccine H is statistically lower than vaccines 1,2,3,4,5,6,7,8,9 (p<0.05). Regarding ASBL, Vaccine H showed statically lower percentage than vaccines 1,2,3,4,5,6,7,8,9 (p<0.05).

## **Discussion and Conclusion**

Vaccination of piglets against Mh reduced the severity of Ep-like lesions in slaughtered pigs. Lungs from farms vaccinated with vaccine H showed less EP-like lesions than lungs from farms vaccinated with any other vaccine or no vaccinated farms. The vaccine H (Hyogen) showed, in this study, its superiority in the reduction of lung lesions over the rest of the vaccines included in the study.

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# DEVELOPMENT OF A LIVE ATTENUATED VACCINE CANDIDATE FOR FMDV BY A COMBINATION OF POINT MUTATIONS IN VP1, 3C, AND IRES

A. Subasinghe <sup>1</sup>, S.Y. Hwang <sup>2</sup>, J. Park <sup>2</sup>, <u>L. Jong-Soo</u><sup>1</sup> <sup>1</sup>College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea <sup>2</sup>Animal and Plant Quarantine Agency, Gyeongsangbuk-do, South Korea

## **Background and Objectives**

Foot-and-mouth disease (FMD) is a highly communicable viral infection. The objective of the study is to design and evaluate the safety and efficacy of a novel live attenuated vaccine (LAV).

## Material and Methods

Site-directed mutagenesis mediated point mutations of specific sequences in IRES (C351G), VP1 (E83K), and 3C (C142T) was implemented generating three LAVs as FMDV 3C(C142T), FMDV VP1(E83K) 3C(C142T) and FMDV IRES(C351G) VP1(E83K) 3C(C142T). In vitro, virus replication, plaque formation, and ISG transcription levels were evaluated. C57BL6 mice were allocated into four groups (n=5), vaccinated intramuscularly with the prepared LAVs and control. They were intraperitoneally challenged with WT virus 7 days post-vaccination (dpv) and tested for survival rate, changes in body weight, percent inhibition of SP-ELISA, with virus neutralization titers (VNT). FMDV seronegative pigs were divided into three groups and vaccinated with double and triple-mutated LAVs (n=6) with control (n=4). Each swine group was divided into two groups where one group was vaccinated intradermally (ID), and the rest were contact inoculated. All the animals were challenged with WT virus (ID) 7 dpv and were tested for clinical score, viremia, VNT, and SP-ELISA.

#### Results

The plaque size reduces with the increase in the mutations in the LAV, indicating attenuation, confirmed with virus replication data. Minimum transcription level of the ISGs was identified in double-mutated virus. Mice vaccinated with FMDV 3C(C142T) and VP1(E83K) 3C(C142T) indicated 100% survival after challenge. Percent inhibition of SP-ELISA result was close to 75%, and VNT was 2.25 log<sub>10</sub>, while the body weight continued to increase after challenge in LAV vaccinated mice. Only double- and triple-mutated LAV were used for the swine experiments. LAV-vaccinated animals from both routes did not show any clinical signs or viremia. Interestingly, ID-vaccinated swine with FMDV VP1(E83K) 3C(C142T) indicated 100% protection from homologous challenge, while WT FMDV-vaccinated swine did not survive until the challenge. Triple-mutated LAV-inoculated swine indicate FMD symptoms. SP-ELISA and VNT results were par with the mice experiments.

## **Discussion and Conclusion**

The results demonstrate that the FMDV VP1(E83K) 3C(C142T) LAV is a potential candidate to control FMDV. [National Research Foundation of Korea (2021R1A6A1A03045495)]

#### IMM – Immunology and Vaccinology

# EVALUATION OF PIGLET VACCINATION PROTOCOL AGAINST GLÄSSER'S DISEASE ON A FARM SUFFERING FROM POLYSEROSITIS IN ARGENTINA

S. Fernandez <sup>1</sup>, M. Colitto <sup>1</sup>, R. Manzano <sup>1</sup>, E. Daffunchio <sup>1</sup>, L. Nodar <sup>2</sup>, <u>L. De Lucas <sup>2</sup></u> <sup>1</sup>*Hipra Argentina*, S.A. <sup>2</sup>*HIPRA*, Amer (Girona), Spain

## **Background and Objectives**

Glässer's disease causes high economic losses due to expensive antibiotic treatment and mortality of animals in the acute form of the disease. Historically, antibiotics have been the most common strategy to treat the disease, but prevention through vaccination has been shown to be an effective and profitable tool. When Glaesserella parasuis causes clinical signs at early ages, early vaccination is needed to properly protect the animals. The present study investigated the efficacy of piglet vaccination in terms of disease control on a swine farm in Argentina.

## **Material and Methods**

Prior to the study, the postweaning mortality rate due to polyserositis was 2.8 % on a 1000 sow farm (farrow to finish) located in Buenos Aires Province. Glässer's disease was confirmed by PCR from piglets at 27, 36 and 73 days of age. Piglet vaccination at 7 and 21 days of age with Hiprasuis® Glässer was initiated in December 2020. For 13 months preweaning+nursery, total and polyserositis mortality as well as weight at slaughter were monitored in alternating vaccinated and non-vaccinated batches, with 3168 piglets and 3158 piglets respectively. To compare both groups a t.test was performed.

# Results

Total mortality was reduced 1,41 points (p value= 0.125) in vaccinated batches. This lack of significancy was due to the farm suffered from serious SIV and APP problems during this period. However, mortality from polyserositis cases was significantly reduced to 0.25% in vaccinated batches compared to 1.77% from the non-vaccinated piglets (p-value=0.002). As regards the weight of the animals at slaughter, the vaccinated batches weighed 1.41 kg more than the non-vaccinated ones (126.02 kg vs 124.61 kg) (p-value= 0.269).

## **Discussion and Conclusion**

This case report shows the efficacy of the early piglet vaccination strategy in controlling mortality due to polyserositis, overcoming possible interference from colostrum antibodies.

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#### HUMORAL RESPONSE AFTER IMMUNIZATION WITH A RECOMBINANT BIOLOGICAL AGAINST PEDV

M. Zapata-Moreno <sup>2</sup>, <u>J.F. Rivera-Benitez</u><sup>1</sup>, J. De La Luz-Armendáriz <sup>2</sup> <sup>1</sup>INIFAP <sup>2</sup>Facultad de Medicina Veterinaria y Zootecnia. UNAM.

#### **Background and Objectives**

Porcine epidemic diarrhea (PED) was identified in Mexico in 2013, generating a high economic impact. Vaccination is under observation under field conditions, the vast majority of which is using inactivated virus. The objective of this work was to evaluate an experimental biological formulated with PEDV recombinant proteins, to understand its antigenic potential and the protection conferred against the challenge.

#### **Material and Methods**

The recombinant proteins N, M and S of PEDV were expressed in the Escherichia coli BL-21 system, purified, concentrated and the biological was formulated with a commercial adjuvant Montanide. The biological (INI-rPEDV) was applied in two doses parenterally and intranasally. 25 adult pigs were used, distributed into five experimental groups: VacR/INF: vaccinated and challenged pigs, VacR/No-INF: vaccinated and unchallenged pigs, No-VacR/INF: unvaccinated and challenged pigs, No-VacR/No -INF: unvaccinated and unchallenged pigs and VacInact/INF: pigs vaccinated with a commercial inactivated virus vaccine and challenged. Sera obtained weekly were analyzed using an indirect ELISA for the detection of IgG, using PEDV recombinant proteins as antigen. At 84 days post-vaccination (dpv), the experimental infection was carried out in the INF groups and the animals were clinically monitored.

#### Results

A greater IgG-specific immune response towards the recombinant proteins was evident in both VacR groups, compared to the VacInact/INF group. Infected animals in the VacInact/INF group showed a shorter duration of clinical signs, followed by the VacR/INF group, compared to the No-VacR/INF group.

#### **Discussion and Conclusion**

The efficacy of the recombinant biological was proven through the induced humoral immune response, as well as the protection conferred when challenged with a virulent strain, leading to it being postulated as a candidate subunit vaccine (INI-rPEDV) against PED. Financing FONSEC SADER-CONACYT 2017-06-292826.

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# INFLUENCE OF NEW VACCINE TECHNOLOGY AGAINST MYCOPLASMA HYOPNEUMONIAE IN PNEUMONIA INDEX AND BACTERIAL DIVERSITY OF LOWER RESPIRATORY TRACT IN PIGS

L.G. De Oliveira <sup>1</sup>, G.Y. Storino <sup>1</sup>, F.M. Petri <sup>1</sup>, B. Bracco Donatelli Muro <sup>2</sup>, A. Pegoraro Poor <sup>3</sup>, M. Carvalho De Abreu Fantini <sup>4</sup>, T. Da Silva Martins <sup>5</sup>, O.A. Sant'Anna <sup>6</sup>, H.J. Montassier <sup>1</sup>

<sup>1</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil

<sup>2</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>3</sup>Department of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine and Animal Sciences, University of São Paulo (USP), Campus São Paulo, SP, Brazil

<sup>4</sup>University of São Paulo (USP), Physics Institute, São Paulo, Brazil.

<sup>5</sup>Department of Chemistry, Federal University of São Paulo (UNIFESP), Diadema, SP, Brazil <sup>6</sup>Butantan Institute, São Paulo, Brazil.

# **Background and Objectives**

Mycoplasma hyopneumoniae infections may affect the diversity and composition of the respiratory microbiota in pigs. New experimental vaccine technology is available and can be a promising tool to control this infection. The present study aimed to describe the abundance of main bacteria present in the lower respiratory tract of animals from different vaccination protocols and its correlation with the pneumonia index.

#### **Material and Methods**

A total of 48 piglets were divided into four groups (n = 12) submitted to the following vaccination protocols, G1: oral adjuvant + commercial vaccine (CV) at 24 days of age (doa); G2: oral vaccine (OV) on the third day of life + CV at 24 doa; G3: CV at 24 doa, and G4: CV + OV at 24 doa. At 73 days, all piglets were euthanized and necropsied for evaluation of the Mhyo-associated lung lesions (European Pharmacopoeia Method) and collection of bronchoalveolar fluid (BALF). The DNA was extracted from BALF and analyzed by Nanopore sequencing to evaluate the lower respiratory tract microbiota.

#### Results

Relative abundance (%) of Mycoplasma in the BALF was 51.9%, 49.4%, 36.3%, and 41.0% for G1, G2, G3, and G4, respectively. Relative abundance of Glaesserella was 2.5%, 1.2%, 0.4% and 7.4% for G1, G2, G3 and G4, respectively. Relative abundance of Bordetella was 3.4%, 34.4%, 17.1% and 6.8% for G1, G2, G3 and G4, respectively. Relative abundance of Streptococcus was 5.3%, 1.5%, 0.4% and 1.8% for G1, G2, G3 and G4, respectively. Relative abundance of Ureaplasma was 9.5%, 1.9%, 13.6% and 8.8% for G1, G2, G3 and G4, respectively. The pneumonia index was 1.07, 0.80, 1.33, and 0.93 for G1, G2, G3 and G4, respectively.

# **Discussion and Conclusion**

The results indicate that G2 and G4 performed better in the pneumonia index and a lower abundance was observed for Ureaplasma and Streptococcus in G2. Analyzing Mycoplasma, G3 and G4 stood out, with a lower relative abundance. Groups including the oral vaccine in combination with the commercial vaccines could reduce the area of pneumonia and probably impact the lower respiratory microbiota, however, it is necessary to analyze global bacterial diversity indices. Grant#2021/11914-0, São Paulo Research Foundation (FAPESP).

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# OBSERVATIONAL FIELD STUDY ON THE USE OF A PCV-2D VACCINE IN A COMMERCIAL FARM IN MEXICO

A. Nava <sup>1</sup>, J. Velasco <sup>1</sup>, P. Alcantar <sup>1</sup>, <u>E. Bousquet</u> <sup>2</sup>, J. Rodriguez <sup>3</sup> <sup>1</sup>Virbac, Av. Inglaterra N. 5070, Guadalajara Technology Park, Zapopan, Jalisco, Mexico, 45010 <sup>2</sup>Virbac SA,13ème rue, 06510 Carros, France <sup>3</sup>31 BX24 y 26 250 Miguel Aleman, CP 97148, Merida, Yucatan, Mexico

## **Background and Objectives**

PCV-2d is now the predominant PCV-2 genotype in major pork producing regions. This study was carried out to evaluate the use of a PCV-2d vaccine on performance of piglets in a Mexican commercial weaning to finishing farm.

#### Material and Methods

Selected farm (3500 sows) is endemically infected with PRRSV, SIV (swine influenza virus), PEDv, BEDv (blue eye disease virus only reported in Mexico) and PCV-2. Weaned piglets were randomly assigned to a treatment (T) or control (C) group according to their weight (6.81 kg and 6.98 kg in average, respectively, 600 piglets per group). T group received SUIGEN<sup>®</sup> PCV2 (genotype d) vaccine and C group received a PCV-2 subunit vaccine, Ingelvac CircoFLEX<sup>™</sup>. Pigs were housed according to conventional management. Mortality cases and injectable antimicrobial treatments were recorded daily from weaning to finishing. Blood samples were taken at weaning on 15 piglets per group and pooled by 5 for PCV-2, PRRSV and SIV detection. Oral fluids were collected by pen at 10, 18 and 22 weeks of age (4 pooled oral fluids per group and date) for PCV-2, PRRSV and SIV detection by RT-PCR. Mortality rate and antimicrobial treatments over time were compared between groups by Fisher exact test and Wilcoxon signed rank test.

# Results

Mortality rate was low in both groups, though numerically lower in T (1.67%) than in C (3.0%) group (p > 0.05). The number of antimicrobial treatments was lower in T than in C group, a reduction of 25.1% being observed (p < 0.05). Half of blood pools were PCV-2 and PRRSV positive at weaning. Most oral fluids pools were PCV-2 positive and PRRSV negative, all were SIV positive, from 10 weeks of age, whatever the group, reaching similar Ct's.

## **Discussion and Conclusion**

Performance of both vaccines was similar when comparing mortality and Ct values, a decrease of antimicrobial treatments being observed with the tested vaccine. PCV-2 circulation from wean to finish may be linked to the lack of sows vaccination. Tested vaccine can be considered as an alternative in weaning to finishing farms for the control of PCV-2 in Mexico.

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# PRRS VACCINES USED AT FIRST WEEK OF LIFE HAVE COMPARABLE SAFETY

<u>K. Koenders - Van Gog</u><sup>1</sup>, T. Wijnands<sup>2</sup>, M. Steenaert<sup>3</sup> <sup>1</sup>Lintjeshof Veterinary Practice and Merefelt Livestock Diagnostics <sup>2</sup>Lintjeshof Vet Group, The Netherlands <sup>3</sup>Boehringer Ingelheim Animal Health Netherlands by

#### **Background and Objectives**

In farms that are not yet successful in delaying the main timepoint of piglet PRRSv infection to at least 5-6 weeks of age it may be indicated to vaccinate in the first week of life. In the absence of a registered PRRS MLV product an alternative PRRS MLV vaccine was used and the safety was evaluated.

## Material and Methods

In 8 sow herds (farrow to grow) the registered product (Suvaxyn PRRS MLV; Before), vaccinated at processing of the piglets 3-5 days of age, was replaced one on one by an alternative product (Ingelvac PRRSFLEX; After). The safety of the product was evaluated by the farming staff and consulting veterinarian by clinical observation, and by a before-after followup of the mortality before weaning. Mortality was calculated per farm as averages of percentages of mortality per week (AgriSyst PigExpert) from 10 weeks 'Before', a 4 week transition period and 10 weeks 'After'.

#### Results

All farms reported that the clinical safety of the product 'After' was comparable to the product 'Before'. Mortality of piglets before weaning, 10 weeks Before, 4 week transition and 10 weeks After, changed from 12.3 + 4.3%, to 14.2 + 3.8%, to 12.9 + 4.3% (avg +/- stdev).

#### **Discussion and Conclusion**

Interactions of commercial PRRSv-1 MLV vaccines with Maternally Derived Immunity has been described and based on that the alternative PRRS MLV vaccine for vaccination in the first week of life was selected. In the participating farms the change of PRRS MLV vaccine took place during summer and autumn of 2022. As in last quarter of 2022 the number of PRRS-outbreaks in the Netherlands increased, the infectious pressure may have had an influence on the results in the 'After' period in the participating farms. Despite this the mortality before weaning was stable or decreased in 4 out of 8 farms.

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# THE EVALUATION OF VACCINATION ELICITED ELISA AND NEUTRALIZING ANTIBODIES AGAINST CLOSTRIDIUM PERFRINGENS TYPE A ALPHA TOXIN IN SOW COLOSTRUM UNDER FIELD CONDITIONS

<u>D. Sperling</u><sup>1</sup>, J. Vanhara<sup>2</sup>, H. Karembe<sup>3</sup>, J. Finzel<sup>4</sup>, S. Springer<sup>4</sup> <sup>1</sup>Ceva Sante Animale, Libourne, France <sup>2</sup>Ceva Sante Animale Slovakia <sup>3</sup>Ceva Santé Animale, 10 Avenue de la Ballastière, 33500 Libourne - France <sup>4</sup>Ceva Innovation Center, Dessau-Rosslau, Germany

## **Background and Objectives**

Neonatal diarrhoea (ND) of piglets is one of the most common diseases on pig farms today, affecting farms worldwide. Clostridium perfringens type A (CpA) is a frequently isolated pathogen from cases of ND. The alpha toxin (CPA) plays a major role as a virulence factor, together with other toxins. The aim of this study was to evaluate the antibody titers against the CPA in colostrum of gilts following primo-vaccination and confirm the presence of neutralizing antibodies against CPA.

## **Material and Methods**

10 randomly selected gilts were vaccinated at 5 and 2 weeks before farrowing with 2.0 ml Enteroporc COLI AC (Ceva Santé Animale, France). Blood samples were collected 1 week before 1<sup>st</sup> vaccination to confirm the status of selected gilts (by ELISA and Lecithinase Neutralization Test- LNT). A colostrum sample was collected from each sow at farrowing and tested for antibodies against CPA (ELISA), as well as for neutralizing antibodies against CPA (LNT). The statistical evaluation was completed using GraphPad Prism. The correlation analysis was performed between levels of Ig obtained by ELISA and LNT (both obtained in colostrum samples).

#### Results

Prior to implementing the vaccination in the study, blood ELISA specific CPA Ig were low (0.367 AU/ml), expressed as mean or absent in selected gilts (5/10), and no neutralizing Igs were detected. Following a series of primo-vaccination, a significant increase in the mean value of colostrum anti CPA Ig (10.935 AU/ml) was reported. All vaccinated gilts except one, showed neutralizing antibodies against CPA (LNT) in the colostrum (titer from 1 to 32) with a mean titer of 8.4 AU/ml at the 1<sup>st</sup> farrowing. The correlation analysis confirmed a significant correlation between the ELISA Ig and neutralizing Ig against CPA toxin, with Pearson coefficient r= 0.9805.

## **Discussion and Conclusion**

Vaccinated gilts developed antibody titers (ELISA) against CPA. Antibodies against the alpha toxin showed neutralizing activity based on results of LNT test and were highly correlated with the results of ELISA CPA Ig. When comparing the antibody titers (ELISA and LNT) in colostrum with those from challenge trials (Springer, et al. 2023), a high protection against CPA can be assumed.

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# SOW VACCINATION AGAINST GLÄSSER'S DISEASE CONFERRED CLINICAL PROTECTION IN THEIR OFFSPRING, IN THE PRESENCE OF VIRAL COINFECTIONS

A. Miralles-Chorro<sup>1</sup>, J. Seva<sup>2</sup>, M. Jiménez<sup>3</sup>, M. Marcos<sup>3</sup>, <u>R. Menjon<sup>3</sup></u> <sup>1</sup>CEFU,S.A., Alhama de Murcia, Spain <sup>2</sup>Dpto. Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria de Murcia, Spain <sup>3</sup>MSD Animal Health

#### **Background and Objectives**

Glässer's disease generates a great productive impact in the swine industry. Lately, its incidence has increased in the first weeks of life where, associated with PRRSV or Influenza, it complicates the resolution of infections. Worldwide, the most prevalent serotypes are 4 and 5. Serotyping is the most used subtyping method for Glaeserella parasuis, being of great relevance for vaccination strategy. The objective of this study was to assess the clinical protection of passive immunization of piglets by sow vaccination against Glässer's disease.

#### Material and Methods

This study was carried out on a production farm housing 1450 sows (site 1) and 5700 weaners (site 2), with a history of Glässer's disease confirmed by clinical signs (high incidence of arthritis, meningitis, and sudden deaths), polyserositis found at necropsies of death piglets and detection by PCR of serotypes 5 and 12 of Glaesserella parasuis. Viral coinfections were also detected at 3 weeks of age by RT-qPCR in lungs (PRRSv ct-value 17; Influenza ct-value 26). Sows were vaccinated with an inactivated Glaesserella parasuis serotype 5 vaccine (Porcilis® Glässer) at 70 and 100 days of gestation. Overall piglet mortality and Glässer's-associated mortality (death piglets with respiratory distress and polyserositis) data were recorded for a total period of 26 weeks, divided in two periods: before (group A; 13 weeks; n=11522) and after vaccination (group B; 13 weeks; n=11880). Data was analyzed using the Mann-Whitney U test

#### Results

The Glässer's-associated mortality of suckling piglets (site 1) was significantly (p=0.001) reduced after vaccination (group B: 0.45 %) compared to the period before vaccination of sows (group A: 2.14%). When considering other causes of mortality, a numerical decrease was also observed for crushed piglets in group B (0.88% less mortality). The overall mortality in site 2 showed highly significant differences (p=0.004) between group A (3.40%) and group B (1.77%). Glässer's-associated mortality in weaned piglets was 1.03% and 0.16% in group A and B (p=0.004), respectively.

#### **Discussion and Conclusion**

Under the conditions of this study, sow vaccination had an apparent effect on reduction of Glässer's-associated mortality in piglets, suggesting a clinical protection (homologous and heterologous serotypes) even in the presence of viral coinfections

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# BEFORE-AND-AFTER COMPARISON OF PRRS VACCINE FIELD EFFICACY IN A 1800 SOW SYSTEM IN VIETNAM

<u>H. Nguyen</u><sup>1</sup>, T. Hoang<sup>1</sup>, V. Tran<sup>1</sup>, C. Maala<sup>1</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Viet Nam

### **Background and Objectives**

PRRS Field Control, especially in Large Systems, is difficult to implement unless a holistic approach is done. This paper aims to present a case-control example of PRRS control particularly in a Large System in Vietnam.

### **Material and Methods**

A total of 14 months, namely a 6-month control period where the farm is implementing a North American strain PRRS vaccine, a 2-month Transition Period and finally another 6 months treatment period using Ingelvac® PRRS MLV, was investigated. The periods were compared graphically using Minitab 19 using Statistical Process Control Charts. For the piglet mortality, a total of 19,778 pigs in 2 groups (Aug 2022-January 2023) and (January 2023-May 2023) were compared via Chi-Square Test.

# Results

Results show an increase in Total born from 12.4 to 13.46 average with lesser monthly variation during the Treatment phase. Average mortality decreased to 7.98 but more importantly with better consistency during the Treatment phase. The difference, however, is only numerivally different.

# **Discussion and Conclusion**

During the period of 6 months using Ingelvac PRRS MLV, there was an increase by almost 1 pig/sow born with lesser mortality. This shows the direct benefits on productivity of the vaccine and showing consistent results in the control of PRRS.

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EFFECT OF VACCINATION AGAINST E. COLI, C. PERFRINGENS TYPE A/C ON PIGLET PRODUCTIVE AND CLINICAL PARAMETERS UNDER FIELD CONDITIONS

<u>A. Dors</u><sup>4</sup>, R. Panek <sup>1</sup>, W. Luzynski <sup>2</sup>, K. Janeczko <sup>3</sup>, A. Augustyniak <sup>4</sup>, H. Turlewicz-Podbielska <sup>4</sup>, E. Czyzewska-Dors <sup>5</sup>, M. Pomorska-Mol <sup>4</sup>

<sup>1</sup>Ceva Animal Health, Poland

<sup>2</sup>Cedrob, Poland

<sup>3</sup>Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Poland; Ceva Animal Health, Poland

<sup>4</sup>Department of Preclinical Sciences and Infectious Diseases, Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences, Poland

<sup>5</sup>Department of Internal Diseases and Diagnostics, Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences, Poland

# **Background and Objectives**

Neonatal diarrhoea (ND) is a cause of major losses in pig herds worldwide. Escherichia coli (E. coli) and Clostridium perfringens (C. perfringens) are one of the most common pathogens involved in piglet ND. The most effective prevention of ND is the vaccination of sows and the transfer of maternal immunity to piglets. The aim of this study was to compare the effect of two vaccines against ND on clinical and productive parameters in suckling piglets.

#### **Material and Methods**

The study was performed in a Polish farrow-to-wean farm with 5,500 sows, already using an E. coli and C. perfringens vaccine, but still suffering ND. The presence of C. perfringens containing beta2 toxin genes was confirmed before the trial. Five consecutive batches of sows were divided into two groups (A and B). Enteroporc Coli AC® (Ceva) was administrated to sows from group A according to the manufacturer's instructions. Sows from group B were vaccinated against ND using two other combined commercial vaccines. In each group piglets from 10 randomly selected litters were ear-tagged and individually weighted at birth, at 8 and 22 days of age. The incidence of diarrhoea, general piglet body condition, and antimicrobial treatment were recorded within 10 consecutive days after birth. Statistical analyses were performed using the Chi-square test, Mann-Whitney test or T-test. Differences were considered significant when p<0.05.

## Results

A total of 234 piglets (119 in group A and 115 in group B) were included. The mean weight gain of piglets from birth to 22 days of age was significantly higher in group A – 4.99 kg than in group B – 4.66 kg (p=0.025). The rest of the recorded parameters such as the presence of diarrhoea, piglet's body condition score, and the number of days with antimicrobial treatment did not differ significantly between groups.

#### **Discussion and Conclusion**

The results of the study confirmed the efficiency of Enteroporc Coli AC® vaccine in the reduction of ND consequences. The vaccinated group showed an improvement in the productive parameters of piglets during the lactation phase.

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# FIELD COMPARATIVE TRIAL OF TWO COMMERCIAL PCV2 VACCINES IN A CANADIAN FARM

<u>R. Jorda</u><sup>1</sup>, M. St-Hilaire<sup>2</sup>, M. De Groot<sup>3</sup>, G. García<sup>1</sup>, G. Ibanez<sup>4</sup> <sup>1</sup>*HIPRA, Amer (Girona), Spain* <sup>2</sup>*Global Porc, St-Bernard (Quebec), Canada* <sup>3</sup>*Hipra Canada, Ottawa, Canada* <sup>4</sup>*Hipra Saúde Animal, Porto Alegre, Brazil* 

# **Background and Objectives**

Porcine Circovirus type 2 (PCV2) is a ubiquitous virus that can lead to significant production losses. Controlling PCV2 virus is essential for swine herds worldwide. The aim of this study was to compare the impact of two PCV2 vaccines on production parameters of a high health status farm from Canada.

## **Material and Methods**

A 600-sow farrow to wean farm positive to PCV2 from Canada was selected, and pigs from this farm were monitored through the finishing period. The selected farm is considered to have a high health status (Mycoplasma hyopneumoniae (Mhyo) negative and Porcine Respiratory Reproductive Syndrome (PRRS) negative) and a high standard of animal welfare. Two consecutive batches were divided into two groups. In the first batch, first group (n=843) was vaccinated with MHYOSPHERE® PCV ID (MH), an intradermal needle-free vaccine against Mhyo and PCV2-associated disease at 4 weeks of age. The second group (n=886) was vaccinated with two doses of Vaccine A, an intramuscular vaccine against PCV2 at 4 days and 4 weeks of age. In the second batch, the same methodology was followed with 830 pigs in MH group, and 830 pigs in Vaccine A group. The production parameters measured included the body weights, days in the fattening unit, growth performance, mortality, and antibiotic treatments. Statistical analysis was conducted for all parameters.

#### Results

No significant differences (p>0.05) were observed on body weights between groups at the time of vaccination. However, significant differences (p<0.001) were observed at slaughter weight with the MH group (128.23±11.35Kg) vs Vaccine A (127.26±11.5Kg). Also, the pigs in MH group were slaughtered 1.38 days earlier (p=0.008) compared to Vaccine A. The average daily weight gain (ADWG) was significantly higher in the MH group, with an increase of 18 g/day (p<0.001). No significant differences were observed in terms of antibiotic treatments and mortality.

#### **Discussion and Conclusion**

These results may indicate that intradermal needle free vaccination with MHYOSPHERE® PCV ID performed better than Vaccine A on this farm. This could be attributed to the vaccine composition itself but also a result of the welfare and health benefits of needle free vaccination that have been reported in previous studies.

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# GLOBAL EVALUATION ON USER-EXPERIENCE FOR A NEW NEEDLE-FREE DEVICE IN PIG FARMS

<u>J. Angulo</u><sup>1</sup>, C. Mah<sup>2</sup>, L. Van De Weyer<sup>3</sup>, J. Abadias<sup>4</sup>, E. Franco<sup>5</sup>, W. Navasakuljinda<sup>6</sup>, T.H. Vo<sup>7</sup> <sup>1</sup>Zoetis Inc, 1040 Swabia Ct, Durham, NC 27703 – United States <sup>2</sup>ZOETIS, Parsippany, NJ, USA <sup>3</sup>Zoetis Canada Inc, Kirkland, QC <sup>4</sup>Zoetis Spain SLU <sup>5</sup>Zoetis Inc <sup>6</sup>Zoetis (Thailand) Limited, Bangkok 10500, Thailand <sup>7</sup>Zoetis Vietnam

# **Background and Objectives**

Despite the clear advantages of needle-free vaccine administration, adoption of needle free devices (NFD's) in the swine industry has not reached high levels due to factors impacting the efficiency of the vaccination process: high maintenance, considerable start-up costs, training requirements, poor ergonomics, and the limited options for vaccines to be administer via Needle-Free. The objective of this global study was to evaluate the practical usability of a new NFD (Pulse FX, Pulse-NF-Systems, KC-US) to assess the user experience during vaccination processes under farm conditions

## **Material and Methods**

Twelve farms were enrolled from nine production companies located in six countries (USA-Canada-Brazil-Spain-Thailand-Vietnam). The duration of the study was 7-months. A 25-question survey was employed to gather user-feedback. The Likert-Scale survey methodology was used to assess four attributes: agreement on ease of use (scale from 1-5: 1-strongly disagree - 5-strongly agree), frequency of satisfaction (scale from 1-5: 1-never - 5-very frequently), relevance for getting vaccination done (scale from 1-3: 1-poor - 3-excellent), and value for increasing vaccination efficiency (scale from 1-4: 1none - 4-high). Evaluations were collected immediately after NFD-vaccination from twenty-three users at several vaccination sessions completing 43 sets of surveys results for the analysis. Data was analysed using frequency distributions, cross tabulation, and hypothesis testing (Minitab-20.1.1-USA). Nonparametric 1-sample sign test was performed against reference scales to identify positive aspects of each attribute evaluated.

#### Results

For ease of use, 53% of users expressed positive scales, 19% neutral and 21% negative. For satisfaction rates, 40% were positive, 21% neutral and 36% negative. Overall, 34% rated the relevance for implementing the vaccination process as excellent, 48% neutral and 16% poor. Thirty-two % considered it high value to increase vx-efficiency, 45% and 16% valued it as moderate and low respectively, 3% considered no value. Most individual scales were statistically significant (p<0.05) towards positive aspects in all areas from the survey.

#### **Discussion and Conclusion**

Overall, positive feedback on the use of Pulse FX was observed; users found it easy to use, neutral to better than syringeand-needle or previously used NFD's. There was a trend in perception that this new NFD helped to increase vaccination efficiency, user safety and improvement of vaccination process.

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# EFFICACY OF SIMULTANEOUS APPLICATION OF IRON/ANTICOCCIDIAL COMBINATION PRODUCT AND OEDEMA DISEASE VACCINE IN SUCKLING PIGLETS

<u>D. Sperling</u><sup>1</sup>, H. Karembe<sup>2</sup>, A. Diesing<sup>3</sup>, J. Morales<sup>4</sup>, L. De Frutos<sup>4</sup>, A. Manso<sup>4</sup>, M. Rodriguez<sup>4</sup> <sup>1</sup>Ceva Santé Animale SA, 10 Avenue de la ballastière, CS 30126 – 33501 Libourne Cedex, France <sup>2</sup>Ceva Santé Animale, 10 Avenue de la Ballastière, 33500 Libourne - France <sup>3</sup>Ceva Santé Animale, Dessau-Rosslau, Germany <sup>4</sup>PigCHAMP Pro Europa

## **Background and Objectives**

Iron deficiency anaemia is the most common deficiency in swine production and neonatal piglets are routinely subjected to parenteral iron supplementation. Oedema Disease (OD) in pigs occurs worldwide and is characterized by acute enterotoxaemia causing substantial mortality on affected farms. The aim of this study was to evaluate the efficacy of simultaneous application of iron/anticoccidial treatment (IAT; Forceris®) and vaccination against OD (ODT; Ecoporc Shiga®) on the development of neutralizing antibodies, hematological parameters, and performance of piglets.

# Material and Methods

Seventy-two (72) piglets from 6 litters were included in the study. Piglets were from sows (parity 3 to 6) negative for Stx2e antibody. At day 2 after birth, piglets were randomly allocated into the following groups: T1) IAT and ODT administered on different days (24-48 and 72-96 hours after birth, respectively) and T2) IAT and ODT administered simultaneously (72-96 hours after birth) in different sites of the neck. Piglets were weighed on days 0, 21 and 60 to evaluate growth, blood samples were collected on days 21 and 60 to determine neutralizing antibodies levels. Faecal samples were collected on day 28 to evaluate the presence of C. suis.

#### Results

There was no difference between the groups in the development of naturalizing antibodies, which was evaluated comparing the number of animals with neutralizing antibodies (response) and animals with protective titer ( $\geq$  15) (P>0.05). There was no difference between treatments in any case of hematinic activity (anemic, sub-anemic and optimal category), neither on day 28 nor day 60 (P>0.05), with only one animal in sub-anemic category in T1 group.Protocol of application did not affect average daily gain (ADG) during lactation. The body weight on day 60 and global ADG (from day 0 to day 60) tended to be higher when Forceris® and Ecoporc Shiga® were applied simultaneously. No oocysts of C. suis were detected.

## **Discussion and Conclusion**

Both protocols can be used under the field conditions without negative effect on efficacy. Simultaneous application on different sites of the body may improve work management in farrowing house during the first days of life of piglets and reduce stress of the animals because of excessive manipulation.

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# IMPORTANCE OF MAINTAINING A LONG-TERM PCV-2 VACCINATION PROGRAM IN SOWS FOR OPTIMAL CONTROL OF EARLY INFECTIONS OVER TIME

<u>S. Oliver</u><sup>1</sup>, S. Cárceles<sup>1</sup>, L. Garza<sup>1</sup>, C. Casanovas<sup>1</sup>, D. Espigares<sup>1</sup> <sup>1</sup>Ceva Salud Animal, Barcelona, Spain

#### **Background and Objectives**

This study sought to evaluate the presence of early PCV-2 infections during the application of a two-year PCV-2 sow vaccination program and after this vaccine protocol was stopped.

#### **Material and Methods**

The selected farm showed PCV-2 PCR positive blood samples at 6 and 9 weeks of age (woa) with loads of 1.2×10<sup>5</sup> and 2.3×10<sup>7</sup> PCV-2 copies/mL, respectively. Piglets were routinely vaccinated with a PCV-2 subunit vaccine and sows remained unvaccinated.Consequently, a new vaccination program was started using Circovac<sup>®</sup> in sows (one dose at the end of lactation) and piglets. In piglets from vaccinated sows, blood samples were taken at 3, 6 and 9 woa (10 samples per age, selecting the smallest piglets from each pen). This sampling was repeated every 6 months during the two years of duration of the sow vaccination program. Samples were processed by PCV-2 PCR (5-sample pools). Moreover, PCV-2 antibody levels at 3 woa were also evaluated by ELISA in samples from the first batch of piglets from vaccinated sows. Two years after the start of the study, the farmer decided to stop vaccinating sows against PCV-2 to save vaccine costs. When the offspring of unvaccinated sows arrived, the same blood sampling was repeated.

#### Results

At 3 woa, piglets from PCV-2 vaccinated sows displayed a statistically significant increase of antibody levels compared to previous piglets from unvaccinated sows.No PCV-2 PCR positive samples at 3, 6 or 9 woa were detected in any of the samplings carried out over two years in piglets from vaccinated sows.When PCV-2 sow vaccination was stopped, piglets from unvaccinated sows arrived again showing significantly lower antibody levels at 3 woa. Additionally, PCV-2 PCR positive blood samples were detected in these animals at 3 and 8-9 woa with loads of 1.0×10<sup>4</sup> and 5.5×10<sup>5</sup> PCV-2 copies/mL, respectively.

## **Discussion and Conclusion**

Sow vaccination protocol with Circovac<sup>®</sup> has proven to be an effective tool for the control of early PCV-2 infections. This study demonstrated the importance of maintaining a vaccination program in sows to achieve an optimal PCV-2 infection status over time.

