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# PROCEEDINGS

April 14<sup>th</sup>–16<sup>th</sup>, 2021

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Dear Ladies & Gentlemen, colleagues, and friends

The ESPHM 2020+1 was jointly organized by the European College of Porcine Health Management (ECPHM) that further engaged outstanding European pig practitioners (Veterinary Practitioner Council, VPC) and a Local Organizing Committee (LOC). However, the current situation regarding the SARS-CoV-2 pandemic has not improved compared to last year, but it is even worse today. Although vaccination campaigns have started all over Europe, the incidence rates remain high, lockdowns have been implemented in many countries, and it has become evident that travelling and/or meeting is neither possible nor even meaningful in this time. Therefore, the organizing committee – namely the ECPHM Board -together with the Professional Conference Organizer (Vet International) has taken the decision to substitute the physical meeting by an online event.

The ESPHM 2020+1 differs from previous editions of the Symposium that started in Copenhagen (Denmark) in 2009 with 220 delegates. Since the beginning, the congress has evolved at all levels, with more than 1,900 delegates in the edition in Barcelona, Spain, in 2018. This is an evident proof of the increasing interest that the scientific contents of the ESPHM offers, not only to European veterinarians but also to the international community. Although settled in Europe, the ESPHM aspires to be a source of updated knowledge and know-how in its field for the whole world.

None of the above has been and is feasible without the contribution of speakers, delegates, and chairpersons, or without the support of the funding of partners, sponsors, and supporters.

Our deep thanks to all of them; they are the core of the ESPHM 2020+1! Moreover, our deep appreciation to the board members of the ECPHM, the members of the Veterinary Practitioner Council (VPC), and the additional members of the International Scientific Committee since they care for the soul and the spirit of the ESPHM.

We provide the scientific content and valuable information from our partners, sponsors and supporters online. This enables all interested persons to update their knowledge and to stay informed about the most recent scientific and practical achievements in Porcine Health Management. Please enjoy the online content and proceedings of the ESPHM 2020+1.

We sincerely hope to see you all again in Budapest (HU) in 2022!

Heiko Nathues President of the ECPHM

Ш

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The ECPHM is a non-profit organization under the umbrella of the European Board of Veterinary Specialization (EBVS). EBVS recognizes veterinary specialist Colleges and maintains a register of European veterinary specialists (Diplomates). Moreover, EBVS encourages and promotes the enhanced utilization and availability of veterinary specialist services to the public and the veterinary profession. Therefore, the ECPHM is the College that 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.
- Nominations Committee manages and reviews the proposals for nominations in the different committees and board.

The ECPHM activities are supported by a permanent secretariat in Parma, Italy.

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#### PORCINE HEALTH MANAGEMENT

Porcine Health Management (PHM) is an open access peer-reviewed journal that aims to publish relevant, novel and revised information regarding all aspects of swine health medicine and production. The journal provides a venue for global research on swine health and production, including infectious and non-infectious 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 **Joaquim Segalés** (Universitat Autònoma de Barcelona and CReSA-IRTA, Spain).

PHM has been publishing articles since 2015 and it is now indexed in different databases, including the MedLine (PubMed). More importantly, PHM got its first impact factor in 2020 (2.190), therefore, within the first quartile journals within the Veterinary Science section. 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**.

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#### **PIG PROGRESS**

IX PROCEEDINGS

ESPHM 2020+1

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Ceva IDT Campus has a strong veterinary biopharmaceutical portfolio and R&D activities which delivers early promise with salmonella vaccine goahead in several European countries.

Ceva, as a gold Sponsor at ESPHM 2021 is taking the opportunity to communicate the strength in swine vaccines in Europe, being 3rd in the SW biologicals ranking and present the Ceva Lung Program, our exclusive tool to monitor PRDC problems.

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# **KEYNOTE PRESENTATIONS**





# Managing compromised pigs – should veterinarians revisit their role?

PROF. DR. ELISABETH GROSSE BEILAGE University of Veterinary Medicine Hannover, Germany

#### Biosketch

Prof. Elisabeth grosse Beilage, DVM, PhD, Dipl. ECPHM

is a veterinarian working at the University for Veterinary Medicine Hannover, Germany, since 1989; at first in the Clinic for Swine and Small Ruminants and later as a senior scientist at the Field Station for Epidemiology. Area of current research is focussed on animal welfare, clinical evaluation and epidemiology (risk factor analysis for inter- and intra-herd transmission) of infectious pig diseases and control/eradication of these infections. Other areas of expertise are pig herd health management, zoonosis control, vaccination and gross pathology.

#### Abstract

Intensive pig farming on large scale, which can be found in many countries today, is inevitably linked to a stronger focus on economics as compared to extensive or small scale pig farming. As herd sizes have increased during the last decades, the daily work of veterinary practitioners specialised in pigs switched from treatment of individual animals to herd health management aimed at disease prevention. In herd health management the pig population as a whole or groups of animals has moved in the focus of the herd attending veterinarian, who is responsible for the monitoring of infectious and non-infectious diseases, the analysis of trends of production data, the development of vaccination programs specifically tailored for the individual herd, the feeding concepts and many other tasks. Veterinarians usually have a firm conviction that pigs can be effectively prevented from disease or cured by treatment. In fact, diseases going along with high mortality usually can be prevented by appropriate measures leading into a situation in which life threatening diseases or injuries are only seen in single pigs of a herd. This is a noteworthy merit of veterinary medicine but unfortunately not a total success as there is a strong evidence that severely diseased or injured individuals sometimes get lost, when the focus is mainly directed to larger groups of pigs or the whole population of a pig herd (Baumgartner, 2016; Magenschab 2012; Mlak, 2012). Population medicine may harbour the risk to focus on an "average pig" or on the majority of pigs in the sense of "the herd is well when most of the pigs are well" or when herd performance meets the expected level. This perception is eligible as long as individuals are not affected by longer lasting substantial pain or suffer. Studies in Germany and Austria yielded in a noteworthy percentage of pigs with pathological findings indicating longer lasting substantial pain or suffer before having been euthanized much too late; in several cases euthanasia was

failed to assist (Baumgartner, 2016; grosse Beilage, 2017). Prevention of these findings should be an urgent matter in veterinary medicine, which so far has been taken up only in a few publications and guidelines for caretaking of compromised pigs (Lessmann und Petermann, 2016; Millman, 2015; Morrow et al. 2001, 2006, 2010; Pig veterinary Society 2013; Turner et al. 2010; Unterweger et al. 2015; Unterweger 2016).

#### Occurrence of pigs with lesions indicative for longer lasting substantial pain or suffer

A baseline study aimed at elaborating lesions associated with longer lasting substantial pain or suffer occurring in pigs and their frequency of occurrence, was performed in four rendering plants in Germany (grosse Beilage 2017). The pigs where submitted from six federal states comprising regions with high pig density and medium sized farms, medium pig density and very large farms as well as low pig density areas and small farms. The prevalence estimation was based on 57 truckloads with a total of 632 breeding and fattening pigs. The number of piglets was always too high to be counted in the available time. For the description of potential lesions 463 conspicuous pigs were selected from these 632 and additional pigs from more than one hundred different truckloads for which a prevalence estimation was not possible due to the high number of pigs, time and space restraints. The evaluation focussed only on severe lesions the farmer should easily have noticed at his/her farm.

Lesions associated with long lasting substantial pain and/or suffer were found in 13.2 % of the fattening pigs and 11.6 % of the breeding pigs. All these pigs have been diseased for a longer time and would have required euthanasia much earlier than actually performed. Many pigs showed no signs of emergency killing indicating that they died without intervention by the farmer. In pigs with lesions and additionally signs typical for emergency killing 62 % revealed deficiencies in the killing technique (no bleeding after stunning, stunning by penetrative captive bolt in the wrong position, incision for bleeding in the wrong place, percussive blow to the forehead in pigs more than 5 kg bodyweight, etc.).

The lesions associated with longer lasting substantial pain and/or suffer comprise claw and dewclaw lesions, arthritis, lesions induced by tail/ear biting, injured hernia umbilicalis, rectal stricture, skin ulcer/decubitus and cachexia. Figures 1 to 6 exemplarily show the grade of lesions that have been scored as causing longer lasting pain and/or suffer.

The high frequency of lesions causing longer lasting substantial pain and/or suffer reveals deficiencies in caretaking of pigs clearly having no likelihood of successful recovery. As the same findings have been made at all rendering plants involved in the study it is very likely that the problem is occurring all around Germany regardless the federal state or region and is likely independent from herd size.

## Suspected reasons for deficiencies in taking care of severely diseased or injured pigs without any chance to recover

A systematic investigation of reasons why farmers euthanize pigs much too late or even fail to assist euthanasia in pigs without any real chance for recovery is not yet done, but based on many discussions with herd veterinarians and pig farmers the following reasons can be considered:

• Deficiencies in education resulting in misestimating the pig's health status ("not as bad as it is").

- Misestimating the chance for recovery ("still hope for healing").
- Missing empathy ("pigs are robust and don't feel much pain").

- Time restraints of the farmer, resulting in non-detection of individual diseased pigs. Time schedules in pig herds usually are designed for healthy pigs and extra time for caretaking of diseased pigs is not calculated.
- Misinterpreting the own responsibilities for the pigs ("the vet is responsible for diseased pigs").
- Discomfort und insecurity up to complete refusal of killing pigs (combined with the unwillingness to call a veterinarian for euthanasia).

#### **Further implications**

According to the German Animal Welfare Act (Tierschutzgesetz) a person inflicting a vertebrate upon longer lasting or repeating substantial pain or suffer can be gated up to three years or amerced by penalty (§172b Tierschutzgesetz). Keeping severely diseased or injured pigs obviously experiencing longer lasting substantial pain or suffer puts the farmer close to criminal law. Seen from the perspective of the animal and the farmer this needs to be avoided.

#### • Improving the farmer's skills in performing euthanasia in pigs

The first reaction to the results of the aforementioned study was to intensify and improve training courses for farmers imparting theoretical and practical knowledge about killing pigs. In the past, euthanasia in pigs did not play a noteworthy role in the education of farmers. A very brief theoretical instruction in the usage of a captive bolt often was all taught to young farmers. This has been changed immediately and advanced training courses for farmers are regularly offered by organisations like the Chamber of Agriculture. These courses are aimed to convey technical skills as well as a mental training to get the farmers prepared to the reaction of the pigs, particularly when shot by penetrative captive bolt. The distinct convulsions and the bleeding are well-known mental challenges for the farmers.

#### • Improving the farmer's skills to decide when a pig has no chance for recovery.

Euthanasia performed too late or completely failed is not only a problem of the technical skills and mental preparedness, but also a result of uncertainties in the decision making process of the farmer about the chance for recovery. In an ongoing project funded by the Ministry of Nutrition, Agriculture and Consumer Protection in Lower Saxony, Germany, a guideline is compiled by an expert-group of researchers in veterinary medicine, agriculture and biology, veterinary practitioners, pig farmers and consultants. This guideline shall enable pig farmers discriminating between "early enough" and "too late" euthanasia. The project encompasses a comprehensive sampling of cases for each relevant disease and injury. Voluntary participants announce pigs they are convinced to have no chance for recovery to University of Veterinary Medicine Hannover. A researcher is instantly going to the farm, recording all clinical findings, doing the euthanasia of the pig and performing the necropsy. All findings from the clinical examination and gross- and histopathology give the basis for the case evaluation and compilation of the guideline by the expert-group.

#### The role of the herd-attending veterinarian

A distinct problem with severely diseased or injured pigs without a chance for recovery that have been euthanized much too late or failed to assist has been identified in studies in Germany (grosse Beilage, 2017) and Austria (Baumgartner, 2016). Similar problems might exist in other countries. As all herds having such pigs also have a veterinarian, it will help when veterinary practitioners and consultants critically evaluate their work and reflect their responsibilities. Crucial questions for this self-evaluation are: Have I clearly communicated what the farmer needs to do when a

treatment is not resulting in recovery? Is the way I am doing herd examinations appropriate to identify these compromised pigs – even when the farmer is not definitely consulting me for this issue? How do I operate when identifying a pig that needs to be euthanized? Do I offer the farmer performing the euthanasia by myself? Do I offer the farmer assistance? Am I sure, the farmer is having the technical skills and is he/she mentally able to kill a pig? Did I give my clients advices how to decide about the chance for recovery? When all answers are "yes" it is likely that your clients are not belonging to those having the problem. If some of the questions are answered with "no" the conditions in the herds of your clients might need a critical revision.

Veterinarians are not only responsible for prevention and treatment of diseases; they also need to feel responsible for every single pig when recovery from severe disease or injury cannot or not any longer be expected.

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Fig. 1 Deep coronary band abscess and crack in the wall in a claw (breeding sow)



Fig. 2 Arthritis (fattening pig)

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Fig. 3 Tail biting induced lesion and arthritis (fattening pig)



Fig. 4 Injured hernia umbilicalis and prolapse of intestine (fattening pig)



Fig. 5 Rectal stricture and cachexia (fattening pig)



Fig. 6 Cachexia, arthritis and multiple abscesses (fattening pig)



## Free farrowing – status quo and future trends

## DR. VIVI AARESTRUP MOUSTSEN University of Copenhagen, Denmark

#### Biosketch

Vivi received her M.Sc. in Animal Science from The Royal Veterinary and Agricultural University in Frederiksberg, Denmark, in 1995, and her PhD in Animal Science from the same University in 2002. Vivi has been focusing on research and development of housing of lactating sows at SEGES Danish Pig Research Centre (SEGES PRC) since 2001. From 2002 onwards the emphasis has mainly been on development of systems for loose housed farrowing and lactating sows. The research includes determination of sow and piglets dimensions, space needed for important behaviours and criteria for pen design. The aim of SEGES PRC is to develop, test and recommend the best technologies for production of pigs in Denmark.

In 2018, Vivi and her colleagues coordinated the workshop Loose Lactating Sows 2018 (LLS18) (https://www.freefarrowing.org/info/2/research/45/free\_farrowing\_workshops; https://www.pigprogress.net/Sows/Articles/2018/6/Loose-lactation-for-sows-fantastic-and-frustrating-292322E/) – workshop number four in a series of the subject of loose housing of lactating sows.

#### Abstract

Should the sows be outdoors like many are in the UK or indoors like in most other countries with a larger scale pig production? Outdoors looks great at least when the pasture is green, but from a more holistic or system approach, outdoor has challenges or weaknesses for instance when it comes to risk of leaching of nutrients. In indoor production systems, it is possible at the same time to consider climate, environment and welfare – and production economy or PPPP: Pigs (welfare and health), Price (economy), Planet (environment and climate) and People (managers, workplace, attracting skilled caretakers). And an important side effect is that caring for pig health and bio security is easier indoors too.

When working with animals, it is a motivation itself to see the animals thrive. And often the possibility to perform natural behaviour can lead to better welfare for the animals. However, loose housing of lactating sows includes challenges especially when it comes to neonatal piglet survival which limits voluntary implementation of the free farrowing.

Should we continue to use crates and keep the sows confined for the entire time they are in the farrowing unit like many countries do? Or should we ban crates and confinement completely like other countries do? Or can we choose a solution in between? There is no simple answer – or most likely will the answer depends whether we ask the sows, piglets, caretakers, owners, retailers, consumers or citizens – and their answers are likely to be different depending on land of origin.

Status quo is that the only countries where farrowing crates for the entire lactation period are not in use, are countries where they are banned like Sweden, Norway and Switzerland. In other countries, like Australia, some recommend that future efforts focus on improving welfare for sows and piglets when housed in crates instead of moving towards loose housing.

There is no doubt though, that there is an increasing interest and awareness of the possibilities of housing lactating sows loose – and a keen interest across borders to overcome the challenges. In 2008, a limited group of scientists and stakeholders from just a few Northern European countries met and discussed free farrowing. In 2011, the group met again and this time with interest and engagement from more countries. Next meeting was in 2016 – and similar pattern – increasing interest and genuine perception that is the future. In 2018, the group met and this time included stakeholders and scientists from Australia, USA and Canada too. It was no longer a question of 'if' but 'how', and there was an acceptance that implementation of loose housing will be increased if there a transition period with an option to confine for the critical period (https://www.freefarrowing.org/info/2/research/45/free\_farrowing\_workshops).

In Austria, it has been decided that by 2033 confinement of lactating sows will only be allowed until the end of the critical phase of life. In Germany, potential legislation of limiting the period lactating sows are confined is being discussed. In Denmark, in the industry have announced that in the future lactating sows should and will be loose housed – but not at the cost of the competitiveness of the industry and the level of piglet survival must be high.

The Danish pig industry set a goal of 10 % of the lactating sows to be loose housed by 2021. For a number of reasons this will not be accomplished. First, the economic situation has led to very few investments in the industry during the last decade. And farmers that have invested have to some extent chosen crates. Why? Because the investment in crates is lower than investment in pens, the productivity in crates is most likely higher and they are unlikely to receive at premium for having loose lactating sows. However, even though there are reasons why not to invest in loose lactating, many have chosen to do so anyway because farmers are also entrepreneurs, they like to develop their enterprise, they like their pigs, they care for their employees and they care about the society's perception of their business. And successful implementation is likely to be when the implementation is done with an understanding of pigs' needs and an interest of the pigs.

There are many challenges when it comes to implementation of loose housing of lactating sows but for the pigs, their caretakers and producers, and citizens, we shouldn't give up just because we're all challenged. When we collaborate and exchange ideas and thoughts across borders, we develop at a higher pace, all of us and many more pigs benefit.



# National, regional and global surveillance of pig health

PROF. KATHARINA D.C. STÄRK Federal Food Safety and Veterinary Office, Bern, Switzerland

#### Biosketch

Prof. Katharina Stärk graduated as a veterinarian from the School of Veterinary Medicine at the University of Zürich, Switzerland, and then completed a PhD at Massey University, New Zealand, in information systems for the prevention and control of infectious diseases in pigs.

Katharina then worked for two years in Denmark as a research officer for the Danish Bacon and Meat Council, and after that took a post as Head of Section of Monitoring at the Swiss Federal Veterinary Office which she held for seven years. During this time, she also worked as a part-time lecturer in Epidemiology and Veterinary Public Health at the University of Bern in 2000, and from 2002 to 2006 she was a Member of the Executive Board at the Swiss Federal Veterinary Office, Bern.

In 2005, Katharina spent four months as a Visiting Professor at the Graduate School of Agricultural Life Sciences, Faculty of Veterinary Medical Sciences, University of Tokyo, Japan. From 2007 to 2017, Katharina was Professor of Veterinary Public Health Policy at the Royal Veterinary College, London, UK. Katharina had an international role as President of the European College for Veterinary Public Health (ECVPH Website) 2007 to 2009. She remains active in the College as a certified Specialist in Population Medicine.

Katharina was the Director of Science and Quality at SAFOSO (Bern, Switzerland), an internationallyactive consultancy in the fields of food safety and public health, from 2010 to 2019 and is currently the Head of the Department Animal Health at the Federal Food Safety and Veterinary Office in Switzerland.

Her research interests are in food safety risk management, surveillance and veterinary public health policy, including evaluation.

#### Abstract

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This paper aims at providing an overview of current challenges in the design and implication of surveillance programmes in the pig sector. Examples from Switzerland and across Europe will be used to illustrate the relevant aspects shown in Figure 1.



Fig. 1 Aspects of surveillance programmes discussed in this presentation.

Disease control efforts of industries and Veterinary Services were traditionally focused on specific diseases and their causative pathogens. Thus, disease or pathogen lists can be found in national legislation as well as in international standards such as the OIE Animal Health Code. Diseases are included in these lists according to specific criteria such as severity of disease, risk of transmission, zoonotic risk and economic impact. For the majority of such disease or pathogen, a surveillance programme is defined and implemented. If accompanied by successful interventions, the occurrence of a disease or pathogen is reduced and the focus of surveillance can move from monitoring without interventions to reduction to elimination. The ultimate challenge of surveillance is the documentation of disease freedom. Switzerland, for example, is free from PRRS and the majority of porcine pathogens listed by the OIE.

Not all hazard-specific surveillance programmes are straightforward in their design or implementation. For example, diseases such as African Swine Fever, which occur in both domestic and wild animal populations, pose specific challenges. Surveillance of wildlife requires cross-disciplinary collaboration. As wildlife move freely across borders, also international data sharing is essential for early warning. Another example of challenging surveillance design relates to antimicrobial resistance (AMR). As AMR is not a specific pathogen, but rather a characteristic of bacteria, special design challenges exist. The selection of bacteria-substance-gene combinations is typically based on risk. AMR is also a good example to illustrate the need to link surveillance with intervention. Using the example of AMR, surveillance results could affect trade and ultimately become a political issue.

After elimination of a disease or pathogen, or in the case of historical freedom, the focus of surveillance is on early detection and documentation of absence of disease. Early detection depends on the observation of clinical signs that can be unspecific; therefore, broader categories of "syndromes" are needed. Over the past years, substantial methodological progress has been made in the field of syndromic surveillance. The use of routinely collected health-related data is attractive and specific aberration detection algorithms can be developed and evaluated to identify signals in such data streams. However, the routine application of such complex methods is still limited. This is partially due to legal challenges such as data confidentiality, some of which have yet to be resolved. Switzerland has explored the utility of data sets from carcass collection services by rendering plants, meat inspections at slaughterhouses, and necropsy reports from veterinary pathologies. The potential of public-private partnerships in the operation of such surveillance systems is explored in this field.

While traditionally, the focus of surveillance was on specific diseases, there is currently a shift to focus on animal health in general. This trend is reflected in the first **Animal Health Strategy of Switzerland**, which was implemented from 2010. Also the European Commission, in its development of the **Animal Health Law**, aims for a more general focus with strong emphasis on prevention. Similarly to Switzerland, the role of stakeholders and the importance of animal health training for farmers are recognized and addressed. The related legal acts will be implemented from April 2021. Such a strategic shift is bound to have impact on health management as well as surveillance. The Swiss Animal Health Strategy 2010+ was evaluated in 2019, and it became apparent that this strategy had been effective. However, more can be done to share responsibilities with stakeholders and to empower farmers to strengthen prevention. These elements are integrated in the revised version of the strategy which is to be implemented from 2022.

Moreover, animal health-related agricultural policies in Switzerland are being reformed. It is proposed to value animal health as a societal contribution by a farmer as it reduces the need for antibiotics and hence contributes to the prevention of public health risks. Such services should be considered when attributing farm subsidies. However, new indicators are needed to assess "health" in this context. Such indicators are currently being developed as part of a research project. Creating an evidence base for such animal health indicators has significant links to the availability of data and thus to surveillance activities.

Data relevant to pig health are also collected by private actors (farmers and veterinarians) to inform management decisions at farm level. Novel methods such as pen-side testing will accelerate the support of such decision-making. In addition, a reduced price may increase the strategic use of diagnostic testing. In turn, such data can contribute to health indicators and population-focused surveillance. Furthermore, negative results have significant value in an industry benefiting from a documented high-health status.

The link between pig health and public health is another relevant aspect. Surveillance of pig health can therefore influence not only veterinary policies, but can also be used as a basis of public health decision making. Such links are the core element of the concept of One Health. The latter is understood to focus on linkages between disease events across sectors. Interventions in one population can have benefits for health in other populations. AMR is currently the most prominent example relevant to livestock – including pigs – in this field. Surveillance data have allowed to link AMR policy interventions in livestock to public health outcomes. Such linkages exist in Switzerland for influenza surveillance in pigs. Samples are screened for emerging virus strains of public health relevance. Novel diagnostic techniques such as whole genome sequencing will further facilitate the interpretation of surveillance results across compartments.



# Quality and feasibility of pen-site tests for surveillance

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#### **Biosketch**

Gerard E. Martín Valls obtained his veterinary degree in 2007 and Ph.D in veterinary sciences in 2012 in the Autonomous University of Barcelona (UAB). He was researcher in the Centre de Recerca en Sanitat Animal (CReSA-IRTA) from 2013 to 2016. Currently, he is a researcher in Animal Health and Anatomy Department of UAB. His research focuses on the epidemiology, molecular characterization and control of with swine viral infectious diseases such as swine influenza and porcine reproductive and respiratory syndrome.

#### Abstract

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In pig production, surveillance of diseases is essential for decision making. It allows to determine the infection dynamics of pathogens present in the farm, helping thus to determine what control and prevention programs are needed and what measures must be prioritized. Further monitoring will be necessary to determine the efficacy of implemented control measures.

Classically, monitoring was performed by sampling individuals. Some good examples of this are: i) the serological screening for Aujeszky disease eradication programs, or ii) the PRRSV classification of the swine farms. Nowadays, individual sample collection still has a high value in diagnosis. However, for surveillance and monitoring purposes (especially when large populations must be evaluated), individual sampling is labour and time-consuming and requires analysis of many samples to reach adequate sensitivity. Additionally, the manipulation and blood sampling of pigs causes stress and fear to the animals. Fearful animals can react with sudden and unpredictable movements being difficult to handle and may injure the operator. Moreover, when the objective is to monitor the efficacy of the implemented control measures detection of the pathogen is more difficult. The more successful the measure, the lower the prevalence of the pathogen and therefore, the larger the sample size needed to assure sensitivity enough. Insufficient sample size may lead to wrong decisions. Finally, pooling individual samples decreases the total cost of the analysis with an acceptable sensitivity reduction. However, pooling does not reduce labour costs as animals still need to be individually sampled. All these reasons impair the feasibility of individual blood sampling, faeces collection or nasal swabbing, pointing out the need for more representative and workable types of sampling.

In 2008, Pricket and co-workers showed that using cotton ropes for collecting oral fluids at pen level could be cost-effective and a sensitive method for sampling pigs after weaning. This method is welfare-friendly, one person can do it by him/herself and, is more efficient than pooling individual samples as larger populations are examined. This type of pen-based sampling has proved to be useful for both detection of the pathogen or antibodies for a wide range of viruses and bacteria such as PRRSV, PCV2-3, swIAV, PEDV, PPV, Mycoplasma hyorhinis, Mycoplasma hyopneumoniae (only antibodies), Actinobacillus pleuropneumoniae, etc. Oral fluids can be applied not only to monitor infection dynamics, but also to determine the successfulness of a control program (e.g. acclimation of gilts, reduction or elimination of a given virus or bacteria), with higher sensitivity than individual sampling (larger populations can be tested). Data from North American laboratories show how this type of sampling has been increasingly used in recent years. Focusing on PRRSV or IAV, where the use of oral fluids has been studied with detail, it was shown that resulting diagnostic sensitivities are high, even in low-prevalence situations. This is particularly true when weaners or growers are sampled. Growing pigs are highly motivated to explore and chew enrichment materials. Thereby, most growers will chew the ropes, and consequently, large volumes of saliva are usually obtained. Recently, the use of oral fluids has been tested for sampling animals at their arrival to abattoirs. This sampling strategy could be extremely useful to evaluate the efficaciousness of an eradication program, reducing significantly the number of staff displacements for sampling reasons. Moreover, oral fluids have been proposed to be a suitable sample for epidemiological surveillance (antibodies or genome detection) for a number of transboundary diseases: African swine fever, classical swine fever or foot and mouth disease viruses.

However, the use of oral fluids shows several limitations. For example, sampling of very young pigs or adult pigs is difficult. Adults need some previous training to ensure that an enough proportion of animals chew the rope; suckling piglets, do not interact easily with the ropes and the obtained oral fluid volume is scarce. Recent sampling experiments with due-to-wean piglets demonstrated that the method can be feasible and sensitive if obtained as a "family oral fluid" (sow + piglets in the litter). For piglets younger than 3 weeks, efficiency has not been determined. Sampling of suckling piglets is important, for instance, to determine if PRRSV vertical transmission is occurring, or to detect the presence/absence of influenza A virus at early ages. Several alternatives to oral fluids in farrowing units have been studied, such as processing fluids (fluids from processing tails and testicles), umbilical cords, tongues from dead piglets, sow udder skin wipes or environmental sampling. For PRRSV, the processing fluids are interesting from the point of view of sensitivity and labour, if those tissues are obtained on a regular basis by the farm staff. However, some of these management practices have raised animal welfare concerns. In the European context, in the last years a high level of political attention has been seen to improve implementation and enforcement of the ban on routine tail docking in the EU (European Union. Council Directive 2008/120/EC). As for castration of male piglets, the "European Declaration on alternatives to surgical castration of pigs" requested that from 1 January 2012, surgical castration of pigs shall only be performed with prolonged analgesia and/or anaesthesia and from 2018 surgical castration of pigs should be phased out altogether. Therefore, obtaining fluids from tails and testicles may not be feasible anymore in the forthcoming years. Alternatives must be explored. Umbilical cords have shown to be equally sensitive or more than bleeding animals for determining PRRSV vertical transmission. Also, this method is useful for risk-based sampling (for example, if the percentage of stillbirths and weak-born animals is monitored). Umbilical cord collection is faster than bleeding and is welfare-friendly; however, fresh tissues are needed and,

therefore, extra labour from the farmer is necessary, limiting its feasibility. Finally, sow udder skin wipes and environmental samples (deposited particles or air samples) are also a sensitive method for detecting influenza A virus in the litter or room.

Another limitation of oral fluids, also applicable to other pooled or aggregated samples - as the ones mentioned above - is related to the use of those samples for sequencing purposes. Firstly, sequencing from aggregate samples may produce results difficult to interpret in cases where more than one strain of a given agent are present in the sample. This is especially important for PRRSV, in cases where modified live vaccines are used as for a period field and vaccine viruses (or different vaccines) could be found in the same sample. Also, this type of samples shows worst efficiency than individual samples. Direct sequencing from oral fluids is possible, but low Ct values are needed, not to mention if isolation of a given PRRSV or IAV strain is required.

Finally, it must be considered that pen-based surveillance systems should not be used for confirmatory diagnostic purposes. Swine nasal and faecal virome and microbiota are extremely complex. Both bacteria and viruses can be recovered from nasal swabs and faeces from both apparently healthy and diseased animals. At the population level, finding a virus or a bacteria explains only the infection dynamics, or the presence/absence of the virus, but in order to link that presence or absence of a pathogen with a given clinical sign, risk-based sampling methods, and determining relative risks are still the election.

In conclusion, pen-based surveillance is a useful, cost-effective and feasible epidemiological tool to evaluate infection dynamics for most viruses and bacteria with economic, or zoonotic importance in swine production. The strategy has become essential in order to make decisions in management, biosecurity, and disease control and prevention.



## Safety issues of veterinary vaccines

## PROF. LARS ERIK LARSEN University of Copenhagen, Denmark

#### Biosketch

Lars Erik Larsen (born 1963) holds a full professorship in veterinary virology and has worked with virus infections in production animals for the last 20 years. He has been involved in research on swine viruses (diagnostic tools, molecular epidemiology, vaccinology and basic pathogenesis) for the last 15 years. Since 2010, he has been responsible for the Danish diagnostic preparedness program and chairs the Danish expert committee for animal influenza viruses. Lars has participated in several national and European projects on enzootic swine viruses with focus on PRRSV, PCV-2 and swine influenza virus. He is the head of the enzootic/zoonotic virus research group at UCPH and is scientifically responsible for the teaching of veterinary students in veterinary virology and is supervisor of a range of PhD, Master and Bachelor students. Lars is currently board member of the Danish Society of Virology and the Danish Pig Veterinary Society. He has published more than 100 international peer reviewed papers on veterinary virology and has co-authored more than 300 other publications.

#### Abstract

#### Introduction

Both inactivated and modified live virus (MLV) vaccines have been extensively used in humanand veterinary medicine for decades and the beneficial effect of swine vaccines to prevent severe viral diseases such as porcine parvovirus (PPV), porcine circovirus type 2 virus (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) is well documented. The structural developments in the pig industry with huge, integrated production systems combined with the increasing demands from consumers and authorities to decrease the use of antibiotics, emphasize that there will be an increasing demand for prevention of infectious diseases in the future. Thus, availability of vaccines will remain a key solution to mitigate the impact of infections in swine herds. However, there is no such thing as a free lunch, and there is an increasing concern among veterinarians and producers related to the safety of especially modified live virus (MLV) vaccines.

The aim of this presentation is to critically evaluate the risks related to the use of vaccines in swine, with focus on modified live virus vaccines.

#### Viruses are moving targets

Many viruses, especially those possessing an RNA genome such as PRRSV, coronaviruses and influenza, has developed mechanism that allow them to adapt rapidly to the host and respond to changed ecologically conditions. Point mutations is a common features of RNA viruses since

the virus-coded RNA-dependent RNA polymerases lack proof-reading capabilities resulting in the generation of a cloud of genetic different progeny viruses (termed quasispecies) each with a slightly modified genome. During subsequent transmission, the virus cloud will undergo selection (bottleneck) allowing the most fit virus to infect the next individual. This is a rather slow process, but over time it will generate viruses that are better adapted to the host, allow the virus to escape immune mediators (i.e. antibodies) or even to switch to another host. Recombination is another mechanism that allow viruses to evolve more rapidly. Recombination happen when two variants of the same virus species simultaneously infect the same cell, which may results in progeny viruses with a genome that contain elements from both viruses (figure 1).



Fig. 1 Recombination e.g. between two virus strains (http://www.virology.ws/2010/09/08/viral-bioinformatics-recombination)

#### **Viral virulence**

Terms like "virulent viruses", "hot strains" and "attenuated strains" are often used in the literature to characterize viruses, however, for almost all relevant swine viruses, there is a lack of reliable "genetic markers" of virulence – meaning that the virulence of a given virus strain cannot be assessed just by sequencing. Instead, virus strains are often characterized according to the degree of disease they induce in infected animals – either in the field or during experimental infections. The challenge with these in vivo trials is that the degree of disease induced depends not only on the inherent virulence potential of the virus, but also on a range of other factors such as animal breed, housing conditions, management, infectious doses and the general condition of the animal. Thus, these factors make it difficult to compare the outcome of studies performed in different settings and, furthermore, results of experimental studies seldom reflect the situation in the field. Taken together, this make evaluation of the virulence of specific virus strains a difficult task.

#### **Requirement for safety documentation**

To get a vaccine licensed for use in Europe, the authorities require a substantial amount of data on the safety of the vaccine including experimental infections and field trials. The requirements differ from virus to virus and between MLV and inactivated vaccines, but in general, the licensing company have to show that the vaccine do not induce sustained clinical signs in the vaccinated animals and that it does not revert to virulence after passage from pig to pig. Low level of transmission from vaccinated to unvaccinated animals are accepted, but if shedding is expected this should clearly be described in the Specific Product Catalog (SPC).

Data on the origin of the vaccine wild type (parent) strain are mandatory; however, there is no requirements for in vivo characterization of the parent strain. Furthermore, there is no requirement for submission of sequence data of the parent nor the vaccine master seed virus; albeit full or partial sequence data of the vaccine strain may be requested by the assessor in specific cases.

In general, the safety issues of all vaccines are listed in the SPC so I highly encourage reading this short document prior to prescription of a new vaccine – these are public available via the European Medicine Agency (EMA) website (https://www.ema.europa.eu/en).

#### Challenges with the use of modified live virus vaccines

Traditionally, the virus strains included in MLV vaccines have been attenuated by repeated passage in vitro to decrease its capability to induce clinical disease in the vaccinated host. Despite the attenuated phenotype, most MLVs strains are excreted from the host for days or even weeks. This in turn possess the risk that the virus are transmitted to unvaccinated animals in the same herd or to other herds by movement of vaccinated animals or even by air. If these animals are naïve or have inadequate immunity they will become infected, replicate the virus and transmit the virus on to other non-immune animals. In this situation, the vaccine strain may undergo repeated pig-to-pig passages` and by that undergo repeated bottleneck selections as described above. This in turn may allow the vaccine strain to regain its ability to replicated to high titers and by that "revert to virulence". Thus, it is of immense importance that practitioners and farmers comply with the instructions included in the SPC of ALL MLV vaccines: "vaccinate all animals in a given epidemiological unit at the same time and do not mix vaccinated and unvaccinated animals until after the end of the shedding period".

During the last 5-10 years, an increasing number of recombined PRRSV strains have been detected in Europe and elsewhere. It is not clear, if this reflects a reel increase in generation of recombinants or if the increased reporting is due to the fact that nowadays many more PRRSV strains are full-genome sequenced by next generation sequencing (NGS). Recombination between PRRSV field strains has been extensively described for PRRSV-2 and less frequently for PRRSV-1. Recombination between PRRSV-1 field strains and strains of PRRSV-1 MLV vaccine strains has only been reported in a few studies, whereas recombination between two PRRSV-1 vaccine strains have been reported once from France and most recently in Denmark.

As described above, recombination requires that a single cell is infected by two different strains which in turn imply that the risk of recombination increase in situation where new strains are introduced into enzootically infected herds. Thus, the repeated use of mass vaccination and the use of vaccination during acute outbreaks may increase the risk of recombination between wild type strains and vaccine strains. Nevertheless, since the majority of the identified recombinant strains are recombinations between two wild-type strains, the increase in the detection of recombinant strains can hardly be explained by the change in vaccination strategies.

Do recombination lead to increased virulence? A few studies have shown that some PRRSV-2 recombinant viruses seem to be more virulent than their parental strains, whereas other studies did not find a correlation between the recombinant phenotypes and pathogenicity. An experimental study from France revealed that the recombinant virus seemed to be more virulent than the two parent PRRSV-1 vaccine strains, however, this study did not assess if the increased virulence of the recombinant virus reflected that the recombinant virus was adapted to swine or if it was a result of the recombination per se. The recent recombinant virus detected in Denmark has caused sustained clinical disease in the affected herds, but the virulence/pathogenicity of this virus has not yet been assessed in experimental trials. Furthermore, several studies have shown that the recombinant viruses often disappear from the herds shortly after being detected which do not support that these viruses have a selective advantage in competition with "pure" wild type strains.

#### **Inactivated vaccines**

Safety issues related to the use of inactivated, killed vaccines are normally related to adverse effect in relation to vaccination – i.e. local skin reactions and hypersensitivity. These effects are well known and will not be discussed further in this paper. During the last decade, however, there has been an increasing focus on two challenges related to the use of human vaccines that may be relevant for veterinary vaccines – unspecific effects of vaccines (NSE) and impact of repeated exposures to the same antigen.

Vaccines against infectious disease undoubtedly have specific disease-protective effects, but there is also increasing evidence that vaccination affect the resistance to other infectious diseases. These effects may be strongly beneficial, but also sometimes detrimental. NSE were initially demonstrated by the high-titre measles vaccine (HTMV) incident. Though protective against measles infection, HTMV was associated with a twofold increased female mortality if the children got diphtheria-tetanus-pertussis (DTP) after the HTMV. In contrast, standard measles vaccine (MV) is associated with a reduction in mortality, which is not explained by prevention of acute measles infection, and with reduced mortality for girls relative to boys. The exact mechanism behind these findings are not completely elucidated, but seem to be due to an altered innate immune response (trained innate response) induced by the vaccines. To the best of my knowledge, NSE in swine or other production animals has never been studied, however, taken into account that breeding animals may receive 5-10 different vaccinations – some in the presence of maternal derived antibodies - it would be interesting to investigate if some of the standard pig vaccination programs induce similar beneficial or detrimental non-specific effects. This information's could in turn be used for the design of more effective vaccination programs.

Nowadays, mass-vaccination against influenza virus and/or PRRSV, three-four times a year is practiced in many herds, which means that older sows may be exposed to identical antigens up to 20-25 times during their life-span. Assessment of the impact of repeated exposure on vaccine safety and efficacy is not required in connection to the approval of new vaccines and there is almost a complete lack of data in the literature on this issue. Repeated exposure to seasonal influenza antigens are subject to an intense debate among human immunologist. A recent study documented a significant impact of repeated vaccination on antibody-affinity maturation following vaccination, which may contribute to lower vaccine effectiveness of seasonal influenza vaccination provided a higher vaccination efficacy compared to single year vaccination. Nevertheless, there is a great need for studies investigating the beneficial and potential detrimental effect of repeated vaccination with the same vaccines in pigs.

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# Are we using drugs in pigs in the right way?

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#### Biosketch

Lorenzo Fraile is a DVM (1992) and obtained a PhD (1996) in Veterinary Pharmacology at the Veterinary Faculty of Zaragoza, Spain. Afterwards, he worked as a swine practitioner for eight years in pig producing companies. From 2004 to 2010, he worked as senior researcher at CReSA (http://www.cresa.cat) focusing his work on porcine bacterial and viral diseases. From 2010 to nowadays, he has been working as Associate Professor of Epidemiology and Pharmacology at the University of Lleida (UdL), Spain. His research interests include epidemiology, pharmacology and immunology focused on developing new tools to control bacterial and viral diseases in livestock. He is member of the European College of Porcine Health and Management and European expert to evaluate drugs and vaccines for animals in the European Union (http://www.ema.europa.eu/ema/index.jsp?curl=pages/about\_us/landing/experts.jsp&mid=WC0b01ac058043244a). He has published ninety-eight papers in peer-review journals.

#### Abstract

#### Introduction

Efficient pig production is based on establishing swine medicine preventive programs for bacterial, viral and parasitic diseases. These programs include the use of vaccines and/or drugs to manage them in an efficient way. In this keynote lecture, it will be revised if drugs are used in the right way in pig medicine with a special focus on management of bacterial diseases. The main families of drugs available for pigs are antimicrobials, antiparasitics, anti-inflammatories and hormones. Under the European Legislation, any drug must be used in a veterinary medicinal product (VMP) that is registered across Europe (through European Medicine Agency) or following national registration procedures. These registration procedures have been developed to assure that the VMP is secure for the user (veterinary or farmer), the target species (pigs in our case), for the environment and for the consumer (food safety). Moreover, the VMP must be efficacious. To meet all these requirements, registration procedure is time-consuming for pharmaceutical companies. After registering the VMP, the use of any veterinary medicinal product is clearly specified in the summary of products characteristics (SPC).

#### Pharmacovigilance

Pharmacovigilance comprises all activities related to the reporting and investigation of any adverse event potentially associated with the use of a VMP, including possible lack of expected efficacy, environmental problems, safety for the user or target species and investigations of the validity of withdrawal periods. Adverse event reporting may include events already included on
the SPC or events that are unexpected. All adverse events occurring in the EU, reported after the use of authorized VMPs, are collected and evaluated both by the marketing authorization holder (MAH), who is responsible for the product, and by the national competent authorities and/or European Medicine Agency (EMA) as agencies that authorized their use. The number of adverse events reported for pigs is extremely low compared with pets (EMA/CVMP/PhVWP/33617/2020) and figure 1). Further work is also needed to get better insights in the pharmacovigilance profiles of VMPs used in pigs for which there is considerable underreporting of potential adverse events. The EMA plan to hold pharmacovigilance focus-group meetings with veterinarians specialized in food producing animals (included pigs) to highlight the relevance of reporting adverse events with the final aoal to aather more information of each VMP.



Fig. 1 Adverse event reports by species received during 2019 following the use of centrally authorised products

#### Residues of drugs in the food chain

The presence of residues of veterinary medicinal products in food may pose a risk factor for public health. The EU legislative framework defines maximum limits permitted in food and monitoring programs for the control of the presence of these substances in the food chain. Regulation (EU) No 37/2010 establishes maximum limits for residues of veterinary medicinal products in food-producing animals and animal products. Council Directive 96/23/EC lays down measures to monitor residues thereof, mainly veterinary medicinal products, in live animals and

animal products. Additionally, Commission Decision 97/747/EC lays down levels and frequencies of sampling for certain animal products. Thus, the legislation clearly establishes the monitoring system for residues in food. Thus, the minimum number of pigs that have to be checked each year for all kinds of residues and substances is 0.05% of the pigs slaughtered the previous year. The minimum requirements for the number of samples to be taken are being fulfilled for the majority of Member States. The percentage of non-compliant samples for residues in pig meat was 0.11%, 0.09% and 0.08% for antimicrobials, antiparasitics and anti-inflammatories, respectively in the year 2018. These percentages were very similar to values obtained the last ten years (EFSA technical report. Doi: 10.2903/sp.efsa.2019.EN-1578) and very similar to the values obtained for other species (figure 2 as an example for antimicrobials). The number of non-compliant samples is extremely low for pigs suggesting that the withdrawal period is almost always respected and the non-compliant samples can be associated to non-deliberately mistakes.



Fig. 2 Number of targeted samples analysed and percentage of non-compliant samples for antibacterials (BI) in animal/product categories

#### Antimicrobials. A critical family of drugs

A new legislation has been recently approved for veterinary medicinal products in Europe (EU/2019/6) where a special attention has been addressed to antimicrobials because the great worldwide concern about one-health issues in the treatment of bacterial diseases in humans. The objective of antimicrobial therapy is to provide an effective drug to obtain a fast clinical recovery from the infection in affected animals but reducing the probability of generating antimicrobial resistance and avoiding the appearance of undesired effects as much as possible (Fraile, 2013). There are many recommendations about prudent use of antimicrobials in Europe coming from official sources (2015/C299/04) or from Federations of Veterinarians in Europe (FVE, 2020). The problem arises how to tackle these recommendations with daily practice.

Antimicrobials should not be used for prophylactic purposes, except in specific cases where they are administered to a particular animal or to a limited number of animals when the risk of infection is very high or its consequences can be serious. On the other hand, antimicrobial drugs

should be used for metaphylactic purposes only when the risk of spreading an infection or an infectious disease in a group of animals is high and adequate alternatives are not available. These restrictions should allow the reduction of prophylactic and metaphylactic use in animals, so that they represent a smaller proportion of the total use of antimicrobials in animals. However, the prescription of antimicrobials for therapeutic purposes (to cure an animal suffering from a bacterial infection) is a mandatory clinical act for a veterinarian in order to ensure the welfare of the animals and comply with their code of ethics. It seems that today there is a pressure to avoid the use of antimicrobials under any circumstances. This point is a conceptual error. The use of antimicrobials to cure sick animals due to bacterial diseases is mandatory and unquestionable. The differential point appears when used for metaphylactic or prophylactic purposes.

Another matter is not only the use of antimicrobials but also how to prioritize or categorize the families of antimicrobials used in animal production because the different antimicrobial families does not have the same risk of generating antimicrobial resistance from a one-health point of view. In connection with this topic, there is a recent recommendation of EMA (EMA, AMEG 2019) that now comprises four categories of antimicrobials, from A to D, each of them with a key action word attributed for more clarity:

- Category A ("Avoid") includes antimicrobial classes not currently authorized in veterinary medicine in the EU. For these medicines, their use in food-producing animals is banned and they may be given to individual companion animals only under exceptional circumstances.
- Category B ("Restrict") refers to quinolones, 3rd- and 4th-generation cephalosporins and polymyxins. Use of these antimicrobials in animals should be restricted to mitigate the risk to public health.
- Category C ("Caution"). These antimicrobials should only be used when there are no antimicrobial substances in Category D that would be effective.

•, Category D ("Prudence") is the lowest risk category. Antimicrobials belonging to this category can be used in animals in a prudent manner. This means that unnecessary use and long treatment periods should be avoided and group treatment should be restricted to situations where individual treatment is not feasible.

This recommendation imply that veterinarians must justify not only the use of antimicrobials but also the selection of the antimicrobial to treat one bacterial disease following prudent use of antimicrobials. The key question is how to carry out it in daily practice. Prudent of use of antimicrobials can be summarized in figure 3.



Fig. 3 Prudent use of antimicrobials

Recommendations on a prudent use of antimicrobials suggest that a microbiological diagnosis should be carried out. It is evident that a microbiological diagnosis will allow us to be sure of the etiologic agent involved in the bacterial infection and select the most suitable antimicrobial in a more efficient way. If these recommendations on prudent use are implemented in a strict way, the need for microbiological isolation for each clinical case could be requested. It is clear that this requirement is not possible to meet in practice because the microbiological diagnosis and the determination of its antimicrobial susceptibility (minimum inhibitory concentration- MIC) requires time to obtain the results that fluctuates between four and six days. Therefore, the results of the referred clinical case can only be useful for that particular case in the event that the first selected antimicrobial option has not properly worked. Thus, we have to carry out prudent use of antimicrobials but we have clinical urgency to select antimicrobials. This "irresolvable" problem has a solution if the concept of epidemiological information is applied for the prudent use of antimicrobials. Thus, the microbiological diagnosis and the determination of its antimicrobial sensitivity can be useful for all clinical cases caused by a certain bacterium within the same production system if an epidemiological link is established. An example of a clinical case of swine actinobacillosis is detailed in figure 4. In this case, we select an antimicrobial (according to our experience) to treat animals affected of Actinobacillus pleuropneumoniae but at the same time, samples are sent to the laboratory. Thus, the diagnosis will be confirmed and antimicrobial sensitivity data will be provided between four to six days. From the results obtained, we will already have information to carry out prudent use of antimicrobials in the following clinical case of swine actinobacillosis coming from the same production system (piglets from the same sow farm). This information about antimicrobial susceptibility may be valid for a period depending on local regulations. Therefore, the clinical diagnosis is acceptable for selecting antimicrobials but updated information on antimicrobial sensitivity should be available in that production system for relevant pathogens.

Fig. 4 Proposal of an epidemiological approach to carry out a prudent use of antimicrobials based on a Actinobacillus pleuropneumoniae clinical case.



Nowadays, this epidemiological approach is being carried out to carry out prudent use of antimicrobials in pigs for respiratory, digestive and systemic pathogens at a large scale in Spain. This initiative has been launched by pig producers and developed by the University (University of Lleida, Spain) and a diagnostic laboratory specialized in swine medicine (http://www.gsplleida. net/e). Finally, this approach has the support of the Spanish Medicine Agency (AEMPS- https:// www.aemps.gob.es/?lang=en) and the Spanish national program to address antimicrobial resistance (http://www.resistenciaantibioticos.es/en). Moreover, the swine sector is leading this approach for food producing animals in Spain.

Swine veterinarian must select a VMP that contains the selected antimicrobial depending on the observed antimicrobial sensitivity (MIC) and antimicrobial category (B, C or D) after carrying out clinical diagnosis and having information on the antimicrobial sensitivity (MIC) against this pathogen (either by data from that own clinical case or by epidemiological data). Finally, VMP must be registered for the species (pig), category of animals (sow or piglet for example) and indication (respiratory problems for example). We must remember that if there is no registered medicine for pigs that allows treating the bacterial infection, the legislation allows using the cascade principle. In this case, we must be aware of filling in the prescription properly and taking into account legal issues such as the withdrawal period.

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## Precision pig feeding: a breakthrough towards sustainability

### DR. CANDIDO POMAR Agriculture and Agri-Food, Canada

#### Biosketch

Dr. Pomar is a research scientist with Agriculture and Agri-Food Canada based at Sherbrooke (Lennoxville), Quebec, since 1991. He also acts as adjunct professor at Laval and Sherbrooke universities in Québec, and UNESP university in Sao Paolo, Brazil. Dr. Pomar obtained his agricultural Engineering degree in Madrid Polytechnic University (Spain) in 1980. After working for two years in the industry, he began studying at Laval University (Quebec) where he obtained a master's degree in 1985. He continued his studies at the University of Michigan, and undertook a term working on the development of a mathematical model for swine production systems at the USDA-ARS Meat Animal Research Center in Clay Center, Nebraska. This model served to complete his PhD presented at Laval University in 1989. In 2002 Dr. Pomar moved to the Scottish agricultural college near Edinburgh, UK, to review actual modelling approaches simulating population responses and predicting nutrient requirements.