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# PROTECTION PROVIDED BY PRRSV MLV (PRRSV1 AND PRRSV2) AGAINST AN ASIAN PRRSV2 FIELD STRAIN

J. Miranda <sup>1</sup>, S. Romero <sup>1</sup>, <u>L. De Lucas <sup>1</sup></u>, M. Fenech <sup>1</sup>, F. Saito <sup>2</sup>, I. Diaz <sup>3</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA Japan, Tokyo <sup>3</sup>Universitat Autònoma de Barcelona

## **Background and Objectives**

PRRSV vaccines have been shown to mitigate the impact of the disease. PRRSV is classified into two species: PRRSV1 is more prevalent in Europe, and PRRSV2 in America and Asia, however, both are circulating in some of the world's leading pork-producing countries. This study assesses the effectiveness of two commercial vaccines - based on PRRSV1 (UNISTRAIN® PRRS; HIPRA) and PRRSV2 (VR-2332) - against an Asian PRRSV2 field strain.

## Material and Methods

23-week-old piglets were divided into four groups: two vaccinated groups (G1, n=5: PRRS1 MLV and G2, n=4: PRRSV2 MLV), and two unvaccinated (INF, n=5 and CTRL, n=5). One month later, G1, G2, and INF were challenged with an Asian PRRSV2 field strain (ORF5 homology with PRRSV1 MLV and PRRSV2 MLV was 63.3% and 85.9%, respectively). Clinical protection was measured in terms of clinical signs and rectal temperatures. Virological protection was measured in terms of PRRSV presence in blood and tissues. Antibodies were determined by ELISAs (PRRSV1 and PRRSV2-antibodies) and specific neutralizing antibodies (NA) by viral neutralization test determined. Statistics were performed using StatsDirect v2.7.7

#### Results

The presence of PRRSV in blood was significantly lower in the vaccinated groups compared to INF, in terms of proportion of positives, titres, extent of viraemia, and overall viral load (p<0.05). At necropsy (21d after the challenge), tissue analysis revealed a similar pattern, with the PRRSV1 MLV showing the lowest viral load in tonsils (p<0.05), a common site of viral presence. One animal (G1) was negative in all tissues. Both PRRSV1 MLV and PRRSV2 MLV induced seroconversion by ELISA. Specific NA against PRRSV2 MLV were detected in G2 at the end of the experiment. Interestingly, PRRSV1 MLV induced NA before the challenge, and a significant NA enhancement appeared after the challenge (p<0.05), contributing to the better protection observed.

## **Discussion and Conclusion**

The vaccine with the lower genetic similarity to the challenge strain provided comparable or even better protection than the vaccine belonging to the same species, suggesting that immunological properties may be more important than genetic similarities for cross-protection. The study highlighted the potential for cross-protection between strains with varying genetic similarities, indicating the complexity of PRRS vaccine efficacy.

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# CASE REPORT: EFFECT OF MATERNAL ANTIBODIES IN PIGLET ANTIBODY RESPONSE AFTER PCV-2 VACCINATION

<u>G. Abella Falcó</u><sup>1</sup>, A. Martinez <sup>3</sup>, L. Rocha <sup>2</sup>, G. Solano <sup>3</sup> <sup>1</sup>Boehringer Ingelheim <sup>2</sup>Gepesa, S.A. <sup>3</sup>Vall Companys S.A. Spain

#### **Background and Objectives**

Porcine circovirus 2 (PCV-2) is the essential etiologic agent of swine diseases comprised under the term of porcine circovirus diseases (PCVDs). Piglet vaccination for PCV-2 is routinely used in pig production. Pig producers cares for serological tests to check a uniform and appropriate antibody response after piglet vaccination. This is a field trial designed to evaluate serological response after PCV-2 vaccination at weaning, in two different scenarios.

## **Material and Methods**

The study was conducted in two different commercial herds in Spain. Farm 1 with no clinical signs observed in finishers; PCR test negative, no histopathological compatible lesions observed.

In Farm 2, the PCVD diagnosis was made based on clinical signs, lesions, viral detection by PCR and immunohistochemistry. Piglets were vaccinated at 21 days of age with Ingelvac CircoFLEX<sup>®</sup>. Sows were not vaccinated in either of these two farms. 50 animals were ear tagged and bled at 21, 69 and 105 days of age in farm 1 and at 21, 58 and 98 days of age in farm 2. ELISA PCV2 Biochek and PCR PCV-2 were run in all the samples. Tongue tips from dead animals at birth were collected in both farms, PCR PCV-2 was analyzed in these samples.

#### Results

In farm 1, tongue tips and all tested animals were PCR negatives at each sampling point. Nevertheless, high levels of maternal antibodies were detected in animals at 21 days of age, before PCV-2 vaccination. At days 69 and 105 antibodies induced by the vaccine were detected.

In Farm 2, PCV-2 vertical transmission was detected by PCR in tongue tips. Viremia was detected in the farrowing unit (21doa), nursery (58doa), and finishers (98doa). The virus circulation caused an erratic antibody response.

## **Discussion and Conclusion**

Protection against PCV-2 is not measured by antibody levels. However when there is no virus circulation at early ages, the vaccination response is solid and homogeneous, like in farm 1.

In farm 2, the antibody response was variable, and it corresponds more to an uncontrolled virus circulation rather than a correct vaccine response. Vertical transmission must be cut to have optimal response to the piglet vaccination.

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# EFFICACY OF AN ORAL MODIFIED LIVE VACCINE AGAINST LAWSONIA INTRACELLULARIS ADMINISTERED VIA A MILK CUP SYSTEM.

L. Eppink<sup>1</sup>, C. Henry<sup>2</sup> <sup>1</sup>Boehringer Ingelheim Animal Health UK and ROI <sup>2</sup>Colin Henry Pig Consultant

## **Background and Objectives**

We report the findings of a field observation (3 stage production site, weekly farrowing, weaning around 3000 pigs/batch) performed to evaluate the efficacy of Enterisol® lleitis given via a cold milk system (Pump'n'Grow) mixed with DanMilk<sup>™</sup> Supreme. The grow-finishing pigs had clinical ileitis (diagnosed by the private vet to be caused by Lawsonia intracellularis by PCR on faecal samples) which resulted in a mortality rate above target levels of in total 3.0%

# **Material and Methods**

Pigs were vaccinated against Lawsonia intracellularis 3 days before weaning at the average age of 25 days. On the day of vaccination, the milk supply was shut off at 5 am. Around 10 am the milk was mixed with the vaccine and the mixture was fed to the piglets by the automated system for 3 hours. After the vaccine/milk mixture was consumed by the piglets, the system switched back to normal milk feeding. After weaning the pigs (whole batch) would first go to 1 of 3 nursery sites and from there to 1 of 25 grow-finishing sites. Data collected was an average for each batch, finishing sites only (FCR, Mortality and ADG g/d), for 3 months without vaccine (slaughtered in the period January-March 2023) and 3 months with vaccine (slaughtered in the period August-October 2023).

# Results

The clinical ileitis disappeared after the vaccination was implemented. As a result, mortality dropped from 3% to 2.3% (p<0.0001). The technical performance of the finishing pigs also improved; after vaccination started the ADG improved by 20g/d from 1012g/d to 1032g/d (p=0.025). The FCR dropped by 0.1 from 2.58 to 2.48 (p=0.0019).

#### **Discussion and Conclusion**

These results demonstrate the efficacy of an oral ileitis vaccine given via a cold milk system, by increasing the ADG (p=0.025) and reducing the mortality (p<0.001) and FCR (p=0.0019).

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# ENHANCED PRODUCTIVITY AND ANTIBIOTIC REDUCTION IN PIGS VACCINATED INTRADERMALLY WITH A PRRSV1 MLV ON A JAPANESE PRRSV2-POSITIVE FARM

J. Miranda <sup>1</sup>, S. Romero <sup>1</sup>, <u>L. De Lucas</u> <sup>1</sup>, A. Puig <sup>1</sup>, F. Saito <sup>2</sup>, R. March <sup>1</sup>, D. Sabaté <sup>1</sup>, I. Diaz <sup>3</sup> <sup>1</sup>HIPRA, Amer (Girona), Spain <sup>2</sup>HIPRA Japan, Tokyo <sup>3</sup>Universitat Autònoma de Barcelona

#### **Background and Objectives**

PRRSV vaccines can reduce the clinical and productive disease outcome. 2 routes of administration, intramuscular (IM) and intradermal (ID), are available for PRRSV vaccines. PRRSV1 is more prevalent in Europe, and PRRSV2 in America and Asia. The objective was to evaluate the efficacy of two commercial PRRS MLV - one based on PRRSV1 (IM and ID), and another on PRRSV2 (IM) - in growing pigs on a Japanese PRRSV2-positive farm.

# **Material and Methods**

At 3 weeks of age, namely 0 days postvaccination (dpv), 180 Kurobuta piglets were distributed into three groups and vaccinated: group V1\_IM (PRRSV1MLV, Unistrain® PRRS; IM); group V1\_ID (PRRSV1MLV, Unistrain® PRRS; ID); and group V2\_IM (PRRSV2MLV VR-2332; IM). Clinical and productive parameters up to slaughter age, the use of antibiotics, detection of PRRSV in blood - and tissues of dead animals -, and humoral responses were measured. Kruskal-Wallis was used to compare averages among groups, proportions were compared with ÷2 test.

#### Results

No vaccine strains were found in blood after one month; however, the PRRSV2MLV strain was found in the lungs from a dead animal (47 dpv) belonging to V2\_IM. After 14 dpv, the V1\_ID group showed significantly fewer days with clinical signs than V1\_IM and V2\_IM (36 < 74 and 77, respectively; p<0.05). During the first month postvaccination, the V1\_ID group showed a significantly higher daily weight gain than V1\_IM and V2\_IM (286.0 > 235.0 and 234.7 g/d, respectively; p<0.05). The cumulative number of days with individual antibiotic injections was lowest in the V1\_ID group (38 days for V1\_ID < 87 days for V1\_IM and 84 days for V2\_IM; p<0.05). PRRSV2 antibodies were detected in all groups.

## **Discussion and Conclusion**

Despite the genetic differences, IM PRRSV1\_MLV and PRRSV2\_MLV provided similar efficacy on a PRRSV2\_positive farm. Conversely, a better outcome was achieved using ID PRRSV1\_MLV. The study suggests that ID vaccination is an effective strategy for PRRS control. Additional advantages of using ID route are: avoiding iatrogenic transmission of pathogens and less stress and pain. Apart from the route of administration, these findings might be explained by the immunological properties of the strains involved and by the interference with maternally-derived antibodies at vaccination

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# EVALUATION OF A ONE-SHOT RECOMBINANT VACCINE AGAINST ERYSIPELAS IN PIGS

L. Karimova <sup>1</sup>, S. Kharief <sup>1</sup>, J. Ben Arous <sup>1</sup>, N. Versillé <sup>1</sup> <sup>1</sup>SEPPIC - AIRLIQUIDE

## **Background and Objectives**

worldwide distributed, Erysipelothrix rhusiopathiae is a causative agent for erysipelas. It can also cause zoonotic infections called erysipeloid. With vaccine failures increasingly reported, and erysipelas emerging in various wild animal reservoirs, investigations into factors associated with protective immunity are warranted, leading to updated vaccine candidates for improved protection against erysipelas. In this study, One-shot recombinant E.rhusiopathiae protein vaccines formulated with MONTANIDE™ ISA 660 VG (water-in-oil (W/O) adjuvant), AIOH or a standard W/O adjuvant are assessed in pig trial.

#### Material and Methods

The safety of the vaccines were evaluated by injecting 4-5 weeks old pigs (5 pigs/group) intramuscularly once in the neck with 4 ml of the 3 vaccines. The safety assessment was performed by monitoring body temperature for 7 days, observation of behavior and local reaction at the injection site for 14 days and after slaughtering. Regarding the efficacy protocol, pigs were injected with 2 ml of vaccines. Antibody titers were dosed from blood samples collected at D0, D20, D27 after injection. Animals were subsequently challenged with a lethal dose of the antigen at D28, the incidence of death observed for 14 days. Control group was unvaccinated.

#### Results

For the safety trial, Pigs showed a weak peak of body temperature (less than 1°C) 24 hours after injection for the two W/O adjuvants. Then, all vaccine groups returned to normal body temperature. Otherwise, no behavior changes were recorded. The scoring of local reactions showed good tolerance for ISA 660 whereas standard W/O induced severe reactions. The results showed that ISA 660 provides a good safety profile compared to standard W/O adjuvant.Regarding efficacy, the two oil adjuvants induce significantly higher antibody titers than AIOH. After the challenge at D28, ISA 660 vaccine induced full protection and all pigs survived whereas standard W/O and AIOH vaccines conferred only 75% and 0% of protection rate respectively.

## **Discussion and Conclusion**

Taken together, these results showed that MONTANIDE<sup>™</sup> ISA 660 VG proves to be a good alternative for vaccination against E. rhusiopathiae. Beside a good safety/efficacy balance, a one-shot vaccination ensures an optimal protection for animals, avoiding booster vaccination with all what it could involve as economic and organizational efforts.

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# MONITORING SALIVARY BIOMARKERS OF HEALTH STATUS IN PIGLETS VACCINATED AGAINST ESCHERICHIA COLI: A PIGMARKSAL APPLICATION IN THE FIELD.

A. Miralles-Chorro <sup>1</sup>, J. Sánchez <sup>1</sup>, P. Fuentes <sup>1</sup>, M. Matas-Quintanilla <sup>2</sup>, <u>A.M. Gutiérrez <sup>2</sup></u> <sup>1</sup>CefuSA,30840, Alhama de Murcia, Murcia, Spain. <sup>2</sup>BioVetMed Research Group, Department of Animal Medicine and Surgery, Veterinary School, University of Murcia, 30100, Espinardo, Murcia, Spain

#### Background and Objectives

Salivary biomarkers are an important diagnostic tool for the detection of some swine diseases, as well as for the determination of stress conditions, the degree of immunity activation and the level of oxidative stress in pigs. This tool can be very useful in weaned piglets when determining the degree of immunity and/or inflammation, including measurements of the body's response to vaccine challenges. The objective of this study was to monitor the levels of salivary biomarkers of innate and adaptive immunity in weaned piglets vaccinated against Escherichia coli.

#### **Material and Methods**

In this study two groups of Landrace-Large White x Duroc weaned piglets were used, piglets vaccinated against E. coli ETEC F4/F18 and unvaccinated piglets. Saliva samples were collected at two different times after vaccination in 10 healthy and 10 sick animals during veterinary clinical examination, specifically at 2- and 4-weeks post vaccination (wpv), from each group of piglets. Individual body weight was also recorded after saliva collection. The levels of immune markers (Adenosine deaminase (ADA), S100A12, C-reactive protein (CRP) and Haptoblogin (Hp)) were measured in saliva samples. To study any possible difference in the behavior of biomarkers between healthy and diseased animals in both groups the results were analyzed statistically using a 2-way ANOVA. Saliva samples were collected and analyzed according to PigMarkSaL recommendations.

## Results

The signs of diseased in animals were growth rate retardation, dyspnea and cough during the whole study but also diarrhea in some animals at 2 wpv. Vaccination of pigs produced a lower level of CRP and Hp but a higher ADA activity in vaccinated than unvaccinnated sick pigs at 2 wpv. Moreover, at 4 wpv the weight loss in sick pigs appeared reduced in vaccinated animals in comparison to unvaccinated pigs with a lower ADA activity.

## **Discussion and Conclusion**

Vaccination against E. coli in piglets should be considered as beneficial since we have showed a less intense acute phase reaction, a lower inflammatory response and a lower weight loss in vaccinated diseased animals compared to non-vaccinated ones. (Granted by PID2020-116310RB-I00; pigmarksal.com)

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# VACCINATION CHECK LIST: CRITICAL POINTS IN THE PROCESS THAT AFFECT VACCINE RESPONSE AND EFFICACY

M. Walter <sup>1</sup>, J. Calveyra <sup>2</sup>, F. Betiolo <sup>1</sup>, P. Filsner <sup>1</sup>, C. Sartori <sup>1</sup>, C. Postal <sup>1</sup>, <u>R. Krejci</u> <sup>2</sup> <sup>1</sup>Ceva Animal Health, Brazil <sup>2</sup>CEVA ANIMAL HEALTH

## **Background and Objectives**

The detail observation of all steps of vaccination is an important tool to guarantee the vaccinal efficacy. The objective of this study was to analyze some vaccines applications in Brazil using a checklist tool and assign points of attention that can influence effectiveness.

# **Material and Methods**

Between February of 2022 and July of 2023, 74 checklists of vaccination were applied in six different states in Brazil. The tool has different areas of observation: points associated with vaccine storage, transportation, application management, equipment, and details after the vaccine process, which can influence directly in vaccinal efficiency. The results are present as descriptive data with the percentage of occurrence by response category.

### Results

Eleven of 74 farms (15%) didn't have storage unit. Most of the farms with storage unit had a minimum-maximum thermometer (77%), but no one has any electronic temperature logger. In some cases, open vaccine bottles were found into the storage unit (29%). 84% of the farms made some kind of preparation before the vaccine was applied. Some of them used a water bath to heat the vaccine to the optimal temperature for application, but others just kept it into room temperature, which ranged from 5 minutes to 3 hours. Apparently, 16% of the farms inject the vaccines at a too cold temperature. Needle specifications for the target animal group to be vaccinated were correct in 64% of the cases. The needle was changed each 50 animals in 25% of the farms, but in some cases (6%) the same needle was used in more than 400 piglets.

#### **Discussion and Conclusion**

Most farms used thermometer to check the temperature of vaccines. This value is higher than what was previously reported, where <10% of farms had a thermometer at the visit. The preparation before application is a common process in Brazil, but it is a point deserving attention, in this study 16% of the farms had vaccines too cold to application. NNeedle change is also a point of attention Needle reuse may increase the chance of hemorrhage and spreading infection. More studies should be carried out to infer the real impact of each point mentioned.

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## COMPARISON OF EFFICACY AGAINST PORCINE CIRCOVIRUS TYPE 2 (PCV2) AND MYCOPLASMA HYOPNEUMONIAE (M. HYO) BETWEEN INTRAMUSCULAR AND INTRADERMAL COMBINED PCV2 AND M. HYO VACCINES UNDER FIELD CONDITIONS.

N. Boonraungrod <sup>1</sup>, I. Nimmansamai <sup>1</sup>, T. Hawungchoo <sup>1</sup>, T. Kawongklang <sup>2</sup>, <u>R. Kreici</u> <sup>3</sup> <sup>1</sup>Ceva Animal Health (THAILAND) Co, Ltd. <sup>2</sup>Bioscience Animal Health Public Company Limited <sup>3</sup>Ceva

# **Background and Objectives**

Porcine circovirus type2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo) are circulating on swine farms worldwide and both are two of major loss-causing pathogens. PCV2 and M. hyo vaccination are commonly used at weaning to control and prevent clinical signs. There are several combined commercial vaccines present in the market. The aim of this study was to compare the efficacy against PCV2 and M. hyo under field conditions, between intramuscular and intradermal PCV2 and M. hyo combined vaccine.

## Material and Methods

This study was conducted in a commercial farm from Thailand. Weaned pigs were divided into 2 groups; group 1 (n=1,166) was vaccinated with intramuscular PCV2 and M. hyo combined RTM vaccine (DUO®) 2.5 ml/pig and group 2 (n=1,149) was vaccinated with intradermal PCV2 and M. hyo combined vaccine 0.2 ml/pig. Serum samples were collected prior to vaccination at 3 weeks of age (WOA), then at 5, 7, 9, 12, 16 and 20 WOA, examined by with ID Screen® M. hyo competition ELISA kits (Innovative Diagnostics, France) and by PCR for PCV2 viremia. Enzootic pneumonia (EP)-like lesions were evaluated by using Ceva lung program (CLP) method to compare the severity of M. hyo lung lesion at slaughterhouse (25 WOA) between two vaccination groups.

#### Results

PCV2 PCR results were negative in both groups since weaning to finishing period. 20% of the pigs in group 1 had M. hyo ELISA positive result at 2 weeks after vaccination and 100% at 4 weeks after vaccination, while 0% of pigs in group 2 had positive result at 2 weeks and 20% at 4 weeks after vaccination. At slaughterhouse, pigs in group 1 had significantly lower prevalence of M. hyo lungs compare with group 2 (43.3% vs 93.3%, p<0.01). For the severity of lung lesions, group 1 also had significantly lower EP index compare with group 2 (1.4 vs 6.0, p<0.05).

## **Discussion and Conclusion**

This study demonstrated that DUO® can control PCV2 viremia until the end of finishing period and however outperformed intradermal combined vaccine in the potency to induce fast immune response and in the protection against the development of lung lesions due to M. hyo.

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# EVALUATION OF POLYMER AND EMULSION ADJUVANTS FOR STREPTOCOCCUS SUIS VACCINATION

L. Karimova <sup>1</sup>, S. Kharief <sup>1</sup>, J. Ben Arous <sup>1</sup>, N. Versillé <sup>1</sup> <sup>1</sup>SEPPIC - AIRLIQUIDE

#### **Background and Objectives**

Streptococcus suis is a major swine pathogen, worldwide distributed, causing considerable economic losses in the porcine industry. Developing a safe and effective vaccine is the key to preventing and treating S. suis. Inactivated vaccines remain the best strategy today to fight against the disease but their effectiveness is controversial and potent adjuvants are needed. In this study, MONTANIDE™ Gel 02 and MONTANIDE™ ISA 201 VG adjuvants formulated with a trivalent (serotype 1 strain Z1, serotype 2 strain Z2, serotype 7 strain S7) inactivated S. suis antigens were assessed in pig trials.

## **Material and Methods**

The trivalent inactivated S. suis vaccine is formulated with Gel 02 (polymer) or ISA 201 VG (water-in-oil-in-water (W/O/W) emulsion) and compared to AIOH and homemade water-in-oil (W/O) adjuvants. The safety is first evaluated by vaccinating pigs intramuscularly with double dose (4 ml) of the vaccines, 5 pigs per group. The body temperature and local reactions at slaughter 14 days after vaccination were collected and scored. In order to assess efficacy, pigs were vaccinated with 2 ml twice 3 weeks apart. Antibody titers from blood samples taken at D0, D21 and D35 were monitored by ELISA. Two weeks after the boost, a challenge was performed by injecting one lethal dose of S. suis (serotype 1 Z1 or serotype 2 Z2). Clinical signs, mortality rate and specific organ lesions after necropsy are observed.

#### Results

Regarding the safety protocol, a weak increase of the body temperature (less than 1°C at 24 hours) was observed in all groups except in the AIOH group. The local reactions score showed that W/O/W emulsion provided an improved safety profile compared to W/O emulsion and that polymer adjuvant demonstrated an excellent safety profile comparable to AIOH. In terms of efficacy, Polymer provided highest efficacy among all adjuvants tested, with 100% protection against both challenged S. suis serotypes, all pigs of the group survived.

### **Discussion and Conclusion**

These results showed that polymer adjuvant is suited for formulating highly efficacious S.suis inactivated vaccines, providing high protection and balanced efficacy/safety profile, constituting a good alternative to AIOH.

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# EVALUATION OF PRRS PIGLET VACCINATION IN A MULTI-ORIGIN NURSERY UNIT IN SPAIN

S. Sánchez <sup>1</sup>, R. Martínez <sup>1</sup>, L. Gordaliza <sup>1</sup>, S. Diaz <sup>2</sup>, <u>L. De Lucas</u> <sup>3</sup>, L. Nodar <sup>3</sup> <sup>1</sup>Piensos la Plata, SL <sup>2</sup>Hipra España <sup>3</sup>HIPRA, Amer (Girona), Spain

## **Background and Objectives**

Vaccination of piglets against PRRS has been shown to be a useful and profitable measure to not only decrease PRRS infection pressure in the nursery phase but also to provide reasonable protection for piglets, that is usually seen as a reduction in mortality and in secondary respiratory infections. In this study, the efficacy of piglet vaccination was assessed in a multi-origin nursery unit in the south east of Spain.

# **Material and Methods**

A postweaning phase unit located in one of the highest density swine production areas in the country received 850 weaned piglets weekly from 3 different sow farms (O1: 700, O2: 650 and O3: 800 sow census farms). All 3 sow farms were PRRS-stable, checked by a negative PCR result from weaned piglets. When the piglets were moved together into a single nursery unit, they became positive for PRRS by PCR with variable Ct values between 30-35 at 8-9 weeks of age. This positivity was reflected in an increase in the mortality rate from 2.5% to 5%. To reduce this horizontal virus circulation and its impact on production, PRRS piglet vaccination with Unistrain® PRRS IM at 3 weeks of age was introduced at entry into the nursery phase. Mortality in the nursery phase amongst the vaccinated animals from 3 different origins (n = 35,672) was recorded over a period of 6 months and compared to the previous 3 months for non-vaccinated piglets (n = 17,836). The differences were tested by Wilcoxon test.

#### Results

After the introduction of PRRS piglet vaccination, the mortality rate was reduced in the vaccinated piglets from 3 different origins, significantly in O1, decreasing from 2.63% to 1.82% (p-value = 0.028) and from 6.07% to 4% in 02 (p-value = 0.024). The reduction in the mortality of the piglets from the third origin (O3) went from 4.89% to 4.05% with a p-value of 0.067.

#### **Discussion and Conclusion**

The results obtained show the effectiveness of PRRS piglet vaccination applied at the correct time as a tool to reduce mortality in a multi-origin nursery phase. Vaccination is just one of the pillars of a complete PRRS control strategy that should be complemented by other measures such as biosecurity, management and monitoring measures.

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# EVALUATION OF THE NASAL MICROBIOTA WITH AN EXPERIMENTAL ORAL VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE

L.G. De Oliveira<sup>1</sup>, G.Y. Storino<sup>1</sup>, F.M. Petri<sup>1</sup>, M. Carvalho De Abreu Fantini<sup>2</sup>, T. Da Silva Martins<sup>3</sup>, O.A. Sant'Anna<sup>4</sup>, B. Bracco Donatelli Muro<sup>5</sup>, A. Pegoraro Poor<sup>6</sup>, H.J. Montassier<sup>1</sup>

<sup>1</sup>São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil <sup>2</sup>University of São Paulo (USP). Physics Institute, São Paulo, Brazil.

<sup>3</sup>Department of Chemistry, Federal University of São Paulo (UNIFESP), Diadema, SP, Brazil

<sup>4</sup>Butantan Institute, São Paulo, Brazil.

<sup>s</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>6</sup>Department of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine and Animal Sciences, University of São Paulo (USP), Campus São Paulo, SP, Brazil

# **Background and Objectives**

Understanding the interplay among respiratory agents is crucial to determining the function of nasal microbiota role in subsequent disease development. Increased mucous immune response in the respiratory tract would affect the nasal and/or oropharyngeal microbiota in pigs. Thus, the present work aimed to study the nasal microbiota using an experimental oral vaccine against Mycoplasma hyopneumoniae.

#### Material and Methods

A total of 48 piglets were divided into four groups (n = 12) submitted to different vaccination protocols as follows, G1: oral adjuvant + commercial vaccine (CV) at 24 days of age (doa); G2: oral vaccine (OV) on the third day of life + CV at 24 doa; G3: CV at 24 doa, and G4: CV + OV at 24 doa. Nasal swabs were collected on the 3rd day (D3), 41 (D41), and the 73 doa (D71) of life. The DNA was extracted from individual samples and submitted to Nanopore sequencing for evaluation nasal microbiota.

#### Results

The average of relative abundance for Glaesserella was 9.3%, 16.9%, 7.7%, and 7.9% for G1, G2, G3 and G4, respectively. The relative abundance of Sthaphylococcus was 2.5%, 5.2%, 3.9% and 3.9% for G1, G2, G3 and G4, respectively. The relative abundance of Streptococcus was 24.2%, 25.3%, 37.4% and 36.3% for G1, G2, G3 and G4, respectively. The relative abundance of Clostridium was 2.2%, 4.6%, 4.9% and 3.3% for G1, G2, G3 and G4, respectively. The relative abundance of Moraxella was 19.6%, 15.7%, 10.3% and 16.2% for G1, G2, G3 and G4, respectively. The relative abundance of Lactobacillus was 2.2%, 2.1%, 3.0% and 2.1% for G1, G2, G3 and G4, respectively.

# **Discussion and Conclusion**

Bacterial diversity was observed in nasal microbiota, highlighting Streptococcus, which can be worrying. Moraxella appears to play a fundamental role in the colonization of the nasal microbiota, together with Lactobacillus, which demonstrated little participation. When we analyze the samples separately, we observe great diversity in D3. Nonetheless, it was not possible to detect the influence of vaccination protocols, still is necessary to analyze global bacterial diversity indices. Grant#2021/11914-0, São Paulo Research Foundation (FAPESP).

#### IMM – Immunology and Vaccinology

# FREQUENCY OF FINDING MAJOR PATHOGENS IN PIGLETS VACCINATED WITH TWO DIFFERENT MESOMYCOPLASMA HYOPNEUMONIAE VACCINES

<u>G. Ramis</u><sup>1</sup>, A. Vela<sup>2</sup>, F. Murciano<sup>3</sup>, B. Gonzalez-Guijarro<sup>1</sup>, A. Cuellar-Flores<sup>3</sup>, R. Krejci<sup>4</sup>, D. Espigares<sup>5</sup> <sup>1</sup>Departamento de Producción Animal. Universidad de Murcia <sup>2</sup>Thinkinpig, Zaragoza, Spain <sup>3</sup>Dpto. Producción Animal,. Facultad de Veterinaria., Universidad de Murcia, Spain <sup>4</sup>Ceva Sante Animale, France <sup>5</sup>Ceva Salud Animal, Barcelona, Spain

# **Background and Objectives**

For more than three decades, vaccination against Mesomycoplasma hyppneumoniae has been a standard part of prevention schemes in pigs, together with other vaccines against various pathogens such as Aujeszky's disease, PRRSv, swIAV or PCV2. The aim of this work was to determine the presence and quantity of major pathogens in two batches of pigs with two different vaccination programs against M. hyppneumoniae.

## Material and Methods

Sixty animals were randomly assigned to two batches of 30 animals each, separated by stables within the same house. One batch was vaccinated once with Hyogen (Ceva Santé Animale, France), which is an inactivated M. hyopneumoniae vaccine based on strain 2940 (HYO Group), while the other was vaccinated once with an inactivated M. hyopneumoniae strain J vaccine (CON Group). Both groups were vaccinated at 4<sup>th</sup> week of life as indicated by manufacturers.Samples were taken by tracheobrobronchial swabs (TBS) at 4th, 9th, 14th and 19th weeks of age (S1, S2 S3 and S4). The first sampling was performed immediately prior to vaccination. hyopneumoniae, M. hyorhinis, PRRSv, swIAV and PCV2 presence and quantity was determined in the TBS by q-PCR.

#### Results

hyopneumoniae was not detected in any of the TBS in the HYO group while it was found in the 56.7%, 60% and 28.5% of CON group in S2, S3 AND S4 (P<0.001). There was no difference in the frequency of M. hyorhinis, PCV2 or swIAV, but PRRSv was found in higher frequency in CON group at S3 (CON=53.3% vs HYO=0%, p<0.001) and in higher frequency in HYO at S4 (CON=17.2% vs HYO=80%, p<0.001)

## **Discussion and Conclusion**

In this study, M. hyopneumoniae was not found in the HYO group, in contrast to the CON group where it was observed in 3 of the samplings. It seems that there is some interaction with PRRSv since in the CON group there has been an early circulation with respect to the HYO group where it has been later. Under the conditions of this study, Hyogen appears to have prevented infection by M. hyopneumoniae.

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# THE USEFULNESS OF PROCESSING FLUID AS A POTENTIAL MATRIX FOR ASSESSING THE CONCENTRATION OF IMMUNOGLOBULINS IN PIGS

<u>A. Augustyniak</u><sup>2</sup>, E. Czyzewska-Dors <sup>1</sup>, I. Kucinska <sup>2</sup>, M. Pomorska-Mól <sup>2</sup> <sup>1</sup>Department of Internal Diseases and Diagnostics, Poznan University of Life Sciences, Poland <sup>2</sup>Department of Preclinical Sciences and Infectious Diseases, Poznan University of Life Sciences, Poland

## **Background and Objectives**

Processing fluid (PF) has piqued scientific interest as a new alternative matrix for the efficient assessment of piglets' immune status, as its collection does not require additional effort and stress for piglets compared to blood sampling. It has already been demonstrated that PF can serve as a useful tool for the monitoring of important porcine pathogens, including PRRSV, PCV2, and Mycoplasma hyopneumoniae. Therefore, the present study aimed to evaluate the usefulness of PF for immunoglobulin determination in newborn piglets.

# **Material and Methods**

For the purpose of this experiment, 146 serum and 146 PF samples from male piglets, 87 serum samples from female piglets, and 31 serum and 31 colostrum samples from their mothers were collected. Sampling was performed 2-5 days after farrowing (except for colostrum, which had been taken 12-24 hours following farrowing onset). The concentration of three classes of immunoglobulins – IgG, IgA, and IgM was evaluated in each type of sample using the ELISA assay. The determination of statistical differences in the concentration of measured parameters between piglets' serum and PF was analyzed using the Mann-Whitney U test. The Spearman's rank correlation coefficient test was used to determine the correlations between particular sets of matrices.

#### Results

The presence of IgG, IgA, and IgM was revealed in all matrices, including PF. The concentrations of IgG, IgA, and IgM in PF ranged between 2.56 - 31.28 mg/ml, 0.61 - 4.35 mg/ml, and 0.39 - 1.95 mg/ml, respectively. Statistical analysis did not show any significant differences regarding the concentration of IgG and IgA between piglets' serum and PF (p>0.05). Moreover, a positive correlation between PF and sows' serum, has been found, however only with respect to the concentration of IgA (r=0.25, p<0.05).

## **Discussion and Conclusion**

In conclusion, the results of the present study indicate that PF could serve as a promising alternative to serum for immunoglobulin concentration assessments in piglets. In addition, the indirect assessment of IgA in lactating sows using PF is probably possible. Nevertheless, further analyses in this area are required. The study was financed by NCN project UMO-2020/37/B/NZ7/00021.

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# THE USEFULNESS OF PROCESSING FLUID TO ASSESS CYTOKINE AND ACUTE PHASE PROTEIN CONCENTRATIONS

<u>A. Augustyniak</u><sup>2</sup>, E. Czyzewska-Dors <sup>1</sup>, M. Pomorska-Mól <sup>2</sup> <sup>1</sup>Department of Internal Diseases and Diagnostics, Poznan University of Life Sciences, Poland <sup>2</sup>Department of Preclinical Sciences and Infectious Diseases, Poznan University of Life Sciences, Poland

## **Background and Objectives**

Collection of blood samples from piglets in their first days of life is time-consuming and associated with the risk of complications. According to previous studies processing fluid (PF) is suitable for the detection of antibodies and genetic material of pathogens. PF is serosanguineous exudate obtained from tissues after piglets' castration and tail docking during processing procedures (mainly 3-5 days of piglets' life). Thus, the study aimed to expand the knowledge of the possibility of using PF as a matric for the detection of immunological indices such as cytokines and acute phase protein (APP).

# **Material and Methods**

Samples from male piglets (146 sera, 146 PF), female piglets (87 sera), and their sows (31 sera and 31 colostrum samples) were tested using ELISA assay, to determine the concentration of cytokines (IL-1â, IL-4, IL-6, IL-8, INF-ã, and, TNF-á), and APP (haptoglobin (Hp), C-reactive protein (CRP), pig major acute phase protein (Pig-MAP) and serum amyloid A (SAA)). The Mann-Whitney U test was used for the determination of statistical differences in the concentration of tested indices between piglets' serum and PF. Spearman's rank correlation coefficient was used for the determination of correlations in the concentration of measured parameters between particular sets of matrices.

#### Results

The IL-1â, IL-4, IL-6, IL-8, INF-ā, CRP, Hp, and Pig-MAP were detected in all sets of matrices, including PF. The concentration of IL-1â, IL-4, IL-6, IFN-ā, and Hp reached comparable levels in piglets' serum of both gender and PF (p>0.05). In addition, positive correlation between PF and sows' serum regarding the concentration of IL-1â, IL-4, IL-6, IL-8, and IFN-ā, and between PF and colostrum, regarding the concentration of IL-1â, IL-4, IL-6, INF-ā, Hp, and, Pig-MAP were diagnosed.

## **Discussion and Conclusion**

In conclusion, the present study indicated that PF represents a promising alternative matrix to serum for cytokine and acute phase protein detection in neonatal piglets. Furthermore, through PF testing, the response of cytokines and acute phase proteins in lactating sows can be indirectly monitored.

The study was financed by NCN project UMO-2020/37/B/NZ7/00021.

#### IMM – Immunology and Vaccinology

# CONCOMITANT USE OF DYSGUARD-S® AND PORCILIS®, MSD VACCINE AGAINST PIG ILEITIS UNDER FIELD CONDITIONS

<u>J.G. Durón Viveros</u><sup>1</sup>, J. Bošnjak-Neumüller<sup>1</sup>, B. Vejnovic<sup>2</sup>, L. Gomez-Osorio<sup>1</sup>, J. Raj<sup>1</sup>, M. Vasiljević<sup>1</sup> <sup>1</sup>PATENT CO DOO., Serbia <sup>2</sup>Department of Economics and Statistics. Faculty of Veterinary Medicine. University of Belarade. Belarade. Serbia

#### Background and Objectives

This study aimed to test the compatibility of a plant-based animal health premix (PHP) with the concomitant use of a vaccine against pig ileitis.

#### Material and Methods

The 111-day study started on a total of thirty 50-day-old piglets on the farm with a history of pig ileitis. Piglets were alloted equally into 3 treatments, NV- non-vaccinated, V- vaccinated against pig ileitis, and VD- vaccinated against pig ileitis and supplemented with 2 kg/ton of PHP ( DYSGUARD-S®). All the groups of vaccinated pigs received the MSD Porcilis® Lawsonia ID vaccine on day 50. of age. Fecal samples were taken from all the pigs in the trial, on days 0., 28., and the last day of the trial. Fecal samples were pooled from the same two pigs at each time of sampling before being analyzed with quantitative real-time PCR analysis of Lawsonia intracellularis. On the same days of fecal sampling, the animals were bleeding for ELISA analysis of antibodies against L. intracellularis. Performance and mortality outcomes were also recorded.

#### Results

There were 3 pigs dead during the trial in NV treatment 3. The NV treatment also had the lowest body weight (BW) and highest FCR at the end of the trial (BW  $89.94\pm9.34$ ; FCR 2.7) in comparison to the V treatment (BW  $97.84\pm15.79$ ; FCR 2.5) and VD group (AW  $93.50\pm7.48$ , FCR 2.4). Fisher's exact test did not find statistical differences among groups in L. intracellularis number (p>0.05), during the trial. Twenty-eight days after the vaccination, the VD group had a statistically significantly (p<0,05) higher number of positive samples (50%) in comparison to the NV group (0%) and a numerically higher than in the V group (40%). At the end of the trial, both V and VD treatments were statistically significantly higher in several positive samples in comparison to the NV group (p<0,05).

## **Discussion and Conclusion**

The application of PHP concomitantly with a vaccine to control pig ileitis may lead to faster reaching Ab-protection against ileitis. PHP's positive impact on the uniformity of pigs and FCR values needs to be further confirmed in large-scale trials because production trial results obtained on a small number of animals could mislead.

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# EVALUATION OF 3 VACCINATION PROGRAMS FOR THE CONTROL OF ACTINOBACILLUS PLEUROPNEUMONIAE IN A BRAZILIAN FARM

J. Calveyra <sup>1</sup>, C. Postal <sup>2</sup>, M. Lisgara <sup>1</sup>, <u>P. Mortensen <sup>3</sup></u> <sup>1</sup>CEVA ANIMAL HEALTH <sup>2</sup>Ceva Animal Health, Brazil <sup>3</sup>Ceva Animal Health. France

### **Background and Objectives**

Actinobacillus pleuropneumoniae (A.p.) is the etiological agent of swine pleuropneumonia. For the control, different vaccines are available, and reliable evaluation of their efficacies can be performed according to the presence of A.p.-like lung lesions. The aim of this study was to evaluate 3 different vaccines available in the Brazilian market by scoring A.p.-like lesions, using the Ceva Lung Program (CLP).

## **Material and Methods**

The study was conducted on a 5,000-sows farm located in the Santa Catarina region, the main swine producing state in Brazil. The farm has a clinical history of laboratory confirmed A.p. serotype 1. Between April 2018 and October 2022, 32 separate batches pigs were investigated at the time of slaughter according to the CLP-methodology described in previous studies. 2669 lungs in total were scored on lung lesions. Three different vaccines were evaluated and applied according to the manufacturer's recommendations. Group1: Animals vaccinated with Coglapix® (Ceva, France) 2 mL at 7 and 10 weeks of age (WOA) (n=1465). Group 2: Animals vaccinated with commercial vaccine, 2 mL at 7 and 10 WOA (n=562) and Group 3: Animals vaccinated with national autogenous vaccine, 2 mL at 7 and 10 WOA (n=642). The groups of 3 different treatments were administered in alternate batches. Comparison of frequency of Ap-like lesions and the APP Index (incidence and severity of dorsocaudal pleuritis, which is generally indicative of pleuropneumonia) was carried out using chi-square test. A significant difference was considered when the P value <0.05.

#### Results

G1 had lower rates of Ap-like lesions, dorsocaudal pleurisy, with 6.67%<sup>a</sup> when compared to G2 (8.36%<sup>a,b</sup>) and G3 (11.11%<sup>b</sup>) respectively p-value=0.017. In addition, G1 showed a lower numerical APP-Index (0.2) when compared to G2 (0.3) and G3 (0.27).

## Discussion and Conclusion

Special scoring systems, like lung injuries, are used to generate numerical data from clinical and pathological observations. In this study it is possible to use CLP as a tool to monitor health challenges, vaccination programs and indicate the best health measures to be adopted. In this study, within the evaluated groups, the G1 vaccine showed statistical difference when compared to G3. The G2 did not present significant differences in relation to the G1.

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# FIELD STUDY OF THE SAFETY OF THE SUISENG® AND SUISENG® DIFF/A VACCINATION COMBINATION IN THAI COMMERCIAL FARMS.

S. Krasaesub <sup>1</sup>, N. Chavaranggul <sup>1</sup>, C. Ananratanakul <sup>1</sup>, K. Lohaprom <sup>1</sup>, <u>S. Traiyarach <sup>1</sup></u> <sup>1</sup>HIPRA Thailand

#### **Background and Objectives**

Neonatal diarrhoea is a disease causing an significant estimated cost per sow per year. Its prevention is based on management and vaccination. The transfer of immunity from sow to piglet post sow vaccination is key for its control. The objective of this study was to observe clinical signs after vaccination with two neonatal diarrhoea vaccines, injected as a combined injection, in pregnant sows.

#### **Material and Methods**

The study was conducted on two commercial farms in Thailand (Farm-A and Farm-B). SUISENG® and SUISENG® Diff/A, were injected intramuscularly in a combined 4 ml. shot containing 2 ml. of each vaccine. Farm-A had vaccinated the combination twice at 6 and 3 weeks before farrowing in 200 sows. Farm-B had vaccinated the combination twice at 6 and 4 weeks before farrowing, intramuscular injection of RHINISENG® was also performed at different parts of the neck area of these sows. Side effects monitored after vaccination were as follows: Swelling at injected area, increased respiratory rate and/or panting, mouth chewing and/or vomiting, redness of skin, seizure and/or weakness, refusal of feed intake, death and abortion. 6 time-points were monitored as follows: Before vaccination, 15 to 30 minutes, 1 to 6 hours, 1 day, 2 days and 3 days post vaccination, respectively.

#### Results

None of the clinical signs were observed in both farms.

## **Discussion and Conclusion**

Based on the results observed, the combination of both vaccines did not cause any adverse effects after administration. These results support the safeness under field conditions in Thailand.

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FORMULATION OF A VACCINE FOR THE PREVENTION OF SWINE BACTERIAL RESPIRATORY DISEASES(A. PLEUROPNEUMONIAE TYPE 1, 2, 5, RECOMBINANT A. PLEUROPNEUMONIAE TOXOID I, II, III, G. PARASUIS TYPE 1, 4, 5, P. MULTOCIDA TYPE A)

Y. Park 1

<sup>1</sup>Daesung Microbiology Labs. co. LTD.

## **Background and Objectives**

Bacterial respiratory diseases are a major cause of reducing the MSY in pig farms. Pig respiratory diseases have diverse causes and preventing the diseases with monovalent vaccine is challenging. Therefore, there is a need for the development of a multivalent vaccine that can offer prevention and treatment effects for multiple bacterial respiratory diseases.

#### Material and Methods

The vaccine used in the study contained App type 1, 2, 5, rApx I, II, III, Gps type 1, 4, 5 and Pm type A. Field trials were conducted in three farms. To evaluate the vaccine's efficacy antibody levels for each antigen were measured at regular intervals after vaccination. Also, clinical symptoms and mortality rates were observed. Additionally, an analysis of antigen excretion in the nasal cavity from both the vaccine group(Vac) and the unvaccinated group(UnVac) were conducted to confirm the vaccine's protective effects. Furthermore, for economic assessment, the pigs were weighed periodically and at 13 weeks of age random necropsies were performed to assess lung conditions.

#### Results

As a result of evaluating immunogenicity, the antibody titer increased in the Vac following the second dose of the vaccine but it decreased in the UnVac which is statically significant(p<0.05). For the productivity evaluation, the weights were greater for Vac compared to UnVac. In addition, specific antigens were not detected in the Vac but specific antigens(Farm 1: PM type A, Farm 3: App type 1, Gps type 5, and Pm type A) were detected in the UnVac. When it comes to pathological analysis, specific findings were not observed in the Vac but fibrinous pleurisy and edema were observed in the UnVac. And, mortality rate was 0% in Vac and 15% in UnVac.

## **Discussion and Conclusion**

Through above the test, it was confirmed that the administration of 'DS APH PigVac' in pigs resulted in an increase in antibody levels, a reduction in lung lesions and a decrease in the mortality and excretion of antigens(App, Gps, PmA). Furthermore, it was also observed that antibodies were formed and maintained for each antigen without interference. In conclusion, it is believed that this respiratory vaccine can prevent or alleviate infections caused by bacterial respiratory diseases not only in Korea but also worldwide.

IMM – Immunology and Vaccinology

# GENETIC DIVERSITY OF PRRSV AND INGELVAC PRRSV MLV VACCINATION

<u>A. Uribe</u><sup>1</sup>, J. Ochoa<sup>1</sup>, G. Marika<sup>2</sup>, C. Martin Del Campo<sup>1</sup>, A. Esquer<sup>1</sup>, F. Pinal<sup>1</sup> <sup>1</sup>BOEHRINGER INGELHEIM ANIMAL HEALTH MEXICO <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH, Germany

#### **Background and Objectives**

The Porcine Reproductive and Respiratory Syndrome (PRRS) is a disease caused by PRRS virus (PRRSV) (Christianson et al., 1993). A significant measure of the effectiveness of MLV vaccines is the ability to reduce the amount of viral shedding and thus reduce the transmissibility of PRRSV (Chae, 2021). This study aimed to analyze the genetic diversity of PRRSV on a vaccinated farm in Mexico for 6 years (2017-2022).

## **Material and Methods**

The study was conducted in collaboration with a producer in Mexico, with 10,000 sows, using Ingelvac PRRS MLV (2 ml) vaccination, in sows and pipeline. Additionally, 8570 serum samples were collected from 5,7,9 weeks of age. The total pool samples were 1714 and were tested by Real-Time PCR. The negative samples were 52% and 48% positive samples. Samples with CT below 30 were sequenced (110 samples). The ORF5 Sequence alignment was performed to determine the lineage according to Paploski, 2019. In addition, the lineages were analyzed by the "chi-squared" statistical method to observe statistical differences during the years.

## Results

A total of 110 sequences were classified by lineages and sub-lineages (2017=15, 2018=18, 2019=15, 2020=20, 2021=30, 2022=12), where Ingelvac (L5) was detected in 72 cases (65.5%), L1G with 21 cases (19.1%), L1A with 10 cases (9.1%), L7 occurred 6 cases (5.5%) and L8 with 1 case (0.9%). Ingelvac and field variants have an 82% and 90% of homology between them. According to the statistical analysis performed it was observed that there is a statistical difference between the vaccine versus the field variants from 2017 to 2021 ( $p \le 0.05$ ). For 2022 there is no significant statistical difference (p > 0.05).

### **Discussion and Conclusion**

The highest percentage of lineages found were from Ingelvac PRRS. The percentage homology of the field variants versus Ingelvac was between 84-90%. On the other hand, although indeed, Ingelvac did not displace the different lineages present on the farm, nor was it observed that the variants increased in the years, which leads us to conclude that the vaccine did not allow lineages to increase the field variants on the farm from 2017-2021.

IMM – Immunology and Vaccinology

# USING T-CELL EPITOPE CONTENT COMPARISON TO PREDICT VACCINE COVERAGE AGAINST PORCINE CIRCOVIRUS TYPE 2 ISOLATES IN THAILAND.

A. Prapattanakullawong <sup>1</sup>, P. Wongchanapai <sup>1</sup>, S. Nantakhruea <sup>1</sup>, <u>W. Navasakuljinda <sup>1</sup></u>, C. Muangpaisarn <sup>1</sup> <sup>1</sup>Zoetis (Thailand) Limited, Bangkok 10500, Thailand

### **Background and Objectives**

Porcine Circovirus type 2 (PCV2) strains have been categorized into 8 genotypes, ranging from PCV2a to PCV2h. While PCV2d is identified in recent years as the prevailing genotype worldwide, it remains crucial to explore the genetic diversity within PCV2. Various commercial PCV2 vaccines are available to safeguard the pigs against PCV2 infections. Utilizing EpiCC, an immunoinformatics tool that compares T-cell epitope cross-conservation the study aims to predict immunological coverage of four commercial vaccines against PCV2 field strains circulating in the farms.

## **Material and Methods**

Tissue samples from PCV2 clinically infected commercial farms in Thailand from Year 2022 were submitted for PCR tests. All PCV2-positive PCR samples were subjected to whole genome sequencing. The similarity between PCV2 sequences was assessed using the NCBI Basic Local Alignment Search Tool (BLAST). A total of 18 PCV2 sequences were analyzed and the shared T-cell epitope content between vaccines and field strains was identified using EpiCC. Subsequently, the correlation of T-cell epitopes between field isolates and various commercial vaccines—specifically, three monovalent PCV2a vaccines (VacAlt1, VacAlt2 and VacAlt3) and a bivalent PCV2a-PCV2b vaccine (VacAB; Fostera® Gold PCV) was compared. An average EpiCC score was calculated for the comparison of a given vaccine and all the field strains.

#### Results

The PCV2 sequences revealed that all 18 sample (100%) belonged to the PCV2d genotype. The EpiCC analysis results indicated that the bivalent VacAB displayed the highest EpiCC scores (8.21), on average, the broadest epitope coverage based on its score at 77.98% matched for PCV2d field strains. In contrast, the monovalent VacAlt1, VacAlt2 and VacAlt3 showcased lower EpiCC scores, ranging from 6.13 to 6.74 (equivalent to 58.22 % to 64.03 % T-cell epitope coverage against PCV2d field strains).

## **Discussion and Conclusion**

Based on the EpiCC analysis results comparing T-cell epitope content of four commercial PCV2 vaccines against contemporary PCV2d field strains circulating in these Thailand farms, VacAB has an averaged 27% more in T-cell epitope coverage than monovalent PCV2a vaccines.

#### IMM – Immunology and Vaccinology

# CYTOKINE GENE EXPRESSION DEPENDING ON SEROPOSITIVITY AGAINST MESOMYCOPLASMA HYOPNEUMONIAE AFTER VACCINATION.

<u>G. Ramis</u><sup>1</sup>, A. Vela<sup>2</sup>, B. Gonzalez-Guijarro<sup>1</sup>, A. Cuellar-Flores<sup>3</sup>, R. Krejci<sup>4</sup>, D. Espigares<sup>5</sup> <sup>1</sup>Departamento de Producción Animal. Universidad de Murcia <sup>2</sup>Thinkinpig, Zaragoza, Spain <sup>3</sup>Dpto. Producción Animal,. Facultad de Veterinaria., Universidad de Murcia, Spain <sup>4</sup>Ceva Sante Animale, France <sup>5</sup>Ceva Salud Animal, Barcelona, Spain

## **Background and Objectives**

Cytokines are molecules of the immune system that are critical in determining whether a predominantly humoral, cellular, mixed or innate immunity-based response occurs. In this study, seropositivity to Mesomycoplasma hyponeumoniae has been determined and linked to the gene expression for cytokines in tracheobronchial swabs from vaccinated piglets with two vaccines.

## Material and Methods

Sixty animals were randomly assigned to two batches of 30 animals each, separated by stables within the same house. One batch was vaccinated once with Hyogen (Ceva Santé Animale, France, which is an inactivated M. hyopneumoniae vaccine based on strain 2940 (HYO Group), while the other was vaccinated once with an inactivated M. hyopneumoniae strain J vaccine (CON Group). Both groups were vaccinated at 4<sup>th</sup> week of life as indicated by manufacturers.Samples were taken by tracheobronchial swabs (TBS) at 4<sup>th</sup> and 9<sup>th</sup> weeks of age (S1 and S2). Simultaneously, blood was obtained and serum isolated.The antibodies were investigated by Elisa ID Screen® IDVet Mycoplasma hyopneumoniae (IDVet, Montpellier, France) and gene expression for 15 cytokines was performed on TBS.

#### Results

No animal was seropositive in S1, but 93.3% of the HYO group and 43% of the CON group were seropositive in S2 (p<0.001). There was higher frequency of amplification in the HYO group compared to CON in IL10 (46% vs 20%, p=0.028), IL1p35 (90% vs 70%, P=0.052), IL12 p40 ((100% vs 86.7%, p=0.038), IL8 (67% vs 6.7%, P<0.001), IL1á (96.7% vs 76.7%, P=0.023) and lower for IL6 (16.7% vs 50%, p=0.006). Taking all data together, gene expression for IL12p35 (p=0.041), IL8 (p=0.048) and TGFå (p=0.068) showed difference comparing seropositive and seronegative samples, however only 2 seronegative samples in were in the HYO group.

# **Discussion and Conclusion**

Hyogen resulted in a much higher frequency of seropositive animals 4 weeks after vaccination, and higher frequency of animals expressing IL10, IL12p35, IL12p40, IL8 and IL1a and lower for IL6, compared to CON. There is detectable immune stimulation in more animals in the HYO group which could account for the difference in seroprevalence observed.
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# EFFICACY OF DIFFERENT COMBINED PORCINE CIRCOVIRUS TYPE 2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES UNDER FIELD CONDITIONS IN THAILAND

I. Nimmansamai <sup>1</sup>, N. Boonraungrod <sup>1</sup>, W. Prucksayoungyuen <sup>1</sup>, <u>R. Krejci</u><sup>2</sup> <sup>1</sup>Ceva Animal Health (THAILAND) Co, Ltd. <sup>2</sup>Ceva Santé Animale, 10 Avenue de la Ballastière, 33500 Libourne - France

#### **Background and Objectives**

Porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhyo) play important role causing economic losses in weaning to slaughter pigs. The concept of combined vaccination has been explored. The aim of this study was to evaluate the efficacy of different PCV2/Mhyo vaccines under field conditions.

#### **Material and Methods**

This study was performed in a 5,000 sows farm. A total of 4,800 pigs were divided into four groups (1,200 each). At 6 weeks of age, Group A and B were vaccinated with various PCV2/Mhyo ready to use vaccine (RTUA and RTUB) 2 ml. Group C was vaccinated with PCV2/Mhyo mixed vaccine 2ml and group D were vaccinated with DUO® (Circovac® and Hyogen® mixed, Ceva, France) 2.5 ml. Serum samples were collected at 8, 18, 22, 24, 26 WOA for PCV2 viremia detection using conventional PCR. Lung scoring was performed at slaughter for EP-like lesions using Straw et al (1986). Loses as death, culling due to wasting, weight loss, respiratory sign, icterus, and pale skin and performance data at finishing phase were recorded and the statistical analyses were performed using SAS. Return on investment (ROI) was calculated using actual profit and total PCV2/Mhyo vaccination cost.

#### Results

PCV2 detection 40% was found in group A at 26 WOA, while all other groups had negative results in all collected samples. Losses in group C and D (1.00% and 0.83%) were significantly lower compare with group A and B (2.05% and 2.50%, p<0.01), while there is no different between group C and D (p>0.05). No statistical different observed in others performance parameters. PCV2/Mhyo mixed vaccine (group C and D) shown higher percentage of healthy lungs (without EP-like lesions) at slaughter; group A = 39.2%, group B = 28.0%, group C = 52.0%, and group D = 54.0% and the average lesions score were 6.0%, 7.0%, 5.7%, and 3.4% respectively. The ROI were 42.0%, 44.0%, 42.0%, and 54% respectively.

# **Discussion and Conclusion**

This study demonstrated that PCV2/Mhyo mixed vaccine perform better in reducing overall losses. DUO® provides high efficacy in control of PCV2/Mhyo and generates best return on investment under field conditions.

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# EFFICACY OF A PRRSV1 MLV IN A PRRSV2-INFECTED FARM IN JAPAN

<u>T. Shimada</u><sup>1</sup>, Y. Uchiyama<sup>2</sup>, C. Uchiyama<sup>2</sup> <sup>1</sup>Katori Livestock Clinic, Chiba, Japan <sup>2</sup>JELLY BEANS Ltd., Chiba, Japan

#### **Background and Objectives**

Although PRRSV2 vaccines are available in the market in Japan, PRRS is still a headache for vets and producers. New vaccines and alternative vaccination routes, such as intradermal, are options to improve PRRS control in Japan. The aim of this study was to compare the efficacy of an intradermal PRRSV1 MLV with an intramuscular PRRSV2 MLV in a PRRSV2-infected farm in Japan

# **Material and Methods**

This study was conducted on a 2 sites 1200-sow PRRSV2-infected farm in Japan. The PRRS vaccination protocol was 4 mass vaccinations per year for sows and 2 times for piglets at 25 and 55 days of age with a PRRSV2 MLV (VR-2332). To improve the PRRS control in the farm, in April23 they switched to UNISTRAIN® PRRS (PRRSV1 MLV, HIPRA) by intradermal route with a needle-free device (Hipradermic®). To evaluate the efficacy of the new vaccine, reproductive performance was compared by assessing abortion rate (AR), total born piglets (TBP), born alive piglets (BAP) and mortality in nursery 6 months before and after the vaccine change. These data were analyzed performing a proportion test or T-test. Moreover, the PRRS status of the farm was monitored from March to September 2023 by PCR weekly at birth (processing fluids) and monthly 30 piglets at weaning (pools of 5 blood samples). All positive samples to RT-PCR were sequenced.

# Results

Regarding reproductive parameters, there were significant differences in the AR (from 4.57% to 2.19%, p<0.001), TBP (from  $15.13\pm4.07$  to  $16.25\pm3.93$ , p<0.001) and BAP (from  $14.08\pm3.82$  to  $14.61\pm3.6$ , p<0.001). About PRRS status, piglets were PCR positive to field strain at birth and weaning at March and April. From May onwards, all the animals at birth and weaning were negative to the field strain.

### **Discussion and Conclusion**

In Japan, PRRSV2 infections are common, and PRRS2 vaccines are used until now to control the disease. In this trial UNISTRAIN® PRRS proved efficacy in a PRRSV2 farm by reducing the circulation of PRRSV2 field strain and improving the reproductive performance, with more piglets born and fewer abortions after the switch to PRRSV1MLV. This confirms that intradermal PRRSV1 vaccination is effective in PRRSV2-infected farms.

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### EVALUATION OF A RESISTANT OIL-IN-WATER ADJUVANT FOR RECOMBINANT PRRSV VACCINE

L. Karimova <sup>1</sup>, S. Kharief <sup>1</sup>, J. Ben Arous <sup>1</sup>, N. Versillé <sup>1</sup> SEPPIC - AIRLIQUIDE

#### **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is the causative agent of PRRS, a viral disease widespread globally. The swine industry reports large economic losses due to variable morbidity and mortality with reproductive disorders in sows and respiratory symptoms often with co-infection in growing and finishing pigs. Oil-in-water (O/W) based vaccines are commonly used against PRRSV, known as a destabilizing antigen hence the increased need for highly stable vaccines. This study aims to compare a recombinant gp 5 protein and recombinant M protein with inactivated cultural PRRSV based vaccines formulated with a "resistant" O/W adjuvant (MONTANIDE™ ISA 28 R VG), or carbomer.

# Material and Methods

The safety and efficacy of the vaccines were evaluated in production conditions by injecting intramuscularly 42-45 days healthy pigs with a negative serology for PRRS, with 2 ml of the vaccines at D0 and D32, 10 pigs per group. Safety was assessed by palpation, in vivo and post mortem visual assessment of the site of injection and thermometry of all animals before vaccination, 24, 48 and 72 hours after vaccination. In order to evaluate efficacy, blood sampling was performed at D0, 32, 64 and 115 to assess specific antigen antibodies and virus-neutralizing antibodies by ELISA

#### Results

For the safety trial, the body temperature monitoring showed a slight increase (less than 1°C) 24 hours after vaccination for the two adjuvants. Then, all vaccine groups returned to normal body temperature. Otherwise, no behavior changes were recorded in both groups. Finally, Post-mortem autopsy and visual examination of the injection site did not reveal any tissue changes in piglets in the two groups. The results showed that the O/W based vaccine demonstrated a good safety profile. Regarding efficacy assessment, from D30, the O/W based vaccine induced statistically higher antibody titers than carbomer and presented 100% of sero-conversion. On the other hand, a higher level of neutralizing antibodies against PRRSV was registered after the boost with O/W adjuvant vaccine compared to the carbomer vaccine.

#### **Discussion and Conclusion**

Taken together, these results showed that MONTANIDE™ ISA 28 R VG adjuvant is well suited for formulating highly destabilizing antigens, providing a balanced safety/efficacy profile compared to carbomer.

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# EVALUATION OF A STX2E TOXOID BASED VACCINE AS A TOOL TO CONTROL STEC LOSSES IN SCENARIOS WITH OR WITHOUT ZINC OXIDE

<u>S. Oliver</u><sup>1</sup>, M. Cabetas <sup>2</sup>, A. Del Valle <sup>1</sup>, D. Espigares <sup>1</sup> <sup>1</sup>Ceva Salud Animal, Barcelona, Spain <sup>2</sup>Vall Companys S.A., Spain

### **Background and Objectives**

Since the ban of zinc oxide (ZnO) in June 2022, swine veterinarians in the EU have one less tool to control pathologies associated with E. coli. The objective of this study was to evaluate the effect of a Stx2e toxoid based vaccine on postweaning mortality in scenarios with or without ZnO.

#### **Material and Methods**

This trial was conducted in a two-site farm with recurrent sudden deaths of good body condition pigs. These problems appeared 2-3 weeks after weaning and sometimes eyelid oedema was also observed. Shiga toxin-producing E. coli (STEC) strain was detected in samples from affected animals. Creep and pre-starter feeds were routinely supplemented with ZnO. Piglets were not vaccinated against enterotoxemia caused by STEC. To carry out the study, five consecutive batches were distributed in 3 treatment groups. First batch (n= 1076) was designated as the control group (G1) since the piglets were not vaccinated against shiga toxin and ZnO was not administered in the diet. Piglets from second (n= 1256) and third (n= 1062) batch (G2) were vaccinated with Ecoporc Shiga® during the first week of age and were supplemented with ZnO in the diet (creep and pre-starter feeds). Piglets from fourth (n= 987) and fifth (n= 1050) batch (G3) were vaccinated with the same product and protocol, but they did not ingest ZnO in their diet. Mortality during the nursery period was registered and analysed using a Chi-square test. Significance level was set at p<0.05.

#### Results

Postweaning mortality percentages from batch 1 to 5 were: 12.45% (G1), 6.85% (G2), 7.82% (G2), 7.09% (G3) and 6.38% (G3).All vaccinated batches (G2 and G3) regardless of the use of ZnO showed statistically significant lower mortality than the control group (G1).No significant differences were observed between vaccinated batches regardless of whether ZnO was used (G2) or not (G3).No suspected cases associated to oedema disease were observed in any of the vaccinated batches (G2 and G3).

# **Discussion and Conclusion**

Under the conditions of the present study, Ecoporc Shiga® proved to be an effective tool for the control of oedema disease. When the pigs were vaccinated, the additional use of ZnO did not offer better results.

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# ANALYSIS OF TWO VACCINATION PROGRAMS FOR CONTROL OF PCV2 AND MHP IN A SPANISH PRODUCTION SYSTEM

<u>M. Genzow</u><sup>1</sup>, S. Figueras<sup>2</sup>, G. Abella<sup>2</sup>, R. Garcia<sup>3</sup>, F. Gonzalvo<sup>4</sup>, V. Rodriguez<sup>2</sup>, E. Sanchez Tarifa<sup>4</sup> <sup>1</sup>Boehringer Ingelheim Vetmedica <sup>2</sup>Boehringer Ingelheim Animal Health España <sup>3</sup>Boehringer Ingelheim Animal Health España, S.A.U. <sup>4</sup>Boehringer Ingelheim Animal Health España, S.A.

#### **Background and Objectives**

The swine industry continues to experience substantial economic losses due to respiratory disease outbreaks in modern pig production worldwide impacting productivity, antibiotic usage, and animal welfare. The objective of this study was to investigate both lung health as well as animal welfare as measured by side effects two different Mycoplasma hyopneumoniae vaccine combinations in a Spanish production system.

### **Material and Methods**

A total of 599 piglets from a single source were split into two groups within the same wean to finish barn. One group was vaccinated with 2 ml/IM of a fresh mixture of Ingelvac CircoFLEX®and Ingelvac MycoFLEX®, (FLEXCombo) Boehringer Ingelheim (group A). The other group was vaccinated in each neck side with 0,5 ml/IM of Circovac® and 2 ml/IM Hyogen® (Ceva Santé Animale) (group B). Both side effects after vaccination as well as lung lesions (scores 0-4) at slaughter were measured. The study was blinded by having people assessing pigs/lung not being aware of the vaccines given.

#### Results

Natural challenge was detected for both pathogens during the study. There were no side effects after vaccination observed in the group A, whereas there were significantly more side effects (n=10) seen in the group B ( $p \le 0.05$ ). The mode for lung lesion score was 1 for group A and 2 for the group B, providing evidence for a better lung health for group A.

#### **Discussion and Conclusion**

Vaccination with FLEXCombo® resulted in better lung health and significantly less side effects compared to a vaccination with Circovac® and Hyogen®. In view of increasing animal welfare demands it is pivotal to choose vaccines with superior efficacy and safety profile.

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# TRANSITIONING TO SINGLE TIER VACCINE LABEL CLAIMS: MYCOPLASMA HYOPNEUMONIAE

<u>B. Thacker</u><sup>1</sup>, E. Larsen<sup>1</sup>, E. Russell<sup>1</sup> <sup>1</sup>Lincoln Memorial University College of Veterinary Medicine, Harrogate, TN, USA

#### **Background and Objectives**

Recently, USDA's Center for Veterinary Biologics (CVB) changed vaccine label claims from a multi-tiered scheme to a single tier scheme. Multi-tiered claims were ordered from highest to lowest: infection prevention (Level 1), disease prevention (L2), aid in disease prevention (L3) and aid in disease control/reduction (L4). Single tier claims state that "this product has been shown to be effective for the vaccination against (disease)." Firms provided raw licensing study data to CVB for generating publicly available Product Summaries. Study data wasn't required for products approved before 2007. Our objectives were to relate the previous multi-tiered claims to licensing study data and identify factors that influence study results for U.S. licensed Mycoplasma hyopneumoniae vaccines.

# **Material and Methods**

Information was obtained from CVB Product Summaries, the Compendium of Veterinary Products and other trade publications. Licensing study data (pig level or group averages) was available from Product Summaries (n = 11) or other publications (n = 4). Thirteen of the 15 studies had a multi-tiered label claim while two were recently licensed. From studies with raw data, group mean lung lesion scores (% pneumonia) were calculated while percent reductions in pneumonia afforded by vaccination were calculated for all 13 studies.

#### Results

For L3 vaccines (n = 6), the percent reduction in pneumonia ranged from 72.6% to 90.6%. For L4 vaccines (n = 7), the percent reduction ranged from 33.4% to 71.1% for 6 studies and was 95.1% in one study. For factors that might influence percent reduction, dosing regimen (1 vs. 2) had no impact (67.4% vs. 69.6%, respectively) while differences were found between monovalent and polyvalent products. For monovalent products (n = 5), the average percent reduction was 86.1% (range = 74.3 to 95,1%); for polyvalent products (n = 8), 57.3% (range = 33.4 to 81.8%) (P value < 0.05).

### **Discussion and Conclusion**

CVB provides a precautionary statement in the Product Summaries to avoid comparing one product to another due to differences in study design and execution. The information reported here provides a broader overview of licensing study data but with the added precaution of applicability to field situations, especially regarding the expected efficacy of monovalent versus polyvalent vaccines.

# ANIMAL WELFARE AND ETHOLOGY

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### NEST MATERIAL DECREASES FARROWING DURATION IN CRATED SOWS

R. Carnevale<sup>1</sup>, M.S. Monteiro<sup>2</sup>, B. Bracco Donatelli Muro<sup>3</sup>, M.V.B. Nicolino<sup>2</sup>, L.K.S. Alves<sup>3</sup>, R.Y. Hoshino<sup>3</sup>, C.A.F. Melo<sup>3</sup>, J.S. Motta<sup>3</sup>, C.A. Pospissil Garbossa<sup>3</sup>

<sup>1</sup> Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

<sup>2</sup>Nerthus Pesquisa e Desenvolvimento, São Carlos, Brazil

<sup>3</sup>Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

#### **Background and Objectives**

The process of swine domestication did not alter the instinctive of nest-building behavior prior to farrowing in sows. However, housing sows in farrowing crates restricts the expression of this behavior due to the lack of space and/or access to materials. This physical and psychological restriction can cause a hormonal disbalance jeopardizing farrowing kinetics and colostrum production. The aim of this trial was to provide gilts a biodegradable, water soluble, and edible material designed for nest building and its effects on farrowing outcomes and colostrum production.

### Material and Methods

Animals were kept in crates for the total duration of the trial. Farrowing was synchronized with prostaglandin; all gilts received a split dose protocol on day 113 of gestation. Gilts were randomly allocated at entrance of the farrowing room in one of the two groups: CON (n = 6; gilts that did not receive any material) and NES (n = 6; gilts that received nesting material after 7 hours from first prostaglandin dose). Farrowing duration was calculated as the interval elapsed from the first and last piglet born, the individual intervals for each piglet were recorded. The number of total born, liveborn, stillborn, and mummified were recorded. Colostrum intake was calculated as described by Theil et al. (2004), and colostrum production was calculated as the sum of litter colostrum intake. Variables were analyzed with generalized linear models and statistical differences were set at p < 0.05.

#### Results

NES gilts had a farrowing duration of 182.3 min that was shorter (p < 0.05) than gilts sows that had farrowing duration of 274.5 min. All the other variables related to farrowing outcome and colostrum were similar (p > 0.05) between the groups.

### **Discussion and Conclusion**

The results support the notion that supplying nesting material can have beneficial effects in farrowing traits for crated females. Even for animals with restricted movement due to the crates the opportunity of performing nest building behavior seems to have a positive effect. This can be a solution for countries where free-farrowing systems are not yet a reality.

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# EFFECTS OF DIFFERENT TEETH GRINDING PROCEDURES ON SKIN LESIONS, BODY WEIGHT AND BEHAVIOUR OF SUCKLING PIGLETS

F. Kleine Kruthaup 1, M. Fels 3, E. Grosse Beilage 2

Veterinary Practice Dümmerland, Steinfeld, Oldenburg, Germany

<sup>2</sup>Field Station for Epidemiology in Bakum, University of Veterinary Medicine Hannover, Germany

<sup>3</sup>University of Veterinary Medicine Hannover, Foundation, Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour

### **Background and Objectives**

In most conventional German piglet production farms, grinding of teeth (Id3 and Cd) is routinely performed to prevent injuries in piglets and the sow's udder. Routine teeth grinding is conducted with a roller head. The use of roller heads is frequently causting in pulp cave openings, as Id3 and Cd, that are different in length, need inevitably to be grinded simultaneosly. A newly designed tea-cup head allows to grind single tooth, resulting in only few pulp cave openings. The aim of this study was to compare pulp cave openings, skin lesions behaviour and body weight in piglets that got their teeth not grinded (CG) or grinded with tea-cup (TCG) or roller head (RG).

# Material and Methods

In total, 110 sows and their litters (n=1400 piglets) were randomly allocated to the treatment groups and followed from birth to weaning. The teeth were grinded after birth. Piglets skin was scored (head, ears, trunk, limbs) and body weight was measured at five times until weaning. Behaviour (lying, suckling, running, sitting) was recorded by video.

#### Results

Pulp cave openings were not found in CG but in 7.59% of TCG and 64.55% of RG (p<0.001). During the entire suckling period, facial skin lesions were more frequently seen in CG compared to the piglets with grinded teeth (p=0.008). Lesions on ears, trunk, limbs revealed no significant differences. The body weight of RG was significantly lower than TCG (p=0.0116). The behaviour of the piglets did not differ significantly in the pairwise comparison of suckling vs. lying and sitting vs. lying calculated over the entire suckling period. The behaviour running was more frequently seen on RG and the pairwise comparison running vs. lying showed a significant difference between the three groups over the entire suckling period (p=0.029).

# **Discussion and Conclusion**

The study indicates that tooth resection with the conventional roller head should be critically scrutinized as pulp cave opening is a frequently occurring side effect. In RG a positive effect on facial skin lesions was confirmed, but this does not exist in relation to body weight and behaviour. The piglet treated with the tea-cup grinder showed significantly less pulp cave openings and higher body weight.