Dr. Pomar is heading a research program in swine nutrition, mathematical modelling and carcass evaluation systems. Dr. Pomar pioneer the development of an innovative precision feeding and farming approach which will enhance profitability and durability of the livestock industry. Dr. Pomar has a large network of collaboration nationally and internationally and has contributed to training many graduate students. His publication record consists of 115 scientific papers, 69 of them peer reviewed, 16 book chapters, and 7 patent depositions and inventions.

#### Abstract

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#### Implications

- The utilization of precision feeding techniques in growing pig operations can significantly reduce production costs (>8%), protein and phosphorous intake (25%) and excretion (40%) and greenhouse gases emissions (6%) by increasing individual nutrient efficiency.
- Additionally, precision feeding allows real-time off-farm monitoring and intelligent management of feeds and animals for improved economic efficiency, significant reduction of labour requirements, and early identification of animal environmental and health stressors and, thus, reduce antibiotics use.
- Precision feeding is a major breakthrough in pig nutrition and one of the most promising avenues to promote high-quality and safe pork, high animal welfare and low environmental impact.

*Keywords* Modern Livestock Production, Nutrient Utilization Efficiency, Precision Feeding, Precision Livestock Farming

#### Introduction

Swine production systems have dramatically changed in the last three decades. Today main challenges for the hog industry are to maximize feed efficiency while minimizing production and environmental costs. With regard to environmental costs, the issue lies mainly with nitrogen (N) and phosphorus (P) excretion which are reaching alarmingly high levels in most intensive pig production areas (Strid Eriksson et al., 2005; Garcia-Launay et al., 2014). The high relevance of environmental load has forced swine producers and nutritionists around the world to reassess the nutritional and feeding programs in use. The excretion of nutrients can be reduced by providing an individual animal with its required dietary levels. This practice also improves nutrient efficiency and reduces production costs (Pomar et al., 2015; Andretta et al., 2016).

Conventionally, pigs have their nutrient requirements estimated using factorial methods (NRC, 2012) while fed in large groups that receive the same feed for extended periods throughout their production cycle. In growing-finishing pigs for example, their appetite increases faster than nutrient requirements and therefore, the optimal concentration of nutrients in the diet decreases as the pig gets older. These pigs are often fed with three feeds, that is, three feedings phases. The number of feeding phases can be increased to avoid oversupplying pigs with nutrients. Preferably, diets should be adjusted daily to account for the nutritional requirements of pigs more accurately, and therefore improve the efficiency of nutrient utilization. However, increasing the number of diets complicates feed management and increases production costs.

Precision livestock farming is an innovative production system approach that can be defined as the management of livestock using the principles and technologies of process engineering (Wathes et al., 2008). Precision animal nutrition or precision feeding (PF) is part of the precision livestock farming approach and involves the use of feeding techniques that allow the proper amount of feed with the suitable composition to be supplied in a timely manner to a group of animals (Parsons et al., 2007; Cangar et al., 2008; Pomar et al., 2014) or to individual animals (Andretta et al., 2014; Andretta et al., 2016) to enhance farm profitability, efficiency, and sustainability (Hauschild et al., 2012; Pomar and Pomar, 2012; Pomar et al., 2017). In this production system, the inter-animal variability is taken into account, by feeding pigs with diets tailored daily to their individual requirements (Pomar et al., 2009; Hauschild et al., 2012; Andretta et al., 2019).

The practical application of PF, especially individual precision feeding, can have great impact in livestock sustainability. Precision feeding is a promising feeding technique to reduce the environmental footprint of pig production systems (Gerber et al., 2013). Precision feeding offers immediate and tangible benefits to the pork producer given that feeding pigs individually with daily tailored diets reduces lysine (Lys) intake by more than 25%, feeding costs by more than 8%, N and P excretion by nearly 40% (Andretta et al., 2014; Andretta et al., 2016) and greenhouse gases (GHG) emission by 6% (Andretta et al., 2017). Still, the actual on-farm application of PF requires better understanding of variability among individual animals in terms of their physiological, behavioral and production responses. Advanced scientific knowledge in animal sciences should be integrated with information and communication technologies for the development of PF.

#### Improving Nutrient Efficiency Reduces the Environmental Impact of Pig Production

Farm animals are raised to produce commodities such as food (i.e., meat, dairy products), fibre and labour. The energy and nutrient losses associated with the conversion of feed nutrients to animal products increase the production costs and the environmental load (i.e.,N, P, trace minerals, carbon and methane). Feed costs may represent between 60 and 70% of the overall production costs in various livestock such as pigs, poultry and cattle. However, the efficiency by which domestic animals transform nutrients in feed into animal products is generally low. For instance, protein (i.e., N), which is among the most limiting and expensive nutrient in livestock feeds, is retained by growing pigs with efficiency normally ranging from 15% (Flachowsky and Kamphues, 2012) to 33% (Dourmad et al., 1999). Similar figures are found for converting dietary protein into meat protein in beef cattle and broilers where the efficiency ranges from 10 to 20%, and from 30 to 40%, respectively (Flachowsky and Kamphues, 2012). The protein in the feed that is not incorporated into animal products is excreted and can result in environmental problems such as nitrate pollution of aquifers, and pollution of surface water with problems such as algal bloom. Improving nutrient efficiency is essential because of the challenges associated with the expected increase in the human population, limited arable land, and the environmental problems that are frequently associated with farm animal production (Niemann et al., 2011).

There are various sources of nutrient inefficiency within the animal. First, portions of the ingested nutrients are used for basal metabolic processes involving degradation (catabolism) and synthesis (anabolism), or are lost in the digestive tract through desquamation and endogenous secretions. These losses are generally referred to as maintenance losses. Nutrients are also lost during the production of animal products (e.g., body lean). In growing animals, the losses associated with the utilization of the first-limiting amino acid for body protein deposition can largely be attributed to its inevitable catabolism (Mohn et al., 2000). These inevitable amino acid losses should be differentiated from other metabolic losses related to the preferential amino acid catabolism, which results from the catabolism of amino acids given in excess, from the excretion of chemically unavailable absorbed amino acids (e.g., heat damaged proteins) (van Barneveld et al., 1994), and from the use of amino acids for the synthesis of non-protein body compounds (Moughan, 1989). In growing animals fed with cereal-based diets, the sum of the undigested N and the losses associated with digestion, maintenance functions and body protein deposition may represent 33% of the total ingested N and similar values are obtained for dietary P (Dourmad et al., 1999). These sources of nutrient inefficiency are difficult to minimize because they occur during digestion and metabolic processes.

Besides the inevitable nutrient losses associated with digestion and metabolism, growing pigs may receive more nutrients than they need and all nutrients given in excess are excreted and contribute to the overall nutrient inefficiency. Pigs are raised and fed in groups, usually with the same feed which is provided to all animals of the group during a given period of time. However, nutrient needs largely vary among animals in a population (Figure 1) and these needs evolve over time following individual patterns (Hauschild et al., 2010). Therefore, two important sources of variation need to be controlled to improve animal production efficiency. These sources of variation are the variation between animals within the group receiving the same feed, and the changes in individual or group nutrient requirements over time. Given that for most nutrients underfed animals will exhibit reduced performance whereas the overfed ones exhibit near optimal performance, nutrients have to be provided to satisfy the requirements of the most demanding animals in the group to obtain optimal production performance (i.e., growth) (Pomar et al., 2003; Brossard et al., 2009; Hauschild et al., 2010). In this situation, almost all animals receive

more nutrients than they need. Providing animals with high level of nutrients to maximize herd performance is common practice in commercial livestock operations, even though maximum growth does not ensure maximum economic efficiency (Hauschild et al., 2010; Niemi et al., 2010). Furthermore, to account for the variability between animals, feed ingredient composition and other uncontrolled and unknown factors (e.g., environment, health), nutritionists include safety margins when formulating diets for maximum population responses. The need of these safety margins can be seen as an admission of our inability to precisely estimate the nutrient requirements of groups of animals (Patience, 1996).



Figure 1. Estimated standardized ileal digestible lysine requirements of individual pigs (thin coloured lines) and minimal standardized ileal digestible lysine levels to be provided to pigs fed in a conventional group 3-phase-feeding system (bold red line) without affecting body weight gain according to Hauschild et al. (2010).

Nitrogen and P conversion efficiency as mentioned earlier can vary between 10% and 40% depending on the animal, diet and farm management. The conversion of dietary N into animal protein is on average more efficient in monogastric than in polygastric animals (Flachowsky and Kamphues, 2012). Taking into account the efficiency and the number of animals, the largest N manure producers are cattle, sheep and pigs contributing 60, 12, and 6%, respectively, of total manure N (Oenema and Tamminga, 2005). It is important to note that these differences in N efficiency can be as large within a type of animal as between production systems. The management of animals and animal feed are the most important factors to be controlled to reduce N excretion (Oenema and Tamminga, 2005).

Production efficiency, and particularly nutrient efficiency, and environmental impacts are strongly correlated. In fact, the dietary nutrients that are not retained by the animal or in animal products are excreted via the urine and faeces but also some greenhouse gases, mainly methane. Reducing nutrient intake without limiting animal performance is therefore the most efficient way to reduce nutrient losses. For example, for each percent unit of reduction of protein intake N excretion can be decreased by 1.5%. Besides the reduction in protein intake and excretion, feeding costs are also reduced (Andretta et al., 2016). Fortunately, PF significantly improves nutrient efficiency by controlling the two identified sources of evitable nutrient losses, that is, those related to the between-animal variation and those related to the individual evolution of nutrient requirements. Furthermore, PF needs to include much lower safety margins than conventional feeding.

#### The Implementation of Precision Feeding

Precision feeding concerns the use of feeding techniques that provide animals with diets tailored according to the production objectives (i.e., maximum or controlled production rates), including environmental and animal welfare issues. Precision feeding is presented in this document as the practice of feeding individual animals while accounting for the changes in nutrient requirements that occur over time and for the variation in nutrient requirements that exists among animals. The accurate determination of available nutrients in feed ingredients, the precise diet formulation, and the determination of the nutrient requirements of individual animals or group of animals should be included in the development of PF (Van Kempen and Simmins, 1997; Pomar et al., 2009). The operation of PF in commercial farms requires the integration of three types of activities: 1) automatic collection of data, 2) data processing according to the established control strategy and 3) actions concerning the control of the system (Aerts et al., 2003; Banhazi et al., 2012a). Application of PF at the individual level is only possible where measurements, data processing, and control actions can be applied to the individual animal (Wathes et al., 2008).

#### Data collection

Measurements on the animal, the feeds, and the environment are essential for PF; and these parameters have to be measured directly and frequently (if possible, continuously). In fact, we cannot manage and control a system without appropriate measurements. Essential measurements for PF in growing pig operations include feed intake and body weight. The availability and the rapid development of new devices and emerging sensor technologies offer to PF a great potential for other measurements (e.g., body composition, physical activity, interactions among animals) that will allow more precise estimation of requirements and real-time animal monitoring.

#### Data processing

Collected data has to be processed according to the farm production objectives. There are several potential control strategies available for the application of PF in swine operations. In animals offered feed ad libitum, the only way to control nutrient intake is by varying the composition of the feed to be served. In this situation, both the between-animal and the time-dependent nutrient requirements variation can be controlled. In contrast, in animals that are offered feed restrictively the amount and the composition of the feed can be easily controlled.

Mathematical modelling is a methodology used to understand and to quantify complex biological phenomena involved in animal production and it is the basis for data processing in PF systems. Mathematical models developed for PF, however, have to be designed to operate in real-time using real-time system measurements. Therefore, they are structurally different

from traditional nutrition models, which are developed to work in a retrospective manner and to simulate known production situations. The first mathematical model developed to estimate in real-time individual pigs nutrient requirements was proposed by Hauschild et al. (2012). The required daily concentration of Lys is estimated in this model using individual feed intake and body weight information. Using these data, an empirical model component estimates the expected body weight, feed intake, and weight gain for the next day, whereas a mechanistic model component uses these three estimated variables to calculate with a factorial method the optimal concentration of Lys that should be offered that day to each pig in the herd to meet its requirements. Other amino acids and nutrient requirements are assumed proportional to the Lys requirements.

#### Control of the system

The information collected and processed is used to control the production system. In the context of PF, automatic precision feeders are used to provide individual pigs with the right amount and composition of the feed at a given time (Figure 2). Ear plastic button tags containing passive transponders (RFID) are used for pig identification. At least 2 feeds (named A and B) are needed for PF. These 2 feeds should be formulated on the basis of net energy, standardized ileal digestible amino acids and other essential nutrients. Feed A (high nutrient density feed) is formulated for the most demanding pigs at the beginning of the growing period, whereas feed B (low nutrient density feed) is formulated for the less demanding pigs at the end of the finishing period. Blending feeds A and B at different proportions allows the feeders to provide individual pigs with the right feed (Figure 3). The feeders (Figure 4) consist of a single space trough in which precision Archimedes' screw conveyors deliver and blend simultaneously volumetric amounts of two feeds contained in independent feed containers. The feeder identifies each pig when their head is introduced into the feeder and the feeds are blended and delivered upon the animal request according to the estimated optimal Lys concentration. A serving is composed of the amount of feed delivered upon each effective serving request. A time lag is imposed to ensure that pigs eat each serving before requesting a new one. Serving size is progressively increased and ranges between 15 and 25 g (Pomar et al., 2011). A meal includes all the servings delivered during each feeder visit. Pigs tend to leave the feeder trough empty or leave very small amounts of feed after each visit, thus ensuring that each pig receives the assigned amount of blended feed. Feed density needs to be measured weekly and this information used to convert feed volumes to feed weights.



Fig. 2 Scheme of the automatic precision feeding system operation using individual pig actual daily gain (DG) and daily feed intake (DFI) to predict individual standardized ileal digestible (SID) lysine requirements.



Fig. 3 Calibrating the precision feeding mathematical model (Cloutier et al., 2015) using four feeds in each feeder. Feeds A1 (130% of lysine requirements) and A2 (70% of lysine requirements) are formulated to meet the pig's highest lysine requirements, and B1 (130% of lysine requirements) and B2 (70% of lysine requirements) the pig's lowest lysine requirements.

Real-time modelling-control approach was used by Pomar et al. (2014) to control the timedependent variation of group-housed pigs offered feed ad libitum. Comparing the traditional three-phase feeding system to the daily-phase feeding system, these authors concluded that protein intake could be reduced by 7% while nitrogen excretion was reduced by 12%. Controlling the time-dependent and the between animal variation can further help the reduction of nutrient intake and excretion. The modelling approach proposed by Hauschild et al. (2012) was used to estimate real-time nutrient requirements in individual pigs was calibrated (Figure 3) in two animal trials (Zhang et al., 2012; Cloutier et al., 2015;), and the overall approach of estimating realtime amino acid requirements was challenged in 2 validation trials (Andretta et al., 2014; Andretta et al., 2016; Figure 4). The latter authors showed that daily adjustment of the diet resulted in a 27% reduction in total Lys supply, without detrimental effects on growth. This additional 20% reduction in Lys intake in relation to group-fed pigs could be obtained by feeding the animals individually and thus controlling simultaneously the time-dependent and the between-animal variation. Although feed cost reduction depends to a great extent on feed prices, it is expected that feed cost can be reduced by 1-3% when only controlling the time-dependent variation while an 8-10% reduction can be obtained when controlling both sources of variation. Nitrogen excretion was reduced by nearly 30% when pigs were fed with daily tailored diets.



Fig. 4 The individual feeders allow one pig at time to request feed and identify each animal due the ear plastic button tags containing passive transponders, providing pigs with daily tailored diets to each pig individual requirement.

#### **Future perspectives**

To further develop PF systems, it is necessary to improve our actual understanding of several animal metabolic processes. Precision feeding is still based on mathematical models and nutritional concepts developed for average population responses. When feeding individual pigs with daily tailored diets, these traditional nutritional concepts are not accurate and even sometimes incorrect (Remus et al., 2017; Remus, 2018). It is necessary to distinguish the nutritional requirements of a population from those of an individual. Individual pigs are able to modulate growth and the composition of growth according to the level of available amino acids (Remus, 2018). Also, pigs can respond differently to the same amount of ingested amino acid, due to differences in the efficiency of amino acid utilization. These aspects are not considered in current nutritional models, which assume that the efficiency by which animals use the available amino acids is constant. Similarly, the amino acid composition of whole body protein is assumed to be constant as well, whereas it has been shown that it can vary. Similar results have been found for the efficiency of calcium and phosphorus utilization (Gonzalo et al., 2018). Understanding the metabolic processes responsible for the observed variation between individual animals in their ability to use dietary nutrients is challenging nutritionists and modellers but is required to further improve the efficiency of livestock production. Advances in PF rely on the development of sound nutritional concepts and comprehensive biological models developed to more precisely estimate individual real-time nutrient requirements. The new understanding of individual metabolism and nutrition will allow animal science to move forward, opening up new opportunities for individual nutrition. Continuous and automatic monitoring of animals and farm resources will support production decisions at the farm level, the early detection of diseases and thus, decrease the use of antibiotics and avoid diseases to spread around. This will ultimately enhance farm profitability, efficiency, and sustainability of the overall production system (Banhazi et al., 2012b).

The mathematical model developed to estimate daily Lys requirements in individual growingfinishing pigs (Hauschild et al., 2012, Zhang et al., 2012, Cloutier et al., 2015) is being updated to take into account the variation in amino acid efficiency and the requirements of amino acids other than Lys (Remus et al., 2017, Ghimire et al., 2017). Further developments will also include new knowledge concerning the genetic capability of pigs to efficiently use nutrients and integrate in the daily estimation of individual pigs' optimal nutrient requirements the interaction between feeding patterns, diet composition and the digestive and metabolic dynamic availability of dietary nutrients. These model improvements will further reduce the environmental footprint of the swine industry with estimated reductions of feed cost of more than 12%, N and P excretion of more than 60% and GHG emissions of over 12%.

#### Conclusion

Precision feeding is a major breakthrough in pig nutrition and one of the most promising avenues to promote high-quality and safe pork (optimal fatness) with the lowest environmental impact (60% less nutrient excretion) and high animal welfare standards. Fewer pollutants would mean improved population wellness and health, given the resulting reduction in odours, harmful waste, and the risks of water, air (e.g., ammonia and greenhouse gas emissions), and soil pollution. Managing feeds and animals by means of advanced computerized technologies make it possible to identify diseases early and apply individual treatments precisely, thus improving herd performance, dramatically reducing antibiotic use and thus contributing to improving public safety.

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## 'Controlled fermentation' – a feeding concept for pigs with diverse effects of veterinary interest!

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#### **Biosketch**

Josef Kamphues was born in Saerbeck (Westfalia, Germany) in 1952. He studied Agricultural Science at the University of Bonn (Dipl. Ing. agr. Animal Production) and thereafter Veterinary Medicine in Hannover (Vet. Degree). After obtaining his doctoral degree and habilitation, he became Professor and Head of the Institute for Animal Nutrition at the Free University of Berlin in 1990. Three years later, he moved back to Hannover as the Director of the Institute for Animal Nutrition at the University of Veterinary Medicine Hannover, Foundation. Josef Kamphues is a Dipl. ECVCN and Dipl. ECPHM. Since 2019, he retired from the official position but still engaged in research as he was during his whole career. He focused on feeding strategies against gastric ulcers/Salmonella in pigs, foot pad dermatitis in broilers/turkeys at different dietary treatments, quality/standards in drinking water for food producing animals, pancreatic duct ligated pigs for studies on human exocrine pancreatic insufficiency; feeds and feeding for special purposes (dietetics), re-evaluation of rye for swine diets, etc.

Prof. Kamphues is member of the Society of Nutrition Physiology, European Society of Veterinary and Comparative Nutrition; the European College of Pig Health Management; the German Committee for Setting Requirement Standards of the Society of Nutrition Physiology; chief editor of the German Journal: Übersichten zur Tierernährung; member of the editorial board of different scientific veterinary journals

He was awarded with "James G. Morris Lectureship in Companion Animal Nutrition" (University of California, Davis) in 2012 and the Main prize/award of the Henneberg-Lehmann-Foundation (University of Göttingen, Germany) in 2014.

#### Abstract

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#### Introduction

For veterinary practitioners engaged in pig health management it is necessary to know the

principles of the feeding concept established on farms because there are advantages but also some risks, more or less related to distinct conditions when diets are produced, treated, stored and finally offered to the pigs (KAMPHUES et al. 2014).

Since decades in pork production diets are fed in a liquid form, here diverse cheap by-products with higher moisture contents can be used, substituting other expensive ingredients like cereals. On farms with a liquid feeding technique there is a trend – since about 20 years – for offering the liquid diet not directly after mixing the ingredients, but after a period of fermentation. Different advantages regarding the nutritive value (higher digestibility and nutrient utilization) are expected, also it is said that this feeding concept might result in further benefits like lower prevalence of enteral disturbances, reduced prevalence of Salmonella and lower needs for antibiotic treatment (CANIBE and JENSEN 2012; HEINZE et al. 2013; LAU et al. 2017; BUNTE et al. 2019).

The main goal of this contribution is a more detailed information and deeper characterization of this modern feeding concept. Based on diverse experimental studies including feeding trials with young fattening pigs and laboratory studies on the different diets that were offered in general in a liquid form, with and without a fermentation prior to feeding (BUNTE, 2018).

#### Materials/Methods

The diets were based on rye (~ 50 % of diet's DM), canola meal (extracted, ~ 30 % of diet's DM) and supplemented by further ingredients (wheat, barley) and a mineral supplement containing minerals, vitamins and amino acids. The diets for the experimental group were fermented during 24 hours; at the process' start a culture of lactic acid producing bacteria was added, the temperature was maintained at about 36° C; for cleaning the empty fermenter steam was used before each new filling with ground rye and the canola meal (extracted). It needs to be underlined that the mineral supplement did not undergo the fermentation, it was added after fermentation to the liquid diet that was offered finally.

Two feeding trials were performed with young pigs (body-weight at trial's start: ~ 20 kg), in two groups each with 5 pigs, housed individually. One diet was fed without fermentation to the controls whereas the pigs in the experimental group were fed the diet after 24 hours of fermentation. Both liquid diets (with/without fermentation) were offered ad libitum, it means in such amounts that on the following day some 'refusals' did occur. After this different dietary treatment was used for 28 days the pigs were dissected to investigate the stomach health, to obtain samples of digesta for diverse chemical analyses (nutrient content, pH, lactic acid and volatile fatty acids) and microbiological investigations (BUNTE et al. 2020; KELLER et al. 2020).

In further laboratory studies the focus was laid on potential effects of the fermentation and of the fermented diet on its 'hygienizing potential'.

Details regarding the laboratory methods, the used techniques and agents, the statistical evaluation and the description have been published in the doctoral thesis of BUNTE (2018).

#### Results

To ease an overview and understanding of the most interesting results it makes sense to differentiate the outcome regarding the effects of fermentation/fermented diet for the feed itself, the animals fed the different diets and finally the results when different pathogenic microorganisms (Salmonella/ E. coli/ Clostridia/ Candida) were exposed to the fermentation process or to the final fermented diet (for about 6 hours) to test the potential 'hygienizing effects' of the fermentation (the fermented diet regarding Salmonella, E. coli and Clostridia).

#### - Characterization of the fermented diet

In table 1 the typical changes in a liquid diet exposed to a 'controlled fermentation' for 24 hours are obvious: Low pH values due to high concentrations of lactic acid as well as high counts of lactic acid producing bacteria describe the most relevant changes during fermentation.

Furthermore it needs to be underlined that the nutrient content of both diets at offering was almost identical, with the exception of sugars (lower content in the fermented diet) indicating that sugars were the preferred substrates for lactic acid producing bacteria, used as starter culture.