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# EFFECT OF TYPE AND AMOUNT OF ORGANIC ENRICHMENT MATERIAL ON DAILY WEIGHT GAIN IN WEANER PIGS

<u>P. Heseker</u><sup>1</sup>, J. Probst<sup>1</sup>, S. Ammer<sup>2</sup>, U. Hartmann<sup>3</sup>, I. Traulsen<sup>4</sup>, N. Kemper<sup>1</sup> <sup>1</sup>Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour (ITTN), University of Veterinary Medicine Hannover, Foundation, Germany <sup>2</sup>Department of Animal Sciences, Livestock Systems, Georg-August-University Göttingen, Germany <sup>3</sup>Chamber of Agriculture Lower Saxony, Bad Zwischenahn, Germany <sup>4</sup>Institute of Animal Breeding and Husbandry, Christian-Albrechts-University Kiel, Germany

#### **Background and Objectives**

Providing organic enrichment material to pigs is a common method for supporting the pigs' foraging needs and prevents aggressive behaviors such as tail biting. However, feeding too much material may reduce the average daily weight gain (ADG), due to a possible replacement of the commercial diet. This study aimed to investigate if the ADG of weaner pigs was influenced by automatically supplied organic material.

# Material and Methods

Six batches of undocked weaner pigs (n=810) housed in one weaning compartment with six pens were included in this study. Alfalfa pellets (AL), oat bran pellets (OB) or a mixture of both (MI) were regularly provided to the pigs (240 g/pen/supply) with two pens receiving the same material. The frequency of supplies changed between 2, 4 or 6 supplies per day (20, 40 or 60 g/day/pig) in a batch. Pigs were weighed individually at weaning and after 39 days (end of rearing period) for calculating the ADG. Effects of enrichment material and frequency on ADG were analyzed using a linear mixed-effect model in RStudio software.

#### Results

The results showed a mean ADG of 461 g/day. For 2 supplies/day, mean ADG was 462 g/day, compared to 457 g/day for 4 and 464 g/day for 6 supplies/day, averaged over the materials. Lowest ADG of 439 g/day was observed when AL was provided twice daily. Highest ADG was calculated for MI and two supplies/day with 480 g/day. However, no significant effects of the different treatments on ADG of weaner pigs were observed in the model (p>0.05).

#### **Discussion and Conclusion**

This study showed that ADG was not affected by providing different types and amounts of organic material to the pigs. Therefore, increasing animal health and welfare by supporting foraging behavior and reducing tail biting can be achieved without decreasing performance.

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# REDUCTION OF LAWSONIA INTRACELLULARIS SHEDDING, IMPROVEMENT OF CARCASS QUALITY AND PARTIAL PREVENTION OF TAIL BITING AFTER INTRADERMAL VACCINATION AGAINST THIS BACTERIUM

<u>R. Del Pozo Sacristán</u><sup>1</sup>, H. Swam<sup>2</sup>, S. Von Berg<sup>3</sup>, A.E. Taylor<sup>4</sup> <sup>1</sup>MSD Animal Health <sup>2</sup>Center for Diagnostic Solutions, MSD AH Boxmeer, The Netherlands <sup>3</sup>MSD Animal Health, Munich, Germany <sup>4</sup>University of Leeds, UK

# Background and Objectives

The aim of this study was to assess the efficacy of an intradermal vaccination against Lawsonia intracellularis on a herd with a subclinical infection.

# **Material and Methods**

A randomized, controlled, blind, side-by-side trial was performed in a herd with a history of subclinical ileitis. At 4 weeks of age (woa), 240 piglets were allocated to Vaccination (V; n=120; vaccinated with Porcilis®Lawsonia ID mixed with Porcilis®PCV ID and concurrent with Porcilis®M Hyo ID Once; administered with IDAL® Twin) or Control (C; n=120; same PCV2 and Mycoplasma hyopneumoniae vaccines) group. Faecal samples (n=30/group) were taken (4, 7, 10, 13, 16, 19woa) from the same individual pigs directly from the anus. Bacterial load in faeces was assessed by qPCR (Ingenetix®). Ileitis-associated mortality %, treatment incidences, scour incidences and tail biting % were registered. At slaughter, carcass quality was assessed by recording carcass weight, back fat level (at P2 level; cm) and Lean Meat % (LM%). Kruskal-Wallis was performed to evaluate bacterial shedding and carcass characteristics analyzed using the mixed linear model procedure. Mortality, antibiotic treatment, signs of scour, or tail bitten animals were assessed by Chi-square analysis.

#### Results

No bacterial shedding was detected before 13 and 16woa for controls and vaccinated pigs, respectively. A significant lower bacterial load (log10 copies/il) was detected at 16wk in vaccinated pigs (V:1.70 $\pm$ 0.66; C:3.31 $\pm$ 1.65; P<0.05). Average AUC (bacterial shedding log10 copies/il) from 4-22woa was significantly lower for vaccinates (20.72 $\pm$ 25.93) compared to controls (40.23 $\pm$ 39.10) (P<0.05). Vaccinated pigs had a significantly lower prevalence of tail biting (31.67%) compared to control pigs (54.17%) (P<0.05). Vaccinated pigs had less back fat (10.5 vs 10.9  $\pm$ 0.14 mm; P<0.1) and greater LM% (62.7 vs 62.1  $\pm$ 0.12%; P<0.05) when compared to non-vaccinated pigs. No significant differences were seen for the other parameters.

#### **Discussion and Conclusion**

In this study, a significant reduction of bacterial shedding and increase of carcass Lean Meat % was demonstrated after intradermal vaccination against L. intracellularis. This is the first scientific study that reports a partial prevention of tail biting after vaccination against L. intracellularis. Further research is needed to elucidate this finding.

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# COLOR PREFERENCES IN THE RESTING BEHAVIOR IN NURSERY PIGLETS TESTED UNDER EXPERIMENTAL HOUSING CONDITIONS

T.J. Nicolaisen <sup>1</sup>, F. Lohkamp <sup>2</sup>, B. Reckels <sup>3</sup>, C. Visscher <sup>2</sup>, I. Hennig-Pauka <sup>1</sup>

<sup>1</sup>Field Station for Epidemiology, Bakum, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany <sup>2</sup>Institute for Animal Nutrition, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany <sup>3</sup>Vets Wettringen, Wettringen, Germany

#### **Background and Objectives**

Pigs are dichromats and there is evidence that they perceive light and color differently than humans. In this study, color preferences of nursery piglets in resting pens were tested in an experimental housing system.

# Material and Methods

Twelve nursery piglets were kept in an experimental housing system for 32 days. Four resting pens existed, facing each other in a 2 x 2 design. Each resting pen was shielded against external light and was illuminated with one of the four tested colors: blue, green, red, and yellow. The colors rotated between the four pens every 24 hours to avoid a possible pen effect on the results. In total, each pen was illuminated for 8 days with each tested color. The level of occupancy of each pen was recorded individually for all twelve piglet between 06:00 PM and 06:00 AM using scan-sampling technique with 10-minute intervals (in total 28,032 data points [dp]).

#### Results

Piglets were observed in the resting pens on 73% of all dp. The four pens were used in varying proportions (pen 1: 21.2%, pen 2: 34.2%, pen 3: 31.8%, pen 4: 12.8%). The individual pen preference differed among the piglets. Significant differences were detected for color preferences in resting pens (p>0.05). Most of the piglets were detected under yellow light (6,979 dp), red light (6,051 dp) and green light (5,836 dp). No significant differences were detected between these three colors. Significantly lower numbers of piglets were counted under blue light (1,632 dp) compared to all other colors (p<0.05). Color preferences differed among individual piglets.

### **Discussion and Conclusion**

One possible explanation for the found results is that pigs perceive colors in the blue spectrum well and avoided these bright pens for resting, whereas colors such as red and green are likely to be perceived weakly or as grayish and are therefore preferred for resting. Illumination of pens with different colors could be used as a tool for structuring pens in future pig husbandry.

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# EFFECTS OF HIGH-CONCENTRATED CO2-STUNNING IN FATTENING PIGS – PULMONARY HEMORRHAGES DURING SLAUGHTER

<u>T. Frey</u><sup>1</sup>, K. Schwalm-Wunsch<sup>2</sup>, M. Kaschny<sup>1</sup>, A. Richter<sup>1</sup>, K. Danowski<sup>3</sup>, F. Löwenstein<sup>3</sup> <sup>1</sup>*Pig Health Service Baden-Württemberg, Fellbach, Germany* <sup>2</sup>*Chemical and Veterinary Analysis Agency Stuttgart, Germany* <sup>3</sup>*State Institution for Swine Breeding of Baden-Württemberg, Boxberg, Germany* 

#### **Background and Objectives**

In the European Union, the  $CO_2$ -stunning is a widely used method for pig slaughter. High concentrations ( $\geq$  90 %) are often used for rapid stunning success. Incorrect stunning can lead to blood aspiration and is officially objected as relevant to animal welfare. In a slaughterhouse in Baden-Wuerttemberg, Germany 5 – 10 % of lungs per slaughter day were officially rejected for blood aspiration over a five month period in 2020.

# **Material and Methods**

Health status of 89 fatteners on one slaughter day were examined by rectal temperature measurement, cough index and slaughter lung check following Madec and Kobisch (1982). Preslaughter stunning was performed using a dip-lift  $CO_2$ -system and a  $CO_2$ -concentration of 90 – 95 % for 140 seconds. Stunning success and slaughter influences were assessed based on epistaxis, corneal reflex, respiratory movements, vocalization, excitation and post-stunning. From 13 animals, that were rejected due to blood aspiration, lungs (n = 13) and heads (n = 5) were collected for pathological and microbiological examinations.

#### Results

The fatteners were clinically unremarkable. Epistaxis (2.67 %), respiratory movement with post-stunning (5.34 %) and excitations (0.89 %) were observed. Corneal reflexes and vocalization were absent. Slaughter lung check with total scores  $\ge$  5 in 0.89 % and  $\ge$  10 in 1.78 % of lungs ruled out suspicion of infectious diseases. 14.61 % of lungs were officially condemned with blood aspiration. Pathomorphologically, heads and tracheae of rejected lungs showed no slaughter injuries. Main and tip lobes of the lungs showed petechial to extensive hemorrhages. Pathohistological, perivascular and peribronchiolar hemorrhages and focal blood pooling in the bronchi with probable spillover into the alveolar lumen were observed.

# **Discussion and Conclusion**

The present study shows that the probable cause of pulmonary haemorrhage was the stress-induced increase in blood pressure during  $CO_2$ -stunning with a high  $CO_2$ -concentration. It is possible to differentiate between blood aspiration and pulmonary hemorrhages in slaughter lungs by clarifying the health status, possible technical slaughter errors and further pathological examinations.

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# CONSEQUENCES OF NO TAIL DOCKING IN PIGS IN BRAZIL: AN ANALYSIS OF THE EFFECTS ON TAIL BITING.

<u>J. Rego Ribas</u><sup>1</sup>, J. Grajales-Cedeño<sup>1</sup>, V. S. Sobral<sup>2</sup>, I. Gianeis<sup>1</sup>, M. Paranhos Da Costa<sup>1</sup> <sup>1</sup>UNESP Jaboticabal <sup>2</sup>Agroceres PIC

# **Background and Objectives**

The Brazilian animal welfare regulations established banishing piglets' tail docking in 2023. However, this was defined without having studies to understand the impact of this procedure on the Brazilian pig production chain.

# Material and Methods

A total of 854 piglets of synthetic Pietran line were randomly located into two treatments: piglets with the third final part of the tail docked according to Brazilian regulation (Normative Instruction No. 113 of the Brazilian Ministry of Agriculture), establishing a residual length of 4.5 centimeters (DT, n = 442) and those without tail docking (NDT, n = 412). The housing standards were according to IN 113/220, which established the welfare standards for rearing pigs. The occurrence of tail biting was assessed daily using a scoring scale from (0) to (2), where (0) indicated no bites, (1) represented healed minor bites, and (2) bites with fresh blood or missing tail fragments. The assessments were conducted in both the nursery and growing phases.

#### Results

The NDT piglets showed a tendency (p=0.08) towards a higher occurrence of tail biting in the nursery phase, with 89.67% of them scoring (0), 8.03% scoring (1), and 2.45% scoring (2). In the DT group, 84.44% of the animals scored (0) and 15.56% scored (1), and no animals scored (2). In the finishing phase, tail biting was observed only in the NDT animals, with 87.89% of them scoring (0), 8.23% scoring (1) and 4.05% scoring (2).

#### **Discussion and Conclusion**

The results indicated that a higher percentage of pigs from the NDT group showed tail-biting behavior during the nursery and finishing phases than those from the DT group. The tail-biting occurred mainly in the end phase of both housing periods, indicating that raising pigs with intact tails will require more space than the regulation established. The results also showed that its possible to raise pigs just docking the third part of the without impacting tail biting incidences.

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# TEATS BITING IN FARROWING ROOM: IS THE ENVIRONMENT MANIPULATION PART OF THE SOLUTION?

J. Rego Ribas 1, J.C. Baumgartner 2, G. Silva 3, A. Pimenta Siqueira 4, M. Paranhos Da Costa 1

<sup>1</sup>UNESP Jaboticabal <sup>2</sup>Universidade Federal do Paraná, Palotina, Brazil <sup>3</sup>Cristal Springs, Toledo, Brazil <sup>4</sup>Agroceres PIC

### Background and Objectives

Animal welfare regulations stablished a higher weaning age to improve welfare of the litter. In modern farms aggressive behaviors from the litter toward the sow has been seen in longer lactation periods resulting at biting of teats and vulva, impacting direct sow welfare. The objective of this study was to evaluate different environment manipulation to decrease piglet aggressive behavior in lactation period.

# **Material and Methods**

A total of 148 sows, commercial crossbread Landrace x Large white, were randomly divided into three groups at farrowing room: control group CT (50); reducing noise in the room MS (52), reducing noise plus rope to the pigletsM SR (46). The housing standards were all according IN 113/220 that stablish the welfare standards for rearing pigs in Brazil. All the litters were creep feeded from day 7. The presence of teat injury from bites were evaluated along the 4 weeks of lactation. The score scale used was: 0 indicated no injury, 1 represented healed minor bites, 2 denoted bites with fresh blood and 3 missing teat fragments with invalidation of the teat.

#### Results

At the first week of lactation there were no differences (p>0,05). The treatment MSR reduce the total amount of lesions in all weeks when compared to others (p>0,05). The percentage of the total prevalence of lesions scored as 2 and 3 was: For cranial teats: Week three: CT: 8%, MS: 3%, MSR:2%, and week four: CT: 12%, MS: 2%, MSR:4% and for caudal teats: Week three: CT: 25%, MS: 8%, MSR:6%, and week four: CT: 47%, MS: 15%, MSR:15% in caudal teats.

# **Discussion and Conclusion**

Reducing the noise in the farrowing room, by limiting the radio use, showed to be an effective measure to reduce teat biting. Adding an edible enrichment to the pen, was a successful measure to reduce bites towards the mammary gland. This phenomenon show that piglets seek to explore the teats as a relief for stress. And was marked in the end phase of lactation, which indicates that piglets stress could be associated with a decrease of milk production, even with a good creep feeding supply.

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# EVALUATION OF GESTATING SOWS' FIGHT INTENSITY USING FOUR DIFFERENT METHODS

<u>E. Cantaloube</u><sup>1</sup>, A. Taktak<sup>1</sup>, L. Gautier<sup>1</sup>, T. Gin<sup>1</sup> <sup>1</sup>Chêne Vert, 4 rue Théodore Botrel, 22600 Loudéac FRANCE

#### Background and Objectives

Mixing gestating sows in groups can lead to fights with a significant zootechnical impact. Therefore, it is useful to evaluate the intensity of the aggressions to fix a mixing problem or to see the result of a new mixing management. The aim of this study was to evaluate four methods of mixing aggression evaluation.

#### **Material and Methods**

110 sows with an average parity of 4,5 were mixed in 4 rooms. Each rooms had 4 pens and was equipped with a Dictaphone and a camera. Cameras enable the observation of 2 pens per room for a total of 57 sows. From 30 minutes to 30 hours after mixing we measured: the number and the duration of fights observed with cameras, the number of shouts and feet tramples (FT) with dictaphones. The number of fights was also noted in situ 30min, 24 and 36 hours after mixing by an observer during 10 minutes per pen. The same observer gave a skin lesion score for each sow before, 24 and 48 hours after mixing.

#### Results

Skin lesion scores rose during the first 24 hours after mixing (p<0,001) but not anymore after 24 hours. The number and the duration of fights observed with cameras, shouts and FT also increased 24 hours after mixing and then dropped (p<0,001). Skin lesion scores were correlated with the number and the duration of the fights observed with cameras (p value <0,001 and 0,05,  $\tilde{n}$ = 0,5 and 0,3). The number of fights observed with cameras and in situ were weakly correlated (p value <0,005,  $\tilde{n}$ = 0,3). Parity and sow body score significantly increased skin lesions, the number and the duration of fights

#### **Discussion and Conclusion**

To evaluate the mixing aggression intensity, we recommend observing the fights with cameras and scoring the skin lesions only during the first 24 hours after mixing. The comparison of advantages and disadvantages of the four methods used here are discussed in another abstract.

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# EVALUATION OF KEY PERFORMANCE INDICATORS AFTER IMPLEMENTATION OF IMMUNOCASTRATION IN FOUR FRENCH FARMS USING A CONTINUOUS QUALITY IMPROVEMENT TOOL

<u>J. Morin</u><sup>1</sup>, F. Colin<sup>2</sup>, N. Wuyts<sup>3</sup>, J. Angulo<sup>4</sup> <sup>1</sup>Selas Vétérinaire du Gouessant, 3 Rue de la Jeannaie Maroué, 22400 Lamballe, France <sup>2</sup>ZOETIS, Bâtiment VIVA, 10 Rue Raymond David, 92240 Malakoff, France <sup>3</sup>ZOETIS Benelux, Mercuriusstraat 20, 1930 Zaventem, Belgium <sup>4</sup>Zoetis Inc, 1040 Swabia Ct, Durham, NC 27703 – United States

# **Background and Objectives**

Quality Improvement philosophy refers to the use of statistical methods for monitoring and maintaining the quality of production processes. The objective of this study was to use a quality improvement tool like Statistical Process Control charts as a practical and reliable tool to evaluate the immunocastration implementation in four French farrow-to-finish farms.

#### Material and Methods

The study was carried out on four 200 to 400-sows in farrow-to-finish farms (A, B, C, D) from a same French cooperative who opted to produce immunocastrated males as an alternative to surgical castration. All the pigs were identified with a seventh character on the carcass-ID- stamp to allow the analysis at farm and slaughter level. Data from batches before (processed) and after (Improvac) by farm were collected and analyzed using Xbar-S SPC charts (Minitab Inc) to monitor the mean and the variation (StDev) between interventions, and within subgroups at each farm. A total of 118 batches were evaluated as following, Farm-A, N=22, before-9, after-13; Farm-B, N=31, before-15, after-16; Farm-C, N=47, before-15, after-32; Farm-D, N=18, before-8, after-10. Individual pig data were used for inferential statistical analysis using a Student or Aspin Welch test at the 5% level.

#### Results

At the individual pig data, statistical significant improvements were observed in immunocastrated males in all key performance indicators evaluated (P<0.0001): higher lean meat percentage (+ 1.21 %), higher average daily gain (+ 20.9 g/d), higher cold carcass weight (+ 0.66 Kg) and lower backfat thickness (- 2.41 mm).

In addition to the significant improvement in individual pig data, we observed a quality improvement before and after the immunocastration implementation using SPC Xbar-S charts in all farms.

#### **Discussion and Conclusion**

This long-term evaluation study showed a significant increase of average daily gain, cold carcass weight and lean meat percentage, combined with a significant reduced backfat thickness along with a reduction of variation for those parameters between the measurements within each farm and between the four farms.

The use of quality tools helps to generate specific knowledge from production systems and establish a common language to link animal welfare interventions with production outcomes in an objective manner leading practitioners to a data-driven decision-making process.

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# GENOMEWIDE ASSOCIATION STUDY REVEALS CANDIDATE GENES FOR SHORTER TAILS AND KINKS IN PIGS

<u>K. Gerhards</u><sup>1</sup>, C. Egerer<sup>1</sup>, T. Yin<sup>2</sup>, S. Koenig<sup>2</sup>, P. Engel<sup>2</sup>, H. Willems<sup>1</sup>, S. Becker<sup>1</sup>, G. Reiner<sup>1</sup> <sup>1</sup>Department for Veterinary Clinical Sciences, Justus-Liebig-University Giessen, Frankfurter Strasse 112, Giessen, Germany

<sup>2</sup>Department of Animal Breeding and Genetics, Justus-Liebig-University Giessen

#### **Background and Objectives**

The possibility of reducing tail biting by breeding pigs with shorter tails has been discussed by some research groups in recent years, although this does not solve the primary problem of tail biting. Current studies also indicate that in pigs, as in other animal species, selection for shorter tails is associated with an increased rate of deformities of the tail. With the help of genome analysis, it may be possible to identify gene variants with a reducing effect on tail length that are not associated with the occurrence of malformations. This was the aim of the present study.

# **Material and Methods**

The study was based on 140 piglets with significant variation in tail length. The piglets were weighed, photographed and measured to record tail length, tail abnormalities and the presence of inflammation or necrosis. All piglets were fully genomically sequenced and bioinformatically analysed using a genome-wide association study.

#### Results

Phenotypically, tail length varied from 20.2 to 31.3 % of the total length of the piglets. Shorter tails were associated with a significantly higher prevalence of kinks of varying degrees: In descending 20% quantiles of relative tail lengths, the proportion of kinked tails increased from 5% to 6%, 10%, 12% and 28% (P<0.001). The genome-wide association study identified 6 significant SNPs associated with tail parameters. The SNPs were located on chromosomes 1, 5, 6, 11 and 15 in introns and intergenic regions in the region of some candidate genes and explained up to 30 % of the phenotypic variance. Two of the SNPs were additionally associated with tail length.

#### **Discussion and Conclusion**

So far, no SNPs could be identified that were associated with a shortening of the tail but not with the simultaneous occurrence of kinks. In addition to the purely symptomatic effect of shortened tails on biting behaviour, which was not investigated in the present study, selection for shortened tails can therefore also not be recommended due to the associated occurrence of kinked tails.

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# CLASSIFICATION OF NEST BUILDING PROFILES IN LOOSE HOUSED SOWS USING ICETAG ACCELEROMETERS

#### R. Westin <sup>1</sup>, S. Gunnarsson <sup>1</sup>, A. Wallenbeck <sup>2</sup>

<sup>1</sup>Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden <sup>2</sup>Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Uppsala, Sweden

#### **Background and Objectives**

Nest building is a behavioural need of sows. Although domestic sows are kept indoors with resources to keep the piglets warm, still they perform specific behaviour sequences related to nest building. The aim of this project was to study the variation in nest building activity between sows by using IceTag accelerometers and to investigate the association of the nest building activity and piglet survival.

#### **Material and Methods**

The study was performed in a commercial piglet producing herd with loose housed sows in Sweden. Seventeen Yorkshire-Landrace sows (parity 2-3) were included in the study. IceTag accelerometers (IceRobotics, UK) were attached to the distal right hind limb of each sow. The accelerometers logged movement in three axes and a proprietary algorithm automatically calculated a motion index in 15-minute intervals. In addition, production data (number of live born, crossfostering, weaned piglets etc.) for each litter was recorded. Based on the activity levels pre-farrowing retrieved from the motion index data, sows were classified into three nest building profile categories (Low, Intermediate or High).

### Results

In average, sows had 16.6 live born, 0.4 stillborn and 12.4 weaned piglets/litter. The piglet mortality in live born piglets was 17.3 %. Three sows were classified as Low, 8 as Intermediate and 6 sows as having a High nest building profile. Piglet mortality differed numerically between High (10.6 %), Intermediate (19.8 %) and Low (24.4 %), i.e. the highest survival was found in litters where the sow performed the most nest building.

#### **Discussion and Conclusion**

Although originally developed for cows, we conclude that accelerometers are a promising tool for measurement of nest building activities and classification of nest building profiles in sows. The technique is simple to use and data is quick to retrieve and analyse compared to behavioural observations from video recordings, typically used in research on sow behaviour. Accelerometers could also be of future interest in commercial herds for detection of sows with high motivation for nest building. The link between nest building and good maternal abilities makes these sows good candidates for breeding, especially in loose housing systems.

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# CURRENT SITUATION OF ISOFLURANE ANAESTHESIA FROM THE FARMERS' PERSPECTIVE

L. Jahn 1, S. Baier 1 Swine Health Service, LUFA Nord-West, Institute for Animal Health, Oldenburg, Germany

#### **Background and Objectives**

Castration of piglets without anaesthesia has been banned in Germany since January 2021. Farmers are allowed to castrate male piglets up to seventh day post natum with isoflurane without an attending veterinarian. Beforehand a training course with a theoretical and practical test is obligatory as well as refresher training after three years. The aim of this poster was to record the current status of piglet castration in Lower Saxony (Germany) and to identify potential animal welfare or user problems.

### Material and Methods

A questionnaire with decision questions and free text for explanations was used. All farmers received this questionnaire during the refresher training with Swine Health Service in Lower Saxony (since July 2023). Participation was voluntary. Until November 2023 319 questionnaires were completed and evaluated, farmers from the same farm only completed one questionnaire. The evaluation was carried out using Excel 2019 and NCCS 2023.

#### Results

The distribution of the five approved anaesthesia machines showed a preference in Lower Saxony for PigNap 4.0 (BEG Schulze Bremer GmbH, 42 %, n=133), PigletSnoozer (GFS TopAnimal Service GmbH, 31 %, n=98) and Anestacia (GDO GmbH, 20 %, n=64). PigSleeper (MS Schippers, 7 %, n=23) and PorcAnest (Promatec Automation AG, n=1) were less represented. 95 % had already made use of customer services. Of these, 6 % were dissatisfied, citing reasons ranging from high costs, short shelf life and hygiene problems (no porc-free technician) to poor service and long waiting times. 15 participants (5 %) stated that there were problems with anaesthesia. 73 % of these 5 % observed insufficiently deep anaesthesia and 9 % observed piglets that were too deeply anaesthetized or died, while the remainder had a combination of both problems. An increased tendency to bleed was found in 24 % of the farms. This is why the use of an emasculator was introduced at 50 % of theses farms. Further measures relating to castration were reported by 84 % of the farms.

#### **Discussion and Conclusion**

The first evaluation after three years of isoflurane anaesthesia in Lower Saxony (Germany) shows good user satisfaction and is a success from an animal welfare perspective. Correct use must be kept in mind and opportunities must be created to reduce the increased tendency to bleed.

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# THE SINS (SWINE INFLAMMATION AND NECROSIS SYNDROME) SYMPTOMS OF SUCKLING PIGLETS ARE SIGNIFICANTLY ASSOCIATED WITH THE SINS STATUS OF THEIR SIRES

<u>E. Kochendörfer</u><sup>1</sup>, K. Gerhards<sup>2</sup>, C. Egerer<sup>1</sup>, S. Becker<sup>1</sup>, M. Lechner<sup>3</sup>, C. Leiding<sup>4</sup>, G. Reiner<sup>1</sup> <sup>1</sup>Department for Veterinary Clinical Sciences, Justus-Liebig-University Giessen, Frankfurter Strasse 112, Giessen, Germany <sup>2</sup>Justus-Liebig-University Giessen <sup>3</sup>UEG, Hohenlohe, Germany

<sup>4</sup>BVN, Neustadt/Aisch, Germany

# **Background and Objectives**

SINS is a widespread disease in suckling piglets and weaners that is associated with inflammation and even necrosis of the acres. The SINS scores of the offspring of different boars can differ considerably and repeatedly, and various studies confirm the hereditary nature of the syndrome. It has recently been shown that the inflammation/necrosis symptoms of AI boars can also vary considerably. The aim of the present study was therefore to investigate correlations between the SINS symptoms of AI boars and their offspring.

# **Material and Methods**

For this purpose, 80 piglets from 13 different AI boars from an ongoing study were examined and analysed. The boars were not pre-selected. Each litter was produced from mixed semen from two different boars. The piglets were scored before the paternity test was carried out (double-blinded study).

#### Results

The correlation between SINS of the piglets and their fathers was r=0.5 (P<0.001). The offspring of the AI boars with the lowest and highest SINS values achieved 47.6  $\% \pm 17.8$  (Cl95: 39.0 - 56.2) and 78.0  $\% \pm 6.3$  (Cl95: 71.4 - 84.6) of the maximum score points respectively and were statistically highly significantly different (P=0.0001). The piglets of the average boars showed average SINS scores. As a single value, the occurrence of venous congestion on the superficial veins of the hind limbs had the highest predictive value, while the findings on claws and heels did not correlate with the SINS scores of the piglets, as expected.

#### **Discussion and Conclusion**

The results suggest that it is possible to pre-select boars with a low tendency to inherit SINS based on their own phenotypes. In future, the control of inflammation and necrosis in piglets could be supported by selecting the AI boars used.

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# BOAR TAINT VACCINATION EFFECTIVENESS IN A 'REAL-LIFE' HUMAN NOSE DETECTION SYSTEM ON THE SLAUGHTER LINE, OVER 7 MONTHS, IN WESTERN FRANCE

J. Morin<sup>1</sup>, B. Petitpas<sup>2</sup>, E. Bellec<sup>2</sup>, O. Quentric<sup>2</sup>, F. Colin<sup>3</sup>

<sup>1</sup>Selas Vétérinaire du Gouessant, 3 Rue de la Jeannaie Maroué, 22400 Lamballe, France <sup>2</sup>COOPERATIVE LE GOUESSANT, Z.I. 1 rue de la Jeannaie, BP 40228, 22402 Lamballe-Armor Cedex, France <sup>3</sup>ZOETIS, Bâtiment VIVA, 10 Rue Raymond David, 92240 Malakoff, France

#### **Background and Objectives**

Boar taint vaccination has repeatedly shown to be as efficacious as surgical castration of piglets to control boar taint in modern swine production. Nevertheless, many French slaughterhouses have been reluctant to accept entire vaccinated males unless a proper boar taint detection by human nose is performed on the slaughter line.

# **Material and Methods**

In a swine farm cooperative from Brittany (western France), boar taint vaccination was progressively implemented in 11 farrow-to-finish farms (150 to 480 sows) between October 2021 and March 2022. All finishers were marketed to the same slaughterhouse, where a standardized human nose method for boar taint detection was progressively introduced in the spring and summer of 2022. The method scored each carcass on a scale of 1 to 5 (1: no boar taint, 4: boar taint, 5: strong boar taint). The main outcome of the study was the proportion of carcasses with score 1. Secondary outcomes were the covariables: type of feed, feed distribution method and hygiene of the loading dock. Statistical analysis of the data was carried out using the Fisher exact test.

#### Results

All batches from the 11 farms that were slaughtered between December 1, 2022 and June 30, 2023 were included in the study (n=15,316 pigs). The proportion of carcasses with score 1 (no boar taint) was 99.22%. The proportion of carcasses with scores 4 and 5 were minimal (0.09% and 0.05%, respectively). For pigs with scores 4-5, there was no significant effect of the type of feed (farm-made vs. commercial), the distribution mode (dry vs. liquid) and the hygiene of the loading dock (systematic vs. sporadic cleaning).

# **Discussion and Conclusion**

In this swine organization and during the study period, boar taint vaccination proved 99.8% efficacious in reducing boar taint. Although, analysed covariables did not have a significant impact on the proportion of carcasses with scores 4-5, systematic cleaning of the loading dock after departure of finishers significantly increased the proportion of carcasses with no detectable boar taint, from 98.85% to 99.42% (p=0.0001). Boar taint vaccination proved to be a reliable alternative to surgical castration under these farming conditions, allowing the sensory quality of carcasses to meet consumers' expectations.

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# COMPARISON OF TWO NEEDLE-FREE DEVICES IN VIETNAM

P. Nguyen <sup>1</sup>, B. Nguyen <sup>1</sup>, G. Pham <sup>1</sup>, V. Tran <sup>1</sup>, C. Maala <sup>2</sup>, <u>H. Nguyen <sup>1</sup></u> <sup>1</sup>Boehringer Ingelheim Animal Health Viet Nam <sup>2</sup>Boehringer Ingelheim Animal Health Philippines Incorporated

### **Background and Objectives**

The objective of this multicenter field study is to compare the following parameters between 2 different needle free devices (needle free intramuscular vaccination and needle free intradermal vaccination) in terms of percent vaccine volume left on pig's skin.

#### Material and Methods

A total of 0.2 ml was used for the needle free intradermal vaccinator while 2 ml was the dispensing volume of the needle free intramuscular vaccinator as recommended by the respective Manufacturers. The volume of vaccine left on the pig's skin is measured by means of a capillary tube. Commercially available micro capillary tubes (e.g. microhematocrit tubes) of 75mm length was used to collect the remaining volume of vaccine administered either with intramuscular or intradermal needle free devices. The volume left on the skin was measured immediately after vaccination in 30 animals per group. Microcapillary tubes that are intended for single use was used. All measurements were recorded, and data were analyzed using descriptive statistics (T-test and Box & Whisker Plot).

#### Results

The volume left on the skin with the needle-free intramuscular vaccinator was on the average 0.011304 ml out of 2 ml (0.56%) while the needle-free intradermal vaccinator had an average 0.033535 ml out of the 0.2 ml (16.8%) volume left on the skin.

# **Discussion and Conclusion**

It has been a quandry on the accuracy of volume being injected into the pig's body using intradermal or intramuscular needle-free vaccination. This is one of the studies that show the actual volume and percentage of vaccine being left on the skin of the pig. It is important that enough antigenic dose is being administered especially in farms where hundreds if not thousands of pigs are being immunized on a regular basis.

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# PRELIMINARY STUDY: FEASIBILITY OF CHECKING THE WEANING AGE OF PIGLETS BY OBSERVING DECIDUAL DENTAL ERUPTION

<u>A.M. Maisano</u><sup>1</sup>, S. Raineri<sup>1</sup>, A. Gazzola<sup>1</sup>, E. Arioli<sup>2</sup>, V.U. Santucci<sup>3</sup>, D. Castelluccio<sup>3</sup>, G. Santucci<sup>1</sup>, M. Tonni<sup>1</sup>, F. Scali<sup>1</sup>, V. Lorenzi<sup>1</sup>, G.L. Alborali<sup>1</sup>, F. Vezzoli<sup>1</sup> <sup>1</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy

<sup>2</sup>Freelance veterinarian, Italy <sup>3</sup>Italian Ministry of health, Rome, Italy

#### **Background and Objectives**

The European animal welfare legislation has two age cut-offs for piglets' weaning: 21 and 28 days old. The deciduous dentition in pigs consists of incisors (I), canines (C) and premolars (P) for a total of 28 teeth (2x I3/3, C1/1, P3/3). This study aimed at checking the timing of deciduous dental eruption in commercial crossbreed piglets in order to find a feasible indicator for estimating piglets' weaning age.

# **Material and Methods**

Dental occlusion, presence/absence and dental eruption of 22 piglets from two sows, housed in a northern Italian farm, were evaluated at 16, 20, 23, 27 and 30 days old. Sex and weight were also recorded. Subsequently, 32 piglets (two per sow) of different ages (17-35d) from another farm were selected to verify the previous results. A linear regression model was performed, considering the individual piglet as analytical unit and combining the observations between right and left hemiarch for each type of tooth.

#### Results

All the 22 piglets enrolled at first showed maxillary P3, C1, I3 and mandibular C1, I1, I3 at 16d. Maxillary I1 was observed in 77.3%, 95.5%, and 100% of the piglets respectively at 16d, 20d, and 23d. Mandibular P4 was observed in 97.7% of the piglets at 16d, and in 100% at 20d. Mandibular P3 was found in 0%, 18.2%, 97.7%, and 100% of the piglets respectively at 16d, 20d, 23d, 27d. No piglets had maxillary P4 at 16d and 20d; instead, it was recorded in 65.9% of the animals at 23d, and in 100% at 27d. These results were confirmed also on the 32 piglets subsequently enrolled: all the animals had the maxillary I1 and mandibular P3 at the age of 21d, and the maxillary P4 at 28d.

#### **Discussion and Conclusion**

Complete eruption of maxillary I1 and mandibular P3 showed a significant difference between 16-20d and 20-23d (P<0.05). Complete eruption of maxillary P4 showed a significant difference between 23d and 27d (P<0.05). Although these results should be confirmed on a larger sample, our study suggests that the observation of dental eruption may be a feasible indicator for assessing piglets' weaning age and thus verifying the respect of the animal welfare law.

WEL - Animal Welfare and Ethology

# SOUND MONITORING TECHNOLOGY ENABLES THE REDUCTION ON TREATMENTS AND IMPROVEMENT OF ENVIRONMENT MANAGEMENT WITHOUT AFFECTING PERFORMANCE IN ONE FINISHER SWINE FARM IN JAPAN

<u>T. Tani</u>, F. Kyutoku <sup>2</sup> <sup>1</sup>Japan Farm Co., Ltd <sup>2</sup>Boehringer Ingelheim Animal Health Japan Co., Ltd.

#### **Background and Objectives**

SoundTalks® is a monitoring device for pig's respiratory health and temperature/humidity that provides an objective soundmetric (Respiratory Health Status, ReHS) translated into LED alarms within the farm for caregivers to early react in the face of respiratory problems. Demonstrated to be the gold standard for respiratory clinic signs monitoring, ReHS's efficiency has never be tested in Japan where traditional farms have a real challenge to manage environment and disease challenges. Therefore, this study aims at evaluating this sound-technology to support caregivers and to measure its impact in production performance under Japanese swine traditional commercial conditions.

# Material and Methods

This study was performed in two 800-finishers, from October16<sup>th</sup>2023 to November19<sup>th</sup>2023, receiving 13<sup>th</sup> week-old pigs from a single sow source following them until slaughter. The treatment unit consisted in one single air space equipped with 4 sound-monitoring devices. The control group was a similar parallel unit not sound-monitored. Weekly production performance (i.e. mortality) as well as health parameters (i.e. ReHS, % animals treated) where monitored and classified by skilled veterinarians and chi-square test used for statistical analysis.

#### Results

Results demonstrated a saving in the number of total treatments applied during the study period in the treatment group when compared with the control group (1% vs 3.4%, respectively; p<0.05%); however, no differences were observed in mortality rates (2.0% vs 1.4%, respectively; p>0.05) (Figure1 and 2). A strong correlation between ReHS and mortality was observed resulting in a K Pearson coefficient regression of R=-0.73, with a coefficient of multiple determination of R<sup>2</sup>=0.55 (P<0.01) (Figure 3).

# **Discussion and Conclusion**

In this study, there was a negative correlation between the weekly ReHS and the mortality registered during the study period. Real-time feedback from the sound-monitoring technology resulted in the application of early treatments and better environment management by caregivers (i.e. ventilation, temp/humidity management) impacting the overall antibiotic usage without affecting production performance. Further studies are needed to replicate this experience at a larger scale, and to further understand and measure how this technology could improve work efficiency of caregivers, reducing the overall treatment, and production costs for Japanese traditional farms.

#### WEL - Animal Welfare and Ethology

# HUSBANDRY PRACTICES IN SUCKLING PIGLETS ON ALTERNATIVE FARMS AND FARMERS' PERCEPTION OF THE PIGS' PAIN

M. Delsart<sup>1</sup>, S.P. Parois<sup>3</sup>, B. Dufour<sup>1</sup>, F. Pol<sup>2</sup>, E. Boudin<sup>3</sup>, V. Dorenlor<sup>3</sup>, F. Eono<sup>3</sup>, E. Eveno<sup>3</sup>, S. Kerphérique<sup>3</sup>, G. Poulain<sup>3</sup>, M. Souquière<sup>3</sup>, <u>N. Rose<sup>3</sup></u>, C. Fablet<sup>3</sup>

<sup>1</sup>Anses, École Nationale Vétérinaire d'Alfort, Laboratoire de Santé Animale USC EPIMAI, 94700 Maisons-Alfort, France <sup>2</sup>ONIRIS, 101 Rte de Gachet, 44300 Nantes, France

<sup>3</sup>Anses Ploufragan-Plouzané-Niort, Unité Épidémiologie, Santé et Bien-Être, 22440 Ploufragan, France

#### **Background and Objectives**

Alternative pig farms, in which animals have outdoor access or are raised in enriched environments, are believed to provide improved animal welfare compared to confined farms. Breeding procedures performed on piglets such as castration, teeth clipping and tail docking may cause pain. The aim of this study was to describe the frequency of these practices in alternative farms and how they were perceived by farmers.

#### **Material and Methods**

The study took place from June 2020 to February 2022 on 92 French alternative pig farms including at least 30 sows. On each farm, it was recorded whether the piglets experienced castration, teeth clipping or tail docking, and any pain management procedures set up. The farmers were also asked about their perception of the animals' pain levels during these procedures.

#### Results

Piglets were castrated in 85/92 farms, with an average age of 6.3 days (minimum = 1, maximum = 28). Teeth clipping (grinding, mean age = 1.3 days) and tail docking (mean age = 5.2 days) were performed respectively in 6 and 13 farms. Regarding pain management around castration, anaesthesia was used in 7% of the farms, analgesia in 60% and cold spray was applied in 33%. Anaesthesia was neither administered during teeth clipping/grinding nor caudectomy. Only three out of the 13 farms used analgesia during tail docking. Cold spray was applied in 15% of the farms for this procedure. Farmers rated castration as the most painful operation for male piglets and considered it to be arduous (mean score=5.9/10). Among the 85 farmers practicing castration, 63 expressed their desire to stop it, with 23% of them preferring to adopt immunocastration, a technique already used on one of the 92 farms.

# **Discussion and Conclusion**

This study highlighted that teeth clipping/grinding and tail docking were not widely practiced on alternative pig farms in France, in accordance with organic specifications, a system mostly represented in the studied sample. However, male castration remained a prevalent practice that farmers wish to cease, primarily for welfare concerns and because of the tediousness of the procedure.

#### WEL - Animal Welfare and Ethology

# PAINLESS METHOD FOR INDIVIDUAL AND PERMANENT IDENTIFICATION OF PIGS

K. Gerdes 1, T. Handt 1, I. Hennig-Pauka 1, N. Kemper 2, M. Wendt 3, E. Grosse Beilage 1

<sup>1</sup>Field Station for Epidemiology, Bakum, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany <sup>2</sup>Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour (ITTN), University of Veterinary Medicine

Hannover, Foundation, Germany

<sup>3</sup>Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

#### **Background and Objectives**

Scientific investigations often require a permanent and individual identification of pigs. The conventional practice of ear tagging or ear notching is known to result in pain and stress. Within the framework of a project designed for the individualized longitudinal observation of animals, a technique was sought to effectuate a permanent and individual marking in a manner characterized by simplicity and painlessness.

### **Material and Methods**

Commercially available washers designed for ear tags were prepared, by a precision cutting to facilitate easy placement over the pre-existing ear tags. Ten distinctively coloured washers were used by linking each colour to a numerical value. In cases involving two-digit numbers, two washers were stacked upon each other. Approximately one-quarter of the upper washer was excised to enable the facile identification of the colour of the underlying washer. The truncated upper washer represented the first digit, while the intact washer below corresponded to the second digit (e.g., violet [top] and blue [bottom] resulted in the number 12). This methodology underwent exclusive testing on individual piglets, weaners, and fattening pigs within pens comprising 12 to 120 animals. The testing period ranged from 10 to 90 days.

#### Results

The attachment of washers onto pre-existing ear tags emerged as a painless, expeditious, and uncomplicated procedure. Some animals exhibited a transient defensive reaction during the washer insertion process, which typically lasted between 10 to 30 seconds. The pre-established colour code facilitated animal identification without the necessity of entering the pen. The loss of one or both washers occurred in 7.5% of the pigs.

#### **Discussion and Conclusion**

This method allows a painless and non-invasive individual marking of animals. The transient stress induced during the washer insertion process can be attributed to the unfamiliarity of pigs with human handling. Post-insertion, the animals exhibited no restrictions and endured no further stress. While some animals experienced washer loss, similar occurrences are also observed with traditional ear tags.

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WEL - Animal Welfare and Ethology

# SCREENING OF NOVEL PRECISION BIOTICS TO MODULATE THE MICROBIOME-GUT-BRAIN AXIS TO ADDRESS STRESS AND ANIMAL WELFARE

W. Ren <sup>1</sup>, J. Schmeisser <sup>2</sup>, E. Canet Martinez <sup>3</sup>, R. Aureli <sup>2</sup>, C. Sester <sup>2</sup>, <u>E. Perez-Calvo <sup>3</sup></u> <sup>1</sup>dsm-firmenich Animal Nutrition Research Center, Bazhou, China <sup>2</sup>dsm-firmenich Animal Nutrition Research Center, Village-Neuf, France <sup>3</sup>dsm-firmenich, Kaiseraugst, Switzerland

#### **Background and Objectives**

Stress-driven stereotypic abnormal behavior remains an economically relevant problem for the swine industry. One such solution would be to leverage the existing microbiome-gut-brain interaction as it is a major driver of the host behavior. Precision biotics (PB) are nutritional products that modulate specific microbiome metabolic pathways and the synthesis of metabolites (such as neurotransmitters), they have the potential to act as mediators of neuroendocrine function and animal behavior. The objective of this study was to select 3 PB leading to up- and down-regulation of neurotransmitter production in an ex-vivo fermentation model and then to investigate the translation of effect in growing pigs.

# Material and Methods

The ex-vivo screening featured four treatments with PB1, PB2, PB3 plus a control (glucose). After 24h of fermentation, supernatants were collected to analyze the concentration of gamma-aminobutyric acid (GABA). GABA fold change (FC) of PB/control was calculated to identified high and low responders. In the 14 days study, 48 grower pigs (Large-White x Redon, 27.90±2.15 kg BW) were housed in 12 pens. Each pen received either the control feed (NC) or one of the three PB candidates. Blood samples were collected, GABA, neuropeptide Y (NPY) and cortisol concentrations were measured.

#### Results

In the ex-vivo study PB2 was identified as a high responder with low variation (GABA FC=2.07, CV=18.44%), whereas PB1 was identified as a high responder with high variation (GABA FC=1.83, CV=39.58%). PB3 was considered a low responder (GABA FC=0.75). Similar ranking was obtained in the in-vivo study, as PB2 demonstrated significantly higher GABA plasmatic concentration compared to NC (P<0.05). Furthermore, PB2 showed significantly lower cortisol and higher NPY plasmatic concentrationcompared to NC (P<0.05).

# **Discussion and Conclusion**

PB2 with the characteristic of low variation showed consistent effects in both ex-vivo and in vivo trials. The effect of PB1 in ex-vivo could not translate into in-vivo due to its characteristic of high variation. The consistency in selecting PB2 as the best candidate between ex-vivo and in vivo models validates our screening approach, this new screening system selected PB2 with the ability to up regulate the production of neurotransmitters. This gives us now the influence ability to cope with stress and drive swine behavior.

#### WEL - Animal Welfare and Ethology

# SATISFYING THE ANIMAL WELFARE PRINCIPLES IN PIG HUSBANDRY AFFECTS MEAT QUALITY INDICATORS AFTER SLAUGHTER BY SCALE

N.B. Duy <sup>2</sup>, N.T. Hai <sup>1</sup>, N.K. Cuong <sup>2</sup>, D.N. Khang <sup>2</sup>, D.T.N. Anh <sup>2</sup>, <u>T.D. Do <sup>3</sup></u> <sup>1</sup>Nong Lam University, Ho Chi Minh City, Vietnam <sup>2</sup>Faculty of Animal Science & Veterinary Medicine, Nong Lam University, Ho Chi Minh city, Vietnam <sup>3</sup>Faculty of Animal Science and Veterinary Medicine, Nong Lam University, HCMC, Vietnam

#### **Background and Objectives**

Although issues of animal welfare and farming scale play an important role in pig industry from productivity and meat quality to economic efficiency, the attention and application of animal welfare in pig-farming practice seem to be still restricted as the scale transformation from households to large-scale farms.

Aims of the current investigation was to assess levels associated with animal welfare and meat quality parameters after slaughter by scale.

# Material and Methods

Firstly, data associated with animal welfare at 45 farms under three different scales, including small-scale (SF, 10-30 units), medium-scale (MF, 30-300 units) and large-scale farming (LF, >300 units), were collected by completing the questionnaire (04 principles and 12 individual criteria of animal welfare assessment) through directly observing and contacting pigs at farms. Furthermore, a total of 18 pigs were randomly chosen from these three scales for meat quality assessment after slaughter (pH, color, water-holding capacity, shear force and fatty acid profile).

# Results

Results showed that the principles of animal welfare were significantly different among three scales (P < 0.01). Score of the Good Feeding principle was higher in LF (80.28) than that in MF (67.89) or SF (59.95) (P < 0.01) while Good Health principle score was higher in SF (7.80) than that in LF (7.74) or MF (7.51) (P < 0.01). The Good Health principle was very low and below acceptable levels probably caused by the pain in management procedures (castration, teeth clipping and tail docking), whereas the other of animal welfare principles were at the acceptable range from 40 to 60. Furthermore, the percentage of crude protein in meat was highest in LF as compared with MF and SF (P < 0.01), while the ultimate pH at 45 minutes after slaughter (SF, MF, LF at 6.54, 6.55, 6.65, respectively), meat color, water-holding capacity, shear force and fatty acid profile were not significantly different among three scales (P > 0.05). There seemed to be an improvement in fatty acid composition and color meat in pigs raised on large-scale farms.

# **Discussion and Conclusion**

Briefly, some indicators of animal welfare and meat quality seem to be significantly better in large-scale farms in Vietnam's pig production.

WEL - Animal Welfare and Ethology

# HOW TO BALANCE SELECTION FOR LITTER SIZE IN PIGS WITH SURVIVAL, HEALTH AND WELFARE

P. Knap <sup>1</sup>, A. Huisman <sup>2</sup>, C. Sørensen <sup>3</sup>, <u>E. Knol <sup>4</sup></u> <sup>1</sup>Genus-PIC, Hannover, Germany <sup>2</sup>Hendrix Genetics, Boxmeer, Netherlands <sup>3</sup>Danish Agriculture & Food Council. Aarhus, Denmark

<sup>4</sup>Topigs Norsvin, Helvoirt, Netherlands

#### **Background and Objectives**

In EU, future housing conditions may involve free farrowing. Another worldwide development is increasing shortage of labour, especially on farms. Both require increased selfsufficiency of sows and piglets. Breeding goals that emphasize production efficiency give an increase in litter size with unfavourable correlations to piglet survival, via changes in birthweight and physiological maturity, gestation length, and farrowing duration. Such antagonisms can be neutralized by balanced selection based on proper understanding of underlying biology; this gives simultaneous favourable trends in litter size and survival. This involves recording of piglet survival (at farrowing and during lactation), birth weight and its within-litter variation, teat number, weaning weight, gestation length, farrowing duration (relevant for colostrum intake), and/or maternal behaviour and mothering ability.

# **Material and Methods**

We present realized phenotypic and genetic trends (2001-2022) of reproduction traits and how they relate to each other, past and future breeding goals, and developments in data recording, of four leading breeding organizations. The data includes phenotypes of litter size, birthweight and mortality, as used in routine breeding value estimation; and the estimated breeding values (EBVs). Mortality and birthweight (2009–2022) phenotypes were related to litter size by year. EBVs for mortality traits were regressed on those for litter size by birthyear (2012–2022).

#### Results

Average litter size is weakly correlated to mortality (R2≤0.06) and birthweight (0.07≤R2≤0.26); those correlations are unfavourable within each year. However, all traits show favourable simultaneous time trends: the antagonisms are neutralized. Above the annual mean litter size, piglet mortality increased with increasing litter size in every year (unfavourable), but the annual intercepts and the slopes decreased (favourable). Average litter birthweight decreased with litter size in every year (unfavourable), but the annual intercepts increased and the slopes decreased (favourable). Within-litter birthweight

variation increased with litter size in every year (unfavourable), but the annual intercepts decreased (favourable). The proportion of <0.9kg birthweights for a given litter size decreases over time; the critical birthweight level (below which mortality increases) is population dependent and changes over time.

#### **Discussion and Conclusion**

Selection for litter size can be balanced with survival, health, and welfare. This improves both animal welfare and efficiency within the pork value chain.

WEL - Animal Welfare and Ethology

# INFLUENCE OF DIFFERENT GESTATION HOUSING SYSTEMS ON SOW MORTALITY DURING FIRST WEEKS OF PREGNANCY

P. Cybulski<sup>1</sup>, R. Kondratiuk<sup>1</sup> Goodvalley Agro S.A., Przechlewo, Poland

#### **Background and Objectives**

According to the Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs, housing sows in stalls from 4 weeks after the service to one week before the expected time of farrowing is prohibited. Having implemented group housing during the entire gestation period, some of farm owners provided pregnant sows with significantly higher welfare standards. The aim of this paper was to evaluate the impact of two different gestation housing systems on sow mortality during first 7 weeks of pregnancy.

# **Material and Methods**

The study was performed in 2022 (January-December) in 2 high-performing PRRSV- and APP-negative sow farms located in Poland. Animals in Farm A (4000 sows) were reared following 2008/120/EC standards. In Farm B (5000 sows) group housing during the entire gestation period was implemented. The animals were offered the same feed and medication strategy. Data regarding the number of dead sows were tracked and analysed, taking into account the period of 7 weeks (W) since the service. The mortality was expressed as a ratio of the number of dead sows to the average number of sows in the herd. Statistical evaluation was completed using Chi-Square test. The results at p<0.05 were considered significant.

#### Results

The total number of 66 and 47 deaths was analysed in Farm A and Farm B, respectively. Results obtained in the investigation indicated the following mortality pattern: W1: 0 % W2: 0.08 %, W3: 0.13 %. W4: 0.28 %, W5: 0.75 %, W6: 0.25 %, W7: 0.18 % in farm A, and W1: 0.02 %, W2: 0.06 %, W3: 0.12 %. W4: 0.28 %, W5: 0.16 %, W6: 0.10 %, W7: 0.20 % in farm B. A statistically significant difference (p=0.00002) between mortality rates in W5 was observed.

#### **Discussion and Conclusion**

Group housing of sows during the entire gestation period can have a positive effect on reduction of mortality in first weeks of pregnancy; nevertheless, ethological and immunological criteria allowing thorough evaluation of the effect of improved welfare standards in sows should be established.

WEL - Animal Welfare and Ethology

# STEREOTYPICAL BEHAVIORS CAN BE REDUCED WITH FIBER SUPPLEMENTATION IN THE BOAR DIET

V. S. Sobral 1, J. Rego Ribas 2, A. Pimenta Siqueira 1, W. Ribeiro 1, B. Quintiliano 2, S. Bunzen 3

<sup>1</sup>Agroceres PIC <sup>2</sup>Unesp Jaboticabal <sup>3</sup>FEEDIS, Salto, SP

# **Background and Objectives**

Boars presents particular behavior needs than other pigs categories and are subjected to different levels of stress during the production routine, such as longs periods of fasting due to feeding management. Few studies consider the effect of synthetic fiber to male diets in order to improve animal welfare and semen production. This study aimed to evaluate the influence of supplementation of partially fermentable eubiotic fiber on the behavior and productive performance of boars

# **Material and Methods**

sixty boars of three different genetic lines (16 Landrace (LD), 14 Large White (LW) e 30 synthetic Pietrain(SP)) were subjected to two groups of feeding: (1) FIB, feeding with 35g/animal/day eubiotic partially fermented fiber addition and (2) CON, control, feeding with no fiber addition, during three months. Behavior, health and seminal parameters were evaluated during the experiment.

#### Results

Despite males from LD showed lower significant stereotypical behavior incidence over time (19% FIB vs. 34% CON) compared to LW (25% FIB vs. 23% CON) and SP (11% FIB vs. 18% CON), fiber supplementation did not show behavioral differences (p>0,05) between FIB and CON. The number of possible doses of semen showed a significant increase in FIB group at the third month of data collection (32.8±1.33 vs. 35.4±1.48 and 35.0±1.29), whilst total sperm count and ejaculation volume decreased during the second and last month of evaluations, respectively (78.2±3.54 million vs. 89.9±3.21 and 84.4±3.29 million and 210±8.33mL vs. 219±9.02 and 210±8.33mL). Other seminal and health parameters were neither affected by the eubiotic fiber supplementation (p>0,05).

### **Discussion and Conclusion**

Those results show that dietary fiber supplementation reduce stereotypical behaviors, especially in LD boars, through extension of satiety, which corroborates data observed in gestating sows fiber supplementation studies. However, more research should be done in order to investigate the impact in boars welfare, health and seminal parameters, since in this study weren't found strong correlations. It can be concluded that partially fermentable eubiotic fiber may be an important resource to animal welfare improvement but not affect health and seminal parameters of boars.

WEL - Animal Welfare and Ethology

# THE WELFARE OF PIGS IN DIFFERENT REARING SYSTEMS: THE ROLE OF ENVIRONMENT ON PIG WELFARE

<u>E. Nadlucnik</u><sup>1</sup>, I. Golinar Oven <sup>1</sup>, J. Plut <sup>1</sup>, T. Steferl <sup>1</sup>, M. Stukelj <sup>1</sup> <sup>1</sup>Clinic for Reproduction and Large Animals, Clinic for Ruminants and Pigs, Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia

### **Background and Objectives**

Pigs are highly motivated to exhibit natural behaviours such as rooting, exploring environment and nest building. Environment in intensive indoor pig farming is generally poor, pigs may develop stereotypic and other adverse behavioural patterns, which indicate poor welfare. The aim of this study is to investigate whether by using enrichment materials and increasing floor space, we can achieve the welfare of pigs kept indoors comparable to the pigs, reared outdoors.

#### **Material and Methods**

The study was conducted on an indigenous Slovenian breed: the Krškopolje pig. Sixty adult pigs were used in the study (N = 60), divided into three groups of twenty pigs. The first group (A) was bred indoors, pigs were kept in a large pen (100 m<sup>2</sup>) with straw bedding. The stocking density was 5 m2 per pig and thus exceeded the European standards of at least 1.00 m2 per pig weighing more than 110 kg (2001/88/EC). The second (B) and third (C) groups were reared in outdoor systems, led by different farmers. We examined all groups over the four seasons. Using a survey, we assessed various aspects of animal welfare: (1) animal behaviour, (2) health status, (3) living conditions and, for the indoor group, (4) environmental conditions.

#### Results

The preliminary results show that the welfare of the pigs was high in all groups. The aspects (2) health status and (3) living conditions of the group A were similar to the group B and higher than group C, as the farmer used different farming equipment – e.g., water was provided in large water tanks, shelter had no bedding. Surprisingly, the biggest anomaly was found in the outdoor pigs, as they all got severe sunburns during the summer months, despite having the possibility to retreat from sunlight.

#### **Discussion and Conclusion**

Our study demonstrates that by using straw and increasing floor space, we can improve the welfare of pigs to the extent that it is comparable to the welfare of pigs in outdoor breeding systems and may even exceed it.

WEL - Animal Welfare and Ethology

# INFLUENCE OF AGE, SEX, VENTILATION SYSTEMS, AND NOISE ON AGGRESSION IN PIGLETS.

D. Yanguas <sup>1</sup>, V. Rodriguez-Vega <sup>2</sup>, D.A. Arroyave <sup>2</sup> <sup>1</sup>AGROCESA S.A.U. <sup>2</sup>Boehringer Ingelheim Animal Health España, S.A.U.

#### **Background and Objectives**

Tail and ear biting of pigs has both a negative impact on production and animal welfare. The aggressive behaviour has a multifactorial origin linked to stressors such as poor ventilation, high housing density, excess of lighting and poor water quality or supply. Aggressive behaviour has been associated to genetics, nutritional deficiencies in the diet and parasites. The objective of this study was to analyse the correlation of noise, age, sex and ventilation systems with the cannibalism behaviour.

# **Material and Methods**

The study was performed in a Spanish 2.200 multi-site sow farm. Two nursery rooms with different ventilation systems and 3830 piglets each, 50% males, and 50% females, from 6 different batches were analysed from June 2022 to March 2023. The correlation between the percentage of pens with skin lesions and gender, ventilation system, age (measured in weeks post-weaning (wpw)), and noise inside the rooms (measured in percent of time (%t) above 70 decibels (dB), 80dB, 90dB, 100dB, 2 standard deviations (2SD), average and maximum noise). To measure the noise, PCE-SLD 10® devices were used. Values were compared between groups by Mann-Whitney U test and ANOVA.

#### Results

Average noise was 70,10dB and maximum noise 95,92dB. No differences or correlation between noise and tail biting were found: 50,17% above 70dB 3,22% above 80dB 0,14% above 90dB and 0% above 100dB (p>0,05). Differences were found between the percentage of pens with bite marks and age, 0% at weaning and 1 wpw; 3,1% 2 wpw; 17,18% 3 wpw; 28,1% 4 wpw; 27,5% 5 wpw and 20,3% 6 wpw (p<0,001) No correlation was found between the percentage of tail biting and gender, 12,00% in males vs. 13,97% in females, or between tail biting and the ventilation system, e. g. room 7 11,63%, room 9 14,24%.

# **Discussion and Conclusion**

Under the conditions of this study, the intensity of noise in the week prior to the occurrence of biting incidents does not affect their onset. Neither do other factors, such as gender or different ventilation systems, have an influence. However, the age of the animals does influence cannibalism, possibly due to a greater need for space among larger animals compared to smaller ones.

# PRECISION LIVESTOCK FARMING

# PLF-PP-01

#### PLF - Precision Livestock Farming

### AUTOMATIC INFRARED THERMOGRAPHY IN WEANER PIGS

<u>J. Probst</u><sup>1</sup>, P. Heseker<sup>1</sup>, G.-.F. Thimm<sup>2</sup>, U. Hartmann<sup>3</sup>, N. Kemper<sup>1</sup> <sup>1</sup>Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour (ITTN), University of Veterinary Medicine Hannover, Foundation, Germany <sup>2</sup>Thünen Institute of Agricultural Technology, Bundesallee 47, 38116 Braunschweig, Germany

<sup>3</sup>Chamber of Agriculture Lower Saxony, Herrmann-Ehlers-Straße 15, 26160 Bad Zwischenahn, Germany

#### **Background and Objectives**

Recording the individual body temperature is valuable for detecting disease events in pigs. However, rectal temperature (RT) measurements are stressful for the animals and time-consuming for the farm staff. The study investigated whether non-contact infrared (IR) thermography is suitable to detect febrile pigs automatically.

#### **Material and Methods**

Six batches of weaner pigs (n=1,002) housed in one compartment with six pens were included in this study. For individual identification, each pig received an RFID-ear tag. Modified drinking stations with RFID-antennas and IR-cameras (thermoIMAGER TIM 41, Micro-Epsilon Messtechnik GmbH & Co.KG) automatically assigned the RFID to the measured hotspot in the IR-video. The localisation was confirmed manually (n=4,232). In addition, RT were taken weekly from half of the pigs and compared with the maximum (max\_IR) and mean (mean\_IR) IR-temperatures recorded in a four-hour timeframe around the RT measurement (n=2,634). Implausible IR-data were excluded and a subgroup of RT >39.5°C (n=1,258) was established. A pearson correlation (r) between RT and IR was calculated and the mean difference (md) with standard deviation (sd), minimum (min) and maximum (max) determined.

# Results

IR-measurements were mainly located at the base of the ear (90%). A low correlation (p<0,001) between max\_IR and RT (r=0.268, md=0.482, sd=0.634, min=-2.4°C, max=4.1°C) and mean\_IR and RT (r=0.246, md=0.954, sd=0.602, min=-2.4°C, max=4.1°C) was detected. Correlation was even lower (p<0,001) for comparing RT >39.5°C with max\_IR (r=0.192, md=0.591, sd=0,610, min=-1.8 °C, max=4.1°C) and mean\_IR (r=0.205, md=1.097, sd=0.580, min=-1°C, max=4.1°C).

#### **Discussion and Conclusion**

No direct conclusion could be drawn from the IR-temperature to the rectal temperature with the selected equipment. Next, air temperature and humidity will be included in the plausibility analysis to explore the potential of automatically generated IR-data to improve pig health and to detect heat stress.

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#### PLF-PP-02

PLF - Precision Livestock Farming

THE NEXT GENERATION OF USER-FRIENDLY OPERATIONAL TOOLS FOR SURVEILLANCE AND MANAGEMENT OF SWINE INFECTIOUS DISEASES AND ANTIMICROBIAL RESISTANCE INTEGRATES BIOINFORMATICS, EPIDEMIOLOGY, AND ARTIFICIAL INTELLIGENCE

<u>R. Rupasinghe 1</u>, M.J. Clavijo 2, R. Robbins 3, R. Mugabi 2, P. Harms 3, J.P. Gómez-Vázquez 1, C. Gonzalez-Crespo 1, X. Liu 4, B. Martínez-López 1

<sup>1</sup>Center for Animal Disease Modeling and Surveillance (CADMS), Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, CA, United States

<sup>2</sup>Department of Veterinary Diagnostic & Production Animal Medicine (VDPAM), Iowa State University, Ames, IA, United States

<sup>3</sup>PIC North America, Hendersonville, TN, United States

<sup>4</sup>Department of Computer Science, University of California, Davis, Davis, CA, United States

### **Background and Objectives**

Swine production significantly contributes to global food security, job creation, and the economy. Infectious diseases and bacterial antimicrobial resistance (AMR) are among the main constraints to the productivity of the swine industry and pig health. Therefore, the development and use of intelligent real-time surveillance and decision support systems are key to better preventing and controlling these swine health problems. Here, we present new tools within our Disease Bioportal platform to gain insights into the spatio-temporal epidemiology and evolution of swine infectious diseases and AMR in near real-time by integrating various state-of-the-art methodologies.

#### Material and Methods

We integrated a new set of operational tools of bioinformatics, spatial-temporal analyses, and artificial intelligence (AI) into Disease BioPortal (https://bioportal.ucdavis.edu), which allows rapid processing and analysis of complex data and easy visualization and interpretation of results. Production systems, veterinary clinics, and diagnostic laboratories can easily add proprietary data, such as disease status, production data, animal movement, biosecurity, AMR, etc., using APIs or manual uploads. Disease/AMR prevalence is evaluated at multiple spatial and temporal scales by space-time analyses (e.g., time-series analysis, scan statistics, phylogeography). Genetic diversity and the evolutionary relationship of pathogens are estimated using maximum likelihood-based phylogenetics or core-genome phylogenomics. AMR patterns are assessed using phenotypic (e.g., Minimum inhibitory concentrations) and genotypic (e.g., whole genome sequences) data. The presumed resistance determinants (e.g., AMR genes) are determined using CARD and ResFinder databases.

#### Results

We demonstrate the value of this tool using de-identified datasets of Streptococcus suis and Porcine Reproductive and Respiratory Syndrome (PRRS) from the United States to understand the spatio-temporal trends of these pathogens and their heterogeneity and resistance to antibiotics, which will be beneficial to develop effective preventive and control strategies. Genomic surveillance of S. suis and PRRS offers insights into the pathogen evolution, population dynamics, and resistance mechanisms at different spatial and temporal scales.

#### **Discussion and Conclusion**

This Al-assisted tool allows users to generate interactive dashboards and facilitates the visualization, analysis, and interpretation of disease/AMR patterns of various swine pathogens promptly. It better informs users of prevention and control strategies appropriate for the target condition (e.g., selecting correct therapeutics/vaccines for circulating variants) and contributes to antimicrobial stewardship.

# PLF-PP-03

PLF - Precision Livestock Farming

# CORRELATION OF RESPIRATORY CLINICAL SIGNS AND MYCOPLASMA HYOPNEUMONIAE DISEASE DETECTION IN FINISHING PIGS UNDER FIELD CONDITIONS

E. De Conti<sup>1</sup>, R. Tesche Lippke<sup>1</sup>, <u>C. Alonso<sup>2</sup></u> <sup>1</sup>Boehringer Ingelheim Animal Health Brazil <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH

#### **Background and Objectives**

Respiratory diseases are a major concern in swine production. Digital technologies, such as those based on sound and artificial intelligence (SoundTalks®, Respiratory Health Status metric ReHS), are now available to support the industry in this aspect. Therefore, this study aimed to investigate the correlation between ReHS and Mhp disease prevalence.

#### **Material and Methods**

Two PRRS negative finishing groups (n= 1000 pigs, 44 pigs x 23 pens barn1 (flow A); n= 1350 pigs 67 pigs x 20 pens barn2 (flow B)) were sound monitored (6 and 7 monitors covering a 20 m radius in barn1 and barn2, respectively). Spatial random diagnostics were taken at 0-60-90 days post-finishing placement. They included six (barn 1) or seven (barn 2) oral fluids (OF, always in the same pens, even distributed along the barn) and 23 (barn 1) or 20 (barn 2) deep tracheal swabs in each time point. For the deep tracheal swabs, one pig was ear-tagged in each pen and sampled longitudinally. The samples were tested by qPCR for Mhp and IAV, and the results were consolidated for analysis.

#### Results

Despite starting with a similar Mph prevalence of positive deep tracheal swabs (8.7% and 10% for flow A and B, respectively, p value>0.05), pigs from flow A had significantly lower prevalence at 60- and 90-days post-placement compared to flow B (17% and 69.6% vs. 100% and 100%, respectively, p value<0.05). Similar results were observed for Mph OF with a significantly lower detection of positives in flow A (0%, 33.3% flow A vs. 42,8%, 57,1% flow B, days 60 and 90, respectively). This correlated with a lower percentage of days in alarm (ReHS) also in flow A compared to flow B (35.64% vs. 76.59%, respectively). All IAV OFs were negative (except day 0 of flow A, with 2/6 positives OF).

### **Discussion and Conclusion**

The data shows how the sound-monitoring metric ReHS correlates with the detection and disease prevalence of Mph under field conditions. Further studies are needed to investigate such correlation for other major pathogens in respiratory disease under similar conditions.
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### DATA ANALYSIS COMPARING THE USE OF SOUND-BASED MONITORING OF RESPIRATORY HEALTH STATUS AND SEASONAL MORTALITY TO REDUCE ANTIBIOTIC USE IN NURSERY PIGLETS.

F. Erusalimsky <sup>1</sup>, E. Vranken <sup>1</sup>, W. Buyens <sup>1</sup>, D. Berckmans <sup>1</sup>, A. Jorge Peñas <sup>1</sup>, T. Guiu <sup>2</sup>, S. Figueras <sup>3</sup>, <u>C. Alonso <sup>4</sup></u> <sup>1</sup>SoundTalks nv, Leuven, Belgium

<sup>2</sup>Piensos Costa S.A, Fraga, Huesca, Spain
<sup>3</sup>Boehringer Ingelheim Animal Health España
<sup>4</sup>Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany

#### **Background and Objectives**

As antimicrobial resistance has become a worldwide problem, threatening both livestock and public health, measures and actions must be taken to reduce the use of antibiotics. Digital monitoring solutions, such as those based on sound, are being implemented and their efficacy well demonstrated, guiding producers to early intervene in the face of respiratory outbreaks (i.e. targeting and timing diagnostics and interventions). The objective of this study was the evaluation of the respiratory health status of pigs by using sound analysis in order to optimize farm diagnostics and antibiotic treatment implementation strategy in growing pigs.

### **Material and Methods**

The study was conducted in a large nursery site in Spain with 32 rooms continuously sound-monitored by SoundTalks® over a 1-year-period (191 batches). Pigs received 7 days of prophylactic water medication (Amoxicillin product) after weaning and Tilmicosin in feed from week 3 for 4 weeks, following previous diagnostics and vet advise. Respiratory health data (ReHS metric: 0-100, being 100 the healthiest value), mortality, and farm antibiotic usage were consolidated at a batch level and further processed and analyzed.

#### Results

Batch respiratory health data demonstrated that summer and autumn were the healthiest respiratory seasons in contrast with spring and winter. Average batch mortality was negatively correlated with ReHS and significantly influenced by season, being average mortality 6.6% (SD±4.4) in winter and spring (lowest ReHS values) and 2% (SD±2.9) in summer and autumn (p value < 0.01). No differences were observed on antibiotic usage.

### **Discussion and Conclusion**

Results from this study support the usage of precision-sound-monitoring technologies could help producers reducing antibiotic consumption by eliminating continuous prophylactic protocols by targeting actions at a pen level. Calculating the potential yearly savings in antibiotics by stopping the systematic respiratory feed treatments (i.e. Tilmicosin, 11 cents/pig/treatment) during healthy seasons (summer and autumn) was calculated to be  $\epsilon$ 7,425 for a farm that produces 135,000 piglets/year.

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### THE USE OF AUTOMATED REAL TIME RESPIRATORY HEALTH MONITORING IN SURVEILLANCE OF PRRSV IN A NURSERY AFTER ELIMINATION OF PRRSV IN THE SOW HERD

L.K. Tolstrup<sup>1</sup>, L.M. Jensen<sup>2</sup>, P.H. Rathkjen<sup>3</sup> <sup>1</sup>Boehringer Ingelheim <sup>2</sup>PORCUS Oerbaekvej 276, DK-5220 Odense <sup>3</sup>Boehringer Ingelheim Animal Health Denmark

#### **Background and Objectives**

The objective was to evaluate a sound-monitoring technology (SoundTalks®, Respiratory Health Status, ReHS 0-100) as a surveillance tool after PRRSV elimination.

#### **Material and Methods**

A Danish sound-monitored nursery site, receiving pigs from a PRRSV eliminated PCR-negative SPF sow herd (Myc, APP negative), collected weekly oral fluid (OF) samples from ten batches of pigs during a ten-month period, testing for PRRSV and IAV by qPCR. In each room, all pens were sampled splitting one rope/two pens. Ropes were pooled into two PCRs/room. ReHS was measured as weekly average ReHS/week/group (ReHSav) as well as the difference between ReHS in one week and the week before (ReHSdiff). A respiratory alarm occurred if ReHS<60. The diagnostic value (Cohen's kappa) of an alarm for detecting PRRS with OF as reference standard was evaluated. Furthermore, logistic regression was conducted for ReHSav and ReHSdiff with OF as the dichotomous statistical outcome (PRRS neg/pos) and batch as covariate. Room was considered study unit. OFs were considered positive if either of the two OF pools for each room were positive on PCR.

#### Results

Of 72 weekly observations, 7 and 25 were positive for IAV and PRRS respectively, 22 cases held only results for PRRSV. Cohen's kappa for at least one alarm in a batch compared with neg/pos OF sample was 0.1 [-0.12; 0.32]. In the logistic regression models, batch was non-significant hence excluded. For batches with negative and positive OF samples ReHSav were 89.02 (SD=13.41) and 78.89 (SD=20.08) respectively with OR = 0.96 [0.93;0.99] and for ReHSdiff the results were - 0.54 (SD=14.15) and -9.47 (SD=20.03) respectively with OR = 0.97 [0.94;1.00].