During the fermentation there was an obvious change in the viscosity of the liquid diet (GRONE, 2018). In the first 6 hours of fermentation markedly increased viscosity values occurred, but afterwards the values declined to a level comparable to the non-fermented die

#### Table 1: Composition of both diets as offered to pigs (control diet without fermentation; fermented diet for the experimental group; trial 1)

Diet Fermentation	Control Diet without	Experimental Diet with
Ingredients, % of DM - Rye - Rapeseed meal, extr. - Wheat - Barley - Mineral supplement	48.2 29.4 9.84 9.80 2.75	48.2 29.4 9.84 9.80 2.75
Chemical Composition - DM content (g/kg) - Crude ash (g/kg DM) - Crude protein (g/kg DM) - Starch (g/kg DM) - Sugars (g/kg DM) - L-lactic acid (g/kg DM) - D-lactic acid (g/kg DM) - Acetic acid (g/kg DM) - pH - Calcium (g/kg DM) - Phosphorus (g/kg DM) - Phytate-P (g/kg DM) - Structure, particles' size (µm) - < 0.2 mm (%)	213 48.5 199 422 71.2 0.103 0.052 0.723 5.95 6.69 6.51 2.04 476 32.8	213 49.5 201 425 18.4 26.2 27.5 8.28 3.67 6.28 6.64 < DL 203 65.5
Bacteria (cfu/g log 10) - Lactic acid producers	4.91	9.31

Table 2: Total tract digestibility rates (%) of distinct nutrients from the two diets (without vs. with fermentation) in young pigs fed ad libitum as well as performance and gastric health

Trial	1		2	
Diet	Control	Experimental	Control	Experimental <sup>1)</sup>
Fermented Ingredients (%)	0	~ 100	0	~ 60
<b>Digestibility rates (%)</b> - Organic substances - Crude protein - Starch - Calcium - Phosphorus	83.2 <u>+</u> 1.64 ° 73.7 <u>+</u> 3.24 ° 98.6 <u>+</u> 0.318 ° 38.7 <u>+</u> 6.41 ° 54.2 <u>+</u> 4.94 °	84.8 ± 0.760 <sup>b</sup> 78.5 ± 1.71 <sup>b</sup> 98.8 ± 0.108 <sup>a</sup> 54.0 ± 7.20 <sup>b</sup> 72.8 ± 3.02 <sup>b</sup>	83.2 <u>+</u> 0.757 <sup>a</sup> 74.0 <u>+</u> 2.09 <sup>a</sup> 98.1 <u>+</u> 0.264 <sup>a</sup> 47.5 <u>+</u> 6.79 <sup>a</sup> 62.3 <u>+</u> 2.68 <sup>a</sup>	81.9 <u>+</u> 1.41 <sup>a</sup> 75.3 <u>+</u> 1.99 <sup>a</sup> 94.6 <u>+</u> 0.807 <sup>b</sup> 50.7 <u>+</u> 3.12 <sup>a</sup> 66.9 <u>+</u> 2.51 <sup>b</sup>
Performance - Daily gains - FCR	971 <u>+</u> 103 1.97 <u>+</u> 0.091	979 <u>+</u> 88.7 1.99 <u>+</u> 0.108	959 <u>+</u> 75.7 1.98 <u>+</u> 0.082	921 <u>+</u> 107 2.09 <u>+</u> 0.104
<b>Gastric health</b> - Score (Pars nonglandularis)	2.70 <u>+</u> 1.30 ª	5.0 <u>+</u> 0 <sup>b</sup>	3.06 <u>+</u> 1.48 ª	1.13 <u>+</u> 1.44 ª

<sup>1)</sup> it means 40 % of the diet consisted of rolled cereals (rye, barley, wheat) that were not fermented, but added to the liquid fermented ingredients (60). Types and shares of ingredients in the final diets were identical in both trials and each group of each trial.

In spite of optimized conditions for fermentation there was also a low formation of acetic acid (here negligible).

A further obvious change in the fermented diet was the loss of 'coarser' particles, more than 65 % of the fermented diet passed the sieve with 0.2 mm holes, in the liquid control diet 32.8 % only.

After the digestibility study demonstrating unexpectedly high phosphorus utilization in pigs fed the fermented diet, it was decided to analyze the phytate content in the diet before and after fermentation. Without having added any phytase the phytate content decreased markedly, i.e. was lower than quantifiable (< 0.2 g/kg DM).

#### - Effects in pigs fed the fermented diet

The high formation of lactic acid and therefore low pH did not impact the feed intake compared with the non-fermented control diet. But the fermentation of the liquid diet resulted in significantly improved changes regarding the digestibility of the organic substances, crude protein and – most obvious – of phosphorus, although in trial 1 no phytase was added. But also in the second trial (with adding phytase in both groups) the values for phosphorus digestibility were significantly higher in pigs fed the diet in which 60 % of DM were fermented.

But in the first trial there were unintended effects regarding the pars nonglandularis, i.e. gastric

health: Each pig of the group supplied by fermented feeds only had a gastric ulcer (score: 5.0). These findings of trial 1 gave the onset to change the share of fermented materials. Thus the liquid diet in trial 2 consisted of 60 % fermented ingredients and of 40 % non-fermented cereals, not ground in a hammer mill but in a roller mill to achieve a coarser structure in the 40 % share of the final diet. The lower intensity of grinding a part of the cereals (40 %) resulted in a significantly lowered digestibility rate for starch and tendentially decreased values for the organic matter.

On the other side the markedly coarser structure of the fermented liquid diet favoured the gastric health efficiently. In trial 2 the pigs fed the fermented diet had even better scores regarding the mucosa at stomachs' entrance. Finally it is worth to be mentioned that in the trial 2 there was a level of performance not significantly lower as in the control group although about 40 % of the diet had an uncommon rough/coarse structure (~ 25 % of particles with a size > 2 mm in the wet sieve analysis).

#### - Potential 'hygienizing' effects of the fermentation/the fermented diet

Due to the development of a flora with high counts of lactobacilli and due the accumulation of lactic acid (I- and d-form in similar shares) within the liquid diet low pH values (< pH 4) were found in the final diet in general. By adding the starter culture at the beginning of fermentation there was a faster drop of the pH than observed without adding lactic acid producers.

The high counts of active bacteria and their formation of lactic acid, accumulating during 24 hours with subsequent drop of the pH had marked effects on the flora of the diets present before fermentation (more or less eliminated), but also on bacteria that were added to the liquid diet.

Zoonotic bacteria like Salmonella were more or less eliminated when these were exposed to the process of fermentation for 24 hours. In E. coli and C. perfringens an exposure to the fermented diet for 6 hours was already sufficient to achieve counts of < 102/ml of the diet. Only the tested yeast (Candida krusei) was not affected as intended. After 6 hours exposure the same or even higher counts were detected.

Bacteria, added experimentally		Exposure	Final counts
Type/species	cfu, log 10/g	time, h	cfu, log 10/g
- Salm. typhimurium - E. coli - C. perfringens - Candida krusei	7 6 5 7	24 6 6 6	< 2 < 2 ~ 2 > 7

## Table 3: Effects of the fermentation/fermented diet on bacteria added experimentally to test potential 'hygienizing' properties (exposure test in vitro)

The 'hygienizing effect' of the fermented diet was also measurable in the alimentary tract of pigs fed the fermented diet.

In the digesta of the small intestine of pigs fed the non-fermented liquid diet a normal broad spectrum of bacteria was detected (KELLER et al. 2020), whereas in pigs of the 'fermentation group' the normal flora was almost lost, here the microflora of the fermented diet was the predominant flora, it means consisting more or less by Lactobacillaceae only.

Last but not least there were also marked changes in the microflora of the colon digesta of pigs fed the fermented diet (including ~ 40 % of coarsely ground cereals). Here, the presence/counts of Lactobacilli, Bifidobacterium and of Clostridium butyricum were significantly favoured.

#### **Discussion:**

Although there are lots of effects of the fermentation of a liquid diet prior to feeding regarding the nutritive value (digestibility of protein and phosphorus, HEINZE et al. 2013; LAU et al. 2017), the performance (gains and feed conversion ratio) as well as the consequences for the management (maintaining a sufficient hygiene in the technical equipment) and for the economy (costs of operating) described by BUNTE (2018) and BUNTE et al. (2019) in a review, here the discussion is focused on effects of specific veterinary interest.

#### - Changes in the diet by the fermentation process

As described repeatedly under optimized conditions (use of a starter culture/higher temperature) there is a more or less pure lactic acid formation due to a fast multiplication and activity of the added lactic acid producers. These microorganisms use preferably soluble sugars as substrate, as it was observed here.

The losses in sugars correspond quite well to the amounts of lactic acid measured in the fermented diet after 24 hours fermentation. During this process the losses of energy are quite low, thus other nutrients are not degraded and lost; for example it is known that amino acids might be degraded via formation of ammonia or biogenous amines (like histamine). Therefore it is recommended to add required amino acids after fermentation is completed, otherwise added amino acids can be lost by microbial degradation (LAU et al. 2017; BUNTE et al. 2019).

If specific veterinary interest is the loss of 'coarse' dietary particles due to the soaking process, the repeated mixing of the liquid diet during the process; soluble constituents of the ingredients will be transferred in the liquid phase favoured by the temperature (~ 36° C) and enzymes released from ground ingredients as well as from active bacteria (BUNTE et al. 2019), these changes in 'structure' of the liquid diet will enhance the risk for the development of gastric ulcers in pigs fed higher amounts/shares of fermented ingredients.

In the first trial, the proportion of 'fines' (particles passing the 0.2 mm sieve) increased to more than 65 %, thus it was not astonishing, that each pig was affected by an ulcer at the stomachs' entrance. Without any doubt those ulcers in pigs are caused mainly by high shares of fines and/ or missing particles in the diet > 2 mm or > 1 mm (WOLF and KAMPHUES 2007). Comparing the results regarding the scores for gastric health in trial 1 and trial 2 it is evidenced that the inclusion of roller mill-ground cereals improved markedly pigs gastric health. Also in the field study of BUNTE et al. (2019) it was found that the inclusion of coarse ingredients – established not on each farm in similar/required extent – is a suitable tool for improving gastric health in pig production.

Furthermore, it is worth to be mentioned that – in spite of optimized conditions – there was also a formation of acetic acid, but a low level. At increased concentration of acetic acid in the fermented liquid diet the consumption will be lowered, even a complete refuse of the diet might occur when the acetic acid level exceeds values of 10 g/kg DM.

Regarding the hygiene in the fermenters, pipelines and troughs it also noteworthy that – in spite of the rapid formation of lactic acid and drop of the pH – specific yeasts are not eliminated. A

high contamination of a diet by yeasts might result in intestinal disturbances associated with highest gas formation in the pig's gastrointestinal tract. On farms successfully operating with the controlled fermentation the use of steam is recommended for cleaning and for maintaining an adequate hygiene in the system (BUNTE et al. 2019).

#### - Effects of the ingestion of fermented diets within the gastrointestinal tract

Since years it is repeatedly said that fermented diets result in welcome effects regarding intestinal dysbiosis and distinct infections like Salmonella, the most important zoonotic bacteria in pork production (JENSEN et al. 2019). Here by the experimental exposure of Salmonella to the fermentation evidenced clearly the preventive effects on this zoonotic agent. Within 24 hours of fermentation there was a marked drop to levels lower than 102 cfu/ml diet, it means to non-quantifiable counts. Whether it is an effect of the pH, the lactic acid concentration or the high levels of competitive bacteria with their capacities (producing diverse substances) that could harm the Salmonella or inactivate them is an open question (BUNTE et al. 2019). It does not matter, field experiences support the results observed here; not seldom the continuously high Salmonella prevalence gave the onset to switch from the liquid feeding technique to a feeding concept including the 'controlled fermentation' in large sized units for fattening pigs.

But besides the preventive effects against Salmonella the ingestion of higher shares of fermented ingredients has further advantages. The flora of the small intestine was dominated by the counts and species of bacteria that were ingested via the fermented diet (BUNTE et al. 2020). These changes could be described as 'probiotic effects', it means, the fermented diet is a way for an efficient application of welcome bacteria that result in marked changes within the flora, gram-negative bacteria like E. coli or further Enterobacteria will be suppressed or even eliminated. Those effects are intended by the use of alternative feed additives, resulting also in costs. The fermented diets contain such high levels of lactic acids that a dietary use of other organic acids is not required, it means those costs are not occurring.

Last but not least there were also effects of the fermented diet in the hind gut of pigs, as demonstrated by investigations on the microbiome of the colon digesta (BUNTE et al. 2020; KELLER et al. 2020). But it needs to be underlined that there were overlapping effects, because the fermented diet - fed in trial 2 - had a high share of coarse particles, due to inclusion of roller mill-ground cereals (~ 40 % of diet's dry matter). At these conditions it can be expected that higher amounts of starch will enter the large intestine, thus the substrate available to the hind gut flora is markedly favoured. Thus, the observed higher occurrence of distinct Lactobacilli, of Bifidobacterium spp. as well as of C. butyricum in the colon digesta might be due to the 'physical form' of the ingested fermented diet. These changes in the diet by using the roller mill treated cereals will accompanied by elevated starch losses via faeces, that were undesired, but the principle is worth to be mentioned, whenever a 'modulation' of the microflora in the hindgut is on debate. The coarse particles of ground cereals might act as a 'prebiotic' substances favouring the formation of lactic acid and volatile fatty acids (KELLER et al. 2020). Especially the production of butyric acid might benefit from the augmented influx of starch into the hind gut (KAMPHUES et al. 2019), with diverse welcome effects within and outside the alimentary tract. Maybe especially some combinations of ingredients (for example rye plus rapeseed extracted meal) are highly recommendable - especially for favouring gut health and animal wellbeing.

#### Literature

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# ORAL

## PRESENTATIONS



#### PURCHASING POLICY, QUARANTINE AND ACCLIMATION PRACTICES OF BREEDING GILTS IN BELGIAN PIG FARMS

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#### **Background and Objectives**

The breeding population is very important in pig herds, for productivity, health and profitability. Replacement of breeding animals can be accomplished by own rearing of breeding gilts or by purchasing them. Purchasing breeding gilts is a hazardous event in terms of biosecurity and introduction of pathogens into a farm. However, in literature, little is known about gilt introduction in a herd. The present study investigated the introduction procedures of purchased breeding gilts in Belgian pig herds, and the compliance of these herds compared to optimal introduction procedures.

#### **Material and Methods**

A questionnaire consisting of twenty questions related to farm characteristics (n=2), purchasing policy (n=6), quarantine period (n=5), and acclimation practices (n=7) was designed, and 68 farms completed the questionnaire during an on-farm interview.

#### Results

The median (min. – max.) number of sows on the farms was 300 (85 - 2500). Fifty-seven per cent of the farms purchased breeding gilts, and there was a lot of variation in frequency of purchase and age at which gilts were purchased. On 95 % of those farms, a quarantine unit was used, and on most of these farms the quarantine was located on the farm itself. The median (min. – max.) duration of the quarantine period was 42 (14 - 140) days. The most common acclimation practice was vaccination against Porcine Parvovirus (96 %) and Erysipelothrix rhusiopathiae (94 %), although in some farms exposure of gilts to farm-specific microorganisms was done by providing faeces from suckling piglets (18 %) and bringing gilts in contact with sows that will be culled (16 %). Only 10 % of the farms fully complied with the optimal introduction procedures, i.e. purchasing policy, quarantine building and quarantine management.

#### **Discussion and Conclusion**

This study showed that in many farms, practices related to purchasing, quarantine and acclimation could be improved to maintain optimal biosecurity.

## CAN RISK FACTORS AT PIGLET LEVEL PREDICT THE DEVELOPMENT OF UMBILICAL OUTPOUCHINGS IN DANISH PIGS?

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#### **Background and Objectives**

Umbilical outpouchings (UO) are common in pigs. The main pathological manifestations are: 1) hernia umbilicalis, 2) enterocystoma, 3) proliferations, 4) subcutaneous abscess, and 5) subcutaneous fibroses. These manifestations appear either alone or in combination. The majority of pigs with UO never reaches the abattoir. Some die due to complications, others are euthanized due to welfare concerns. At birth, some piglets may have a higher risk of developing UO than others. The main objective of the study is to identify risk factors at individual piglet level associated with development of UO.

#### **Material and Methods**

A longitudinal study following pigs from birth to slaughter were carried out in two commercial Danish farrowto-finisher herds with a history of high prevalence of pigs with UO. Piglets (n=3031) born during a period of three weeks were included. At farrowing ( $t_0$ ), the following was registered for each piglet: sow-id, sow parity, littersize, gender, birth-weight, sign of immaturity, length of umbilicus and umbilical characteristics (bleeding, detachment, rupture, omphalitis and atypical wet umbilical cord). Pigs were inspected in the second week ( $t_2$ ) after birth and every month afterwards for the development of new UO. Animals with OU were clinically examined monthly until slaughter.

#### Results

In total 255 piglets (8.41%) developed an UO. The odds of developing an UO were significantly lower for males than females (OR=0.7, p=0.004). Pigs born immature (OR=3.4, p-value=0.008) or observed with specified umbilical characteristics in the umbilical area at  $t_0$  (OR = 5.1, p=<0.001) or  $t_2$  (OR= 1.9, p=<0.001), were at higher risk for developing an UO. No significant association (p>0,05) was found between development of UO and sow parity, littersize, birthweight and length of umbilicus.

#### **Discussion and Conclusion**

Identifying piglets with a high risk of developing UO make it possible to identify to which pig special attention should be paid during the daily management.

#### AN INNOVATIVE INDICATOR OF THE LEVEL OF INTRA-UTERINE GROWTH RETARDATION (IUGR) OF PIGLETS

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#### **Background and Objectives**

Selection for increased litter size has resulted in an increased number of piglets affected by intrauterine growth retardation (IUGR) linked to immaturity of many organs, impaired cognition and often low weight, with consequences on survival, well-being, growth, muscle percentage, feed conversion ratio. Identifying IUGR piglets relies on (i) ratios of brain weight relative to that of other organs (liver, heart, lungs) or (ii), head morphology of newborn piglets. This study aims to propose an innovative indicator: quantitative, repeatable, achievable on live piglets, inexpensive and simple to implement on farms ; the head to breast perimeter ratio (Rtp). We hypothesize that the higher this ratio, the greater the immaturity.

#### **Material and Methods**

In a 375 Largewhite x Landrace sows (terminal boar: pedigree n/n) farrow-to finish farm, 118 piglets born from 8 sows were individually monitored. Were recorded: birth weight (Pn in Kg), weight at 24 hours to estimate colostral intake (Pcol in g), perimeter of the head (at eye level), breast perimeter (behind the tip of the elbow), weaning weight. Data was analyzed performing regressions using R v. 3.4.4.

#### Results

Rtp had a 0.818 mean (SD: 0.043) and unimodal distribution. Pcol, Pn and lactation average daily weight gain (ADWG<sub>L</sub>) were significantly associated to Rtp (P<0.001). In the multivariate analysis Rtp was negatively associated to ADWG<sub>L</sub>(P=0.052), and Pn positively (P<0.05).

#### **Discussion and Conclusion**

Results suggest that an increase of Rtp is associated with poorer colostral gain, poorer growth and lower birth weight, as is immaturity. The effect of Rtp on ADWG<sub>L</sub> seems to be confirmed at equal birth weight and colostral gain. Validation of Rtp being an estimator of the ratio of brain weight to lung weight is ongoing. If true, IUGR could be considered as continuous and not simply bimodal. Rtp could also be used to quantify the effect of measures to prevent immaturity, and its consequences.

#### SOS – SWINE, OBJECTIVE SURVEILLANCE – A NEW DIAGNOSTIC TOOL FOR MONITORING PATHOGENS IN PIG HERDS

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#### **Background and Objectives**

There is a need for an inexpensive and simple concept for health monitoring of pig herds which can identifying pathogens circulating in herds, and whether their presence relate to clinical symptoms and reduced growth. Centre for Diagnostics (CfD) DTU has developed a new concept, Swine Objective Surveillance (SOS) for the continuous monitoring of relevant infectious diseases in pig herds based on a PhD-study [1]. CfD has conducted a SOS test project in 11 integrated pig herds for further validation of the concept. The project was completed September 2020.

#### **Material and Methods**

SOS builts on a high-throughput real-time PCR system (Fluidigm), which holds the capacity for testing 192 samples for 17 respiratory and enteric viruses and bacteria simultaneously [2]. The SOS concept is as follows; the farmer collects oral fluid and sock samples eight times during a year, sampled from the same age groups each time. The laboratory results are sent to the veterinarian to be used for health management in herds.

#### Results

The dataset from the project contains 9976 PCR analyses. An excerpt of the results revealed 4 out of 11 herds negative for PCV2, while swine influenza A virus was widespread in all the herds. Furthermore, influenza A virus was most prevalent after weaning, where 51% oral fluid samples were positive.

#### **Discussion and Conclusion**

The use of SOS will result in focused and evidence-based intervention and optimized use of vaccines and antibiotics. The collection of large amounts of diagnostic data allows for identification of the characteristics and criteria for production of robust animals with low disease frequency, mortality and high growth rate.

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## COMPARTMENTALIZATION IN COMMERCIAL SWINE HERDS - PREPARING FOR BUSINESS CONTINUITY IN THE FACE OF ASF

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#### **Background and Objectives**

The current pandemic of African Swine Fever (ASF) is causing major losses to the swine industry worldwide. The World Organization for Animal Health (OIE) allows for the use of compartmentalization to address the threat to business continuity posed by infectious animal diseases. Although compartments have been implemented for various diseases and production types, no ASF-free swine compartment has been published by OIE to date. The purpose of our study was to identify the requirements for an ASF-free swine compartment, provide guidance for implementation, and discuss advantages and challenges of the approach.

#### **Material and Methods**

We performed a comprehensive review of the available scientific literature and international standards to define the requirements for an ASF-free swine compartment. Then, we conducted a desk-based and on-site assessment of a vertically integrated commercial swine production system of a North American producer to develop practical, ASF-specific recommendations for implementation of compartmentalization.

#### Results

Our study identified that the implementation of compartmentalization for ASF first requires the development of a standard for compartmentalization in coordination with all relevant stakeholders including a governance structure, biosecurity standards, animal traceability, auditing, and a national ASF surveillance system. At the compartment level, biosecurity sufficient to mitigate the risk of introduction of virus via people, fomites, live pigs, pork products and proximity pathways must be implemented and documented. Barnbased observational surveillance and testing of surveillance samples can be designed to support early detection and demonstrate freedom from ASF. Last, biosecurity, segregation and traceability ensure that compartment products are not exposed to contamination with ASF virus outside the compartment boundaries.

#### **Discussion and Conclusion**

Integrated production systems lend themselves well to compartmentalization for ASF purposes. Although substantial work is required to define standard programme, development of compartmentalization showed benefits beyond ASF preparedness, such as strengthening overall systems and building trusting partnerships between industry and government as well as trading partners.

#### **EXPLORATION OF NEWBORN PIGLETS MORTALITY BY A 10 POINTS AUDIT**

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#### **Background and Objectives**

The current pre-weaning mortality rate is around 20% of total born piglets (mostly within the first days of life). Reducing this early mortality is a challenge. Both farrowing process and colostrum intake are key issues that must be considered thoroughly when investigating piglet mortality cases. Examining and necropsying dead piglets using a fast and well-defined method would be an excellent tool for determining main causes of mortality and thus discussing management around farrowing with the farmer.

#### **Material and Methods**

Dead piglets within the first 48h of life have to be submitted for external body examination and necropsy. While the vet performs examination and necropsy, the farmer records the following criteria on a preestablished paper grid:

Five external ones: a) global aspect, b) piglet's weight, c) head shape (normal / a bit round / round), d) diameter and aspect of umbilical cord (big- medium – thin / wet-dry), e) fetal bead on claws (present / diminished / absent).

Five necropsic criteria: f) presence of liquid or fibrin in abdomen / thorax, g) lungs sink in water or not, h) content of stomach (fetal liquid / meconium / empty / some milk / full of milk), i) colon's content, j) piglet's management (teeth clipping, tail docking,...).

At the end of the session, both the vet and the farmer comment on the results and discuss about the causes of mortality.

#### Results

The audit was performed in farms exhibiting piglet mortality problems in 2019. Dead piglets were classified as light (< 0,6 kg), stillborn, dead in first hours, dead in days 1-2. Piglets considered as stillbirth were frequently alive during farrowing (meconium in stomach). Lack of colostrum intake was the most frequent issue

#### **Discussion and Conclusion**

This method proved to be very educational and helpful to heighten farmer awareness about farrowing practices.

## COMPARATIVE EFFECT OF TWO DIFFERENT PRODUCTS FOR IMMUNOLOGICAL CASTRATION OF PIGS ON PRODUCTIVE PERFORMANCE AND TESTIS SIZE

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#### **Background and Objectives**

Physical castration of young male pigs to prevent boar taint in pork meat is the common industry practice, but increasingly controversial due to growing animal wellbeing concerns. Immunization against GnRF is an effective alternative to prevent boar taint. This study focuses on the comparative effect of two different products anti-GnRF on productive performance and testis growth.

#### **Material and Methods**

A total of 44 pigs were randomly allocated to one of three groups: 14 entire males received placebo (EM), 14 received Improvac<sup>®</sup> (IM1) and 16 the alternative product (IM2). All pigs received two doses, first (V1) at 14.5+2.9 kg and the second (V2) 10 weeks later at 90.9+9.9 kg. Four weeks after the second dose pigs were slaughtered (132.4+8.1 kg). Animals were scanned at V2 with computed tomography to determine testis volume. Statistical analyses were performed with the GLM procedure of SAS software.

#### Results

Feed consumption was significantly higher in IM1 and IM2 than EM (3.6 and 3.6 vs 3.0 kg) between V2 to slaughter. Feed conversion was higher (P<0.05) in both IM1 and IM2 than EM from V2 to slaughter and during the overall study period. Testis volume at V2 were lower (P<0.05) in IM2 than IM1 and EM (381.5 vs. 504.9 and 525.5 cm<sup>3</sup>). At slaughter testis weight was significantly higher in EM (895 g) than in IM1 (347 g) and these were significantly higher than IM2 (237 g). Testosterone levels tended to be lower in IM2 (P=0.06) at V2, and lower (P<0.05) in IM1 and IM2 than EM at slaughter.

#### **Discussion and Conclusion**

This preliminary work shows that both vaccines were effective castrating pigs, increased feed consumption after second dose and reduced testicle size. However, testicles in IM2 were significantly smaller compared to Improvac and controls before the second dose, indicating a premature castrating effect of the first IM2 dose.

## DETAILED ANALYSIS OF FARMERS' RECORDS OF PIGLET MORTALITY IN FREE FARROWING SYSTEMS IN SWITZERLAND

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#### **Background and Objectives**

Crushing by the sow is commonly assumed to be the most important reason for piglet mortality. As free farrowing systems are mandatory in Switzerland, a major breeding goal is to reduce crushing losses. Therefore, most farmers record not only the number but also the cause of piglet losses. However, farmers usually are not trained to reliably distinguish different causes of piglet mortality. Consequently, piglet losses due to crushing might be overestimated. In the present study, we analysed whether farmers misclassified piglets without external signs of trauma as well as weak piglets as crushed.

#### **Material and Methods**

The study included records of 12 farms and a total of 879 litters with 11'339 live-born piglets. Farmers were instructed to keep records of all piglet losses occurring in the first week after birth. According to their common knowledge, they specified the cause of death of every live-born piglet by classifying them as crushed, euthanised, death by weakness, or cause unknown. In addition, the piglets' age, sex, and weight were recorded. For piglets classified as crushed, the records also included detailed information regarding external signs of trauma (body colour, body shape, and lesions). Therefore, farmers were instructed how to identify these signs by means of a photo documentation.

#### Results

In total, records of 1214 live-born losses were analysed. Farmers classified 64 % of these as crushed, 5 % as euthanised, and 23 % as death by weakness. Of the piglets classified as crushed, 28.1 % had no external signs of trauma, indicating an overestimation of crushing losses. Moreover, 49.2 % were underweight (< 1 kg body weight or < 200 g daily weight gain) and 3.2% weak.

#### **Discussion and Conclusion**

In conclusion, farmers should be trained to distinguish different causes of piglet mortality more reliably to improve the quality of data used for breeding purposes.

#### RES-OP-01

#### A CASE OF ABORTION OF SOWS IN MID-GESTATION - A DIAGNOSTIC MARATHON

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#### **Background and Objectives**

Abortion in sows can have various infectious and non-infectious causes. The diagnostic work-up is timeconsuming due to the long list of potential differential diagnoses, including bacterial and viral infections as well as and parasitic diseases.

#### **Material and Methods**

In a piglet producing farm with a three-week batch farrowing interval, an episode of abortions of sows during mid-gestation (gestation days 60-80) started in June 2019. In three consecutive groups of fifteen sows each, three, zero and seven sows (all parities) aborted and two pregnant sows died.

For diagnostic purposes, aborted foetuses and placentas of one sow, blood samples, cervical swabs and feed samples were collected. Additionally, back fat of sows was measured. Feed was analysed for mycotoxins and general nutrient content; progesterone concentrations and antibodies against several infectious agents were measured in serum. Placenta and foetal tissues were tested for PRRSV, PCV2, PCV3, EMCV, pestiviruses, enteroviruses, herpesviruses, ASFV, bacteria, and coccidia.

#### Results

Histopathologic evaluation of the placenta revealed multiple cysts and oedema of the allantochorial tissues. Foetal organs were unsuspicious for infectious agents, except for PCV2, which was detected by qPCR but not by ISH. Analyses of all other samples resulted in unsuspicious findings. Initial investigation of sow sera resulted in unsuspicious influenza A virus (IAV) antibody titers using HI-test. Only the HI-test of paired sow sera in a specialised lab for antibodies against several different subtypes of IAV resulted in the most probable cause of this case of abortions: pandemic HINI IAV.

#### **Discussion and Conclusion**

Influenza A virus infection in swine often is caused by H1N1, H1N2 or H3N2 subtypes. Clinical symptoms in sows may include fever, anorexia and apathy, but pandemic H1N1 infections may not always induce apparent clinical symptoms. This case illustrates the difficulties in diagnosing infections with pandemic H1N1 in sows with abortion.

#### RES-OP-02

## PREVALENCE OF LAWSONIA INTRACELLULARIS IN SIX EUROPEAN COUNTRIES IN PIG HERDS WITH A HISTORY OF DIARRHEA

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#### **Background and Objectives**

Lawsonia intracellularis causes large economic losses in the pig industry worldwide. Pigs suffer from reduced daily weight gain, poor feed conversion and increased mortality. The number of affected animals and herds in Europe remains unknown. This study will provide an overview of the prevalence of Lawsonia intracellularis in herds with a history of diarrhoea in six European countries and thereby identify country specific differences.

#### **Material and Methods**

Therefore, 6'480 faecal and 1'800 blood samples of not pre-treated nursery to finishing pigs were taken in Germany, Denmark, Spain, the Netherlands and the United Kingdom.

#### Results

Out of the 144 herds sampled, 90.3 % presented at least one L. intracellularis positive faecal sample by quantitative polymerase chain reaction (qPCR). The within-herd prevalence was 26.2 %. Overall, 91.7 % of blood samples from 60 herds contained L. intracellularis specific antibodies determined by an enzyme linked immunosorbent assay (ELISA). The within-herd prevalence was 31.6 %. Differences between the countries were found regarding:

**Within-herd prevalence- qPCR**: Samples from Denmark were more often positive than samples of Spain or the United Kingdom.

**Within-herd prevalence- ELISA**: Samples from Denmark were more often positive than samples from Spain and the Netherlands.

Affected age category- qPCR: Nursery pigs in Denmark were more often positive and shed more genome equivalents than nursery pigs in all other countries.

**Concentration of detected genome equivalents- qPCR**: The concentration of genome equivalents of Lawsonia intracellularis in herds in Denmark was higher compared to all other countries.

#### **Discussion and Conclusion**

A widespread of Lawsonia intracellularis was confirmed, whereby a large part of the positive animals only excreted small amounts of genome equivalents. Country specific differences were found with Denmark in particular diagnosing more L intracellularis then the other countries. Herd data collected in this study will be analysed to get more information about possible reasons for the differences found between the countries.
### RES-OP-03

# IMPLEMENTATION OF "BEST PRACTICE" AT FARROWING – ARE SWEDISH FARMERS DOING WHAT THEY SHOULD DO TO INCREASE PIGLET SURVIVAL?

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## **Background and Objectives**

To increase the chances of piglet survival, it is important to provide optimal environment and management. To see if and how "best practice" is implemented around farrowing, a survey was conducted in 200 Swedish piglet producing herds during the yearly visit of a herd health veterinarian.

# **Material and Methods**

Farmers answered a questionnaire about their management routines and a fact sheet with management advice was discussed. Measurements of water flow and surface temperature (IR-thermometer) were made in five pens in one farrowing section with suckling piglets.

#### Results

Herd size ranged between 9–1100 sows. Farmers reported an average litter size of 11.6 pigs/litter at weaning (~5 weeks age) and a piglet mortality of 18.4%. A correct water flow for sows (3–4 L/min) was obtained in 84% of the farms but only 41% had a correct water flow in all tested pens. Temperature in the piglet corner was on average 27.1°C. Only 39% of the herds had a roof over the piglet corner. Farms with well-designed roofs (20%) weaned +0.5 piglets/litter compared to farms without roofs over the piglet corner (p=0.02). In 188 herds, nestbuilding material was provided to the sows before farrowing, usually chopped straw. Cross-fostering was carried out in all herds except one and 90% completed this within 48h hours. One third of the respondents stated that they use nurse sows. Split suckling was practiced in 64% of the herds, mainly in large and uneven litters.

## **Discussion and Conclusion**

The results of this study show that there is room for improvement of management around farrowing in Swedish pig farms, especially regarding water supply and providing an optimal temperature in the piglet corner. To install well-designed roofs over the piglet corners and to establish routines for regular check-up of temperature and water flow could increase piglet survival.

### RES-OP-04

## A COMPREHENSIVE INVESTIGATION OF PIG LAMENESS ASSOCIATED TO MYCOPLASMA HYOSYNOVIAE

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## **Background and Objectives**

Arthritis associated to Mycoplasma hyosynoviae is an increasing concern to swine producers. It is still difficult to correlate diagnostic data to the actual disease prevalence in the field. The aim of this work was to investigate the connection between detection of M. hyosynoviae in oral fluid (OF) and synovial fluid (SF) samples and the clinical presentation of lameness in growing pigs.

### **Material and Methods**

Five wean-to-finish farms reporting recent history of lameness were enrolled in the study. Oral fluids were collected by pen (9–10 samples collected by site). Real-time PCR was performed on each OF to detect M. hyosynoviae. Lameness scores (0–4) were recorded in pigs from pens where OF were collected. In addition, three finisher pigs, one healthy pig (score 0) and two lame pigs (score ≥3), were identified at each farm and euthanized to collect synovial membrane and SF samples from multiple joints, which were evaluated by histopathology and real-time PCR, respectively.

### Results

Mycoplasma hyosynoviae was detected in all OF from the five enrolled farms. However, lameness (score >2) was observed in 4.42% of the assessed pigs across all five sites. Nine out of the 10 clinically lame pigs were positive to M. hyosynoviae in SF, at least in one of the sampled joints. From healthy pigs, three tested positive to M. hyosynoviae in at least one of the joints sampled. Lameness scores were not correlated with detection of M. hyosynoviae in OF and SF. Detection of M. hyosynoviae in SF was also poorly correlated with histological alteration of the synovial membrane; positive results were obtained in joints with no microscopic lesions and vice versa, both in lame and healthy pigs.

# **Discussion and Conclusion**

Detection of M. hyosynoviae in SF as well as in OF samples was not necessarily associated with the presentation of clinical lameness, neither at the individual nor at the population levels.

## RES-OP-05

# WHY VACCINATION TIMING MATTERS: A CASE STUDY ON THE IMPORTANCE OF VACCINATION AGE AND DIFFERENT PRODUCTION FLOWS TO CONTROL ACTINOBACILLUS PLEUROPNEUMONIAE ON A FARROW-TO-FINISH FARM

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# **Background and Objectives**

When vaccinating to control Actinobacillus pleuropneumoniae (APP) infections, timing(/age) of vaccination is a key factor. However, in many farms delaying (smaller)pigs from its normal flow is still common practice, leading to sub-optimal vaccination timings. The aim of this study was to investigate the effect of vaccination timing on APP sero-conversion, and on pleurisy prevalence at slaughter of pigs in a farrow-to-finish farm.

## **Material and Methods**

The study took place on an Irish 600 sow farrow-to-finish farm with history of APP. A commercially available vaccine was used to vaccinate pigs at 10 and 12wk of age, based on previous sero-profiling. A total of 495 piglets born within four days were individually tagged and weighted at birth. At vaccination, three groups were formed: 1)pigs timely vaccinated (OPTIMAL;n=10); 2)pigs vaccinated at older ages (DELAYED;n=20); and 3)pigs not vaccinated (CONTROL;n=20). Study-pigs were blood-sampled before vaccination (10wks) and after the vaccination of DELAYED pigs (14wks). Serology analysis was performed using IDEXX APP-ApxIV AbTest kits. All animals were sent to the slaughterhouse at 21wk and pleurisy prevalence was assessed. Fisher's exact tests, Kruskal-Wallis tests and Nemenyi-tests for multiple comparisons were used to examine differences between groups.

#### Results

Before vaccination, no differences were found in SP-values between groups (P=0.846). After vaccination, OPTIMAL SP-values were higher than CONTROL (P=0.015), and tented to be higher than DELAYED (P=0.088); no differences were found between CONTROL and DELAYED (P=0.762). At slaughter, pleurisy prevalence tended to be different across groups (P=0.074): DELAYED, CONTROL and OPTIMAL pigs had 35, 38 and 0% of pleurisy lesions, respectively.

## **Discussion and Conclusion**

Vaccinating pigs at sub-optimal timings had a significative effect on immunity development, leaving pigs exposed to disease, and it was associated with pleurisy lesions. This study emphasizes the usefulness of sero-profiling and the importance of good husbandry to control disease in pig farms.

# FREQUENCY OF PORCINE CIRCOVIRUS 3 (PCV-3) GENOME IN SERA FROM GILTS AND SOWS AND THEIR RESPECTIVES STILLBORN TISSUES FROM SPANISH PIG HEALTHY FARMS

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## **Background and Objectives**

Porcine circovirus 3 (PCV-3) has been detected in serum and/or tissues from pigs affected by different disease conditions as well as in healthy animals. Although a number of reports suggest a potential association of PCV-3 with reproductive disease, no information is available on the virus detection rate in farms with good reproductive parameters. This study aimed to assess the frequency of PCV-3 in sera from gilts and sows from farms without reproductive problems, as well as in tissues of their respective stillborn piglets.

### **Material and Methods**

Sera from 60 primiparous and 64 multiparous sows belonging to 3 different farms (A n=47, B n=37 and C n=40) without reproductive problems were collected at two time points (peri-mating and close to farrow). Tissues (brain and/or lung) from stillborn piglets (n=264) from these sows were collected. Tissues were homogenized by separate. DNA extracted from sera and tissues were tested by PCR and by qPCR when positive.

### Results

All sera from multiparous sows were PCV-3 PCR negative, while 19/60 (31%) primiparous ones were PCV-3 PCR positive at the first or second sampling. From the 264 stillborns, 90 (34%) had at least one tissue positive to PCV-3 PCR. The rate of detection of the virus in stillborns from primiparous sows (77/98, 79%) was significantly higher than that from multiparous sows (13/166, 8%). The percentage of lungs PCV-3 PCR positive was higher (84/258, 32%) than that of brain (68/261, 26%).

#### **Discussion and Conclusion**

PCV-3 DNA was detected only in sera from primiparous but not from multiparous sows of the studied herds. The prevalence of PCV-3 in both tissues was significantly higher in stillborns from primiparous than from multiparous sows. These results indicated that PCV-3 can be vertically transmitted causing intrauterine infections in absence of reproductive problems in the farm.

# GENETIC CHARACTERIZATION OF A TYPE 1 PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV-1) STRAIN EMERGED FROM RECOMBINATION BETWEEN TWO MLV VACCINE STRAINS

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## **Background and Objectives**

In July 2019, an outbreak of PRRSV-1 was detected in a Danish PRRSV negative boar station. The virus subsequently spread to more than 40 herd that had received semen from infected boars. The objective of the present study was to characterize the PRRSV strain from the outbreak by complete genome sequencing and successive bioinformatics analysis.

#### **Material and Methods**

Total RNA was extracted from a serum sample obtained from the boar station and the PRRSV genome was amplified from long range PCRs in four overlapping fragments. Complete genome sequencing was performed on the MiSeq platform and the assembly of reads and phylogenetic analysis was conducted using CLC Genomics. The Recombination Detection Program 4 (RDP4) was utilized to predict any minor or major parent and to examine putative recombination breakpoints.

## Results

The complete genome sequencing resulted in a sequence of 15,098 nucleotides including the 5'- and 3'-UTRs. Phylogenetic analysis showed the case virus to group with the Amervac strain, the virus strain included in the Unistrain MLV vaccine, in ORF5 with 99.01% similarity, and to group in ORF2 with 98.80% similarity to the Belgian 96V198 strain, the virus strain used in the Suvaxyn PRRS MLV. Recombination analysis predicted one breakpoint at nucleotide position 12,383 which corresponds to nucleotide position 201 in ORF3 with 96V198 as the major parent (nt. pos. 1-12,383) and Amervac as the minor parent (nt. pos. 12,384-14,763).

#### **Discussion and Conclusion**

The present study identified a new PRRSV strain that had evolved from recombination between two PRRSV MLV vaccines. The results emphasize that sequencing of only a small part of the PRRSV genome, often only ORF5, may lead to false conclusions to the origin of new strains.

# IMMUNITY FAILS INDUCING PROTECTION AGAINST A HOMOLOGOUS PORCINE EPIDEMIC DIARRHOEA VIRUS CHALLENGE IN A FIVE-MONTH LAPSE

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## **Background and Objectives**

The aim of the present study was to evaluate the length of protection against a PEDV S-INDEL European strain homologous challenge.

## **Material and Methods**

The study was conducted using 90 four-week old piglets divided in two groups: challenge (CH; n=76) and control (C; n=14). Piglets in CH were inoculated orally at day 0 post-infection (0 dpi) with a PEDV S-INDEL European strain (2ml/animal; 10<sup>4</sup> TCID<sub>50</sub>/ml). At 154 dpi, both CH and C groups were inoculated again as described above. Presence of PEDV was determined in faeces by RT-qPCR. Two commercial ELISA (A and B) based on the spike glycoprotein were used to measure antibodies. Virus-specific IgA were measured in sera and faeces using an in-house ELISA.

#### Results

After the initial challenge, loose stools/diarrhoea and PEDV were detected in all challenged pigs. All animals were positive by day 21 and by day 28 by ELISA A and B, respectively. At 154 dpi, only 27.4% of pigs in CH were still positive in ELISA A, while 91.9% were positive using ELISA B. Regarding PEDV-specific IgA, they were constantly detected in all sera from CH, while in faeces showed an irregular pattern. After the second challenge, PEDV was detected again in all pigs from both groups, although not all the animals showed diarrhoea. For both ELISA and for PEDV-specific IgA, a significant increase was observed in CH.

#### **Discussion and Conclusion**

According to our results, sterilizing immunity is not present five months after the initial challenge. This suggests that a homologous reinfection at older ages can occur with no clinical signs. Further investigation concerning the role of these animals in the maintenance of the infection in the farm is needed.

# THE BENEFIT OF LFD TESTING FOR AFRICAN SWINE FEVER OUTBREAK INVESTIGATIONS – FIELD STUDY IN THREE PIG FARMS

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# **Background and Objectives**

African swine fever (ASF) remains a growing threat to the pig farming industry, affecting new countries and regions every year. In case of ASF outbreaks in pig farms EU legislation requests a thorough epidemiological investigation aiming to identify the likely origin and the estimated length of time that ASF was on the farm (high risk period, HRP). For estimating the HRP it is important to detect the epidemiological units affected. This is a challenging task, particularly on larger farms and under the time pressure of control measures. So far, random sampling for epidemiological investigations during the culling was the common approach. However, due to the biological characteristics of ASF (slow spread), targeting sick and dead animals has shown to be more efficient.

Our study aimed to set up a targeted sampling strategy combined with the use of lateral flow devices (LFD) for epidemiological investigation purpose. LFDs are commercially available for antigen and antibody detection and have been tested under experimental conditions.

## **Material and Methods**

We carried out a field study using antigen and antibody LFDs in comparison to PCR, ELISA and IPT, to estimate the time and entry point of ASF virus and further spread within the holding in three Latvian pig farms of different sizes.We propose a three-step sampling approach: (i) definition of sampling units, (ii) classification of sampling units and (iii) targeted selection of animals to be sampled.

## Results

Despite certain limitations in the use of LFD, the findings demonstrate conditions and situations when LFD testing is suitable.

## **Discussion and Conclusion**

This strategy enables prioritization of control measures within the farm and facilitates tracing of the infection. Furthermore, the strategy provides information to improve the quality of outbreak epidemiological investigations and presents potential for modifications in control approaches.

## RECONSIDERATION OF THE DIAGNOSTIC CRITERIA REQUIRED FOR PCV2 REPRODUCTIVE DISEASE

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## **Background and Objectives**

For a correct diagnose of PCV2-RD, a combination of specific lesions in foetal material with detection of PCV2 in the lesions (middle to high grade) have to be observed. For routine workup at Vetmeduni Vienna two weak points emerged: while the literature proposes >10<sup>7</sup> GE/500 ng extracted DNA from myocardium to be the cut-off for PCV2-RD, the unit used for routine diagnostics is "GE/g tissue" and histological lesions are rarely found. The objective of this study was to determine a comparable cut-off, applicable for the present diagnostic settings (real time qPCR of heart tissue using the AVID-method VIR02, histopathological examination and ISH).

# **Material and Methods**

During 2017–2019, 300 aborted, stillborn, autolytic or mummified piglets from 103 Austrian farms were analysed for the involvement of PCV2 in fertility disorders with a 10.5 % prevalence (positive, if qPCR result > 10<sup>5</sup> copies/g tissue). Pathohistological examinations and subsequent ISH of 30 hearts from 10 different farms, positively tested for PCV2 by qPCR and negatively tested for PRRSV, PPV, Leptospira spp. and Chlamydia spp., were performed.

### Results

We determined the viral load at which the ISH results positive signals with as 10° copies/g heart tissue. There was no evidence of histopathological lesions in any sample. The highest viral loads (10<sup>12</sup> copies/g tissue) were detected in autolysed and mummified piglets and were identified as PCV2d. Co-infections of PCV2a and b as well as PCV2a and d were also found in individuals. In all cases with high viral load (>10° copies/g tissue) subsequent PCV2 vaccination of sows was successful according to herd veterinarians.

## **Discussion and Conclusion**

In case of PCV2-RD, it should be reconsidered if pathohistological lesions are a prerequisite. 10^9 GE/g tissue could be handled as the cut-off value for this specific diagnostic setting (ISH + qPCR).

## **PORCINE TESCHOVIRUS – A REEMERGING AGENT?**

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## **Background and Objectives**

The Teschoviruses are small, single-stranded RNA viruses belonging to the Picornaviridae. Porcine Teschoviruses (PTV) infections are associated with different clinical outcomes including polioencephalomyelitis, reproductive disorders, intestinal diseases and pneumonia. Sporadically occurring individual cases and endemically recurring herd problems are increasingly reported from Germany.

### **Material and Methods**

More than 40 piglets were clinically examined following a PTV outbreak in a conventional piggery. Six animals with severe clinical symptoms were necropsied. Brain and spinal cord samples were taken for virological examination. All samples were analyzed using RT-PCR and virus isolation in cell culture.

### Results

The piglets in the rearing unit (7-12 weeks old) were particularly affected. Morbidity ranged from one to 17%. Clinical signs such as ataxia, dog-sitting position and paralysis of the hind limbs were observed in severe cases. Up to 70% of the affected animals had to be euthanized. Histopathological examination revealed a moderate, subacute, multifocal, non-suppurative polioencephalomyelitis and meningitis in these animals. PTV was detected in the brain and spinal cord samples by RT-PCR. Cell culture isolation and nucleotide sequencing revealed the genomic sequence of a so far unknown PTV strain. An inactivated herd-specific vaccine was prepared and administrated because the intended spread in the herd did not produce satisfactory immunity.

#### **Discussion and Conclusion**

Typical clinical presentation and pathological findings referring to infectious paresis led to the molecular detection and sequence determination of a so far unknown, virulent PTV strain. The Teschovirus encephalomyelitis is currently a rare disease in Germany, although severe outbreaks with large economic losses occurred in the past. It is believed that these outbreaks ended when the pig population had developed sufficient immunity against circulating PTV strains. However, since the pig population remains susceptible to infection with PTV strains to which they have not previously been exposed, special attention should be drawn to the emergence of novel, divergent Teschoviruses.

# VIREMIA AND SHEDDING OF PRRSV AUT15-33 ("ACRO" PRRSV) BY VACCINATED AND NON-VACCINATED PIGLETS

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## **Background and Objectives**

The aim of the present study was testing of the efficacy of Ingelvac PRRSFLEX EU in decreasing viremia levels and viral shedding in piglets after a challenge with a virulent PRRSV-1-isolate (PRRSV AUTI5-33) causing severe clinical problems in the field.

# **Material and Methods**

Vaccinated and non-vaccinated piglets (4 groups, n=16 per group) were intranasally infected with a low dose (1x10^3 TCID50) or a high dose (1x10^5 TCID50) of PRRSV AUT15-33 28 days post vaccination (study day 28). Serum samples and oral swabs were collected at different time points (study days 0, 14, 21, 28, 31, 33, 35, 37, 39, 42, 49, 56, 63, and 70) throughout the study to assess the viremia levels and viral shedding by qRT-PCR.

### Results

Viral load in serum increased in all infected piglets after challenge. Slower increase was measured in the low dose infected groups than in the high dose infected groups. Viral load of the respective non-vaccinated group increased faster compared to the vaccinated group. On study day 39 all infected pigs reached approximately same viremia levels.

All infected animals shed virus in oral fluids. There was a significant reduction in the AUC of PRRSV RNA load in oral swabs of vaccinated, low dose infected pigs compared to non-vaccinated, low dose infected pigs (p < 0.05) as well as between vaccinated and non-vaccinated high dose infected pigs.

# **Discussion and Conclusion**

All infected animals reached approximately same viremia levels post challenge and all animals shed virus through oral fluids. Nevertheless, vaccinated infected groups shed less virus than the non-vaccinated animals. Furthermore, the duration of shedding was lower in vaccinated pigs than in non-vaccinated pigs.

# THE SWISS PIG TRANSPORT NETWORK: IMPLICATIONS FOR DISEASE TRANSMISSION BETWEEN ANIMAL HOLDINGS

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## **Background and Objectives**

The topology of animal transport networks can contribute substantially to how fast and to what extent an infectious disease spreads between animal holdings. While previous research has mostly focused on animal movement data from government databases, we also consider more granular transport data from a Swiss livestock transport company, which reveal additional forms of contact between animal holdings through shared trucks and truck contamination. The primary goal of our work is to investigate the topology of the network and to assess its impact on disease spread.

## **Material and Methods**

Our investigation was based on two datasets. First, we examined the topology of the government database, which contained animal batch movements. Second, we examined a sample of transport data from which we inferred additional contacts between holdings based on (i) batches from different holdings that shared the same truck and (ii) truck contamination. We used social network analysis tools to measure individual and overall network connectedness in both datasets. We focused specifically on temporal network measures.

### Results

For the analysis, we considered all monthly networks over a 4-year period. By considering the additional contacts due to truck sharing and truck contamination, both individual and overall network connectedness of those networks increased substantially. Nevertheless, the networks remained strongly disconnected as even in the worst-case, only 0.34% of all holding pairs were connected through time-respecting paths.

## **Discussion and Conclusion**

We conclude that the Swiss pig transport network is strongly fragmented, which naturally prevents largescale disease outbreaks. However, the analysis of the transport data demonstrated that truck sharing and especially truck contamination increased the connectedness of the network which may lead to an underestimation of the outbreak size if only government administered databases are considered. Based on these results, we expect a worst-case outbreak size of 70 to 80 animal holdings in one month for Switzerland.