### **Discussion and Conclusion**

These results show the potential for the ReHS as a PRRS surveillance metric in a negative or low-prevalent herd. More studies are needed to investigate the specific use of ReHS in relation to PRRS real-time detection. Real-time surveillance can reduce disease impact and thereby potentially lower antibiotic use. ReHS values and PCR testing should be combined at relevant timepoints and adapted to individual herds.

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### COMPARISON OF MIXABLE PORCINE CIRCOVIRUS 2 + MYCOPLASMA HYOPNEUMONIAE WITH A READY-TO-USE VACCINE ADMINISTERED USING 2 DIFFERENT NEEDLE-FREE VACCINATORS IN A MULTIPLIER FARM IN THE PHILIPPINES

L. Romabon<sup>1</sup>, A.I. Bulay<sup>1</sup>, J. Elemia<sup>1</sup>, C. Maala<sup>2</sup>, M. Genzow<sup>3</sup> <sup>1</sup>Boehringer Ingelheim Animal Health Philippines Incorporated <sup>2</sup>Strategic Business Unit Swine, Boehringer Ingelheim Animal Health, <sup>3</sup>Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany

### **Background and Objectives**

Several studies have shown the advantage of a Mixable PCV2 and Mycoplasma hyopneumoniae vaccine against a Readyto-Use vaccine. Some studies show the advantage of an adjustable 1-2 ml intramuscular Needle Free Device NFD) against an intradermal NFD. The objective of this trial was to compare two needle free devices (NFD) for proper administration of two approved PCV2 and Mhp vaccines with regard to early harvest of Breeders (%) as well as heavier Market Pigs.

#### **Material and Methods**

This study was done in a multiplier farm located in Central Island of the Philippines. Nine hundred forty nice (949) pigs 3-4 weeks of age were vaccinated with either a Mixable PCV2 and Mycoplasma hyopneumoniae vaccine using an intramuscular NFD (Group A=475) while another set of pigs were vaccinated with a Ready-to-Use vaccine given via an intradermal NFD Group B= 474). The Breeders were selected at 13, 18 and 23 weeks of age. Breeder selection criteria include good body conformation, at least 500g/day ADG, and lack of visible physical deformity. The performance of pigs of both groups were compared up to market age using average values as well as variation using Distribution Graph (Minitab 19). Market weight and ADG were analyzed using T-test

#### Results

In terms of % Selection for Breeders 30% were selected as Breeder in Group A, while only 14% for Group B at 13, 18 & 23 weeks. Conversely, 70% were selected as Fatteners for Group A, while 86% for Group B at 23 weeks

### **Discussion and Conclusion**

While there are multiple traits that determine Breeder selection, it was observed in this study that more animals were selected in the Mixable PCV2 and Mycoplasma hyopneumoniea vaccine using an intramuscular NFD compared to the other group. Further studies are needed to confirm this causation.

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### ENHANCING PIG FARM MANAGEMENT AND RESPIRATORY HEALTH MONITORING WITH SOUND-BASED TECHNOLOGY: AN ACTINOBACILLUS PLEURONEUMANIAE CASE STUDY IN THAILAND

N. Young <sup>1</sup>, <u>C. Alonso</u> <sup>2</sup>, S. Jamawat <sup>3</sup>, S. Samanrak <sup>4</sup>, K. Charatpaew <sup>4</sup> <sup>1</sup>Boehringer Ingelheim Animal Health (Thailand) <sup>2</sup>Boehringer Ingelheim Vetmedica GmbH, Germany <sup>3</sup>Boehringer Ingelheim Animal Health Singapore Pte Ltd <sup>4</sup>Mittraphap Phokaphan

### **Background and Objectives**

Pig farm management is crucial for maintaining the health and productivity of the animals. Respiratory health monitoring is key in daily management when dealing with acute endemic diseases (i.e. APP) and can be very challenging due to lack of trained personnel and subjectiveness in its assessment. SoundTalks<sup>®</sup> aims to improve pig farm management by providing objective sound-surveillance using a respiratory health metric (Respiratory Health Status, ReHS, 0-100 scale) as well as environment-surveillance (temp/humidity) for farmers to early intervene. This study investigates the effectiveness and cost benefits of such a sound-technology to improve pig farm management in Thailand.

### Material and Methods

The study was conducted in 8 wean-to-finish facilities in central-Thailand filled with pigs from same sow source (PRRS, PCV2, M. hyo, and APP positive status, 300-400 pigs/barn). Barns 1-4 (Grupo1) were sound monitored (2monitors/barn) and actions were driven by ReHS-alarms. Barns 5-8 (Group 2) were not sound monitored, and pig-management and respiratory assessment was traditional as usual. Batch production parameters (i.e. average daily gain (ADG), mortality, and culling rate) were consolidated and analyzed at a batch level between groups.

#### Results

Results demonstrated production performance advantages in Group 1 compared to Group 2 regarding ADG (656.9 g/day vs 651.2 g/day, p = 0.85), mortality rate (5.27% vs 7,16%, p=0.006) and culling rate (3.65% vs 5.09%, p = 0.046). A costbenefit ratio for this technology of 6.14:1 was obtained considering the improvement in mortality and culling rates.

#### **Discussion and Conclusion**

Objective respiratory alarms based on sound-sensor technology guides farmers to early intervene in farm areas with respiratory issues (e.g., adjust ventilation and humidity, targeted zone-diagnostics, targeted individual treatment) recovering the pigs sooner. Particulary in this case, this early intervention allowed caregivers to decrease APP mortality of Group 1 compared to Group 2 as reflected in the ReHS scores recovery leading to an overall better production performance.

A Ferreira Paulo, 403 A Silva Gefferson, 403 Abadias Javier, 626, 863 Abarca M. Lourdes, 236 Abd El Wahed Ahmed, 718 Abd El-Wahab Amr, 140 Abed Mouna, 237 Abella Falcó Gloria, 581, 726, 759, 810, 867, 891 Abércio Da Silva Caio, 90, 179 Abesinghe Sachini, 276 Abily Erell, 53 Abuoun Manal, 62 Aburjaile Flávia Figueira, 28 Acal Lucia, 812 Acebes Fernández Vanessa, 58, 826 Adsuar Marc, 850 Aerts Rob, 537 Agerlin Marianne Viuf, 169, 230 Agnoletto Ribeiro Vitória, 488 Aguarón Turrientes Alvaro, 58, 364, 802 Aguiar Fabiana, 285, 500 Aguiar Gabriel A., 657, 808, 830 Aguiar Vanilda, 638 Aguilera Eduardo, 739 Aguirre Kimberly, 743 Aguirre Laia, 807, 834 Ahlqvist Kristina, 107, 730, 763 Ahmed H Ghonaim, 324 Aiyimaphan Saowaporn, 605 Alapi István, 589 Alba Mary Grace, 610, 663 Alban Lis, 678 Alber Gottfried, 183 Alberton Geraldo C., 97 Albin Michael, 218, 613 Alborali Giovanni Loris, 21, 318, 472, 909 Alcantar Patzimba, 856 Aldaz Alvaro, 39, 91, 144, 436, 443, 531, 771, 795, 832 Alexsander Rojas Moreno Danny, 90, 179 Aljets Ethan, 65, 134 Allepuz Alberto, 70 Allerson Matt, 213 Allison Grant, 238 Almeida Marcelo, 55, 65, 69, 134, 165, 429, 473, 495, 497, 519 Almerheim Pontus, 41 Almond Glen, 157 Alonso Garcia-Mochales Carmen, 112, 199, 200, 201, 379, 922. 923. 926 Aluwé Marijke, 39 Alvarez Carlos, 741 Alvarez Gabriel, 509, 591, 603, 630, 654, 665 Alvarez Julio, 524 Alvarez-Martín Úrsula, 158 Alvarez-Norambuena Joaquin, 5, 217, 280, 297 Álvaro Mañas Aurora, 406 Alves Laya Kannan Silva, 106, 180, 893 Alves Ferreira Felipe Norberto, 775 Amalraj Arthi, 190

Amarante Victor Santos, 815 Amaya Hernández María José, 384 Ambroso Adib Donato Henriques Mariana, 31, 149 Amilcar Rossi, 843 Am-In Nutthee, 35, 118, 146, 392, 671, 721, 727 Ammer Stefanie, 895 Amodie Deb, 453 An Vanderzeypen, 301 Ananratanakul Chonlada, 118, 159, 539, 577, 882 Anderson M.D., 615, 629 Anderson Tavis K., 11, 245, 292, 312 Anderson Reever Amanda, 704 Andraud Mathieu, 169 Andrés Leiner, 628 Andretta Ines, 374, 376 Andriani Laura, 23 Ang Dian-Wen , 207, 427 Angel Diana C, 383, 410 Angelats Daniel, 363, 801 Angelichio Michael, 244 Angjelovski Branko, 70 Angulo Jose, 832, 863, 902 Anh Dang Thi Ngoc, 914 Anilkumar Vinithakumari Akhil, 162 Ansolin Alisson Leandro, 390, 394, 398, 407 Antão Cavalcanti Pizani Mariana, 839 Antônio Guizzo João, 839 Apolloni Andrea, 751 Aragon Virginia, 275, 471, 481, 558, 593 Arasil Aurea, 714 Araujo Matheus, 496, 639 Archer Courtney, 110 Ardies Cathy, 700 Arevalo Sureda Ester, 776 Arguello Hector, 256, 491, 572, 580 Arharat Rujira, 609 Arioli Enrico, 909 Armenta-Leyva Betsy, 214, 215, 250, 287, 333 Arnal Bernal José Luis, 272, 545 Arroyave Diego Alonso, 581, 919 Arruda Andreia, 242 Arruda Bailey, 11, 162, 187, 241, 288, 312, 495, 497, 622 Arruda Laíza, 657 Asanger Sophie, 17 Assavacheep Pornchalit, 474 Astrid Pausenberger, 805 Atthaapa Waranya, 7 Auer Angelika, 286, 326 Augustojusto André, 31, 149 Augustyniak Agata, 239, 428, 861, 878, 879 Aulinas Elisabet, 3, 204, 247 Aumann Kai, 452 Aundrup Jakob, 147 Aunpad Ratchaneewan, 150 Auping Nienke, 642 Aureli Raffaella, 913 Ausejo-Marcos Raquel, 158 Autret Kristell, 700 Azeitona Martins Vasco Daniel, 713, 831

Azevedo Vasco, 28 B. Martins Gabriel, 376 Bacci Cristina, 23 Badia Sergio, 669, 714 Bae Chaewun, 460 Bago Fabian, 684 Baier Sylvia, 905 Bak Hanne, 153, 613, 689 Baker Amy L., 245 Baker Julia, 130, 315, 327 Bakkegård Goecke Nicole, 305 Balasch Mònica, 39, 799, 832 Balbinot Adriana, 500 Baldasso Débora, 184, 798 Baldessar Pricila, 37, 413 Baliellas Jordi, 3, 204, 292, 477, 726 Balka Gyula, 14, 226, 286 Ballarà-Rodriguez Isaac, 189, 336, 512, 794, 806, 807, 814, 834,838 Ballester Maria, 299 Bandrick Meggan, 799 Bangkeaw Thanthip , 721, 727 Bano Luca, 444 Banyard Ashley, 223 Barbosa Ivan, 714 Barbosa Jessica, 496, 639 Bárcenas-Morales Gabriela, 664 Bardini Roberto, 434 Bariselli Simone, 418 Barta Leonard, 569 Bartelt Stefanie, 415 Bata Zsófia, 27, 139, 385 Baudouard Marie-Agnès, 237 Bauer Benjamin U., 456 Bauerfeind Rolf, 587 Baumgartner Jean Carlos, 900 Baums Christoph Georg, 163, 183, 185, 482, 513 Bearson Shawn, 480 Bearth Angela, 103, 170, 197 Beasley Shea, 673 Beatriz Da Rosa Aline, 374 Bechmann Larissa, 415, 421 Becker Jens, 381 Becker Sabrina, 191, 193, 903, 906 Beek Josine, 269, 365, 512, 694, 731 Beer Martin, 338 Behrens Sven-Erik, 94 Beilvert Fanny, 264 Belecz Nikolett, 535 Bellec Elise, 907 Belloc Catherine, 43 Ben Arous Juliette, 870, 874, 889 Benaben Stéphane, 89, 789 Benito Zuñiga Alfredo Ángel, 545 Bennewitz Stefan, 94 Berckmans Dries, 923 Berg Stephan Von, 97 Bergmann Rene, 513 Bernaerdt Elise, 70

Bernal Ignacio, 603, 651, 654 Bernaus Xavier, 581 Bernreiter-Hofer Tanja, 19 Berte Alexis, 309 Berton Pauline, 284, 314, 530, 755, 768, 835 Betiolo Felipe, 461, 529, 559, 872 Betlach Alyssa, 61, 168, 172 Bettini Ruggero, 383, 410 Beulen Henri, 717 Beumer Marius, 155, 161, 690 Bhojwani Rahul, 225 Bianchi Ivan, 37, 84, 394, 413 Bianchini Emilie, 220 Biasibette Debora, 496, 639 Bidewell Cornelia, 62 **Biebaut Evelien**, 70 Bienert-Zeit Astrid, 456 Bilecka Magdalena, 375 Billy Thibaut, 645 Birch Andreas, 125 Bisesti Petra, 770 Björkman Stefan, 32 Blach Nielsen Gitte, 271 Blanc Aurore, 279 Blanchard Yannick, 99 Blanco Patricia, 693, 714 Blanco López Sandra, 406 Blanco-Fuertes Miguel, 481 Blome Sandra, 9, 338 Blömer Henri, 189 Blümlinger Michael, 64 Boerboom Gavin, 774, 776, 781 Boeters Marloes, 706 Bohmer Jan, 292 Bohrer Ben, 91, 436, 443, 771 Boivent Benoit, 487 Boix Oriol, 600, 601, 623, 742, 750, 831, 843, 848 Bona Preisler Juliana, 138 Bonardi Silvia, 23 Bonckaert Caroline, 75, 243, 672 Bonillo-Lopez Laura, 593 Boniotti Maria Beatrice, 318 Bonto Francis Xavier, 334 Boonprakob Rafa, 146, 401 Boonraungrod Naritsara, 393, 873, 887 Boonsoongnern Alongkot, 345, 438, 457, 588, 633 Boonsoongnern Prapassorn, 438, 457, 633 Bordignon Diego, 798 Bordin Luiz Carlos, 313 Borges Da Silva Jéssica, 648 Borobia Belsue Jesus, 310 Bortolozzo Fernando, 145, 564 Bošnjak-Neumüller Jasna, 496, 639, 655, 772, 880 Bossé Janine T., 599 Botet Fernando De Mergelina, 118 Botía María, 338 Boucher Christina, 13 Boudin Edouard, 668, 911 Boulbria Gwenaël, 34, 53, 284, 314, 400, 755, 835

Boulot Sylviane, 690 Bourry Olivier, 99 Bousquet Eric, 284, 314, 530, 755, 835, 836, 856 Bouwknegt Martijn, 141 Bowen Craig, 242, 351 Boyen Filip, 447, 489 Bracco Donatelli Muro Bruno, 31, 83, 90, 149, 174, 179, 192, 455, 855, 876, 893 Bradner Laura, 479, 495 Braga Eduarda R., 525, 550, 681, 808, 820 Brandalise Luciano, 274, 390 Braselinoborges Lucas, 31, 149 Braun Sascha, 64 Bravo Jonathan, 13 Breitfelder Annika Katharina, 183 Brenig Bertram, 28 Bresolin Eduarda, 488, 564 Breukers Eveliina, 325 Brilland Sophie, 34, 49, 339, 355, 757, 846 Bringas Joaquim, 585, 607 Brissonnier Mathieu, 53 Brockmeier Susan, 479, 540 Broekaert Nathan, 88 Brossé Charlotte, 75, 243, 672 Brown Ian, 223 Brown Justin, 57 Bruguera Sergi, 551 Bruner Laura, 213 Brunthaler René, 42, 52, 286, 289 Buakhiew Alongkorn, 118, 159, 539 Buch Juhle-Marijke, 54 Buchallik-Schregel Johannes, 430, 456 Buchan Jordan, 231 Buchebner Birgit, 718 Buckley Alexandra, 11, 270, 298 Budzisz Petri Rogério, 564 Bühler Kathrin, 777 Bui Thi To Nga, 212 Bukowska Paulina, 722, 816 Bulay Andres Iii, 925 Bundee Nontaprecha, 605, 609 Bundgaard Hans, 424 Bünger Moritz, 52 Buntenkötter Vitus, 532 Bunyakida Belle, 146 Bunzen Silvano, 917 Burlet Hannah, 634 Burlot Vincent, 380, 422, 640, 645 Burrell Alison, 197 Burrough Eric, 165, 479, 495, 497, 519 Burton Brandi, 704 Burton, Dvm Brandi, 683 Bussi Riccardo, 728 Buter Rianne, 292 Butkhot Namphueng, 320 Buxeda Marc, 832 Buyens Wim, 923 Búza László, 24, 369 Buzoianu Stefan Gabriel, 175

Byrne Alexander, 223 Byun Jeongjae, 591, 630 C Cardoso Lucas, 403 C. Paiva Rodrigo , 55, 65, 165, 309, 519 C. V. Furtado Júlio, 376 Cabal Rosel Adriana, 45, 64 Cabana Marta, 832 Cabañes Jose Quirino, 760 Cabetas Marta, 890 Caekebeke Nele, 677 Caetano Monti Guedes Rodrigo, 845 Caiafa De Alkmim Tulio Cezar, 775 Calderon Diaz Julia Adriana, 669, 693, 714 Calero-Herrera Ibrain, 785, 786, 787, 788 Calixtro Shella, 567 Calveyra Juliana, 441, 449, 461, 462, 529, 549, 559, 599, 637, 638, 667, 739, 872, 881, Camacho-Medina Juan, 664 Camarasa Jordi, 848 Cameron Schmitt, 57 Camp Montoro Jordi, 669, 714 Candeias Joice, 461, 638 Canet Martinez Estel, 913 Cano Jean Paul, 711 Cantaloube Emma, 196, 676, 901 Cantão Maurício E., 37 Canturri Albert, 59, 60 Cao Xu, 586 Caon Jose Antonio, 475 Capern Lena, 556 Cárceles Sonia, 186, 266, 321, 840, 865 Cardoso Renato Mendes, 398 Cardoso-Toset Fernando, 494 Carlos Crestani Luiz, 648 Carmo Kellem Lopes Do , 507, 525, 541 Carmona Marta, 56, 627, 644, 851 Carneiro Daniela Oliveira, 478, 505, 518 Carnero Jesus, 330 Carnevale Rafaella, 31, 83, 90, 149, 174, 179, 192, 455, 893 Carnino Bruno Braga, 31 Carr John. 135 Carranza Alejandro, 205 Carrasco Librado, 253 Carrau Tessa, 338 Caruso Claudio, 143 Carvajal Ana Maria, 256, 491, 572, 580 Carvajal Mario, 389 Carvalho Rodrigo Dias De Oliveira, 28 Carvalho De Abreu Fantini Marcia, 808, 820, 855, 876 Casal Carmen, 802 Casal Nicolau, 656 Casanovas Carlos, 218, 266, 321, 850, 865 Casanovas Granell José, 726, 850 Case Daniel, 695 Cassart Dominique, 446 Castelluccio Domenico, 909 Castillo-Espinoza Ana Fiorella, 160 Castillo-Pérez Jaime José, 259

Castro Arthur Silva, 478, 518 Castro João Humberto T., 550 Castro Natalia, 441 Catania Salvatore, 472, 486, 535 Cavalcante Filho Rosiel Moreira, 390, 394, 398, 407 Cay Ann-Brigitte, 101 Ce Taís Michaelsen, 500 Cerón Madrigal José Joaquín, 296, 323, 338, 432, 452, 502, 533, 562, 592 Cerutti Dazzi Cláudia, 839, 845 Ceyssens Kristel, 416, 419 Cezar Guilherme, 242, 309, 351 Cha Ji-Won, 790, 828 Chabeauti Elisabeth, 448 Chacon Fernando, 462 Chae Heejin, 460 Chagas Sarah, 534, 803 Chaionnom Chayanin, 660 Chama Francisco, 739 Chanapiwat Panida, 18, 150, 151 Chang Chia-Jung, 825 Chang Chia-Yi, 268, 316 Chang Hui Wen, 316 Chang Jennifer, 210 Chantziaras Ilias, 70, 190, 489, 677 Charatpaew Kwanchai, 926 Charles Mathieu, 744 Charrot Julie, 221, 361 Chathuranga Kiramage, 790 Chaudhary S., 319 Chavaranggul Nichada, 118, 882 Chavez Amelia, 596 Che Lianqiang, 783 Cheah Eric Heng Chow, 207, 427 Chen Hsin, 65 Chen Huanchun, 516 Chen Ping, 595 Chen Xiongnan, 2103 Chen Yu-Cheng, 257, 331 Cheng Ting-Yu, 234, 250 Chevance Celine, 34, 53, 284, 314, 400, 755, 835 Chiang Hsin Heng, 498, 560 Chiapponi Chiara, 169 Chiu Wen Cheng, 542, 566 Cho Ho-Seong, 232, 265, 282, 291, 493, 705 Cho Jeong-Jun, 705 Cho Ki-Hyun, 248 Choi Minsoo, 591, 630 Chompuming Rattaphon, 610, 624, 653 Christensen Charlotte Sonne, 340 Christiaens Isaura, 761, 824 Christoforou Marios, 388 Christy Diego, 695 Chuang Wen-Yang, 341 Chunqing Sun, 702 Ciacci Zanella Giovana, 241, 288 Ciacci Zanella Janice, 245, 285, 313 Ciprian-Carrasco Abel, 515, 571, 664 Claerhout Lieven, 356, 358, 424, 486, 615, 616, 629

Claeyé Eva, 623 Cláudio Da Silva Bueno Ives, 90, 179 Claver Mateos Miguel, 585, 607 Clavijo Maria Jose, 57, 198, 471, 476, 497, 507, 546, 596, 711, 743, 921 Clemens Noelie, 446 Clilverd Hepzibar, 275, 300, 807 Climente-Retornano Andrea, 158 Clive Stephanie, 261 Cloutier Laetitia, 96 Cobos Alex, 10, 246 Coelho Ana Cláudia, 710 Coelho Flávio Aguiar, 180 Coldebella Arlei, 559 Coldebella Luciane, 500 Cole Aleah, 92 Colin Frederic, 902, 907 Colitto Mariano , 555, 600, 843, 853 Coll Teresa, 581 Comin Arianna, 325 Comtet Loïc, 219, 220, 359, 490 Cong Li, 665 Conraths Franz Josef, 67 Cons Luis, 850 Consoni Wagner, 117 Consuelo Arancibia Paulina, 839, 845 Conter Mauro, 23 Cools An, 455 Cooremans Sven, 717 Cordeiro Rui, 713 Cordioli Benedetta, 444 Cordon Cassidy, 59, 522 Cornelis Bert, 48, 218, 273, 575, 707 Coronado Liani, 169 Correa-Fiz Florencia, 236, 275, 481, 558, 593 Correge Isabelle, 70, 373 Correia Paula, 496, 639 Correia Gomes Carla, 70 Correia Lima Linhares Daniel, 50, 80, 108, 235, 238, 429, 431, 442, 680 Correia da silva Eric, 149 Correia-Gomes Carla, 197, 715 Cortey Martí, 4, 275, 300 Corzo Cesar, 2, 5, 50, 131, 168, 206, 213, 225, 260, 262, 280, 315, 327, 729, Costa Francisco, 218 Costa Mara, 20, 722 Cota-Valdez Angel, 739 Cotta Higor, 753 Couteau Mathieu, 422, 530, 768 Crawford Kimberly, 480 Creach Paul, 751 Cregan Brendan, 231 Cregenzan Sergio, 850 Crespo Sara, 404 Cristani Jose, 117, 138, 368, 370 Cristine Penha Letícia, 397 Cros Silvia, 189, 794, 814 Croubels Siska, 88

Cruz Christopher, 617 Cruz Luis, 829 Csikó György, 27 Cudal Roselle Falconite, 610, 663 Cuellar-Flores Amanda, 89, 267, 296, 302, 323, 360, 420, 647, 738, 789, 877, 886 Cugno Andrés, 843 Cujic Svetlana, 655 Culhane Marie, 206, 217, 297, 729 Cuong Nguyen Kien, 914 Curto Paula, 3, 204, 247 Cvjetkovic Vojislav, 415, 421 Cybulski Piotr, 14, 305, 344, 375, 391, 402, 568, 649, 762, 769,916 Czanderlova Linda, 411, 506, 520, 561 Czyzewska-Dors Ewelina, 861, 878, 879 D Kich Jalusa, 394 Da Rocha Bonera Dos Santos Roberta, 374 Da Rosa Ulguim Rafael, 564 Da Silva C.A., 144 Da Silva Daniela Gomes, 830 Da Silva Aleksandro, 368 Da Silva Martins Tereza, 808, 820, 855, 876 Da-Costa Jean-François, 53 Daffunchio Esteban , 555, 600, 843, 853 Dagorn Dominique, 448 Dahl Jan, 167 Dahlin Lisa, 70, 737 Dai Jianghe, 412 Dalmina Rech Rodrigo, 145 Dalpozzo Ildo, 559 Dalsgaard Sara, 747 Daluzeau Laurent, 448, 484, 579, 594 Daly Stéphane, 361 Dang T. X. Thiep, 720 Daniel Amanda G., 97 Daniel A Domínguez-Olvera, 459 Danowski Katrin, 898 Dauphin Gwénaëlle, 169, 308, 751 Dauvier Alexandre, 752, 767 Davi Traverso Sandra, 117, 138, 368, 370, 374, 376 Davis Laura, 297 De Almeida Bruno Albuquerque, 274 De Arriba María Luisa, 364, 802 De Backer Peggy, 48, 273, 329, 417, 537, 575, 642, 703, 707, 735, 753 De Bruijn Anne, 181 De Bruijn Naomi, 152 De Cacqueray Preden, 745 De Castro Lucas Teixeira, 274 De Castro Renzi Owell, 435 De Conte Elisa, 598, 804 De Conti Elisa, 636, 841, 844, 922 De Cuyper Carolien, 776 De Freitas Costa Eduardo, 431, 507 De Frutos Laura, 864 De Grau Francisco, 227 De Groot Mike, 862 De Jong Sanne, 416, 419, 423

De Jonghe Eva, 218, 329, 417, 703, 735 De La Luz-Armendáriz Jazmín, 203, 254, 304, 346, 854 De Laat John, 121 De Lara Anne Caroline, 285, 500 De Lorenzi Giorgia, 418 De Lucas Lidia, 343, 555, 617, 793, 814, 837, 853, 866, 869,875 De Mergelina Fernando, 834 De Meyer Dimitri, 190 De Moraes Reis Josiane, 621 De Nardi Marco, 70 De Oliveira Luís Guilherme, 447, 489, 505, 525, 550, 558, 657, 681, 808, 820, 830, 855, 876 De Oliveira Costa Matheus, 634 De Oliveira Telesca Camargo Nathalia, 374 De Paula Teixeira Angelica, 804, 844 De Quadros Fernando, 290, 598, 636 De Reus Gert-Jan, 753 De Souza Eliane Marucci Baccin, 505 De Souza Manoela Garcia Lino, 478, 518 De Souza Costa Tamanini Mayara, 798 De Souza E Silva Gustavo , 50, 80, 108, 235, 429, 431, 442, 480, 680 De Toro Maria, 580 Deblanc Céline, 169 Deboisséson Claire, 43 Declerck llse, 547 Decoux Marc, 121 Dee-Ampai Wichaya, 414, 474, 573, 574, 584, 610, 620, 658.766 Deelum Nattawut, 118 Deesuwan Jirapinya, 146 Defois Justine, 386, 387 Deitmer Ricarda, 611, 646 Del Pozo Sacristán Rubén, 73, 222, 510, 635, 849, 896 Del Valle Ana, 890 Delaunay Isabelle, 448 Delgado Laeticia, 364, 802 Delgado García Mario, 58 Delsart Maxime, 668, 911 Dembowski Martin, 163 Dénes Lilla, 14 Deng Ming-Chung, 268, 316 Denisova Alena, 692 Denzin Nicolai, 67 Deon Kich Jalusa, 598, 636 Depondt Wouter, 356, 358, 486, 615, 616, 629 Dereu Andre, 307, 597 Derscheid Rachel, 479, 495, 497 Descamps Frédéric, 39 De-Sousa-E-Silva Gustavo, 66, 69, 77, 242, 312, 497 Despois Lea, 220 Deutschmann Paul. 338 Devreese Mathias, 88 Devriendt Bert, 447, 489 Dewolf Brad, 126, 328 Dewulf Jeroen, 677 Dhaka Pankaj, 677 Di Bianco Soazig, 386, 387

Diamondidis Yvette, 799 Diaz Andres, 342, 389, 711 Diaz Ivan, 866, 869 Diaz Marta, 299 Diaz Sergio, 875 Diaz-Rayo Concepción, 348 Didara Mislav, 70 Diesing Anne-Kathrin, 864 Dieste-Pérez Lucía, 15, 93, 197 Diesterbeck Ulrike, 94 Díez Jesús, 840 Dijkman Reinie, 289 **Diness Line Hummelmose**, 47 Dinh Xuan Phat, 229 Dion Kate, 429 Diosdado-Vargas Fernando, 304 Dittrich Barbara, 92 Do Thi Hong, 614 Do Tien Duy, 212, 255, 353, 631, 914 Do Thi Hong, 322 Dodantenna Niranjan, 294, 306 Domingo Ivan, 4 Domingo Mariano, 10 Dominiek Maes, 447, 489 Donescu Daniel, 347 Dongqi Xie, 324 Donin Daiane G., 97 Donneschi Anna, 21, 318 Doolittle Kent, 214, 215, 250 Dorenlor Virginie, 668, 911 Döring Steffen, 122 Dors Arkadiusz, 239, 428, 861 Dos Santos Igor Ribeiro, 274 Dos Santos Laura, 145 Doupovec Barbara, 42, 408 Driemeier David, 274 Drigo Ilenia, 444 Droce Aida, 129 Du Plessis Louis, 141 Duarte Mirtha, 639 Dufay-Lefort Anne-Christine, 386, 387 Dufour Barbara, 668, 911 Duivon Didier, 448, 484, 579, 594 Duong Nhat, 577, 631 Duque Elisabeta, 831 Duran Oliver, 823 Dürlinger Sophie, 824 Durónviveros Jaime Gustavo, 880 Duval Pauline, 422, 768 Duy Ngo Ba, 914 Eberle Kirsten, 540 Echtermann Thomas, 691 Eckey Isabell, 784 Eclercy Julie, 264 Eddicks Lina, 40 Eddicks Matthias, 40 Edler Roy, 485, 544 Eduardo Zanoni Nubiato Keni, 397 Eenink Karlijn, 197, 240

Egerer Christiane, 903, 906 Egerszegi Istvan, 38 Eggink Dirk, 15 Ehricht Ralf, 64 Ekawatpanich Jaturong, 320 Elemia Jane, 925 Eliasson- Selling Lena, 41 Ellerma Leonardo Domingo Ellerma, 510, 849 Emeric Chassaing, 347 Engel Petra, 903 Engel Sebastiaan, 433 Engelen Bart, 416, 419, 423 Eon Loïc, 484, 579, 594 Eono Florent, 668, 911 Eppink Lysan, 868 Erik Larsen Lars, 249 Erusalimsky Fabian, 199, 923 Eschke Maria, 183 Escobar-González Maria, 236 Escoda Alberto, 726, 850 Escuder Margarita, 445 Espigares David, 186, 266, 267, 296, 302, 321, 323, 360, 627, 644, 738, 809, 818, 840, 850, 851, 865, 877, 886, 890 Esquer Alejandro, 884 Esteves Alexandra, 710 Estruch Josep, 236 Euring Belinda, 116, 317, 326 Evelsizer Robert, 59 Eveno Eric, 668, 911 Everaert Nadia, 776 Everett Helen E., 169, 223 Ewers Christa, 587 F Hernig Luciana, 290 Fabà Lluís, 93 Fablet Christelle, 169, 668, 911 Fabros Karl Alexander, 610, 624, 653, 663 Fairbrother John Morris, 565 Faisant Lucile, 640 Falcão Da Costa Heni, 839, 845 Falceto Maria Victoria, 36, 754 Faldyna Martin, 114 Falkenberg Shollie, 298 Fall Nils, 737 Fang Jie, 604, 610 Fang Shuhe, 606, 847 Fangman Thomas, 616 Fano Eduardo, 61, 102, 172, 497, 524, 632, 800, 833 Faris Richard, 121 Farkas Hunor, 655 Farmaki Rania, 439 Farre Clara, 626 Farzan Vahab, 565 Favrel Justine, 53 Feehan Brandy, 319 Fei Wentao, 173, 363, 577, 612, 651, 665, 709, 801 Feitosa Leal Diego, 83, 90, 174, 179 Fellag Mustapha, 530 Fels Michaela, 894 Fenech Mar, 806, 866

Feng Baohai, 363 Ferino Lucia, 209 Fernandes Saffir Dominique, 28 Fernandes Vinicius, 531, 795 Fernandes Lenhard Bianca, 488 Fernandez Ana, 545 Fernandez Camino, 802 Fernandez Sebastian , 555, 600, 843, 853 Fernández-Fontelo Amanda, 111, 56 Fernández-Rodríguez Raguel, 44, 148, 352, 464 Fernand-Genty Jeanne, 380, 422, 640, 645 Fernando Dos Santos Lucas, 164, 377, 483, 501, 543, 548, 625 Feronato César, 97 Ferraz Maria Eugenia, 553 Ferreira Soraia Viana, 778 Ferreirataka Filipe, 621 Ferreiro Julian, 507, 541 Ferronato Cesar, 78 Fertner Mette, 6, 228 Ficagna Cassio, 368 Fidler Gabriele, 51 Fiers Jorian, 101 Figl Sophie, 42 Figueiredo Tadeu Chaves, 778 Figueras Sebastián, 199, 759, 813, 891, 923 Filek Klara, 756 Filippi Jorge, 349 Filippitzi Maria Eleni, 70 Filsner Pedro, 461, 529, 559, 638, 872 Fily Bernard, 380, 422, 640, 645 Finatto Arthur, 634 Finzel Jacqueline, 858 Fiorin Hernig Luciana, 598, 636, 804, 844 Firminiano Pedro Do Vale, 31 Firth Ava Marie, 175 Fischer Egil, 141 Fiuza Aparecida, 841 Fleischer Lena. 381 Flores Alejandro, 596 Floros Dimitrios, 439, 551 Floyd Tobias, 261 Fochesatto Naiara, 500 Foiani Greta, 434 Földi Dorottya, 535, 466 Folens Evie, 177 Folque Pedro, 724 Fong Lai Yee, 542, 566 Fong Michelle Wai Cheng, 207, 427 Fonseca Eliane M.E. 97 Fontes Dalton Oliveira, 778 Fontes Maria Da Conceição, 710 Fontseca Mireia, 336, 794 Formanowski Aleksander, 649 Formenti Nicoletta, 21, 318, 472 Fornari Barbara, 285 Fouchier Ron, 15 Fraile Lorenzo, 3, 204, 247, 299, 477, 670, 726 Franchi Luigi, 418

Francisco Ernesto Martínez-Castañeda, 396, 458, 459 Franco Erico Granado, 795, 863 Franco Martínez Lorena, 338, 432 Franco-Castillo Isabel, 158 Frandoloso Rafael, 97, 184, 475, 488, 564, 798, 839, 845 Frandsen Dorthe Poulsgård, 98 Franz Kairon Adam, 531, 795 Franzo Giovanni, 272, 481 Frederick Bussy, 745 Frederico Giovani, 84 Fregulia Priscila, 92 Freitas Thiago De Faria, 507, 541 Frey Tanja, 371, 898 Freymüller Georg, 185 Freytag Sarah, 161, 690 Fried Henning, 193 Friedrichs Virginia, 9, 338 Friendship Robert M, 565 Frio Arturo Jose L., 780 Fristiková Karola, 253 Fritz Scott. 92 Frossard Jean-Pierre, 261 Fuchs Andreas, 45 Fuentes Pablo, 76, 166, 699, 871 Fujiwara Takahiko, 570 Fuke Naoyuki, 570 Fullick Edward, 223, 261 Funes Ivan, 36 Fux Robert, 40 G A Vidigal Izabelle, 409 Gabner Simone, 42 Gabrielle De Souza Daniel Amanda, 648 Gadea Joaquin, 404 Gajda Anna, 375 Galán-Relaño Angela, 494 Gale Christina, 136 Gale Irene, 189, 336, 512, 742, 750, 794 Galemou Yoga Etienne, 94 Galindo-Barboza Alberto-Jorge, 254 Galland Stephan, 317 Gálvez-Pérez Laura, 246 Galvis Jason A, 68, 208 Gamage Nuwan, 211 Gambade Patrick, 487 Ganges Llilianne, 299 Gantelet Hubert, 43, 523, 537, 642, 827 Ganter Martin, 456 Garay-Guerra Isamar, 739 Garbuio Manuel, 444 Garcia Daniella Berenice, 617 Garcia Hector, 695 Garcia Javier, 669, 714 Garcia Rocio, 581, 759, 891 Garcia Rodrigo, 843 García Gonzalo, 603, 628, 654, 862 García Simón, 840 Garcia Borgi Lino De Sousa Manoela, 397 García Flores Alberto, 343 Garcia Manzanilla Edgar, 338, 533, 562, 749