# BIDIRECTIONAL HUMAN-SWINE TRANSMISSION OF SEASONAL INFLUENZA A(H1N1)PDM09 VIRUS IN PIG HERD, FRANCE, 2018

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# **Background and Objectives**

In 2009, pH1N1 influenza A virus (IAV) emerged in humans, then spread in pig populations. Phylogenetic analyses suggest de novo human-to-swine pH1N1 transmissions occur during seasonal epidemics. This study, investigated a case of pH1N1 infections in a herd located in a low pig density area in France.

# **Material and Methods**

During winter 17-18, in a farrowing herd (1000 sows), sows exhibited an influenza-like illness (ILI). The veterinarian and a technician handled the animals and collected nasal swabs from 3 sows for IAV detection (M-gene RT-qPCR), molecular subtyping (HA-/NA-gene RT-qPCR), virus isolation and full genome sequencing (NGS). The veterinarian (72 hours later) and technician (48 hours later), both not vaccinated against seasonal influenza, got ILI symptoms. Neither reported close contact with sick humans before their symptom onset. On days 5 and 6 after handling the pigs, the veterinarian self-collected nasal swab samples for IAV analyses.

## Results

All samples were positive for IAV genome. The HA and NA genes identified were exclusively those of pH1N1. The nucleotidic sequences of the viruses isolated from sows and veterinarian showed 100% of identity. These virus sequences were compared with those of pH1N1 strains available in the GISAID database using the integrated BLAST program; the highest similarities (up to 99.94% identity) were found with a pH1N1 isolate identified in France during the 2017–18 winter. Isolates from our case study were more closely related to seasonal influenza isolates than isolates from the swine specific lineage identified in 2015–2016 in France.

# **Discussion and Conclusion**

Considering that before outbreak, no gilts were introduced but that a stockperson got ILI, the virus was most probably transmitted to sows by an infected person. Afterwards, it was reversely transmitted to the veterinarian. This study provides evidence of bidirectional transmission of pHINI between humans and pigs in a pig herd.

# COACHING AIDS IN REDUCTION OF ANTIMICROBIAL USE AND RESISTANCE IN BELGIAN AND DUTCH PIG FARMS; I-4-1-HEALTH PROJECT RESULTS

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# **Background and Objectives**

Reduction of antimicrobial use (AMU) is the first step in curbing antimicrobial resistance (AMR). However, influencing farmer behavior is challenging. In the i-4-1-health project in the Dutch(NL)-Belgian(BE) crossborder region, 29 pig farms were coached to reduce AMU and study effects on AMR. To assess farmer's attitude and behavior towards AMU the ADKAR coaching tool, scoring for Awareness, Desire, Knowledge, Ability and Reinforcement was adjusted to use for farmers.

## **Material and Methods**

Four farm visits were conducted on 29 farms with high AMU over 18 months. Biosecurity (BioCheck-UGent), technical performance, AMU (treatment incidence/100 days) and AMR were assessed. AMR was determined in Enterobacteriaceae from fecal samples (FecalSwab, Copan Italy) on selective agar plates (ChromID ESBL/CARBA/OXA-48, bioMérieux; McC-ciprofloxacin 2 mg/L, in house). Coaching started four weeks after the first visit, based on a tailor-made action plan. The farms were revisited twice to evaluate implementation and reinforce compliance and collect samples. MLE models with random farm and fixed country and time effects were used.

## Results

The initial AMU in weaned pigs was 65% lower for NL versus BE farms and decreased with 53% in BE, and 7% for NL herds. Biosecurity scores significantly improved in BE farms, but overall were not significantly associated with AMU course on farms. High scores for farmer's Awareness, Desire and Knowledge on AMU were significantly associated with lowering AMU. Ciprofloxacin-resistant (Cipro-R) and ESBL-producing Enterobacteriaceae (ESBL-E) were found more on BE compared to NL farms. No Carbapenem resistance was detected. A significant decrease in Cipro-R was observed over time, but not for ESBL-E. 36% of Cipro-R samples tested ESBL+, in contrast to 16% of Cipro-S samples (adjusted OR=2.4).

## **Discussion and Conclusion**

Coaching towards improvemed infection control and prudent AMU resulted in clear reduction of AMU and the project provided insights in AMR on pig farms.

# SEROLOGY-BASED SALMONELLA MONITORING IN THE NETHERLANDS; SAMPLING AT THE FARM OR AT THE SLAUGHTERHOUSE?

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## **Background and Objectives**

The serology-based Salmonella monitoring program in the Netherlands applies to all farms with more than 30 fattening pigs and is based on the analysis of 12 blood samples/trimester (36 samples/year). Blood samples can be collected at the farm or at the slaughterhouse. Samples are analyzed for the presence of antibodies against LPS from Salmonella (serogroups B-CI-D). Scores are given based on the number of samples with an OD%>40, farms are classified into risk categories based on the results of the last three trimesters.

### **Material and Methods**

From January 2015 until November 2019, Royal GD has evaluated 382.602 serum samples (2015: 98.150, 2016: 82.661, 2017: 73.648, 2018: 70.126, 2019: 58.017) for the presence of Salmonella-specific antibodies with a commercial ELISA-kit (IDEXX Swine Salmonella Ab Test®). Of these samples, 17-36% were collected at the farm, 58-77% were collected at the slaughterhouse, and 4-6% were submitted for diagnostic purposes (not discussed further).

## Results

The mean OD% of the samples collected at the farm (OD% 16) was significantly lower compared to the mean OD% of the samples collected at the slaughterhouse (OD% 28) (p<0.001, each year). The percentage of samples collected at the farm with an OD%>40 (12%) was significantly lower compared to the percentage of samples collected at the slaughterhouse with an OD% >40 (20%) (p<0.001, each year).

### **Discussion and Conclusion**

Although sampling at the farm might result in a lower number of samples with an OD%>40, which is beneficial for the serological status in Salmonella monitoring programs, it should be encouraged to sample fattening pigs at the slaughterhouse. Sampling at the slaughterhouse provides an easy sampling method and avoids stress for the animals. Additionally, since all sampled animals are from the same age-category, this allows for standardized classification of farms into risk categories, which is the main goal of Salmonella monitoring programs.

# FACILITATING HERD LEVEL DIAGNOSES AND PIG HEALTH SURVEILLANCE THROUGH TARGETED POST-MORTEM SAMPLING BY VETERINARIANS – A PILOT STUDY

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# **Background and Objectives**

Post-mortem examination is a powerful diagnostic tool for confirming (presumptive) herd diagnoses in pig herds, thereby facilitating prudent antimicrobial usage and exclusion of notifiable diseases. This information gathered can also contribute to a better insight into the health situation and early disease detection across herds. When sending a pig to a pathological laboratory is impossible, the solution can be that the herd veterinarian opens dead pigs directly on-farm and takes appropriate samples for further analyses.

# **Material and Methods**

In 2019 the Federal Food Safety and Veterinary Office (FSVO) initiated a pilot, in which 13 pig veterinarians, specifically trained in targeted post-mortem sampling ("TPS"), receive financial support for TPS on farms. In return, in compliance with data protection requirements, they provide the FSVO with case documents. Close collaboration with laboratories is part of the project, and veterinarians receive continuous technical support by the university swine clinics (telephone hotline, regular case review meetings).

## Results

Since January 2019, 140 pigs from 76 farms were examined. The distribution of age categories was 35% suckling, 31% weaning, 25% fattening and 9% (sub)adult pigs. Most cases were gastrointestinal (64%), systemic (14%) or cardiovascular disorders (6%). Exclusion examinations for notifiable diseases were initiated on 4 farms. In 80% of cases, the veterinarian identified the cause of the herd problem, and in 81%, the health situation improved according to a follow-up examination.

## **Discussion and Conclusion**

First results suggest good acceptance of TPS by veterinarians and farmers and a good collaboration with laboratories. The high proportion of clear diagnoses indicates a good command of the method by veterinarians. Overriding analysis allowed an informative insight into the general health situation in Swiss pig herds. With the high improvement rate of herd problems after TPS, a widespread use of the method by trained veterinarians can contribute to a further improvement of pig health in Switzerland.

# REDUCED USAGE OF HIGHEST PRIORITY CRITICALLY IMPORTANT ANTIMICROBIALS IN PIGS WITHIN THE SUISSANO PROGRAM IN SWITZERLAND

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## **Background and Objectives**

In order to improve transparency and to monitor antimicrobial usage in pig production in Switzerland, the Suissano program was started in 2015. One important goal of this program was to reduce the usage of Highest Priority Critically Important Antimicrobials (HPCIAs) in participating pig farms. For this reason, treatments with HPCIAs were multiplicated with factor four,when reporting antimicrobial usage to the farmers within the Suissano program. Since April 2016 the use of HPCIA is restricted by legal regulations in Switzerland. The aim of the study was to investigate the impact of both legal regulations and private initiatives in reducing the usage of HPCIAs.

### **Material and Methods**

The number of study farms was 312 in 2015, 483 in 2016 and 598 in 2017. All veterinary prescriptions were assigned to four age groups: suckling piglets, weaned piglets, fattening pigs and sows. Antibiotic usage in the study farms was calculated based on Defined Course Doses ( $DCD_{CH}$ ) per animal per year and analysed by antibiotic classes for the years 2015, 2016 and 2017.

## Results

The relative usage of HPCIAs in all age groups decreased from 25% in 2015 to 10% in 2017. In sows the relative usage decreased from 17% in 2015 to 2% in 2017, in piglets from 20% to 5%, in weaners from 42% to 26% and in fattening pigs from 8% to 3%.

#### **Discussion and Conclusion**

The reduced usage of HPCIAs described in this study was most likely induced by legal regulations and the impact of the multiplication factor of such treatments within the SuisSano programme. Because of the voluntary character of the SuisSano programme participating farmers may be more motivated than others to reduce the usage of HPCIAs on their farms.

## POST-WEANING DIARRHEA OUTBREAKS IN DANISH HERDS NOT USING MEDICINAL ZINC OXIDE

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# **Background and Objectives**

Medicinal zinc oxide (M-ZnO) is widely used in the intensive pig production to prevent post-weaning diarrhea (PWD) caused by enterotoxigenic Escherichia coli, but from June 2022 the use of M-ZnO is no longer permitted in the European Union.

Objectives: To determine the proportion of weaner herds not using M-ZnO in Denmark; the frequency of PWD problems in herds not using M-ZnO; and the within-outbreak morbidity and etiology of PWD in these herds.

## **Material and Methods**

The study included all indoor herds in Eastern Denmark with >200 nursery pigs (7-30kg) according to the Danish Central Husbandry Register in February 2019 (n=638). Out of these, 129 herds were revealed not to accommodate newly weaned pigs. Herds were categorized as using M-ZnO or not-using M-ZnO based on data of M-ZnO purchase from the VetStat-register and telephone interviews. Clinical and microbiological investigations of PWD outbreaks were performed in herds reporting outbreaks of PWD and not-using M-ZnO.

#### Results

Only 4.8% (n=24) of the weaner herds did not use M-ZnO Among these herds, 50% (n=12) experienced problems with PWD, as evidenced by routine use of antimicrobial batch medication (n=2) or frequent metaphylactic antimicrobial batch medication of diarrhea-outbreaks (n=10). Among the latter group, PWD-outbreaks were investigated in nine herds. The within-outbreak diarrhea prevalence was between 12.8% and 95.7%. Hemolytic E. coli was isolated from 66.7% of the diarrheic pigs (n=90) and 56.6% of the non-diarrheic pigs (n=83).

### **Discussion and Conclusion**

M-ZnO was widely used for PWD prevention in Denmark. Antimicrobial batch medication was regularly used in 50% of the herds not-using M-ZnO. Hemolytic E. coli was not detected in a substantial proportion (33.3%) of the pigs suffering from PWD. These findings suggest that Danish swine herds may have to implement preventive measures and perform proper diagnostic procedures to comply with antimicrobial stewardship when terminating the M-ZnO usage.

# NO DIFFERENCE IN OCCURRENCE OR LEVELS OF LAWSONIA INTRACELLULARIS IN A FAECAL SOCK SAMPLING FROM DANISH FINISHER BATCHES WITH OR WITHOUT OUTBREAK OF DIARRHEA.

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# **Background and Objectives**

Lawsonia intracellularis (LI) infections causes loss of production in both nursery and finisher pigs but investigations on LI in finisher herds have been sparse. The objective of this study was to evaluate the association between faecal LI excretion and occurrence of diarrhea at batch level.

## **Material and Methods**

In 60 finisher herds with a history of diarrhea and previous detection of LI at some point in time, sock sampling was carried out from November 2019 till April 2020. All herds contributed with 1 sample either from a batch having experienced outbreak of diarrhea within the first 4 weeks post entry or if such a batch was not found then with 1 sample at a fixed time point 3-4 weeks post entry from a batch without diarrhea. The sock samples were tested for LI by qPCR at the National Veterinary Institute, Technical University of Denmark using BioMark platform (Fluidigm). Lower detection limit being 3 log10 bacteria/gram faeces.

### Results

Of 26 samples from batches experiencing an outbreak of diarrhea, 23 samples were positive to LI with a mean level of LI at 5.8 log10 bacteria/gram faeces (min: 3 log10, max: 7.7 log10, median: 6.2 log10).Of 34 samples from batches without diarrhea, 28 samples were positive to LI with a mean level of LI at 5.7 log10 bacteria/gram faeces (min: 3 log10, max: 7.5 log10, median: 6.0 log10).No significant difference was found neither regarding the occurrence of LI (Fisher's exact test, P=0.71) nor the level of LI (Wilcoxon test, P=0.66) in samples from batches with or without diarrhea.

## **Discussion and Conclusion**

In agreement with previous findings in nursery pigs, it was found that even at high levels, LI does not necessarily cause diarrhea. Occurrence of diarrhea at batch level was not a sufficient diagnostic tool to evaluate the presence or impact of LI in finisher pigs.

# PHYLOGENETIC RELATIONSHIPS BETWEEN SEROVAR 8 ISOLATES OF ACTINOBACILLUS PLEUROPNEUMONIAE FROM THE UNITED KINGDOM, DENMARK AND NORWAY

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## **Background and Objectives**

Actinobacillus pleuropneumoniae (APP) is the major pathogen causing porcine pleuropneumonia, a disease of great impact on pig health and productivity globally. There are 18 known serovars that vary in prevalence between geographic regions. Bioinformatic analysis of whole genome sequences grant us the opportunity to study relationships between APP strains in different countries. The objective of this study was to compare genomes of APP serovar 8 (APP8) isolates from UK, Denmark and Norway to determine their relationships and estimate time of separation from a common ancestor.

## **Material and Methods**

67 UK, 22 Danish and 130 Norwegian clinical APP8 isolates have been collected between 1983 and 2019. Extracted genomic DNA from each isolate was sequenced and assembled into draft genomes. Single nucleotide polymorphisms within the core genome were identified following alignment of the draft sequences to the complete genome of MIDG2331, a UK APP8 isolate. Phylogenetic reconstruction was performed using maximum-likelihood and bootstrap support values. Visualization and annotation of the phylogenetic trees were performed using an online Interactive Tree Of Life (iTol v4.314). The phylogenetic relationships of these isolates will be further compared by means of Bayesian Evolutionary Analysis by Sampling Trees (BEAST) to evaluate the hypothesis of common ancestry and transmission between geographic areas, and the genetic drift within this population of bacteria.

## Results

The APP8 isolates from UK and Norway were completely separated into two distinctive phylogenetic branches. Three Danish isolates clustered with those from the UK, while the remaining 19 Danish isolates clustered with those from Norway. Preliminary results of the BEAST analysis will be presented.

#### **Discussion and Conclusion**

The results from this study indicate that bioinformatic analysis of draft genomes is a valuable tool in surveillance of pathogens and can be helpful when assessing transmission patterns between populations.

#### STOMACH ULCERATION IN PIGS: IDENTIFYING THE SUSPECTS

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#### **Background and Objectives**

The exact mechanism behind porcine gastric ulceration is not completely clear. Our aim was to gain new insights in the role of Helicobacter suis and a novel bacterial species, Fusobacterium gastrosuis.

## **Material and Methods**

The impact of a H. suis infection on gastric acid secretion was investigated in naturally H. suis-infected and non-infected pigs of different age groups. The ability of F. gastrosuis to induce cell death in vitro was determined, its genome was analysed for the presence of virulence factors and its abundance was determined in H. suis-infected and non-infected pigs.

#### Results

During the acute phase of a H. suis infection, gastric acid secretion is unaffected and so no irritation occurs of the Pars oesophagea. Later on, a decreased gastric acid secretion affects the composition of the Pars oesophageal microbiota, which may affect development of lesions in this stomach region. Indeed, higher numbers of F. gastrosuis were detected in the Pars oesophagea of H. suis-infected pigs with gastric acid secretion alterations and lesions. It was demonstrated that F. gastrosuis induces epithelial cell death in vitro and that genes are present in the genome of this bacterium with sequence similarity to genes encoding factors involved in adhesion, invasion and induction of cell death as well as immune evasion. Finally, increased production of gastric acid during the chronic phase of a H. suis infection might further aggravate severity of lesions in this stomach region, which is not protected by mucus.

### **Discussion and Conclusion**

We hypothesize that H. suis is involved in gastric ulceration through its effects on gastric acid secretion and on the gastric microbiota composition. F. gastrosuis might also play a role by inducing epithelial cell death. Inhibition of colonization by these bacteria and/or their induced pathologies can be considered as potential control measures against porcine gastric ulceration.

# IMPACT OF GENOTYPIC VARIABILITY OF MYCOPLASMA HYOPNEUMONIAE ON VIRULENCE AND DYNAMIC OF INFECTION.

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## **Background and Objectives**

Enzootic pneumonia still causes significant economic losses in the pig industry; although the genotypic variability of Mycoplasma hyopneumoniae (M. hyopneumoniae) is well known, little is known about how the different genotypes influence the virulence in field. The objective of the present study was to evaluate the associations between single or multiple genotypes infection and lung lesions at lobe level.

## **Material and Methods**

Seven farms with a history of enzootics pneumonia due to M. hyopneumoniae during fattening phase were selected. Thirty pigs were randomly chosen from each farm to asses lung score at slaughterhouse. The lobes with Mycoplasma-like lesions were collected and analyzed by real-time PCR, the positive samples were genotyped by Multiple Locus Variable number tandem repeat Analysis (MLVA).

# Results

The lungs with lesions were 101 and the lobes sampled were 427, of those 260 resulted positive at real-time PCR (60.9%). The genotyping showed the presence of 22 different genotypes distributed in single (79.2%) and multiple infections (20.8%). The majority of the genotyped lobes scored one (44.6%) or two (29.3%), the remaining scored three (18.8%) and four (7.3%). The magnitude of lesions are not directly proportional to the rate of multiple infections at lobe level.

# **Discussion and Conclusion**

Despite the wide genotypic variability, most of the infections were single. Therefore, several genotypes colonize alone the lung lobes, thus is reasonable to think that the infection with some genotypes overcame others or some genotypes tend to remain more stable and not undergo mutation. Furthermore, different genotypes can have a distinct virulence features. Patterns of infection are complex; genotypes can play a primary role or worsen previous infections. Our preliminary results demonstrate that M. hyopneumoniae is highly variable in the field and a better completion of virulence traits is necessary.

# EVALUATION OF A NOVEL ZINC CHELATE IN THE TREATMENT OF CLINICAL SWINE DYSENTERY UNDER FIELD CONDITIONS IN THE NETHERLANDS

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## **Background and Objectives**

Brachyspira hyodysenteriae is the primary cause of swine dysentery and primarily affects pigs during the grow/finishing stage. A novel non-antibiotic zinc chelate has been reported to demonstrate positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and B. hyodysenteriae excretion after a 6-day oral treatment. The objective was to evaluate the zinc chelate (IntraDysovinol® 499 mg/ml; Elanco) on naturally occurring swine dysentery due to B. hyodysenteriae under Dutch field conditions.

## **Material and Methods**

Two conventional pig farms with clinical signs of swine dysentery were selected and pigs were randomly attributed to control/treatment. Pigs were treated with the oral zinc chelate according to label instructions. Several individual clinical and performance parameters were collected besides individual fecal samples were collected for qPCR analysis of B. hyodysenteriae. Statistical analysis was performed using JMP 14.0 – ANOVA.

#### Results

Oral administration of zinc chelate resulted in improvement of general clinical signs, total fecal score, average daily weight gain during and after treatment and a reduction in B. hyodysenteriae excretion and a high percentage of animals with no excretion of B. hyodysenteriae. No additional antimicrobial treatments were needed in the treated group, whereas 35 percent of the pigs in the control group were treated with an antibiotic at least once. No mortality occurred in both groups.

# **Discussion and Conclusion**

Zinc chelate is a novel non-antibiotic treatment for swine dysentery due to B. hyodysenteriae that reduces B. hyodysenteriae shedding within its 6-day treatment, improving clinical signs and fecal quality within 2-4 days of administration in naturally infected pigs. The treatment resulted in a higher growth rate and improved general health, whereas no mortality was observed and no additional therapeutic treatments were necessary in contrast to the control pigs.

## CHARACTERIZATION OF STREPTOCOCCUS SUIS CAUSING MENINGITIS IN SPANISH PIG HERDS

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# **Background and Objectives**

Streptococcus suis is a worldwide swine pathogen frequently involved in septicemia, meningitis, arthritis, endocarditis and pneumonia. To date 29 different serotypes have been described showing different frequency distribution depending on the geographical area. Serotypes 2, 7 and 9 are predominant in Europe. Nonetheless, there is no updated information about the current situation in Spain. Moreover, although many virulence factors (VF) have been proposed for this bacteria, only SLY, MRP and EPF have been regularly associated with European diseased animals infected with particular serotypes.

## **Material and Methods**

A set of qPCR assays to detect serotypes 1-14, 2-½, 3, 4, 5, 7, 8 and 9 was developed targeting their respective cps locus. Moreover, 3 assays detecting sly, mrp and epf were designed to run simultaneously in a multiplex qPCR reaction.

Ninety-eight different S. suis strains were recently isolated from the brain of diseased animals with nervous signs in Spain. Afterwards, isolates were analyzed by qPCR to determine their respective serotype. Furthermore, 56 of those isolates were characterized for the above mentioned VF.

## Results

Serotype 9 was the most frequently found (47%) and its VF pattern was without exception (sly<sup>pos</sup>epf<sup>neg</sup>mrp<sup>pos</sup>). Afterwards, serotype 2-1/2 (17%) and serotype 1-14 (11%) were reported showing the same chageless VF pattern (sly<sup>pos</sup>epf<sup>pos</sup>mrp<sup>pos</sup>). Just 13 isolates (13,3%) remained as untypeable because resulted negative for all the studied serotypes.

## **Discussion and Conclusion**

Serotype 9 has clearly replaced serotype 2 as the most prevalent serotype in Spain and probably in other European countries. Our findings endorse previous studies which pointed a particular pattern of VF as remarkable to determine the virulence of the strains belonging to serotypes 2 and 9. This set of qPCR assays, which also allows direct detection on tissues such as brain, joints and organs, has proven to be effective for serotyping Spanish S. suis isolates since over 87% of the strains were characterized.

# MOLECULAR CHARACTERISATION OF BRACHYSPIRA HYODYSENTERIAE: DEMONSTRATING THE VALUE TO VETERINARY PRACTITIONERS

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## **Background and Objectives**

A rise in swine dysentery (SD) diagnoses in Great Britain from 2017 prompted investigation using whole genome sequencing (WGS) to find possible links between incidents and predict antimicrobial susceptibility.

## **Material and Methods**

WGS and minimum inhibitory concentrations (MIC) by broth microdilution for six antimicrobials were assessed on Brachyspira hyodysenteriae isolated from GB submissions during 2017-2019. Multi-locus sequence types (STs) and AMR genotypes were determined. Relatedness was assessed through SNP-based core genome phylogenetic analysis.

### Results

B. hyodysenteriae isolates from 61 submissions grouped into nine STs, the majority (91.8%) being ST52, 88, 229, 242 or 251, each of which comprised 9–13 isolates. ST52 was detected in six GB regions and was most commonly detected in smaller pig herds. The other four main STs were detected in 2–3 regions. Several AMR genotypes were observed, for example; ST251 harboured multiple AMR determinants, ST229 carried none and ST88 had diverse AMR genotypes. The tvaA gene, predicting reduced pleuromutilin susceptibility, was present in 64% of isolates. MIC values were raised for valnemulin but not in all isolates for tiamulin, however none were clinically resistant (>4µg/ml).

## **Discussion and Conclusion**

Phylogenetic analysis showed the rise in GB SD diagnoses involved multiple STs in 2017-19. Analysis of WGS data helped identify or rule out potential epidemiological links between incidents and could indicate means of introduction or spread. If disease re-occured, it can differentiate new from previous infection. Some STs were found more commonly in certain regions or in small-scale pig herds and others were not detected prior to 2017. Full value is obtained if information is shared and an interactive MLST dashboard is being developed to facilitate this. Detecting the tva(A) gene and others associated with antimicrobial resistance provided veterinarians early warning of an isolate's potential resistance, but MIC testing remained necessary for definitive assessment of susceptibility.

# EVALUATION OF FINISHING PIG FARMS AT SLAUGHTER FOR ILEITIS DUE TO INFECTIONS WITH LAWSONIA INTRACELLULARIS

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## **Background and Objectives**

llea at slaughter can be investigated without the need to sacrifice animals. The objective of this study is to evaluate the presence of ileitis at slaughter.