García-Flores Alberto, 321 Garcia-Morante Beatriz, 111, 170, 197, 706, 811, 817 Garmon Alba, 356, 358 Garrido Ana Maria, 754 Garza Laura, 56, 266, 321, 754, 809, 818, 865 Gasparini Thais, 793 Gasperin Bernardo G., 37, 413 Gastaldelli Michele, 434 Gaudreau Annie, 827 Gauger Phillip, 16, 65, 66, 80, 134, 210, 235, 238, 245, 281, 292, 312, 495, 497 Gaustad Ann Helen, 32 Gautier Lucie, 196, 901 Gayán Julia, 656 Gazzola Alessandra, 909 Gebhart Connie, 22, 803 Gelasakis Athanasios, 551 Gellermann Maria, 67 Genís Sandra, 39 Genz Berit, 675 Genzow Marika, 460, 521, 611, 891, 925 Gerdes Kathrin, 650, 701, 912 Geremias Henrique Rafael, 192 Gerhards Katharina, 191, 903, 906 Germeraad Evelien, 15 Gerner Wilhelm, 188 Gerszon Joanna, 675 Geurts Victor, 224, 597, 723 Gheller Marina, 798 Giacobbo Igor, 839, 845 Giacomini Enrico . 21 Gianeis Isadora, 127, 899 Gickel Julia, 140 Gielen Chretien, 289 Gijsen Emile, 289 Giller Katrin, 87 Giménez-Laita Concepción, 158 Gimenez-Lirola Luis Gabriel, 74, 110, 160, 214, 215, 234, 250, 287, 293, 333, 480 Gin Thomas, 196, 901 Giralt Dolors, 159, 269, 365, 539, 591, 600, 617, 630, 651, 694, 700, 713, 731, 742, 750, 837 Girotto Elisa, 444 Gleeson Bernie, 675 Gliši Dimitrije, 121, 362 Glisic Milica, 772 Goecke Nicole B., 169, 230, 249 Goesten Bram, 171 Goetz Frank, 317 Gol Sofia. 299 Golinar Oven Irena, 748, 758, 918 Gombos László, 24, 369, 466 Gombosne Szur Veronika, 24 Gomes Bruna C.K., 97 Gomes Nadia De Almeida Ciriaco, 149, 180 Gomes Dutra Tatiana Carolina, 793 Gómez Luis, 272, 304 Gomez Cruz Francisco, 782 Gomez-Duran Charles Oliver, 688

Gomez-Gascón Lidia, 494 Gómez-Laguna Jaime, 253 Gómez-Martínez Samuel, 256, 491, 572 Gomez-Osorio Luis-Miguel, 880 Gómez-Vázquez Jose Pablo, 743, 921 Goncalves Jessica, 479 Gonçalves Rômulo, 841 Gonçalves Mellagi Ana Paula, 145 Gonsalves Ray Nascimento, 407 Gonzalez Alejandro De Jesus, 617 González Bulnes Antonio, 502, 533, 562 González Fernández Alba, 58, 826 Gonzalez-Crespo Carlos, 198, 921 González-Guijarro Belén, 89, 267, 296, 302, 323, 360, 445, 647, 738, 789, 877, 886 González-Martínez Raúl, 346 González-Ramiro Henar, 148, 406 González-Solórzano Karla, 203 Gonzalvo Francisco, 112, 726, 759, 813, 891 Gordaliza Laura, 875 Gorin Stéphane, 169 Goris Kaat, 121 Goslar Mariana Santiago, 285, 500 Gottschalk Marcelo, 55, 57, 95, 96, 471, 472, 792, 827 Gougoulias Christos, 8 Gow Lisa, 244 Goyal Sagar, 5 Goyena Elena, 267, 296, 302, 323, 360, 502, 840 Graaf-Rau Annika, 132, 169, 311 Graage Robert, 740 Graat Elisabeth Anna Maria, 104 Grabkowsky Barbara, 67 Grahofer Alexander, 29, 399, 679, 733 Grajales-Cedeño Joseph, 127, 899 Grau Kristin, 132, 311 Greatrex Anna, 220, 359 Greaves Gillian, 126, 328, 674 Gresakova Lubomira, 70 Griessler Alfred , 42, 52 Griffioen Friso, 14 Grionkova Y., 100 Grosse Beilage Elisabeth, 650, 701, 716, 894, 912 Gründl Julia, 40 Gu Hyunji., 265 Guangyuan Yi, 324 Guarneri Flavia, 21, 472 Guedes Roberto, 157, 496, 639 Guedes Barbosa De Lima Débora, 563 Guerra Manuel. 840 Guerra Nicolas, 303, 415, 421 Guerrero Álvaro, 186 Guinat Claire, 141 Guitart-Matas Judith. 477 Guiu Teresa, 199, 923 Guizzo João Antônio, 184, 488, 798, 845 Gumbert Sophie, 17 Gunnarsson Stefan, 904 Guo Tao, 426 Guolo Angela, 444

Gustafsson Stig, 46 Gutiérrez Ana María, 76, 166, 647, 699, 871 Gutierrez Zuleyka Abigahil, 617 Gutiérrez Martín César Bernardo, 58, 826 Gutierrez Montiel Daniela, 634 Gyuranecz Miklos, 466, 486, 486, 535 Haden Cara, 57 Hägg Grønborg Louise, 382 Hai Nguyen Thanh, 914 Hailong Ma, 324 Hajdinjak Melita, 748, 758 Hakimi Maria, 622 Hakze Renate, 141 Halev Avishai, 198 Hallbauer Josepha, 482 Haller Helene, 124, 163 Haluwana D. K., 295 Hamar Flora, 19 Hamon Philippe, 700 Hams Perry , 711, 743 Handt Tobias, 452, 912 Hankel Julia, 140 Hänsel Stephanie, 723 Hansen Blaine, 91, 436, 443, 771 Hansen Lea Hübertz Birch, 12, 556, 756, 773 Hansen Lisbeth Ulrich, 153, 689, 746 Hansen Marie-Louise, 719 Hansen Olaf, 452 Hansen Tara, 799 Hanson Daniel, 485, 544 Hansson Ingrid, 737 Hanstein Sophia, 163 Hao Wu, 324 Harder Timm, 132, 169, 311 Harders Frank, 141 Harms Perry, 57, 471, 546, 921 Harris Catherine, 522 Hartmann Ulrich, 895, 920 Hartwig Tina, 38 Harzer Maxi, 116, 317, 326 Hassila Martti, 528 Hau Samantha, 479, 540 Haugegaard John, 779 Haugegaard Svend, 153, 230 Hautefeuille Claire, 751 Hawungchoo Thanida, 873 Hayes Dermot, 708 Hazelden Sophie C., 777 Hebbel Cleverson . 37 Heenemann Kristin, 116, 147, 317, 326 Heim Giseli, 774, 781 Heimann Marcus, 155 Heinonen Mari, 107, 673, 763 Heinz Andrea, 799 Heliez Sylvie, 768 Hellige Maren, 456 Hellmann Klaus, 194 Helmer Carina, 54, 161, 690 Helverskov Ole, 228

Hemker Deanne, 711 Henao Alexandra, 389 Henao-Diaz Alexandra, 214, 215, 250, 695, 739 Hendrickx Diedrich, 685 Hennemann Hanjo, 94 Hennig-Pauka Isabel, 147, 189, 430, 452, 456, 513, 532, 650, 701, 897, 912 Henry Colin, 868 Hernández Jon-Iñaki, 656 Hernandez Juan Manuel, 596 Hernández Martín Jonás, 626 Hernández-Andrade Laura, 515, 571, 661 Hernández-Banqué Carles, 299 Hernández-García Juan, 742, 750 Herran Olga, 741 Herrero Juan Manuel, 714 Herve Gwendoline, 690 Hervé Séverine, 169 Heseker Philipp, 895, 920 Hidalgo Alvaro, 244 Hill Tom. 136 Hines Bergeson Nichole, 11 Hinestrosa Ruth Del Carmen, 628, 837 Hirai Takuya, 570 Hirose Flávio, 657, 839, 845 Hjulsager Charlotte Kristiane, 129 Hložková Jana, 816 Hoang Truong, 860 Hofer Andreas, 679 Hoffman Kyle, 298 Højgård Camilla Kaae, 98 Holdefer Adriano, 559 Holenweger Fabienne, 679 Holgado-Martín Rocío, 272 Holmström Anna-Riia, 763 Holtkamp Derald, 50, 78, 429, 442, 708 Holtz Adriane, 798 Holzmann Tina, 399 Homann Christian, 784 Hong Seong-Keun, 248 Honorato Gatto Igor Renan, 839, 845 Hoornenburg Gerben, 467 Horst Andreia, 461 Horta Felipe, 84 Hoshino Roberta Yukari, 893 Hosokawa Masashi, 570 Hou Yanyan, 516 Houben Manon, 15, 240 Hourcg Pascal, 237 Hsun Wu Ming, 577, 665, 801 Hu Qunshan, 797 Hu Xiao, 471, 495, 546 Hua Lin. 516 Huang Chao, 198 Huang Jinhu, 586 Huang Lv, 606, 823 Huang Szu-Wei, 257, 331, 542, 566 Huang Xi, 516 Huang Yu-Liang, 268, 316

Huerta Eva, 10, 246, 593 Huerta Isaac, 669, 693, 714 Huisman Abe, 915 Hulshof Tetske G, 93 Hulzing Johan, 514, 557 Humphrey Nicki, 270 Hung Yu Fan, 283 Husmann Robert, 100, 283 Hutet Evelyne, 99 Hwang Seong Yun, 852 I. Cardenas Matias, 241 Ibanez Felipe, 78 Ibanez Gabriela, 862 Ibelli Adriana M. G., 37, 413 Igelbrink Ralf, 371 Igriczi Barbara, 14 Illas Francesc, 481 Illner Tina, 38 Iriarte Borja, 819 Ishii Koji, 492 Iskrzak Pawel, 762 Isusi Sara, 445 Iturbide Alexis, 389 Ivok Marianna, 589 Jaadar Hamid, 423 Jablonski Artur, 14, 375, 568, 769 Jablonski Eva, 576 Jacobson Magdalena, 41, 737 Jahn Laura, 905 Jaime Jairo, 281, 342 Jakobsen Nadia, 698 Jakovcevic Zdenka, 655 Jamawat Supachai, 926 James Joe, 223 Jamikorn Uttra, 86 Janeczko Krzysztof, 762, 861 Jang Young Dal, 92 Jansen Micah, 576 Jansen Rutger, 517, 547, 602, 606, 611, 646, 688, 717, 765 Janssen Rick, 732 Janssens Geert Paul Jules, 455 Jansson Ellinore, 325 Janzen Garrett, 245 Jardin Agnès, 34, 49, 218, 339, 355, 751, 757, 846 Jardon Samantha, 829 Jayaraman Swaminathan, 431 Jean Christian, 337 Jedryczko Roman, 554 Jefferv Alison, 634 Jeklová Edita, 114 Jensen Henrik Elvang, 125 Jensen Lars Morten, 350, 379, 687, 924 Jensen Peyton, 803 Jensen Rachel, 57 Jensen Tina Birk, 125, 689, 719, 746 Jensen Vibeke Frøkjær, 678 Jeong Jongyeon, 460 Jeong Min A, 258, 263, 666 Jeong Won-Hwa, 828

Jermsutjarit Patumporn, 7, 65, 86, 133 Jesus Alice N., 97 Jeusselin Justine, 34, 53, 284, 314, 400, 755, 835 Jezdimirovi Nemanja, 121, 362 Jheong Weonhwa, 790 Jiménez Marta, 4, 246, 259, 405, 682, 859 Jiranaparat Atthariya, 766 Jirawattanapong Pichai, 345, 438, 457, 588 Jitplodprong Piyachart, 605 Jittidecha Jiramet, 605 Johnson Alaina, 92 Jones Alex, 734 Jong-Soo Lee, 211, 233, 252, 276, 277, 294, 295, 306, 790, 828, 852 Jorda Ramon, 603, 649, 654, 700, 862 Jorge Peñas Alvaro, 923 Jørgensen Benjamin Meyer, 125 José Guadalupe Herrera-Haro, 459 Joudou Louise, 264 Jourguin Jan, 686 Jovancevic Marko, 772 Jové-Juncà Teodor, 299 Joye Paul, 181 Juan Li. 665 Juel Annette Sønderholm, 613 Juhász Akos, 139, 385 Jung Byeong Yeal, 527 Junior Fabiano Vaguero Silva, 31 Junker Karin, 156, 512, 521 Juntanupong Boontarika, 18 Jurado-Martos Francisco, 494 K O Pires Dayanne, 409 K. Anderson Tavis, 241, 288 K. Souza Carine, 288 Kaebisch Lisa, 684 Kaeoket Kampon, 18, 133, 150, 151, 320, 660 Kaewbubpa Surasak, 435 Kaewchomphu Nuanate, 609 Kaewthamasorn Morakot, 255 Kähl Sophie, 163 Kam Kok Yen, 207, 427 Kaminsonsakul Tanyanant, 438, 588 Kamlangdee Attapon, 633 Kang Gijong, 460 Kang Hae-Eun, 248 Kankuntod Somyod, 588 Kanters Miranda, 152 Karembe Hamadi, 415, 725, 858, 864 Karimova Liliya, 870, 874, 889 Karlsen Signe, 756 Karriker Locke, 680 Kartalik Bolazs, 201 Käsbohrer Annemarie, 19 Kaschny Maximiliane, 898 Kauffold Johannes, 124, 163, 183, 736 Kawilaphan Yanee, 234 Kawongklang Tiparpha, 393, 873 Kazlouski Aliaksandr, 292 Keeratikunakorn Krittika, 150

Kemper Nicole, 367, 701, 895, 912, 920 Kerenyi Katalin, 201 Kerherve Vincent, 454 Kerphérique Stéphane, 668, 911 Ketphan Warisa, 573, 574 Khadem Alireza, 8 Khang Duong Nguyen, 914 Kharief Sarah, 870, 874, 889 Khatri Meenakshi, 223 Khine Nwaii Oo, 584, 620, 696 Kiene Frederik, 430, 456 Kiesel Gabriela, 327 Kiesewetter Clara S., 317 Kieu Minh Tuan, 624, 653 Kikuti Mariana, 2, 50, 131, 206, 213, 260, 262, 280, 315, 327 Killian Mary Lea, 11 Killinger Manuela, 408 Kim Aro , 527 Kim Chong-Han, 258, 263, 291, 493, 666 Kim Dae-Min, 232 Kim Geundong, 791 Kim Gyurae, 291 Kim Jeonghoon, 198 Kim Jin Soo, 85 Kim Jineui, 283 Kim Jiyoun, 283 Kim Keun-Ho, 527 Kim Min Ho, 790, 828 Kim Seung-Chai, 232, 282 Kim Won-II. 282 Kim Yeon-Hee, 248 Kim Yong-Gwan, 828 Kim Yongkwan, 790 King Vickie, 39 Kinsley Keith, 12 Kiorala Sulove, 634 Kipschull Kerstin, 415, 421 Kirkwood Roy, 35 Kirwan Susanne, 20, 722, 783 Kittawornrat Apisit, 234 Kjærgaard Boldsen Søren, 228 Klahr Fritz Mikaela, 63 Klarenbeek Sjoerd, 156, 289, 433 Klein Ulrich, 356, 358, 486, 535, 615, 616, 629 Kleine Kruthaup Franziska, 894 Knap Pieter, 915 Knecht Christian, 188 Knol Egbert Frank, 104, 915 Knörr Andrea, 103 Knox Robert V., 395 Kobialka Rea Maja, 718 Koch Michaela, 30, 188, 718 Kochendörfer Eva, 906 Köchling Monika, 218, 278 Kocik Marcin, 554 Kock Jan, 796 Koenders - Van Gog Karien, 240, 301, 538, 811, 817, 857 Koenig Sven, 903

Koger Simone, 30 Koharn Anchitta, 146 Kok Jeanette, 301 Kolesnikov Alexander, 692 Költö Karola, 535 Komolpis Kittinan, 395 Kondratiuk Radoslaw, 391, 402, 568, 916 Kongpanna Panumas, 86 König Emilia, 673 Könighoff Philipp, 532 Kornhoff Thomas, 189 Korntipasasi Jirut, 836 Koskikallio Hilkka, 107, 730 Kouznetsova Alexandra, 236 Kpogo Agbee, 712 Krantz Seth, 198, 711, 782 Krasaesub Sittipoj, 159, 539, 660, 882 Krebs Shelby, 72 Kreienbrock Lothar, 716 Kreizinger Zsuzsa, 535 Krejci Roman, 135, 340, 529, 549, 559, 618, 637, 641, 662, 667, 752, 767, 846, 872, 873, 877, 886, 887 Kreutz Luiz Carlos, 184, 488, 564, 798, 839, 845 Kreutzmann Heinrich, 30, 42, 156, 188, 240, 521, 824 Kristensen Charlotte Sonne, 47, 613, 719 Kroeger Molly, 102, 182, 281, 800 Kroneman Annelies, 15 Krueger David, 652 Krueger Karen, 134, 292, 479, 497 Kuchiishi Suzana, 475, 541 Kucinska Izabela, 239, 878 Kuecker Kurt, 429 Küker Susanne, 70 Kukurtcu Bulent, 785, 786, 787, 788 Kümmerlen Dolf, 87, 381, 691, 740 Kunstmann Lars, 424, 467 Kunzler Souza Carine, 187, 245 Kuo Hung-Chih, 257, 331, 498, 542, 560, 566 Kureljuši Branislav, 121 Kurschilgen Lars, 112 Kutzer Peter, 163 Kvisgaard Lise Kirstine, 129 Kwon Hyeok-II, 790, 828 Kyutoku Fumiaki, 910 L Fiúza Aparecida, 290 L Shinkawa Abner, 403, 409 L. Baker Amy, 241, 288 Labronikou Georgia, 551 Labuscagne Annie, 154 Lacerda Shinkawa Abner, 775 Ladinig Andrea, 19, 30, 42, 45, 52, 64, 157, 185, 188, 226, 286, 718, 824 Lager Kelly, 270, 298, 298 Lai Cong Danh, 353 Laitat Martine, 446 Lakkanasombat Wirod, 414 Lallas Aimilios, 439 Lambertz Jacqueline, 51 Landeau Emmanuel, 89, 789

Landero Jose, 91, 771 Lang Alexander, 378 Langford Paul R., 599 Lantz Kristina, 11, 479 Lara Horacio, 205 Larcher Fabien, 676 Larenas-Muñoz Fernanda, 253 Larsen Erin, 704, 892 Larsen Inge, 698, 719 Larsen Michael Albin, 47, 340 Larsen Lars Erik, 129, 169, 230, 305, 308 Larska Magdalena, 769 Lasa Carlos, 656 Lasierra Mayte, 56, 627, 644, 851 Laszlo Varga, 369 Lau Shu Xin, 207, 427 Launay Ferdinand, 422 Lauterbach Nicholas, 468 Lawananon Phawinwit, 393 Laza Cristina, 682 Lázaro Sofía, 545 Le Thi Thu Ha, 229, 322, 614 Le Van Phan, 8 Le Thi Khanh Dung, 255 Le Dimna Mireille, 99 Le Potier Marie-Frédérique, 99 Le Thanh Hien, 720 Leblanc-Maridor Mily, 386, 387 Lebret Arnaud, 34, 53, 284, 314, 400, 755, 835 Lechner Mirjam, 906 Lecznieski Luiz, 460, 702, 822, 847 Ledur Mônica C., 37, 413 Lee Eungyeoung, 265 Lee Haeyoung, 503 Lee Hyejeong, 503 Lee Jia Xin, 207, 427 Lee Joo Young, 790, 828 Lee Seung-Chul, 790 Lee So-Min, 258, 291, 493 Lee Sungseok, 504 Lee Young-Min, 283 Lee Somin, 263, 666 Leenknegt Stijn, 547 Leetham Shannon, 169 Lefebvre Aline, 744 Lefevre Claire, 238 Lehebel Anne, 43 Lehnert Silke, 185 Lei Ting, 426 Leiding Claus, 906 Leijon Mikael, 63 Leite Fernando, 66, 480, 485, 544, 547 Leitner Natascha, 226 Lelièvre Maïté, 221, 361 Leneveu Philippe, 49, 339, 355, 744, 757 Leppkes Viviane, 736 Leuwerke Brad, 213 Lewandowski Eric, 43, 523, 744, 757 Lewko Renata, 357

Lewko-Wojtowicz Renata, 762 Leyva Baca Ivan, 279, 347 Li Chong, 217, 297 Li Ganwu, 57, 80, 134, 471, 476, 479, 495, 546 Li Jun, 608 Li Peng, 309 Li Wenli, 92 Li Yanli, 300 Li Yanwen, 599 Liang Ting-Yu, 257 Liao Che Wei, 560 Lichterfeld Hanna, 189 Lichty Clint, 126, 328, 674 Lillie Brandon N, 565 Lillie-Jaschniski Kathrin, 132, 169, 218, 266, 267, 278, 296, 302, 311, 323, 344, 355, 357, 360, 583 Lima Ana Clara A. De, 550 Lima Civilo, 510 Lima Isadora De Sousa, 563, 621 Lima Marcelo Dourado, 778 Lima Tarik Mohallem Pereira, 192 Limozin Adrien, 219, 220, 490 Lin Hongyao, 133, 510, 849 Lin Nien-Nong, 268 Lin Pei Ju, 498, 560 Lin Shuangxi, 847 Lin Yuan, 565 Linatoc Marlon, 334 Linhares Daniel, 16, 69, 77, 242, 309, 351, 497, 541 Lino Fiuza Aparecida, 598, 636 Lipori Chiara, 104 Lippke Ricardo, 841 Lippolis John, 540 Lisa Nier, 367 Lisgara Marina, 881 Little Erin, 485, 544 Liu Can, 354 Liu Cathy, 618 Liu Hsin-Meng, 268, 316 Liu Xin, 198, 921 Llamas-Amor Eva, 296, 323, 432, 502, 533, 562 Llopard David, 692 Llopart David, 551 Llorens Anna, 10, 809 Llorens Josep, 581 Lo Dan Yuan , 498, 560 Loewen Matthew, 712 Lohaprom Kriangkrai, 882 Lohkamp Florian, 897 Loncaric Igor, 19, 42, 45, 64 Lopes Idael Matheus Góes, 778 Lopes Pedro , 440, 469 Lopes Ferreira João Vitor, 775 Lopez Itzcoatl, 389 Lopez Sébastien, 484, 487, 579, 594 López Islas Jonathan J , 515, 571 López Martínez María José, 338, 432, 502, 533, 562, 592 Lopez-Moreno Gustavo, 115, 201, 764 Lorenzen Bjørn, 167, 228, 678, 697

Lorenzi Valentina, 909 Lorgere Jean Claude, 744 Lösing Hendrik, 421 Lostao Yolanda, 626 Loucks Aaron, 522 Lourenço Matheus, 496, 639 Low Suet Ee, 207, 427 Lowary Todd, 95 Löwenstein Frederik, 371, 898 Lozano Bernardo, 205 Lu Gang, 2103 Lu Yue, 595, 797 Lucia, Jr. Thomaz, 37, 413 Lúcio Dos Santos Daniel, 164, 377, 483, 501, 543, 548, 625 Lúcio Dos Santos José, 164, 377, 483, 501, 543, 548, 625 Lückstädt Christian, 341 Ludwig Takeuti Karine, 374, 376 Luehrs Adrian, 587 Luevano-Adame José De La Luz, 348, 389 Lüken Caroline, 147 Luo Leo, 783 Luo Tzu-Po, 825 Lupi Jose, 669, 714 Luppi Andrea, 169, 418 Luque Inmaculada, 494 Lütke-Dörhoff Michael, 81 Luyten Nikkie, 450 Luzynski Wojciech, 465, 861 Luzzago Camilla, 21 Lyons Wesley, 92, 782 M. H. Heegaard Peter, 698 MaKa Agnieszka, 842 Maala Carlo, 460, 602, 605, 609, 860, 908, 925 Maas Alexander, 482 Macedo Nubia, 55, 162, 165, 471, 479, 480, 497, 519, 546, 622 Machado Gustavo, 68, 208 Machado Isadora Fernanda, 69, 309, 680 Macías-García Marta, 254, 304, 346 Madigan Jamie, 468 Madrid Jose Antonio, 44 Maeda Chieko, 570 Maes Dominiek, 101, 105, 190, 685 Magalhães Edison, 69, 108, 242, 429, 442 Magallon Pablo, 714 Maglie Luigi, 383, 410 Magnabosco Diogo, 374, 376 Magpantay Ronan, 510 Magstadt Drew, 495, 497 Mah Choewkong, 797, 863 Main Rodger, 80, 210, 242, 351, 471, 479, 495, 497, 622, 711 Mainar Raúl C., 813 Mainquist Christine, 309 Maiqués Ester, 626 Mair Kerstin H., 188 Maisano Antonio Marco, 472, 909 Makau Dennis, 315, 327

Makovska Iryna, 677 Makrai László, 466 Malcher Clarisse Sena, 657, 808 Maldaner Vitória, 798 Malgarin Carolina, 227 Malicharn Suwat, 721, 727 Malik Mateusz, 105 Manche Léa, 700 Manders Vera, 240 Mangarova Nevena, 832 Manoli Claire, 386, 387 Manso Alberto, 864 Manzanilla Edgar Garcia, 502 Manzano Rodrigo , 555, 600, 843, 853 Maprang Roshorm Yaowaluck, 320 March Ricard, 869, 812 Marchand Dominique, 454 Marco Stingelin Giovani, 397 Marcos Marcial, 4, 246, 405, 859 Marcos-Cienfuegos Marcial, 259, 682 Maria Cavalcante Granja Manuela, 501, 543, 548, 625 Maria Ferreira Passos E Prado Marques Brenda, 648 María Lucía Quinto-Aviña, 396 Marika Genzow, 823, 884 Markin Alexey, 288 Marlonpereira Erick, 149 Marois-Crehan Corinne, 43 Margues Mariana Groke, 37, 394, 413 Marra Cristina, 143 Marroco Maria, 111 Martelo Marciano . 313 Marthaler Doug, 172, 319 Martin Elise, 279 Martin Madeleine, 67 Martín Desirée, 272 Martín Marga, 300, 807 Martín Margarita, 834 Martin Del Campo Carlos, 884 Martin Valls Gerard Eduard, 169 Martinez Antonio, 848, 867 Martinez Elena, 545 Martínez Rubén, 875 Martínez Alborcia Maria José, 406 Martínez Gómez Daniel, 515 Martínez Martínez Sonia, 58, 826 Martínez Subiela Silvia, 432, 592 Martínez-Alarcón Laura, 44, 89, 148, 352, 464, 738, 789 Martínez-Bautista Rebeca, 254, 346 Martinez-Castañeda Francisco Ernesto, 348 Martínez-Gómez Daniel, 571 Martinez-Lobo Javier, 259 Martínez-López Beatriz, 198, 476, 546, 743, 921 Martínez-Mercado María José, 254, 346 Martínez-Moreno Anselmo, 148, 420 Martínez-Nicolás Antonio, 44 Martins Sergio, 487 Martin-Valls Gerard, 275, 300 Marucci Baccin Eliane, 397 Marx Jaqueline, 832

Mascher Sebastian, 805 Maschhoff Amy, 77 Masserdotti Marta, 21 Mata Carolina, 405 Matas-Quintanilla Marta, 76, 166, 647, 699, 871 Mateu De Antonio Enrique M., 169 Matias-Ferreyra Franco, 242, 351, 495 Matiašková Katarína, 114 Matiašovic Ján, 114 Matté Fabrizio, 368, 370, 374, 376 Mayer Leonie, 185 Mayer Zoltán, 139 Maynard Bertrand, 744 Mayola Albert, 812 Mazerolles Philippe, 135, 618, 641, 662, 767 Mcauliffe Shane, 749 Mcdermott Katie, 777 Mcdowell Emily, 833 Mcglone John, 110 Mear Xavier, 676 Mebumroong Sunit, 133 Mechler-Dreibi Marina L., 808, 820 Medeiros De Almeida Jonathas, 775 Medina Aurelio, 596 Medina Salvador, 596 Medrano Miranda, 729 Meedom Leif, 424, 467 Meester Marina, 141 Mehmedi Blerta, 70 Mei Xiaowei, 659, 578 Meijer Adam, 15 Melero Mireya, 545 Melini Marcello, 131, 206, 213, 260, 262, 315 Mellagi Ana Paula, 564 Mellencamp Martha, 453 Melo Cecilia Archangelo Ferreira, 893 Membrebe Juver, 349 Mencía Ares Óscar, 826 Méndez Olvera Estela T, 515, 571 Mendez-García Vicente, 785, 786, 787, 788 Mendonça Pascoal Lívia, 22, 534, 563, 621, 803 Mendoza-Elvira Susana Elisa, 384, 515, 571, 661, 664 Mendoza-Magén Noelia, 158 Meneguzzi Mariana, 13 Menin Álvaro, 313 Menjon Rut, 4, 246, 259, 405, 682, 859 Meppiel Laurianne, 725 Merenda Marianna, 434, 535 Mesa Juan Carlos, 449 Messager Ingrid, 237 Metzler-Zebeli Barbara, 30 Meyer Daniel, 155 Meyns Tom, 301 Mezzina Ana Lígia Braga, 180, 192 Michael Alyona, 55, 473, 495, 497 Michalik Edyta, 391, 402, 568 Michiels Annelies, 623 Middendorf Theresa, 482 Miguel Joaquín, 159, 173, 336, 363, 539, 567, 612, 631, 692, 709, 742, 750, 794, 848 Miguélez Pérez Rubén, 58, 826 Migura-García Lourdes, 477 Milano Margot, 219 Mil-Homens Mafalda, 309 MiliEvi Vesna, 121, 362 Miljkovic Vladan, 115, 764 Miller Moana, 701 Millet Sam, 455, 776 Milovanovi Bojan, 121, 362 Mingkai Lei, 324 Miotto Rovian, 313 Miotto Ternus Eduardo, 368, 370, 374, 376 Miralles-Chorro Aida, 76, 166, 562, 699, 859, 871 Miranda Joel, 269, 694, 731, 806, 807, 814, 834, 838, 866, 869 Miranda Da Silva Ariane, 374, 376 Misener Marty, 126, 328 Mitjana Olga, 36, 754 Mo In-Pil, 828 Mogollon José Darío, 342 Moine Sandrine, 221, 279, 337, 347, 361 Molina-Barrios Ramón Miguel, 348 Møller Kristian, 697, 747 Mollett Benjamin, 169, 223 Molnár-Nagy Viviána, 27, 139, 385 Molossi Franciéli Adriane, 274 Mondin Paolo, 434 Monecke Stefan, 64 Monnerais Baptiste, 645 Montané Giralt Jordi, 189, 512, 794, 812 Montassier Hélio J., 808, 820, 855, 876 Monteiro Divanildo, 724 Monteiro Matheus Saliba, 31, 83, 90, 149, 174, 179, 180, 192, 455, 893 Montovani Graziano, 121 Montoya Maria, 3 Moon Sung Hyun, 232 Moorkamp Lars, 650 Mora Franques Jordi, 487 Mora-Diaz Juan Carlos, 110, 160, 293 Moraes Daniel, 16, 309 Moraes Paulo, 78 Morales Joaquin, 864 Moreira Fabiana, 37 Moreira Petri Fernando Antonio, 447 Mores Marcos A.Z., 37, 413 Morgan Jana, 66 Morgenstern Rebecca, 115, 402 Mori Ana Paula, 184, 488, 564, 798 Morillo Alujas Alberto, 314, 835 Morin Jérémy, 902, 907 Morizur Gaetan, 725 Moro Sabrina, 461 Morral Alba, 812 Morris Andrew, 675 Mortensen Preben, 303, 589, 599, 613, 881

Moser Katja, 733

Moser Ralf, 675

Mostert Dorothea, 119 Mostin Laurent, 101 Motta Jennifer Soutter, 893 Motta Pedro, 841 Moura Cesar, 16 Mtaallah Oumaima, 751 Muangpaisarn Chonnatcha, 451, 885 Mück Sophia, 805 Mueller Adam, 12 Mugabi Robert, 57, 471, 476, 497, 546, 711, 921 Muhammad Salman, 7, 727 Muhammed Yusuf Satici, 208 Mulberry Lance, 702 Muller Vincent, 43 Müller Annemarie, 770 Müller Uwe, 183 Mulvenna Christina, 734 Mun Jun Young, 85 Mungthisarn Kwankla, 660 Munguia-Ramirez Berenice, 214, 215, 250, 287, 333 Muñoz Jose Antonio, 760 Muñoz Prieto Alberto, 338, 432, 562, 592 Muñoz-Luna Antonio, 44, 445 Muñoz-Prieto Alberto, 296, 323, 502, 533 Muns Ramon, 734 Münster Pia, 587 Munsterhjelm Camilla, 107, 730 Murciano Francisco, 44, 89, 267, 296, 302, 323, 352, 360, 420, 464, 647, 738, 789, 877 Murillo José Alberto, 445 Murrav Deb. 65 Musse Susanne Leth, 271, 528 Musser Rob , 414 My Yang, 217, 297 N'Guetta Eric, 722 Naderer Lena, 194 Nadlucnik Eva, 748, 758, 918 Nagy Eszter Zsófia, 466, 535 Nai Giorgia, 434 Nally Jarlath, 540 Nalovic Alexis, 454 Nantakhruea Sunaree, 82, 178, 885 Naruekatpichai Gaemgaew, 146 Nascimento Ray, 403 Nathaniel Alec Rogers-Montoya, 458 Nauwynck Hans, 243, 307 Nava Aileen, 856 Navasakuljinda Wichian, 345, 451, 863, 885 Nebbia Patrizia, 142 Nedbalcová Kate?Ina, 114 Nedosekov Vitalii, 70 Neleman Hans, 538 Nelli Rahul Kumar, 160, 293 Nerem Joel, 57, 485, 544, 833 Neto Ricardo, 450, 454, 722, 724, 761, 816, 824 Neubert Saskia, 430 Neveau Thomas Megan, 288 Newkirk Rex, 712 Never Daniel, 532

Ngamwongsatit Natharin, 18, 150 Ngo Thi Ngoc Tram, 212, 255, 353, 631 Nguyen Bach, 908 Nguyen Bang, 725 Nguyen Chau Nguyet Anh, 610, 653 Nguyen Dung, 349 Nguyen Huy, 602, 860 Nguyen Ngoc Hai, 849 Nguyen Non, 725 Nguyen Pham Huynh, 610, 653 Nguyen Phong, 602, 908 Nguyen Thi Bich Nga, 229, 614 Nguyen Thi Dieu Thuy, 229 Nguyen Van Giap, 349 Nguyen Van Tam, 8 Nguyen Huy, 908 Nguyen Minh Nam, 212, 255 Nguyen Ngoc Hai, 229 Nguyen Tat Toan, 212 Nguyen Thi Kieu Trinh, 255 Nguyen Thi Phuong Trang, 212 Nguyen Thi Thu Nam, 212 Nguyen Pham Huynh, 624 Nguyen Thi Bich Nga, 322 Nico Wertenbroek, 643 Nicolaisen Thies, 70 Nicolaisen Thies Jesper, 430, 456, 897 Nicolazo Théo, 34, 53, 284, 314, 400, 755, 835 Nicolino Marcos Vinicius Batista, 192, 893 Niederberger Arthur, 751 Nielsen Daniel, 162, 495, 622 Nielsen Elisabeth Okholm, 129, 230 Nielsen Mai Britt Friis, 98 Nielsen Niels Peder, 125 Nielsen Almeida Ferdinando, 839, 845 Niemyjski Rafal, 428 Nienhoff Hendrik, 122 Niepold Jeanette, 456 Nikaido Sae, 463 Nikolaou George, 388 Nikolic Marko, 70 Nikunen Sanna, 528 Nilubol Dachrit, 7, 86, 133, 721, 727 Nimmansamai Irawin, 393, 873, 887 Ninet Olivier, 753 Niu David, 303 Niyomtham Waree, 366, 584, 620, 696 Njegovec Peter, 748, 758 Nodar Lorena, 269, 343, 509, 555, 603, 623, 654, 694, 713, 731, 742, 750, 843, 848, 853, 875 Nogueira Giovana S., 525 Noh Hyundong, 591, 630 Noh Sanhyun, 591, 630 Nolan David, 616 Nollet Nathalie, 455 Normand Valérie, 34, 53, 284, 314, 400, 755, 835 Norte Ana. 634 Nosach Roman, 634 Novara Roberta, 143