## **Material and Methods**

At slaughter 40 ilea per farm were collected. The first 10 ilea samples were taken (at random selected ilea; ARI). Subsequent ten ilea with the most apparent visible lesions were selected (Macroscopic selected ilea, MSI). A sample of ileum tissue was taken for Lawsonia intracellularis (LI) qPCR analysis. From the first 8 farms IHC was carried out for the MSI. Based on the results of the first 15 farms, it was decided to continue analyzing only 10 ARI.

### Results

In total 376 ilea obtained from 23 different farms were analyzed, seven farms tested negative for all PCR samples. IHC was well correlated with qPCR results (neg <log5, pos>log6; sensitivity 95% specificity 93%). From five farms, all selected ilea were positive by LI-qPCR. From the positive ilea, ARI were significant lower in the amount of present LI bacteria (ARI: 5,40 log GE/mI; n=48 vs MSI: 6,61 log GE/mI, n = 48; p< 0,0001). Detection of presence of LI not different between ARI and MSI.

#### **Discussion and Conclusion**

This is the first report of the use of LI qPCR taken from ileum samples at slaughter for confirmation of disease on farm. The random selection of ilea at slaughter is a useful tool to check the ileitis status of a batch of pigs. Macroscopic evaluation of ilea is not enough to diagnose ileitis, since other (parasitic) infections or stress related events prior to slaughter can give macroscopic visible changes. The evaluation of 10 random selected ilea at slaughter could be a useful tool in assessing the ileitis status at slaughter. The use of qPCR analysis of ileum tissue is a good and relative fast and affordable alternative to IHC.

# EVALUATING MYCOPLASMA HYOPNEUMONIAE NATURAL TRANSMISSION AND DETECTION DYNAMICS IN A RECENTLY EXPOSED NAÏVE POPULATION

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## **Background and Objectives**

Mycoplasma hyopneumoniae (Mhp) continues to be a prevalent and economically important, respiratory pathogen. Replacement gilts can be a risk for pathogen entry into negative farms. Serum and oral fluid samples are commonly tested to clear incoming replacement gilts for several pathogens, including Mhp. However, diagnostic limitations exist with these sample types, particularly due to low sensitivity during acute stages of Mhp infection. Therefore, the objective of this study was to evaluate the early detection of natural Mhp infection in a recently exposed population.

### **Material and Methods**

Twenty-nine, 10-week old Mhp and PRRSV negative gilts were housed with a Mhp naturally infected gilt for 8 weeks (initial 3% prevalence). Deep tracheal catheters (DTC), laryngeal swabs and blood samples were obtained on 0, 1, 2, 4, 6, and 8 weeks post-exposure (wpe), along with the collection of oral fluids from the group. The Mhp natural transmission rate (ß) was estimated using a Bayesian logistic regression model (assuming a SI dynamic model).

## Results

At 8wpe, 27% of the naïve contact gilts became infected (B=0.36). Antibodies for Mhp were initially detected in the naturally infected gilt at 6 weeks post-infection. One contact gilt became seropositive at 8 wpe. At all samplings, Mhp was detected by PCR in DTC from the naturally infected gilt. At 6 and 8wpe, 3% and 17% of the contact gilts were identified as Mhp positive by PCR in DTC. Oral fluids were negative for Mhp at all samplings in the study, regardless of the presence of infected gilts in the group.

## **Discussion and Conclusion**

Overall, these results showed that sample type and diagnostic methods are key for Mhp detection, especially considering acute stages of infection and risk of false negatives. The use of pathogen specific, surveillance protocols is needed to achieve high sensitivity and avoid the introduction of potentially infected gilts into naïve sow farms.

# SCORING AND INFRARED THERMOGRAPHY IN SWINE INFLAMMATION AND NECROSIS SYNDROME (SINS) IN SUCKLING PIGLETS OF DIFFERENT BREEDING POPULATIONS

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## **Background and Objectives**

SINS is a multifactorial event causing inflammation of tail, teats, coronary bands, soles, heels and claws. The aim of the present study is to investigate if clinical evaluation of the syndrome and discrimination of susceptibility in different breeds and breeding lines can be improved by infrared thermography.

## **Material and Methods**

One hundred twenty piglets from Pietrain and Duroc boars mated by mixed sperm insemination to a Yorkshire x Landrace sow herd were scored and thermographed between their first and fourth day of life. The mating system was applied to minimize environmental effects. Paternity was identified with DNA isolated from the tips of the docked tails, after phenotyping was completed (blinded).

#### Results

SINS symptoms were significantly more pronounced in any of the body parts in Pietrain than in Duroc offspring (P<0.001), e.g. 59% of tail bases in Pietrain-offspring, but only 8% of the Duroc-offspring were affected. More severe cases of SINS showed an abrupt temperature drop of more than 1°C within a distance of 1 mm in the tail base. This finding speaks for a circulatory disorder and is located directly before the inflammation. Infrared thermography shows a significantly stronger decrease of the tail temperature from the base to the tip in piglets with inflammation compared to offspring without clinical signs of inflammation.

#### **Discussion and Conclusion**

Pietrain offspring are significantly more affected by SINS than Duroc offspring. Infrared thermography can be successfully used to improve diagnostics of inflammations in the tail at an early stage. This leaves more room for the initiation of countermeasures in the herd. The findings of infrared thermography can be interpreted as an indication of latent to manifest circulatory disorders in the area of the tail affected by SINS.

# EFFECT OF LIVE YEAST SACCHAROMYCES CEREVISIAE SUPPLEMENTATION ON THE PERFORMANCE AND HINDGUT MICROBIOTA COMPOSITION OF SUCKLING AND WEANLING PIGLETS

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## **Background and Objectives**

Today, due to consumer pressure over antibiotics resistance and the demands of high quality products, regulatory bodies are looking on new strategies to have a responsible use of antibiotics in animal feed. As one of the solutions, Live yeast supplementation has proven useful in reducing weaning stress and improving performance parameters of piglets.

## **Material and Methods**

Two independent studies were conducted in suckling and weanling pigs where piglets were fed either control diet or diet with live yeast Saccharomyces cerevisiae (Actisaf Sc 47; Phileo by Lesaffre, Marcq-en-Baroeul, France). Performance parameters were measured every week and cecal content samples collected at the end of each experiment for microbial profiling by the 16S rRNA gene sequencing method

## Results

Results show that yeast supplementation significantly improved ADG (P<0.05) both in the preweaning and post weaning piglets. Alpha and Beta diversity analyses indicated differences among treatments (P = 0.03); Yeast supplementation resulted in development of microbial communities that were phylogenetically more homogenous and less dispersed compared to the microbiota of control piglets. The microbiota of yeast supplemented piglets was enriched with bacterial phylum reported to be beneficial to the host, such as Actinobacteria, specifically family Coriobacteriaceae, as well as Firmicutes families Ruminococcaceae, Clostridiaceae, Peptostreptococcaceae, and Peptococcaceae. Previous reports show that these bacteria are involved in fiber digestion, SCFA production and promoting gut health in piglets. In addition, correlation network analysis on the microbiota of the weanling piglets has revealed that yeast supplementation was associated with enrichment of positive correlations among proportions of different bacterial genera within the hindgut ecosystem.

## **Discussion and Conclusion**

Within the cecal microbiota of supplemented piglets, higher numbers of positive correlations were observed among potentially beneficial genera of the phyla Actinobacteria and Firmicutes, suggesting a mechanism by which yeast supplementation may contribute to regulation of intestinal homeostasis and improved performance of piglets.

# OLIGOELEMENTS OR HEPATOPROTECTORS SUPPLEMENTATIONS TO INCREASE SOWS AND PIGLETS PERFORMANCES : ARE THEY REALLY NECESSARY ?

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## **Background and Objectives**

The aim of this study was to evaluate the effects of oligoelements supplementation with or without support of the liver function on the performances and health of sows and their piglets.

## **Material and Methods**

A total of 84 LargeWhite x Landrace multiparous sows from one farrow-to-finish farm were divided into three equivalent groups based on their parity and backfat thickness (BF). The CONTROL group received the pregnancy and lactating diets classically used in the farm. For the OLIGO and HEPATO groups, an oligoelement supplementation (B09MB2®+B22TEM®, Comptoirdesplantes.com, France) was added to the normal diet during 14 days before farrowing. Plants supporting the liver function (Carestim®, Carephyt, France) were given to the HEPATO group during 7 days before farrowing. The data from sows (weight, BF, parity, breed and duration of lactation) and from their litter (weight at birth, at 24h, at weaning and every week until eight weeks old; mortality and piglets' health) were collected. Daily feed intake was recorded and total feed intake calculated.

### Results

No significant differences were observed on body weight and feed intake of lactating sows with both supplementations compared to the CONTROL group. Nevertheless, for 13 fat sows, the loss of BF after the lactating period is less important for the group OLIGO. All groups presented an equivalent litter size (17.4 piglets/sow on average) and a low preweaning mortality (11.8%). Piglets' performances (weaning weight, average daily gain (ADG) and gain:feed ratio were not significantly different. No diarrhoea or health problems were recorded during the trial.

## **Discussion and Conclusion**

For an homogeneous herd, this study highlights the absence of positive effects of oligoelements or hepatoprotectors supplementations on sows and piglets performances. The positive effect on fat sows (limited number) underlines the importance of a proper veterinary diagnosis to target the specific sows for which a supplementation could be beneficial.

## LOW PROTEIN STRATEGY FOR WEANER DIETS CAN REDUCE DIARRHOEA TREATMENTS

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## **Background and Objectives**

Research has shown reduced protein content post-weaning to be a promising means to control diarrhoea outbreaks among newly weaned pigs. The aim of this trial was to reduce diarrhoea - and thereby antibiotic use - by lowering the protein content in weaner diets without compromising pig productivity.

# **Material and Methods**

Four protein strategies were compared with two control groups with and without 2,500 ppm zinc, respectively, to determine the effect on diarrhoea and on productivity. The trial was conducted at a trial station with high health status. A total of 6,800 DanBred weaner pigs weighing 5.5–9.0 kg were included in the trial until roughly 30 kg. The trial comprised six groups with a total of 75 replicates in 5 groups and 187 replicates in the negative control group without 2,500 ppm zinc.Piglets were fed according to a three-phase strategy: 6–9 kg, 9–15 kg and 15–30 kg. Protein content varied in the four protein strategies: S=Standard=19.0 %, L=Low=16.6%, VL=Very low= 14.0%, M=Medium=17.4%, and H=High=19.3% protein. Free amino acids were added to reach the same level between the groups.

## Results

The pigs given diets including 2,500 ppm zinc had 50% fewer diarrhoea treatments compared to the zerozinc group (p<0.05) with no differences in productivity. The best protein strategy was Low-Low-High with a 29% reduction in diarrhoea treatments (p<0.05), but with a 15 g decrease in daily gain per day from 6-30 kg.

## **Discussion and Conclusion**

Even though a low protein strategy did not reduce diarrhoea treatments as efficiently as 2,500 ppm zinc (29% vs 50%), it is clearly an important part of the solution towards weaning without high levels of zinc. Based partly on this outcome, the protein requirements in Danish pig production are reduced to 17.5% protein in weaner diets (6-15 kg period).

# BUTYRATE PRODUCTS DIFFERENTLY AFFECT PIG HEALTH AND PERFORMANCE, DEPENDING ON THEIR BUTYRATE DELIVERY IN DISTINCT DIGESTIVE TRACT SEGMENTS

<u>T. Goossens 1</u> 1Adisseo

## **Background and Objectives**

Butyrate has been recognized as a feed additive with the potential to increase gut health and animal performance. As it can trigger a plethora of signaling pathways in different cell types along the entire digestive tract, we hypothesized that different butyrate products, with a distinct enteric butyrate release profile, will elicit different responses related to pig health and performance.

## **Material and Methods**

Trial 1; University of Bologna (Italy); 54 piglets, 6 treatments: animals were infected or not with E. coli K88 (ETEC), and received either a diet with no additives, or with 2 kg/T uncoated butyrate (UB, Adimix® Easy) or 2 kg/T precision delivery coated butyrate (PDCB, Adimix® Precision).

Trial 2: 362 sows from a commercial farm in Sao Paulo (Brazil); 3 dietary treatments during 84 d of gestation until weaning (day 23): negative control, 0.33 kg/T butyrate, either as UB or as PDCB.

### Results

Trial I: in the challenged group, 6/12 piglets from the dietary control group died, while mortality was lower (3/12) in both UB and PDCB-fed groups (chi square, p = 0.087). In both challenged as unchallenged piglets, average daily gain was higher in the PDCB-supplemented piglets than in the other groups (p < 0.05), while jejunal villicrypt ratio was higher in this group as well.

Trial 2: During lactation, body weight loss was higher in the UB group (p < 0.05). Colostrum from the PDCB-supplemented sows was higher in IgG (p = 0.086) and IgA (p = 0.081). Body weight gain of the piglets from the PDCB-fed sows, but not those from the piglets of the other two groups, was higher at the end of lactation (p < 0.05).

## **Discussion and Conclusion**

These data emphasize the critical importance of taking into account product-dependent butyrate release kinetics in investigating the effects of exogenous butyrate supplementation in swine.

# THE IMPACT OF MICROBIOLOGICAL PARAMETERS IN LIQUID FEED DISTRIBUTED TO SOWS ON THE OCCURRENCE OF NEONATAL DIARRHOEA IN PIGLETS: A CASE CONTROL STUDY

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## **Background and Objectives**

Microbial colonisation of piglets' intestines starts at birth, especially from contact with sow faeces which composition could be influenced by diet. The objective of this study was to evaluate whether the microbiological flora of sows liquid feed could be associated with the development of neonatal diarrhoea.

## **Material and Methods**

This study was carried out in ten control and ten case farms in France. These case farms exhibited neonatal diarrhoea in more than 20% of litters within a batch for at least two consecutive batches. On each farm, two liquid feed sampling were performed: one approximately two weeks prior to farrowing and one in the week after parturition. In case farms, two diarrhoeic piglets were euthanized for aetiological diagnosis, confronting bacteriological, virological and histological results. Finally, a generalised linear model was used to assess if liquid feed microbiological counts and pH were different between case and control farms.

#### Results

Enterococcus hirae, Clostridium perfringens and rotavirus were identified as enteropathogens involved in neonatal diarrhoea in seven, four and three case herds respectively, sometimes in coinfection. Regarding liquid feed analyses, for thermotolerant coliforms, sulphite-reducing bacteria, heterotrophic bacteria and lactic-acid bacteria counts, there was no significant difference between case and control farms. However, counts of yeasts, total coliforms and Enterococci appear to be relevant criteria in establishing if sow liquid feed poses a risk. The higher the count of Enterococci, the greater the probability of observing neonatal diarrhoea.

#### **Discussion and Conclusion**

This case control study highlighted three criteria worthy of consideration in farms feeding sows with liquid feed systems for the prevention or management of neonatal diarrhoea. These results should be consolidated by further results over time. In particular, the role of Enterococcus hirae in neonatal diarrhoea and the link between the presence of Enterococci in liquid feed and the occurrence of neonatal enterococcal diarrhoea should be further investigated.

# SUPPLEMENTATION OF RESIN ACID-ENRICHED COMPOSITION AT WEANING IMPROVES GROWTH PERFORMANCE AND REDUCES POST-WEANING DIARRHEA IN PIGLETS

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## **Background and Objectives**

The weaning process can be detrimental to piglets often leads to low feed intake, low weight gain, diarrhea, and, eventually, increased mortality. Resin acid-enriched composition (RAC) has been used in feed to improve performance in pigs. This study aimed to determine the effects of RAC supplementation on improving piglet growth and reduce post-weaning diarrhea.

## **Material and Methods**

Forty sows (Yorkshire × Landrace) were balanced for body condition and parity (2-4), and all of them were provided with the same diet differing only for the addition of RAC (R) during pregnancy feed (n=20) or control (C, n=20). Piglets were treated with either RAC in the creep feed (R) or not (C), generating four respective piglet treatments, according to the previous allocation of the mother (CC, CR RC, and RR). At post-weaning, the same piglets were allocated to RAC treatment (R) or not (C), generating eight respective weaner groups, according to the previous mother and creep feed allocations (CCC, CCR, CRC, CRR, RCC, RCR, RRC, and RRP). Piglets were then followed up until seven weeks of age.

## Results

There were no significant differences among the four treatment groups at weaning (p> 0.05). However, by the end of the 3-weeks post-weaning period, piglet body weight was higher for the RRR group than the other dietary groups (p< 0.05). In addition, a higher incidence of diarrhea cases was observed in piglets not fed with RAC after weaning (Control), regardless of the treatment of the mother or earlier treatment during the suckling phase (p< 0.05).

## **Discussion and Conclusion**

In this study, we found that RAC supplementation to piglets improved growth performance and reduced postweaning diarrhea. We therefore, consider RAC as a potential alternative to antibiotics or zinc oxide treatments.

### « O'PORCTUNITE »: A FRENCH WEB AND MOBILE APP FOR PIG WELFARE

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## **Background and Objectives**

Societal demand for more welfare friendly swine rearing system is being tackled by legislation at the European level through measures such as forbidding pig castration (enforced in 2021 in France) and the pork industry is mobilising to meet these requirements. However, although studies suggest significant economic gains from changes in behaviour related to animal welfare, these are difficult to perceive in the short term. Welfare measures are more often perceived by farmers as a constraint rather than an improvement. There is therefore a real need to support farmers in the evolution of their practices and to summarise the regulatory requirements applicable, taking into account the field costs and time constraints. The objective of the app is to provide stakeholders with an easy to use and free of charge tool to assess sow welfare on farms.

### **Material and Methods**

The application was developed using the AGILE/SCRUM approach, an iterative holistic framework based on the repetition of 3-week "sprints". Classical method in IT development, it remains innovative, productive and creative. The self-assessment system is based on a simplified, optimized and updated Welfare Quality® grid. Figures and clickable question marks enable self-training along the process and limit subjectivity and errors.

### Results

Results appear as scores by categories (Good general condition, Appropriate environment, Health, Expression of behaviour) and sub-categories (12) under a radar format but also histograms and evolution curves over time. They lead to a proposal for consultation of short advice sheets which can be downloaded and printed and compare pros and cons of different improvement opportunities. They can be 'liked' or commented making the tool participatory.

# **Discussion and Conclusion**

This innovative, pedagogical and practical tool helps farmers and veterinarians to take into account welfare and summarises the regulatory requirements related to welfare applicable to pig farming. Its proof of concept is being tested in the field.

#### MIS-OP-01

# ANALYSIS OF THE QUALITY OF THE VACCINATION PROCESS IN PIGLETS AT WEANING WITH VACCINOMICS PROCEDURE

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### **Background and Objectives**

Vaccination is one of the most effective tools reducing and preventing the appearance of infectious pathologies in our farms. There are several factors involved in vaccine failure related to the animal, the farm environment or the vaccine application and management procedure.

The aim of this study was to design a semi-qualitative model to evaluate the vaccination process in piglets at weaning, that allows to know the process and conditions of vaccinations on farms and identify risk factors responsible for possible vaccine perceived lack of efficacy to define points of improvement to ensure the correct vaccination.

# **Material and Methods**

A total of 32 Spanish farms were audited using Vaccinomics with a questionnaire that groups aspects that may have an impact on the quality of the vaccination. It includes questions related to description of the farm; vaccination equipment and material; transport, storage and preparation of vaccines; animal handling; work safety; registration and processes taken after vaccination.

Indices evaluated are scored according to the risk they may pose. An univariate and bivariate statistical analysis was performed.

## Results

12,5% of farms evaluated were vaccinated at non-optimal age. 89,3% of farms didn't checked the dosage. 47% of farms, vaccinations were performed by one person penalizing the correct immobilization of animals (p<0.0007). 85% didn't check for reflux or did it but didn't re-vaccinate. Needle change frequency and vaccination material cleaning not done properly; 72% and 76%, respectively.

## **Discussion and Conclusion**

Vaccination is a heavy repetitive physical process and it tends to be performed as quickly as possible neglecting important aspects that will constitute risk factors for vaccine failures. Aspects related to the management procedure are the most committed within those analysed in the study.

Having a tool that allows identification and weighting of these risks is very useful to correct them in order to optimise the vaccination performances.

MIS-OP-02

#### **CLAW HEALTH IN BREEDING SOWS**

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## **Background and Objectives**

Claw health is essential in a sow herd, but methods to objectively assess it are rare. Therefore, we examined whether pedometers originally designed for sheep are suitable for assessing claw health in gestating sows. The correlation between claw health and the behavioural characteristics "laytime", "standtime", "walktime", "standup", "laydown" and the activity index, which indicates how often the sow has changed its activity (e.g. getting up from lying) within an hour, were investigated.

## **Material and Methods**

Based on the Feet First® Lesion Scoring System assessing seven parameters per claw, 35 gestating sows (between 35 and 95 days of gestation) on a Swiss piglet producer farm with group housing were divided into three groups: group "bad" (score 3; n=11), group "medium" (score 2 ≥3 times or score 1 >7 times; n=11), and group "good" (score 2 <3 times or score 1 ≤7 times; n=13). Pedometers were fixed to the right hind leg laterally below the ankle of each sow. Data were collected over 7–10.5 days and statistically evaluated using a mixed linear regression model with fixed and random effects.

## Results

Above all, differences between group "good" and "bad" are visible. The average "walktime" of group "good" (0.45h) between 12 pm and 12 am was significantly longer than the one of groups "medium" (0,36h) and "bad" (0.34h). The average «laytime» of group "bad" (9.92h) in the first half of the day was significantly longer than that of group "good" (9.46h). The opposite tendency was visible for the second half of the day (12 am to 12 pm).

### **Discussion and Conclusion**

Claw health influences the behaviour of gestating sows. Pedometers are a suitable instrument to collect behavioural data of sows. However, a final assessment of claw health based on these data alone is not possible and should be combined with other objective methods.
#### MEASURING THE IMMUNE RESPONSE OF PRRS "NAÏVE" GILTS AFTER VACCINATION WITH DIFFERENT ELISA KITS

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#### **Background and Objectives**

#### Introduction

Gilts quarantine/acclimation includes PRRS immunization in most farms by vaccination. Immunization is usually monitored to verify the viremia status and the antibody response after contact with the vaccine strain and/or the wild type strain.

The following study attempted to test the immune response in PRRS "naïve" gilts with different ELISA kits after the vaccination with Suvaxyn® PRRS MLV (Zoetis).

#### **Material and Methods**

The test site was a PRRS positive sow herd which introduced "naïve" gilts every 4 months from the quarantine facilities, separated 1 km from the main herd. Quarantine took 2 months.40 gilts were vaccinated and bled at 10 days post-vaccination (dpv); 19 gilts were also bled at 17 dpv and 49 dpv.

Sera were checked for viremia by DIVA qPCR (Bio-T kit® PRRSV DIVA RXN) grouping 3 sera in each pool. Antibodies were measured in each serum individually by 5 different ELISA commercial kits (A, B, C, D, E).

#### Results

DIVA qPCR showed that all pools were negative to wild type strains throughout the study. The vaccine strain was found in 14/15 pools at 10 dpv, in 4/4 pools at 17 dpv and 3/7 pools at 49 dpv.

Antibody results by ELISA were not homogeneous between the different kits:A-B-C-D-E kits detected 10,1,0,0,0 positives out of 40 sera at 10 dpv, respectively.A-B-C-D-E kits detected 13,13,3,4,1 positives out of 19 sera at 17 dpv, respectively.A-B-C-D-E kits detected 19,19,12,15,4 positives out of 19 sera at 49 dpv, respectively.

#### **Discussion and Conclusion**

ELISA kits may be measuring different types of antibodies, which are produced at different times in the immunization period.

Therefore, it is necessary to test the different kits to adapt each one to the interval between vaccination and collection of samples to check the immune response. Sensitivity and specificity of each kit may also be considered.

USE OF A NOVEL IMMUNOINFORMATIC TOOL (EPICC) TO DETERMINE T-CELL EPITOPE COVERAGE FROM DIFFERENT PCV2 VACCINES AGAINST FIELD EUROPEAN STRAINS

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#### **Background and Objectives**

The objective of this analysis was to compare T cell epitope coverage from a monovalent baculovirus expressed PCV2a capsid vaccine and a bivalent cPCV1-2a/cPCV1-2b chimeric PCV2 vaccine against circulating PCV2 viruses in Europe.

#### **Material and Methods**

Two PCV2 vaccines, a PCV2a baculovirus-expressed capsid (AltVac) and cPCVI-2a-cPCVI-2b chimeric virus vaccine (VacAB) were assessed for their T-cell epitope relatedness to 112 PCV2 sequences (ORF2) from 10 European countries. Capsid amino acid sequences were used to predict T-cell epitope content of both vaccines and field strains based on predicted SLA binding probability of each T-cell epitope including both SLA class I and class II alleles. The vaccines and field strains were then compared to calculate epitope content comparison (EpiCC) scores based on shared T-cell epitope content (EpiVax, Rhode Island, USA). Scores were normalized by the number of 9-mers in the field strains and the number of SLA alleles used for the analysis. EpiCC-scores quantify how well the T-cell epitope content of field strains was matched by a given vaccine.

#### Results

Sequences analyzed were collected during years 2014 to 2020, and most from reported PCV2 clinical cases. Fifty-nine (53%) were PCV2d, forty-two (37%) PCV2b and eleven (10%) PCV2a. For EpiCC analysis, 109 sequences were included (three excluded due to incomplete data). Bivalent, chimeric PCV1-2a, PCV1-2b vaccine (VacAB) showed a greater epitope coverage based on EpiCC score compare to PCV2-a baculovirus expressed vaccine (AltVac) having a median coverage of 81% compared to 60% respectively (P<0.0001).