Novell Elena, 477 Nowaczyk Szymon, 465 Noyes Noelle, 13 Nuanualsuwan Suphachai, 721, 727 Nubiato Keni Eduardo Zanoni, 478, 518 Nueangphuet Phawut, 570 Nunes Tiago, 269, 365, 694, 700, 713, 731 Nunes Reis Amanda, 31, 149 Nuntapaitoon Morakot, 33, 82, 178 Nuntawan Na Ayudhya Supalark, 773 Nur Mahiza Md Isa, 207, 427 Nuyens Max, 79 O. T. Camargo Nathalia, 376 Obregon-Gutierrez Pau, 275, 481, 593 O'Brien Katie, 213 Occón Armando, 406 Ochoa Johanna, 884 Oehlmann Sophie, 513 Oehme Rainer, 371 Oh Yeonsu, 232, 265, 282, 291, 493, 705 Oh Yusik, 460, 503, 504, 582 O'Hara Kathleen, 74 Olagnon Laura, 219, 359, 490 Oliveira Barbara, 496, 639 Oliveira Nathan, 496, 639 Oliveira Carneiro Daniela, 397 Oliveira Filho João X., 97 Oliver Salvador, 266, 321, 865, 890 Oltedal Aslak, 32 Ono Rafael K., 37 Ooi Peck Toung, 207, 427 Oppitz Ferreira Rodolfo, 798 Orengo Juan, 89, 789 Oropeza-Moe Marianne, 32 Orozco Ignacio Bernal, 591, 630 Orozco Victor, 596 Ortega Anethe, 739 Ortega-Cerrilla María E, 459 Ortin Bustillo Alba, 338, 432 Osemeke Henry, 16, 309 Osemeke Onyekachukwu Henry, 50, 235, 238, 431 Ózsvári László. 70 P Lozano Arturo, 370 P Moreira Leticia, 403 P Pavaneli Ana Paula, 370 P Siqueira Amanda , 390, 394, 398, 403, 407 P Teixeira Angélica, 290 Paboeuf Frederic, 99 Padet Tummaruk, 146 Pages Alicia, 490 Paiano Diovano, 368 Pairis-Garcia Monique Danielle, 106 Paladino Eliana, 22, 168, 534, 803 Palazzolo Luca, 444 Pallarés Francisco José, 253 Palma Mario, 389 Palmai Nimrod, 589 Palócz Orsolya, 27 Palzer Andreas, 684

Pamornchainavakul Nakarin, 2, 315, 327 Pan Weive, 801 Panek Robert, 218, 344, 357, 762, 861 Panichpakdee Tichada, 766 Panisson Josiane, 712 Panneitz Ana Karolina, 525, 550, 681, 808, 820 Panyasing Yaowalak, 234 Papadopoulos Georgios, 439 Paphavasit Termsitthi, 605, 609 Papic Bojan, 508 Paploski Igor A.D., 2, 225, 315, 327 Paraboni Frandoloso Gabriela, 488 Parada Julian, 281 Parameswaran Sirisha, 160 Paranhos Da Costa Mateus, 127, 899, 900 Park Gyeong-Seo, 258, 263, 666 Park Jong-Hyeon, 852 Park Yunjeong, 883 Parois Séverine P., 911 Parra Yenny Lucia, 837 Parys Anna, 101 Pasian Isis Mariana, 553 Pastor Josep, 809 Paudyal Basu, 251 Paula Pavaneli Ana, 648 Paula Teixeira Angélica, 598, 636 Paulo Sato Jose, 370, 374, 376 Paulo Sato Jose, 368 Pavaneli Ana Paula, 84 Payne Brian, 704 Pazinato Dias C., 144 Pedernera Romano Cecilia, 812 Pedersen Kasper, 308 Pedersen Ken Steen, 157, 698, 719, 779 Pegoraro Poor André, 855, 876 Peimbert Pablo, 821 Peixoto Jane O., 37 Peixoto Lucas, 78 Pelck Jeanett Snitgaard, 125 Pellis Caroline, 138 Peltoniemi Olli, 32 Pena Romi, 247, 299, 670 Peña-Calzada Kolima, 785, 786, 787, 788 Pendl Wolfgang, 87 Peng Zhong, 516 Penha Letícia Cristine, 478, 518 Pereira Francisco Alves, 83, 174, 179, 775 Pereira Paschoalini Gabriela, 501, 543, 625 Pereira Silva Maria Clara, 563 Perelló Jiménez Alberto, 826 Peres Rubio Camila, 338 Perez Daniel, 241 Perez Monica, 10, 246 Pérez Héctor, 254 Pérez Esteruelas Lorena, 585, 607 Pérez Zapater Jose Ignacio, 343 Perez-Calvo Estefania, 913 Perez-Perez Lucia, 256, 491, 572, 580 Peric Dejan, 772

Perrucci Alice, 142, 728 Pesch Stefan, 311 Petitpas Brigitte, 907 Petri Fernando M., 525, 550, 657, 681, 808, 820, 830, 855, 876 Petri Rogério, 184, 798 Petrocchi Rilo Máximo, 58, 826 Pettersson Emelie, 46 Petznick Thomas, 309, 680 Pey Ariadna, 3, 204, 247 Pham Giang, 602, 908 Pham Thi Hai Trang, 322 Phan Truong, 631 Phetphloy Narongphol, 120 Photiphat Phatchara, 721, 727 Phuttapatimok Sahathat, 345, 438 Piccinin François Elisa, 20 Piehler Daniel, 326 Pierce James, 782 Pieters Maria, 59, 60, 61, 72, 109, 168, 172, 522, 524, 632, 833 Pigozzo Rudy , 507, 541 Pileri Emanuela, 218 Pillatzki Angela, 242, 351 Pimenta Siqueira Amanda, 900, 917 Pina Nunes Telmo, 70 Pinal Fausto, 821, 829, 884 Piñas Iñaki, 813 Piñeiro Carlos, 699 Piñeyro Pablo, 52, 65, 102, 133, 165, 182, 281, 497, 519, 800 Pinheiro Moreira Letícia, 775 Pinheiro Paes Geísa, 164, 377, 483 Pinsard Gaëtan, 99 Pinyocheep Natchakarn, 146 Pirágine Márcia Richena, 505 Pires Oliveira Dayanne Kelly, 775 Pires-Alvez M., 100 Pissetti Caroline, 274, 290, 313, 507, 525, 541, 598, 636 Pittalis Livio, 62, 261 Platero José L., 445 Plawska Daria, 554 Plessis Mathieu, 454 Plourde Lori, 244 Plut Jan, 508, 748, 758, 918 Poeikhampha Theerawit, 176 Pogranichniy Roman, 319 Poissonnet Alexandre, 386, 387 Pol Françoise, 668, 911 Poleze E., 144 Pollmann Steve, 91, 436, 443, 771 Pollock Yvette, 675 Polo Emilio, 36 Polo Gina, 342 Polutov Dmitry, 692 Polyiam Kanokporn, 320 Polzer Daniel, 569 Pomorska-Mol Malgorzata, 239, 428, 861, 878, 879 Pongpan Tawat, 366

Poolperm Pariwat, 120, 414, 438 Portal Ximena, 429, 442 Pospissil Garbossa Cesar Augusto, 31, 83, 90, 106, 149, 174, 179, 180, 192, 455, 893 Posta Katalin, 139, 385 Postal Cristian, 461, 529, 559, 872, 881 Postma Merel, 685 Poulain Gilles, 668, 911 Poulin Marie-Claude, 96 Pourquier Philippe, 219, 220, 359, 490 Prakobsuk Phakawan, 234 Prapasarakul Nuvee, 366, 584, 620, 696 Prapattanakullawong Apichart, 451, 885 Prarat Melanie, 242, 351 Prataviera Davide, 434 Pratt Emily, 309 Praxedes Campagnoni Igor, 368, 370, 374, 376 Preault Florine, 448 Preesong Pattaveekan, 82, 178 Prenger-Berninghoff Ellen, 587 Preveraud Damien, 88 Prieto Cinta, 259 Prieto Patricia, 321 Prigol Simone, 564 Priller Florian, 224 Prior Keila, 507 Probst Jeanette, 895, 920 Prodanov-Radulovic Jasna, 70, 121 Prodell Marie, 92 Prosperi Alice, 169 Prot Manuel, 371 Prucksayoungyuen Worrapon, 887 Prüst Herman, 200, 332, 514, 521, 557, 765 Przyborowska-Zhalniarovich Paulina, 762 Puente Héctor, 256, 572 Puig Ainhoa, 869 Puigredon Alba, 336, 512, 794, 814, 838 Pupillo Giovanni, 418 Purser Max . 414 Puspitarani Gavrila A., 308 Putrino Callabria, 175 Puyal-Rucine Célia, 43 Pwint Thu Phoo Pwint, 35, 118, 392, 671 Qigai He, 324 Quentric Olivier, 907 Querubin Jefrey, 509 Quijada Anne, 279, 337, 347, 361 Quinonez Angie, 5 Quintero-Ramírez Víctor, 661, 829 Quintiliano Beatriz, 917 Quirino Monike, 37, 413 Qvist Pawlowski Mia, 271 R De Conti Elisa, 290 R. Hutter Carl, 241 Rackova Barbora, 411, 506, 520, 561 Radanovic Oliver, 619 Rademacher Christopher, 57, 77 Radicchi Campos Lobato De Almeida Fernanda, 28, 403, 409, 775, 778

Raele De Oliveira Eduardo, 84, 648 Raff Lena, 12 Rahe Michael, 495, 497 Raineri Camila, 106 Raineri Stefano, 909 Raj Jog, 496, 639, 655, 772, 880 Rajska Magdalena, 14 Ramis Guillermo, 44, 89, 148, 267, 296, 302, 323, 352, 360, 420, 445, 464, 562, 592, 647, 738, 789, 877, 886 Ramos Carolina Pantuzza, 815 Ramos Prigol Simone, 488 Rampon Cendron Natália, 138 Ramsay Andrew, 223 Ran Q, 319 Ranathunga Lakmal, 252, 277 Randt Andreas, 194 Ratanamongkolmas Apichaya, 414, 474, 573, 574, 610, 658,766 Rathkjen Poul Henning, 350, 379, 687, 924 Rattana Manassanan, 451 Rattanavanichrojn Nattavut, 438 Rauh Rolf, 160, 511 Ravichandran Sayujya, 450, 722 Rawal Gaurav, 134 Recchia Matteo, 318 Reck Carolina, 313 Reckels Bernd, 897 Reese Sven, 40 Reever, Ms, Dvm Amanda, 683 Rega Martina, 23 Rego Ribas Juliana, 127, 899, 900, 917 Reif Johannes, 286 Reimert Inonge, 104 Reimus Fenja, 371 Reiner Gerald , 191, 193, 903, 906 Reinmold Jennifer, 701 Reis Adrienny T., 525 Remmenga Marta, 74 Rémond Morgane, 43 Ren Jinglei, 659 Ren Wen. 913 Rendahl Aaron, 72, 168 Rendón Carlos Enrique, 449 Renken Christine, 25, 26, 137, 372, 796 Renoult Christophe, 448 Renzhammer René, 45, 286, 326, 718 Revesz Tamas, 218 Revilla-Fernández Sandra, 51, 378 Revnaga Julia, 821 Ribeiro Willian, 917 Ribeiro Krasilchik Sarah, 138 Ricardo Bárcena-Gama, 459 Richard Gautier, 169 Richt Juergen, 319 Richter Agnes, 898 **Rigaut Martial**, 448 Rigauts Charlotte, 75, 243, 672 Righetti Verdiana, 434 Rigo Natalia, 138

Risco David, 272 Ritzmann Mathias, 17, 40, 132, 311 Rivera-Benitez Jose Francisco, 203, 254, 304, 346, 854 Rizzardi Alessia, 444 Roady Patrick, 283 Robbins Rebecca, 110, 198, 711, 743, 921 Roberts David, 208 Robino Patrizia, 142 Roca Elisabet, 364, 802 Roca Mercè, 812 Rocha Lluisa, 867 Rodeghiero Kendra, 564 Rodrigues João Victor, 390, 394, 398, 407 Rodrigues Luis Candido, 394, 398 Rodrigues Melissa Alves, 710 Rodrigues Chagas Sarah, 22, 563, 621 Rodrigues De Costa Maria, 749 Rodriguez Jorge, 856 Rodriguez Maria, 864 Rodriguez-Fernandez Juan Carlos, 785, 786, 787, 788 Rodríguez-Gómez Irene Magdalena, 253 Rodríguez-Hernández Elisa, 352, 464 Rodríguez-Ruiz Macarena, 253 Rodríguez-Vega Víctor, 759, 813, 891, 919 Roge Julie, 347 Roggeman Fien, 623 Roggow Brian, 213 Roivira Albert, 242 Rojo Alejandro, 832 Romabon Larra, 925 Romanelli Costanza, 70 Romanov Oleksandr. 849 Romeo Claudia, 472 Romero Facundo, 349 Romero Salvador, 577, 801, 806, 838, 866, 869 Romero-Flores Arianna, 515, 571, 661 Romero-Salmoral Antonio, 494 Rooijakkers Elisa, 152 Ros Fuertes Maria, 669, 693, 714 Ros Párraga Ester, 352 Rosager Weber Nicolai, 228 Rose Nicolas, 169, 668, 911 Rosell Rosa, 236 Rosendal Thomas, 63 Rosero David, 429, 442 Rossi Leonardo, 78 Rossow Stephanie, 260 Rostalski Anja, 194 Rotolo Marisa, 57, 74, 711 Rouco-Yáñez Antonio, 459 Rouillier Jessica, 448 Rousseau Thibault, 676 Rovira Albert, 130, 280, 351 Ruampatana Jakavat, 82, 178 Rubico Joseph, 509, 567, 577 Rubio Pedro, 491, 572, 580 Rückner Antje, 116 Ruczizka Ursula, 30 Rudolph Nicole, 611, 646

Ruedas Torres Ines, 251 Ruiz Emilio, 840 Rümenapf Till, 30, 188, 286, 824 Rupasinghe Ruwini, 476, 546, 921 Ruppitzsch Werner, 45, 64, 569 Rushton Jonathan, 706 Russell Erin, 683, 892 Rustvold Justin, 485, 544 Ruy-Sanchez Marco, 695 Rybkowska Weronika, 14, 305 Rynkowski Jedrzej, 391 Ryt-Hansen Pia, 169 Ryytty Sylvén Kaisa, 41, 46 S. Rajão Daniela, 241 S. Silva Gustavo, 309 S. Sobral Vivian, 127, 899, 917 Saalmüller Armin, 188 Sabaté David, 869 Saewong Jariya, 118 Sagrera Mónica, 236, 321, 809, 818 Sahin Orhan, 57, 162, 471, 479, 495, 497, 546, 622 Saito Fumitoshi, 866, 869 Sakamoto Eigo, 463 Sala Echave Ruben, 714 Salazar Allan, 509, 567 Salguero Francisco Javier, 251, 253 Salogni Cristian, 318 Salvador Cardoso Geovanna, 31, 149 Samanrak Sakchai, 926 Samatiwat Kanokwan, 609, 605 Samngamnim Sukuma, 474 Sanchez Eugenio, 581 Sanchez Pedro, 741 Sánchez Jorge, 76, 166, 699, 871 Sánchez Paloma, 656, 818 Sánchez Sergio, 875 Sanchez Tarifa Eugenio, 891 Sánchez-Betancourt Jose Ivan, 571 Sánchez-Carvajal Jose María, 253 Sánchez-Giménez Paula, 44, 148, 186, 352, 405, 406, 420, 464 Sanchez-Osorio Jonatan, 406 Sandamuen Pathavee, 766 Sanden Jesper B., 424 Sanderman Christiaan, 156, 433, 521 Sandoval Gustavo, 517 Sandvang Dorthe, 556 Sanhueza Juan Manuel, 225 Sankankhum Imporn . 584 Sannö Axel, 737 Sant'Anna Osvaldo Augusto, 808, 820, 855, 876 Santamarta Irene, 489 Santos Lesleyane, 496, 639 Santos Thaís Garcia, 28 Santos Streauslin Jessica, 162, 495, 497, 622 Santucci Giovanni, 909 Santucci Vincenzo Ugo, 909 Sanz Celia, 545 Saraiva Luis Henrique Gouvea, 507, 541

Sarfati David, 205 Sartori Cintia, 461, 529, 559, 872 Sartori Laura, 370 Sátiro De Oliveira Isadora Maria, 775 Sato Jose Paulo, 541 Satomi Kuchiishi Suzana, 488 Sattler Tatjana, 183, 378, 526, 569 Sauter-Louis Carola, 67 Savarit Loic, 220 Savi Božidar, 121, 619 Sbardella Pedro Ernesto, 553 Scali Federico, 21, 472, 909 Scalise Xavier De Freitas Rafaela, 90, 179 Schacht Erik, 460 Schatzmayr Dian, 408 Schiavon Eliana, 444 Schibalski Michael, 827 Schlegel Michael, 652 Schleper Marjorie, 102 Schlindwein Da Silva Gustavo, 138 Schmeisser Jerome, 913 Schmid Martina, 691 Schmoll Friedrich, 378, 569, 526, 51 Schneider Claudia, 194 Schnyder Angela, 740 Schobersberger Thomas, 226 Schoder Gottfried, 286 Schulz Jochen, 367, 81 Schulz Lee L., 708 Schulze-Osthoff Jan-Dirk, 770 Schumacher Loni, 495 Schumans Joyce, 48, 575, 707, 273, 642, 537 Schüpbach Gertraud, 399, 679 Schut Corinne, 565 Schuttert Arjan, 731, 269, 694 Schwabe Ingo, 371 Schwalm-Wunsch Katharina, 898 Schwartz Jake, 61 Schwartz Kent, 495, 497, 480 Schwartz Mark, 213, 72, 61 Schwarz Bernd-Andreas, 326 Schwarz Lukas, 42, 45, 19, 64, 52 Schwecke Haley, 109, 833 Schweizer Matthias, 733 Scollo Annalisa, 143, 142, 728 Seesing Eric, 307, 597 Segalés Joaquim, 272, 706, 170, 197, 111, 10, 246, 681, 321, 809, 236, 558 Segovia Carlos Damian, 563, 621 Segura Mariela, 95, 792, 96, 827 Seidel Anne-Marie, 183 Seifert Annika, 40 Sellars Melony, 675 Sena Gil, 713 Seo Byoung-Joo, 493, 291, 263, 258, 666 Serafini Poeta Silva Ana Paula, 497, 511, 242, 711, 77 Serna Andres, 741 Serod Chinda, 660 Serradesanferm Robert, 812

Serrano Emmanuel, 236 Serrano Michelle Joan, 780 Sester Clement, 913 Seva Juan, 859, 352 Sevastiyanova Milena, 8 Sevin Jean-Luc, 640, 484, 594, 579 Shanahan Serena, 222 Shevchenko Serhii, 669, 714 Shibata Mayu, 806 Shimada Takao, 888 Shin Hyun Sook, 527 Shin Sungho, 582 Sia Bang-Wen , 207, 427 Sibila Marina, 272, 170, 197, 10, 246, 681, 321, 809, 236, 481, 593, 558 Sidler Xaver, 679 Siegrist Michael, 103 Siepker Christopher, 495, 497 Siesenop Ute, 535 Sigmarsson Haukur Lindberg, 183, 736 Sigognault Flochlay Annie, 264 Sigwalt Annie, 387, 386 Silva Anna Paula, 471, 57, 495, 622, 479, 680 Silva Francisca, 549 Silva Gefferson, 900 Silva Gustavo, 16 Silva Inês, 724 Silva Joana, 724 Silva Rodrigo Otavio Silveira, 815 Silva Castro Arthur, 397 Silva Minafra E Rezende Cíntia, 563, 621 Silveira Naiara Cristina Santos, 778 Simão Gustavo Manoel Rigueira, 274, 507, 525, 541 Simetzberger Erwin, 45 Simjee Shabbir, 584, 620, 474, 658, 574, 573 Simões Santana Renato, 841 Simon Gaëlle, 169, 308 Simon-Grifé Meritxell, 512, 814, 794, 838, 336, 812 Singer Randall, 168, 534, 22 Singthana Worachet, 766 Sirisinha Kalyanee, 18 Sitjà-Arnau Marta, 812 Sitthicharoenchai Panchan, 495, 497, 479 Sitthinan Susichol, 766 Sittikorn Traiyarach, 118 Siv Meling, 70 Sjölund Marie, 325, 63 Skadborg Karina, 230 Skovgaard R, 47 Slanikova Michala, 520, 506, 561, 411 Sligar S. G., 100 Smits Han, 589 Smutzer Megan, 799 Snider Timothy, 437 Snitgaard Pelck Jeanett, 6 Snow Casondra, 72, 60 Snyder Celeste, 288, 241 Soares Natalia, 176 Soede Nicoline Maria, 104

Soh Shi-Ling , 207, 427 Sol Cinta, 84 Sola Xavier, 222 Solaja Sofija, 362 Solano German, 867 Solé Marina, 709, 512, 665 Soler-Llorens Pedro, 158 Soliani Laura, 169 Sonalio Karina, 657, 447, 489 Sonna Settasart, 836 Soothiluck Kanjanat, 86 Sopin Supasit, 457 Sørensen Christian, 915 Sorrasittsuksakul Burin, 721, 727 Sotillo Juan, 76 Soto Maria, 724 Souquière Marie, 911, 668 Souza Rodrigo, 370 Souza Thayanne Gabryelle Viana, 815 Soy Judit, 812 Spanoudes Kyriakos, 388 Spergser Joachim, 42, 535, 64, 52 Speridião Silva Neta Clarice, 775 Sperling Daniel, 264, 415, 421, 462, 461, 441, 449, 638, 858, 725, 864, 47, 393, 586, 425, 412, 738 Spiekermeier Ines, 54, 690, 465, 155, 161 Spindler Christian, 744 Sponheim Amanda, 632, 524, 800, 102, 833, 485, 544 Spricigo Alessandro, 798 Springer Sven, 858 Srisang Chutikan, 401, 773 Srisuwatanasagul Sayamon, 33 Stadejek Tomasz, 292, 769, 14, 305 Stadler Julia, 311, 132 Stadler Maria, 188 Stadler Tania, 141 Stahl Chase, 213 Stas Melissa R., 188 Steenaert Martijn, 732, 557, 514, 200, 79, 521, 811, 817, 857, 688, 332, 112 Steeneveld Wilma, 706 Steferl Tim, 758, 748, 918 Stegeman Arjan, 141 Šteingolde Žanete, 347 Steinparzer Romana, 526 Stella Maria Cristina, 142 Stewart Chelsea, 102 St-Hilaire Manon, 862 Stingelin Giovani Marco Stingelin, 505, 518, 478 Stoeger Anna, 569 Stoffregen J. D., 716 Stoian Ana Maria, 247, 3, 204 Storino Gabriel Y., 876, 855, 657 Storms S., 100 Streit Barbara, 408 Strunz Anne Mette, 336, 601 Strutzberg-Minder Katrin, 805, 568, 357, 40 Stubert Suzana, 634 Stukelj Marina, 70, 918, 508, 758, 748

Su Chia-Ming, 283 Suárez-Vélez Luis, 664 Subasinghe Ashan, 233, 852 Subramaniam Marina, 712 Sueyoshi Masuo, 492 Sujit Thammarath, 573, 574 Sukchai Sujin, 320 Sun Chunging, 823, 606, 847, 822 Suntisukwattana Roypim, 7 Supimon Natthapong, 366, 696 Susana Elisa Mendoza-Elvira, 396 Suwimonteerabutr Junpen, 401 Suwunwong Monticha, 588 Svennesen Line, 70 Swalla Richard, 453 Swam Hanny, 73, 896, 635, 40 Sykes Abagael, 208 Szabo Istvan, 24, 24 Szur Veronika Gombosne, 369 T Lippke Ricardo, 290, 804, 844 Tabeling Robert, 372, 25, 26, 137, 40 Taechamaeteekul Preechaphon, 773, 395 Taktak Amel, 901, 196 Talaga Piotr, 402 Talummuk Sasiwimon, 414, 658, 574, 573 Tamanini Mayara, 184, 564, 488 Tan Tao, 604, 608, 659, 426, 578 Tang Junyu, 283 Tani Tomotaka, 910 Tansarawut Pakamas, 633 Tantanasarn Stayu, 118 Tantituvanont Angkana, 133, 86, 727 Tapia Edgar, 541 Tarancón Vicens, 477 Tarasiuk Grzegorz, 74 Tarasiuk Michal, 402 Tardío Solans Ignacio, 726, 850 Targhetta Chiara, 209 Tark Dongseob, 265, 232, 705, 291, 493, 282 Tarradas Carmen, 494 Tarrés Joaquim, 299 Tassis Panagiotis, 551, 439 Tavares Meloto Carolina, 149, 31 Taylor Amy Elizabeth, 73, 896, 635, 777 Taylor Lucas, 799 Tchilian Elma Z., 251 Tecles Fernando, 502 Teiga-Teixeira Eduardo, 710 Teiga-Teixeira Pedro, 710 Teixeira-Costa Charlotte, 755, 284, 835, 314, 53, 400, 34 Tejedor María Teresa, 754 Tenk Miklós, 589 Terranova Melissa, 87, 691 Terrones Cristina, 581 Tesche Lippke Ricardo, 598, 636, 922 Tezza Julian Andrés, 256 Thacker Brad, 892 Thanantong Narut, 588, 438 Thanawongnuwech Roongroje, 255, 212, 234

Theuns Sebastiaan, 243, 14 Thi Bich Tuyen, 849 Thibault Eric, 264, 827 Thimm Georg - Friedrich, 920 Thirouard Karine, 448 Thiroux Sarah, 169 Thomas Megan, 312 Thominiaux Stephane, 454 Thomson Jill, 222 Thorup Flemming, 195, 249 Thuring Valentijn, 200 Tignon Marylène, 101 Tippitak Panida, 836 Tirkkonen Taneli, 528 Tobias Tijs, 197, 156, 141, 289, 152, 15, 433, 308 Toft N, 47 Toft Poul, 47 Toledo Leonardo T., 657 Toledo Manuel, 840 Tolstrup Lola Kathe, 687, 924 Tomàs Marc, 628 Tomic Zdravko, 115, 764 Tondo Annalucia, 434 Tonelli Claudio, 434 Tong Hao, 65, 309 Tonni Matteo, 318, 909 Toppari Ina, 70 Torchetti Mia, 11 Torreggiani Camilla, 418 Torremorell Montserrat, 297, 217, 260, 213, 13, 72 Torres Islas Juan Agustin, 205 Torrez Martha, 596 Torrison Jerry, 260, 238 Tóth Gergely, 466 Tóth Lilla, 535 Trabal Alba, 247, 3, 204 Traiyarach Sittikorn, 159, 539, 631, 882, 660 Tran Dai Nghia, 849 Tran Vu. 860. 908 Tran Kien, 631 Tran Vu, 602 Traulsen Imke, 895 Trebault Justine, 448 Tres Dener, 531, 795 Trevisan Giovani, 351, 497, 242, 77, 16, 210, 69, 80, 238, 235, 429, 442, 108 Trittmacher Sara, 189, 650 Trujillo-Ortega Maria Elena, 348, 515, 458, 396, 459 Truven Uwe, 718 Tsai Ching-Ping, 335 Tsai Kuo-Jung, 268 Tsai Shu-Hui, 268 Tschannen Philipp, 87 Tseng Hsin-Hui, 566, 542 Tso Ko-Hua, 139, 385 Tu Pan, 651 Tuanpukdee Keangfha, 660 Tucker Alexander, 57, 471, 711 Tummaruk Padet, 401, 773, 395

Tuominen-Brinkas Miina, 107, 730 Turci Silvia, 237 Turlewicz-Podbielska Hanna, 239, 861 Tvarijonaviciute Asta, 432 Twu Ning Chieh, 160, 293 Tzika Eleni, 551, 439 Ubieto María, 545 Uchiyama Chika, 888 Uchiyama Yuki, 888 Udompoka Santi, 435 Udvarhelyi Eliza, 754 Ueda Toshiki, 492, 570 Uemura Ryoko, 492, 570 Ulguim Rafael, 145, 413, 37 Ullerich Astrid, 805 Ulrich Reiner, 163 Umali Dennis, 334 Unterweger Christine, 226, 718, 185 Uriarte Javier, 475 Uribe Alberto, 821, 884 Ustulin Martina, 209 V Ferreira Soraia, 409 V L Ferreira João, 403, 409 V. Nogueira Danrlei, 376 Vahlenkamp Thomas W., 147, 317, 116, 326 Valcarce Borrás Ferrán, 406 Valdivia-Granda William, 319 Valentine Kobie, 723, 224 Valenzuela Agüí Ceci, 141 Valeris-Chacin Robert, 833 Välimäki Elina, 528 Vallejo Andrés, 441, 449 Valros Anna, 107, 673, 763, 730 Van As Inge Maria, 104 Van Berlo Twan, 301 Van Colen Steven, 703, 753, 48, 575, 707, 273, 537 Van De Weyer Leanne, 436, 443, 771, 91, 863 Van Den Broeke Alice, 39 Van Der Giessen Joke, 15 Van Der Heide Arjan, 79 Van Der Horst Yvonne, 781, 774 Van Der Poel Wim, 141 Van Der Veken Wouter, 467 Van Der Vries Erhard, 15 Van Diemen Pauline M., 223 Van Dongen Katinka A., 188 Van Esch Eric, 731, 269, 694, 365, 512 Van Gelderen Rainier, 450, 761 Van Gisteren Hans. 307 Van Hamme Valentine, 450, 454, 816, 722 Van Hees Hubèrt M. J., 93 Van Herten Wouter, 537, 765, 365 Van Kuijk Sandra, 781, 774, 776 Van Loocke Hilde, 597 Van Schaik Gerdien, 706 Van Soest Jolien, 780 Van Winden Steven, 749 Vandamme Karel, 811 Vande Maele Lien, 780, 85

Vandekerckhove Elise, 547 Vandenbussche Frank, 101 Vandenbussche Geert, 181 Vandendriessche Veerle, 780, 85 Vanderhaeghe Caroline, 121 Vandersmissen Tamara, 75, 243, 672 Vanderwaal Kimberly, 2, 130, 315, 225, 327, 260 Vandoorn Elien, 686 Vangroenweghe Frédéric, 181, 538, 459, 536, 552, 177 Vanhara Jonas, 858, 752 Vannucci Fabio, 274, 534, 525, 803, 22 Vargas Eduardo, 637, 667 Vargas Sánchez Alejandro, 384 Vargas-Bermudez Diana S., 65, 342, 281 Vasiljevi Marko, 496, 639, 772, 655, 880 Vedana Marco, 444 Vega-Munguía Gabriela, 664 Vegter Amber, 66 Veit Dalvan Carlo, 795, 531 Vejnovic Branislav, 880 Vela Alejandro, 714 Vela Bello Antonio, 36, 886, 877 Velarde Roser, 236 Velasco Glorilyn, 334 Velasco Jose, 856 Velazquez Eduardo, 218, 136 Veloso Caroline, 192 Venegas-Vargas Cristina, 799 Venkateswaran Dhithya, 727, 7 Vera Aline, 549 Verbeure Wout, 686 Verduyn Kris, 597 Verhaagh Mandes, 17 Vermeulen Cornelis, 292, 240 Vernunft Andreas, 38 Versillé Nicolas, 889, 870, 874 Veselinovic Aleksandar, 764 Vestergaard Gisle, 756 Veugeois Pamela, 448 Vezzoli Fausto, 909 Viana Fialho Martins Thaís, 543, 548, 625, 501 Vicente-Carrillo Alejandro, 158 Vidakovic Milan, 772 Vidondo Beatriz, 29 Viehmann Miriam, 583 Vieira Furtado Júlio César, 374 Vieira Guimarães Walter, 543, 548, 625, 501, 377, 483, 164 Vigneron Sophie, 380, 640, 422, 645 Viktor De Paula Barros Baêta Jefferson, 377, 543, 548, 625, 501, 483, 164 Vilaca Kevin, 674, 231 Vilalta Carles, 170, 197 Vilaró Anna, 477 Viltrop Arvo, 70 Vincent Baker Amy, 11, 312, 187 Vincenti Valeria, 143 Vink Eline, 70 Vio Denis, 434, 209 Visscher Christian, 784, 140, 81, 897

Visscher Klaas, 307 Vo Thi Hue, 863 Vogelmayr Thomas, 185 Voisin Florian, 448, 594, 579, 484 Volkmann Nina, 367 Von Altrock Alexandra, 430, 456 Von Berg Stephan, 896, 635, 648, 227, 4, 78, 803 Von Und Zur Muehlen Friederike, 372, 137, 171, 25, 26 Vonnahme Kimberly, 453 Voss Thomas, 278 Vraeghe Thomas, 623 Vranken Erik, 923 Vu Huong, 577, 631 Vugts Thea, 419, 416 Vyt Philip, 535 Wache Aurore, 387, 386 Waehner Christoph, 652, 827, 684, 736 Wagener Matthias G., 456 Wahl Lisa, 124 Waldner Karoline, 526 Wallenbeck Anna, 904 Wallgren Per, 46, 63, 41 Wallgren Torun, 41 Walter Marina, 461, 872, 559, 529 Walther Lea, 29 Waltrich Andre Luis, 390 Wang Bin, 651 Wang Chao-Min, 566, 542, 331, 257 Wang Chong, 711, 108 Wang Heng, 2103 Wang Huaishan, 659 Wang Kewen, 797, 354, 216, 595 Wang Liping, 586 Wang Y., 771, 91 Wang Yanjun, 822 Watcharavongtip Parin, 133, 65, 7 Wealleans Alexandra, 816, 722 Webber Hayley, 244 Weber Nicolai Rosager, 698, 230, 697, 169 Wegner Birte, 189 Weide Vinicius, 431 Weinert Lucy, 57 Weiss Marcelo, 488 Weisse Christine, 513 Weissenböck Herbert, 42 Wells Jerry, 57 Wendt Michael, 701, 912 Wentao Li, 324 Werth Courtney, 126, 328, 674, 231 Wesselmann Stefan, 415, 421 Westendarp Heiner, 81 Westin Rebecka, 904 Wideman Greg, 674 Wighton Hayley, 62 Wijnands Thomas, 817, 857 Wilke Volker, 784 Wilkens Mirja, 81 Will Kelly, 309 Will Kelly Jaqueline, 564

Willems Hermann, 191, 903 Williams Todd, 57 Williamson Susanna, 261, 62, 223 Williemsen Marcel, 761, 824 Willkan Mira, 689, 746 Wirasate Supa, 18 Wisser Claudia Salete, 117 Wittenberg-Voges Liza, 456 Woeller Burkhard, 201 Wojciechowski Jaros?Aw, 14 Wongchanapai Pichanun, 885 Wongrujira Krit, 671 Woonwong Yonlayong, 633, 457, 345, 588, 438 Wozniak Aleksandra, 769, 14, 305 Wu Bin, 516 Wu Ching-Fen, 560, 498, 566, 542, 331, 257 Wu Jing, 797, 354, 216, 595 Wu Ming, 709, 612, 173, 363 Wu Pei-Yin, 825 Wuyts Niels, 902, 39 Wymore Brand Meghan, 187 Xiao Haijun, 604, 608, 659, 578 Xiaomei Yue, 50 Yamaguchi Ryoji, 570 Yamsrikaew Unchean, 178, 82, 33 Yang Tianning, 709, 612, 173, 363, 665, 801, 577 Yanguas David, 760, 919 Yasmin Abd Rahman, 427, 207 Ye Danielle, 586, 425 Ye Fangshu, 215, 214, 250 Yen Lu, 160, 293 Yeske Paul, 309, 61 Yim-Im Wannarat, 134, 292 Yimpanfai Luksamee, 609 Yin Chuangcheng, 822 Yin Tong, 903 Yindee Jitrapa, 366, 584, 620, 696 Yong Raquel Li Hui, 427, 207 Yoo Dae-Sung, 705, 291, 493, 282 Yoo Dongwan, 283 Yoo Sung-Sik, 790, 828 Yoon In-Joong, 828, 790 Yoon Soonseek, 527 Young Malachy, 771, 91 Young Nathaya, 605, 926 Yuan Linyong, 354 Yue Xiaomei, 206, 315, 262, 131 Yunbo Jiang, 324 Z. Fraga Alícia, 376 Zaabel Pam. 74 Zablotski Yury, 17 Zaldivar-Lopez Sara, 580 Zandonai Marco Antonio, 407, 390 Zanella Eraldo, 270, 298 Zang Fuyu, 426 Zanin Gabriela, 145 Zanon Caterina, 209 Zapata-Moreno Manuel, 854 Zaragoza Luis, 669, 714

Zaruba Marianne, 30 Zdravkovi Nemanja, 619 Zeeh Friederike, 740 Zein-Phillipson Yasmine, 221, 361 Zeller Michael, 210, 80 Zem Fraga Alícia, 374 Zeng Avery, 135 Zerbielli Fábio, 798 Zhang Danyang, 74 Zhang Jianqiang, 134, 292, 210, 80 Zhang Jun, 586, 425, 618, 412, 135, 641, 662, 303 Zhang Martin, 783 Zhang Ting, 665 Zhang Ze Yang, 425, 618 Zhao Frank, 425 Zhao Yue, 595 Zhou Circe, 783 Zhou Kegang, 363 Zhou Xiao, 170, 197, 103 Zhu Zhanwei, 516 Zimmerman Jeffrey, 287, 215, 214, 74, 511, 234, 250, 333 Zimmerman Silvia, 214, 215, 250, 541 Zizlavsky Marek, 520, 506, 561, 411 Zohari Siamak, 325 Zöls Susanne, 17 Zoppi Simona, 143, 472 Zouharová Monika, 114 Zuckermann Federico, 100, 205, 283 Zuffo João, 500 Zuliani Riccardo, 418 Zurovac Sapundzic Zorana, 362



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