#### **Discussion and Conclusion**

Despite current PCV2 vaccines having demonstrated disease control, PCV2 virus is still evolving. The EpiCCtool offers a way to assess the impact of genetic divergence on T-cell epitope coverage for current PCV2 vaccines. For the set of analyzed field strains, the bivalent PCV1-2a, PCV1-2b vaccine may confer broader cross-reactive cell-mediated immune response and protection compared to the monovalent PCV2a vaccine (VacAlt).

#### EFFECT OF PRRS MLV VACCINATION ROUTE (INTRAMUSCULAR VS INTRADERMAL) ON VACCINE IMMUNE RESPONSE AND EFFICACY

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#### **Background and Objectives**

PRRS is a viral disease of pigs with worldwide distribution. To control PRRSV infection, Modified Live Vaccines (MLV) are widely used, mainly administered intramuscularly (IM). In recent years, some MLV were authorized for intradermal (ID) administration, which has the advantages of greater animal welfare and being more user friendly. The objectives of the study were to compare the immune response and the vaccine efficacy after IM or ID immunization of piglets with a PRRS MLV.

#### **Material and Methods**

SPF piglets were vaccinated at 5 weeks of age with Porcilis PRRS® (MSD) either by IM (V+IM; n=16) or ID route using the IDAL® device (V+ID; n=16), or were left unvaccinated (V-; n=16). Four weeks after vaccination, 8 piglets in each group were challenged intranasally with a PRRSV field strain. Twenty-four hours after challenge, sentinel pigs were mingled with the inoculated pigs to evaluate PRRSV transmission. Control piglets were left unvaccinated and unchallenged (n=8). During the post-vaccination and the post-challenge phases, PRRSV replication (RT-PCR), PRRSV-specific humoral (ELISA) and cell-mediated-immune (CMI) responses (ELISPOT IFNg) were monitored in blood and bronchoalveolar lavages.

#### Results

Post-vaccination (PV), the vaccine viremia was lower in the V+ID compared to the V+IM pigs, whereas the CMI response was higher for the V+ID group at 2 weeks PV. Post-challenge, the vaccine efficacy was similar in the inoculated animals with a partial control of viremia in V+ID and V+IM animals. In sentinel pigs, the vaccine drastically reduced PRRSV transmission for both V+IM and V+ID groups.

#### **Discussion and Conclusion**

Our results show that the tested PRRS MLV was equally efficacious after IM or ID immunization. Considering the practical and welfare advantages of ID vaccination, these data support the use of this vaccination route for PRRS MLV vaccines.

#### SUCKLING PIGLET DIARRHOEA CAUSED BY ROTAVIRUS A - DIAGNOSTICS AND POSSIBLE IMMUNOPROPHYLAXIS

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#### **Background and Objectives**

Rotavirus A (RVA) is a major cause of acute gastroenteritis in suckling piglets. The outer capsid proteins VP7 and VP4 induce neutralising antibodies and form the basis for the G and P typing system. The complex epidemiology, pathogenicity and high genetic diversity of porcine RVA is widely recognized. The proof of an RVA infection is possible by rtPCR of gut or faecal samples gained from piglets suffering from acute diarrhoea. Moreover, sequencing is possible in order to investigate the specific genotypes present in a herd. Viral culture of RVA might also be performed in specialised labs.

#### **Material and Methods**

From 2016-2019 6,368 samples (gut tissue/faecal samples, faecal swabs) of piglets showing acute signs of diarrhoea were investigated for RVA by rtPCR (Kylt® Rotavirus A, AniCon Labor GmbH). Samples positive for RVA with a Ct-value <28 were used for viral culture. Sequencing of the G and P genotypes was performed for 54 RVA isolates. Data analysis was performed using the DNAstar Software from Lasergene, USA. G and P genotypes have been determined using the RotaC<sup>20</sup> automated genotyping tool (http://rotac.regatools.be/) for RVA and the NCBI database.

#### Results

2,260 samples were PCR positive for RVA (36%) and around 70% of those showing Ct-values <28 and were cultured. The 54 sequenced isolates could be assigned to 22 different genotypes. The most frequent genotypes were G9P[7] (n=7), G4P[32] (n=7), G5P[23] (n=5) and G3P[32] (n=4).

#### **Discussion and Conclusion**

Investigating whether RVA is involved in cases of suckling piglet diarrhoea is easily possible by testing gut tissue or faecal samples by rtPCR. Viral culture reveals the possibility to produce an autogenous vaccine against RVA. This kind of immunoprophylaxis (AniVac®) might be of special interest for the practitioner due to the high genetic diversity of RVA strains in between farms but also within pig herds over time.

#### SUCCESS FACTORS FOR PIGLET VACCINATION

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#### **Background and Objectives**

In Dutch pig production both the percentage of vaccinated piglets and the number of vaccinations per piglet have increased, leading to cost of piglet vaccines up to 75 euro per sow per year. In most farms piglets are vaccinated by the farmer or by staff members and the work is often seen as unattractive. Any lack of motivation may lead to less prudent use, more side effects and vaccine failure. By interviewing farmers and staff, we tried to have more insight on factors that may influence successful piglet vaccination in the Netherlands.

#### **Material and Methods**

A total number of 50 questionnaires of 11 multi choice questions each were processed during the year 2019. Answers were given by farmers or staff members responsible for piglet vaccination. The results were analyzed using Excel.

#### Results

Results show that: In 43% of the farms opened vaccine vials are stored for later use 36% of the farms store vaccines in an old refrigerator 34% of the farms change the needle at minimal in between every litter or pen Syringes after use are washed and flushed with tap water only in 80% of the farms Average number of vaccination events per batch: 1.8 (up to 4) Average cost of labor per event per vaccinated piglet: euro 0.15 (assuming labor cost per person per hour of 30 euro)

#### **Discussion and Conclusion**

Cases in which piglets become infected by re-using syringes or opened vials are numerous. Old refrigerators may easily be the cause of the wrong storage conditions. Creating Good Veterinary Practice conditions will not only lead to better welfare for the piglets, but also increases the effectiveness of the vaccines used. Minimizing the number of vaccination events and injections reduces labor costs and keeps motivation up. Any registered mixing of vaccines contributes to that.

#### VACCINE WARMING MAY LEAD TO VACCINE FAILURE

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#### **Background and Objectives**

Some inactivated vaccines require warming up before use. In the Netherlands that resulted in a more general approach to warm up all piglet vaccines before use. Cases of vaccine failure were reported related to warming up of vaccine vials in hot water. This study tries to define a safe and efficient procedure for warming up vaccines before use.

#### **Material and Methods**

The temperature evolution of the content of vials was registered over time when being warmed up in warm water. Before use the HDPE vials, containing 100 ml of phosphate buffered solution representing vaccine, were stored at 4-6 degrees Celsius (°C). The volume of water used for warming up the vials was 3 liter (60 °C) or 12 liter (30 °C, 40 °C). To measure the temperature in the vials (6 vials per temperature) the probe tip of the thermometer (ADE BBQ 1600 Food Thermometer) was stuck in the rubber stopper for a distance of about 5 cm. Temperatures were registered with 2 minutes intervals over 20 minutes starting immediately after putting the vials in the water.

#### Results

In water of respectively 30, 40 and 60 °C it took 8, 4 and 2 minutes for the vial content to reach a temperature of at least 25°C. In water of 60 °C it took 4 minutes to reach a temperature of 40 °C.

#### **Discussion and Conclusion**

Protein denaturation is temperature dependent. For this abstract a critical temperature for vaccine of 40 °C and more is assumed. High vaccine temperatures are likely to affect the quality of the product leading to possible vaccine failures. The conclusion is that the need for warming up inactivated vaccines is product dependent and is both efficient and safe to do this in a water environment of 30 to 40 °C.

#### AN INVESTIGATION INTO UTERINE CAPACITY IN TWO SOW LINES WITH DIFFERENT PROLIFICACY

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#### **Background and Objectives**

Over recent years, litter size in modern so called hyperprolific sows such as of crossbred Danish Landrace x Danish Yorkshire increased considerably, however, often associated with unfavorable side effects, such as decreased piglet birth weight and increased within litter birth weight variability likely due to a limited uterine capacity.

#### **Material and Methods**

This study was conducted to investigate uterine capacity based on litter and placental characteristics in two sow lines with different prolificacy, i.e. crossbred Danish genetic (Landrace x Yorkshire; DG; n = 14) and purebred German Saddleback (GS; n = 12) sows. Litter size, piglet birth weight and vitality, placental weight and surface area and placental vascularization were recorded.

#### Results

Litter size in DG was on average larger than in GS (P < 0.001). DG piglets weighed on average less than GS piglets (P < 0.001), and were less vital (P < 0.001 – 0.142). Increasing litter size was associated with a reduction in piglet birth weight and an increase in within litter birth weight variability in GS, remarkably however, not in DG. Placentae of DG were on average lighter (P < 0.001) and smaller (P < 0.001) than of GS, but placental efficiency (the quotient of piglet and corresponding placental weight) was on average higher in DG compared to GS (P < 0.001). Placental vascularization was on average not or only slightly different between both breeds (P < 0.05 – 0.982). Interestingly, however, vascularization of the lateral and apical chorionic epithelium of the chorionic ridges representing the immediate fetal/maternal interface was on average slightly higher in DG than GS (P < 0.05 – 0.111).

#### **Discussion and Conclusion**

In conclusion, uterine capacity as studied herein was higher in DG than GS sows. Results indicate that a higher placental efficiency as found in DG sows compared to GS may contribute to the large litter phenomenon of DG sows.

#### WHY DO PIGLETS DIE DURING BIRTH: FACTORS THAT IMPACT ASPHYXIA AND STILLBIRTH.

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#### **Background and Objectives**

Data from our research centre were analyzed to identify factors that increase the risk of piglet asphyxia and stillbirth.

#### **Material and Methods**

Multiparous Large White x Landrace sows (Hypor, Hendrix Genetics, n=256) were monitored continuously around parturition. Sows were allowed to farrow naturally with no interventions and the following were recorded: time of birth of each piglet (liveborn or stillborn), sex, patency of umbilical cord, umbilical cord blood acid-base values, meconium staining, birth weight, and 24 h weight gain to estimate colostrum intake. Non-fresh stillborn and litters with less than 12 piglets were not included in the data.

#### Results

Piglets born within 2 h from the start of farrowing had a 2.7 % risk of being stillborn while those born 2 to 4 h, 4 to 6 h, 6 to 8 h, or more than 8 h into the farrowing process had 6.9 %, 10.7 %, 13.4 %, and 27.3 % risk of being stillborn (P < 0.05). The effect of farrowing time was also reflected in measures of asphyxia such as piglet blood pH and lactate. Interval time between piglets had no effect on stillbirth rate, as long as the interval did not exceed 90 min (only 6 % of the piglets). Piglets with a broken umbilical cord (21 %) had a higher risk of being stillborn (16% vs 3%; P < 0.01). Stillbirth rate was not related to birth weight or sex. Meconium staining was not related to asphyxia or stillbirth.

#### **Discussion and Conclusion**

Duration of the total farrowing process had the largest impact on asphyxia and stillbirth rates. This appears logical given the cumulative effect of uterine contractions on reduced perfusion of the placenta and oxygenation of the foetus. Therefore concepts to reduce stillbirth should focus on improving oxygenation or reducing overall farrowing length.

## INFLUENCE OF DIFFERENT SOW TRAITS ON THE EXPULSION AND CHARACTERISTICS OF THE PLACENTA IN A FREE FARROWING SYSTEM

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#### **Background and Objectives**

The last phase of parturition is the detachment and expulsion of the placentae and should not exceed four hours after the birth of the last piglet. Until today, only few information is available about factors influencing the expulsion of the placenta, especially in sows accommodated in free farrowing systems. This study aimed to investigate the influence of sow traits on placenta expulsion in a free farrowing system in Switzerland.

#### **Material and Methods**

In this study, 48 crossbred sows were included and the following traits of the sows were investigated: backfat thickness, birth induction on day 116 of gestation with prostaglandin F2 $\alpha$ , placenta parts and weight, placental efficiency (quotient of litter weight by placental weight) and expulsion of the first placental part in relation to the last piglet. In addition, the farrowing process and litter parameters were recorded. Significance was considered when p<0.05.

#### Results

Sows receiving birth induction showed a significant higher backfat thickness (mm) (13.8 ± 2.9 vs.11.6 ± 1.9) and a total placental weight (kg) ( $4.4 \pm 1.1 \text{ vs.} 3.6 \pm 1.1$ ). Sows with a backfat thickness ≥12.5 mm showed a significantly prolonged farrowing duration (interval between first piglet and last placenta) (min) (683 ± 398 vs. 468 ± 188). Sows with placental efficiency < 5 were characterized by a significantly higher number of live born piglets (17.1 ± 2.7 vs. 13.6 ± 2.8) (. Sows that expelled the first placental part before the last piglet was born showed a significantly prolonged piglet expulsion duration (min) (305 ± 216 vs. 139.0 ± 34.9).

#### **Discussion and Conclusion**

This is the first study that evaluated the placental characteristics in a free farrowing system and compare it with specific sow traits. Especially, the expulsion of the first placenta could be a reliable parameter to detect sow with birth problems and therefore improve sow and piglet health.

#### ULTRASOUND MONITORING OF THE EVOLUTION OF FETAL MORTALITY AFTER A PORCINE PARVOVIRUS 1 CHALLENGE IN GILTS

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#### **Background and Objectives**

Ultrasonography is a wide used technique for early and accurate diagnosis of pregnancy in sows. There is no literature describing the evolution of fetal viability after a challenge with porcine parvovirus 1 (PVVI) in gestating sows by means of ultrasonography. The objective of this study was to assess the ability to identify non-viable fetus by means of ultrasonography at different stages of gestation as well as the ability to predict litter sizes after a PPVI challenge at ~40 days of gestation.

#### **Material and Methods**

Sixty-six pregnant gilts were monitored by ultrasonography 4 times during gestation (~41, ~58, ~70 and ~90 days of gestation). An ultrasound MyLabTMOneVET device with an abdominal probe was used. The number of embryonic vesicles was measured at ~41 days of gestation, and in the following days, the assessments focused on the number of viable and non-viable fetuses. The ultrasound results were compared to the observations at necropsy (~90 days of gestation) and the predictive value of the technique was assessed.

#### Results

From all parameters assessed, the number of embryonic vesicles was the most accurate parameter to predict the litter size. On further dates (gestation days 58, 70, and 90), the number of fetuses observed during the ultrasound assessments underestimated the number of fetuses observed at necropsy. On the other hand, the ultrasonography was able to detect non-viable piglets from day 58 of gestation onwards. In addition, it was able to detect fetal death with 100% accuracy in those litters in which all the fetuses were determined to be non-viable at necropsy.

#### **Discussion and Conclusion**

Ultrasonography has clinical value for diagnosis of pregnancy failure and early embryonic death leading to either fetal mummification or partial embryonic decomposition. Nevertheless, it might not be an accurate tool to predict litter size and number of viable/non-viable fetuses.

#### NO NEED TO ADJUST FOR WEIGHT UNIFORMITY IN LITTERS FROM PROLIFIC SOWS

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#### **Background and Objectives**

In herds with prolific sows a specific management is recommended. Small piglets are collected at smallnurses to support their specific needs, and the largest surplus piglets are transferred to nurse-sows. This reduces the variation in weight in the remaining piglets. It is questioned, if the remaining piglets need to be transferred between litters, to make the weight of the piglets within the litters become uniform.

#### **Material and Methods**

In two commercial herds, 8088 liveborn piglets from 451 DanBred LxY sows mated to Duroc boars (17,9 piglets per litter) were ear tagged and weighed. After colostrum intake, the smallest piglets were collected at smallnurses. Then the litters were reduced to 14 piglets by transferring the largest surplus piglets to two- stepnurse-sows. In the control group piglets were transferred within the group to obtain a uniform weight within each litter. Piglets in the trial group stayed with their mother. After 17 days the surviving piglets were registered and weighed. All piglets born to a sow in the trial but growing up with a nurse sow, were also included in the trial.

#### Results

The survival of the piglets was 92 % for the control group, where the piglets were sorted by size, and 93 % if piglets grew up in the litter, where they were born. Weight at day 17 was 4,5 kg in both groups. When corrected for the effect of birth weight, transferring piglets to nurse sows did not affect survival of the piglets, when compared to piglets staying at the mother.

#### **Discussion and Conclusion**

When litter size in large litters has been adjusted to the optimal number of nursing piglets, there is no need for further adjustment for weight uniformity, as this does not affect survival nor growth rate. This will reduce the work load, the risk of disease transmission, and may reduce fighting among the litter mates.

In total 157 still born piglets obtained from 15 farms were analyzed. 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, this resulted in a sensitivity of 92.3% and a specificity of 94,4%. Pcr testing: in 2 farms were positive for PRRS, 1 farm was positive for PPV, 9 farms were positive for PCV2. Elisa testing for the present of antibodies against PCV2 and PRRS were negative. There was not enough serum for PPV antibody testing.

#### **Discussion and Conclusion**

In nine farms, positive PCR tests proved presence of intrauterine infections with either PRRS, PPV, PCV2 and/or PCV3. The relevance of the presence of PCV3 is still unclear. The immunocrit assay proved to be a useful alternative for predicting the presence of intrauterine produced antibodies. The test is fast and cheap and can be used before more expensive ELISA testing for specific infections is performed.



## FLASH TALKS



#### Welfare and nutrition

#### EFFECTS OF ENRICHING SOW AND PIGLET DIETS WITH N-3 FATTY ACIDS ON IMMUNE INDICATORS OF GESTATING AND LACTATING SOWS AND LACTATING AND POST-WEANED PIGLETS.

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#### **Background and Objectives**

While n-6 fatty acids (FA) are associated with proinflammatory functions, n-3 FA are reported to play an antiinflammatory role. The aim of the current study was to determine the effect of enriching sow and piglet diets with a n-3 FA source on different immune indicators in sow and piglet blood serum.

#### **Material and Methods**

Thirty-six sows were randomly assigned to a control or a n-3 enriched diet from insemination to the end of lactation. A total of forty-eight piglets, two low birth weight (LBW) and two high birth weight (HBW), were selected from twelve sows and at weaning one piglet of each birth weight was randomly assigned a control or a n-3 enriched diet. Blood was sampled from sows one week before farrowing and at weaning and from selected piglets at weaning and 28 days post-weaning. Serum immune indicators were analysed by ELISA.

#### Results

In sows, n-3 FA increased IgM at the end of gestation (P=0.014) and lactation (P=0.008) and IL-6 (P=0.012) at the end of lactation. Piglets from sows fed the enriched diet had increased IgG (P=0.007), IL1 $\beta$  (P<0.001), and IL10 (P<0.001) at weaning, and at day 28 post-weaning, they had increased IgA (P = 0.090), IL1 $\beta$  (P=0.011) and IL6 (P=0.005) and decreased IgG (P=0.069). There was an interaction between maternal diet and birth weight for piglet's IgA concentration (P=0.045) at weaning. The highest IgA concentrations were found in HBW piglets from control sows and the lowest in HBW from n-3 enriched sows.

#### **Discussion and Conclusion**

To conclude, enriching sow diets with n-3 FA during gestation and lactation affects immune indicators of sows and their piglets and this effect is maintained at day 28 post-weaning. Enriching post-weaning diets with n-3 FA had no effect on the parameters analysed.

Welfare and nutrition

#### DYNAMICS IN LACTOBACILLUS SPP. IN THE FECES OF GROWING PIGS

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#### **Background and Objectives**

Genus Lactobacillus includes species that potentially benefit health status of their host. Selection for large litter size has led to increased within-litter variation in piglet birth weight: small piglets may be at higher risk for infectious diseases and antimicrobial treatment due to insufficient colostrum intake and impaired immune development. Especially early-life antimicrobial treatment has long-term effect on gut microbiota development. We studied fecal lactobacilli count and diversity of growing pigs, and immune parameters associated to fecal lactobacilli.

#### **Material and Methods**

Thirty pigs, either small (S) or large (L) at birth were followed from birth to slaughter in two commercial herds (H1 and H2). Sow colostrum quality, colostrum intake, serum immunoglobulins and piglets' growth were determined. Individual fecal samples were taken from piglets after weaning and during finishing. Lactobacillus count was measured and species identified with 16S PCR. Mixed model procedures were used for statistical analyses.

#### Results

The total count of lactobacilli increased between fecal sampling points in H1 and decreased in H2, whereas the species diversity decreased in both herds. S piglets showed higher lactobacilli count in both herds, but the difference was significant only in H2 (p = 0.01). Seven Lactobacillus species were identified with a maximum of five different species found in one herd. Relatively large proportion of lactobacilli remained unidentified with the sequencing technique used. Colostrum quality was numerically better in H1 where colostrum intake was significantly associated with the total lactobacilli count (p = 0.05).

#### **Discussion and Conclusion**

S piglets had more lactobacilli than L piglets in both herds. Change in total lactobacilli count over time differed between herds, suggesting underlying herd-level factors influence the dynamics of lactobacilli. In HI, where Lactobacillus sp. diversity was higher in the first fecal sampling point, sow colostrum quality was significantly better compared to H2 indicating the role of colostrum in microbiota development to be long-lasting.

Welfare and nutrition

#### PREVALENCE OF TAIL LESIONS AND TAIL BITING IN SWISS FINISHING PIGS

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#### **Background and Objectives**

Tail biting and tail lesions in fattening pigs cause animal welfare, animal health and food hygiene issues worldwide. In Switzerland, approximately 2.6 million pigs with undocked tails are raised and slaughtered per year. Tail lesions examined in previous Swiss studies ranged between 0.7 to 21.9 %, whereas a recent study from 2016 reported a prevalence of 41.3 % of non-intact tails in a sample of Swiss fattening pigs. This result is in line with observations made by official veterinarians at the abattoirs mentioning an increasing prevalence of tail lesions in fattening pigs during the last few years.

#### **Material and Methods**

In order to determine the actual prevalence of tail lesions in Swiss finishing pigs, pigs' tails are currently evaluated at four large abattoirs. In every season of the year at each abattoir data collection will be conducted for two consecutive weeks, accounting for repeated origin of pigs and seasonal impacts. Date and time of the slaughter, origin of the animal, length of the tail, condition of the tail tip, indication of broken tail and swelling are recorded by the first author immediately after CO2 stunning and bleeding. The post mortem results will be compared with data from the Swiss pig health service, who is examining tail lesions intra vitam during an annual routine herd visit.

#### Results

Data collection started in August 2019 and will be finished in June 2020. In the first examination period, 51'516 pig tails were evaluated. Preliminary results indicate 62.7 % intact tails, 22.8 % non-intact tails with healed lesions, 13.3 % non-intact tails with signs of proliferation or healing tendencies and 1.1% non-intact tails with acute tissue damage.

#### **Discussion and Conclusion**

This study will provide important information about the actual situation regarding tail lesions and tail biting in undocked pigs and will enable further analysis of country specific risk factors for tail lesions.

Welfare and nutrition

#### MICROBIOLOGY OF SOW COLOSTRUM

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#### **Background and Objectives**

Colostrum is essential for the wellbeing of neonatal piglets. It offers them not only nutrition, but also protection from diseases. In addition to immunoglobulins, piglets may also ingest harmful bacteria in colostrum. The aim of this study was to assess the existence of possibly harmful bacteria in sow colostrum.

#### **Material and Methods**

For this study colostrum samples were taken from 42 farrowing sows within the first six hours after the birth of the first piglet. One person milked the sample using clean disposable gloves, after disinfecting one teat and the section of the udder. Meanwhile, another person held back the already born piglets from the teats for the duration of sample taking. The samples were frozen at -20°C within an hour of sampling and stored at -70°C within a day of sampling until further analyzing. The samples were analyzed by culturing aerobically on blood agar and by PCR, using a cattle mastitis -panel.

#### Results

The aerobic culture showed mixed growth in 16 (38.1 %) of the samples. In seven samples (16.7 %) colonies of resistant coagulase-negative Staphylococci (CoNS) and in one sample (2.4 %) colonies of resistant Staphylococcus aureus were growing. The mastitis PCR-panel identified S. aureus, CoNS, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis and Escherichia coli in the samples. Additionally, 38 out of the 42 samples (90.5 %) contained bacteria with beta-lactamase genes.

#### **Discussion and Conclusion**

Microbes found in the samples by culturing or PCR are all opportunistic bacteria living on skin or in feces. These were not pathogenic for the sows. The bacteria probably originate from the skin or teat canals of the sow. Aseptic sample collection was difficult and, despite the effort, not as sterile as aspired. Nonetheless, these bacteria are a good depiction of the bacteria neonate piglets ingest when suckling: opportunistic and potentially antibiotic resistant pathogens.

Viral diseases

#### GENETIC CONNECTIVITY OF WILD BOAR IN A REGION ENDANGERED BY AFRICAN SWINE FEVER

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#### **Background and Objectives**

Non-affected wild boar areas at risk of African Swine Fever (ASF) introduction from neighbouring regions are obliged to understand the host's dispersion dynamics to combat the spread of ASF by spatial fixation as one of the major aspects of today's plaque-control.

#### **Material and Methods**

Rhineland-Palatinate in Germany was selected as a country with a high density of wild boars and the threat of ASF via infected wild boars from the neighbouring Belgium. On an area of around 20,000 km<sup>2</sup>, almost 1200 samples from 22 wild boar areas strategically located to a network of potential barriers like motorways and rivers were selected. Genetic differentiation between these areas was analysed using one spatial and two non-spatial Bayesian approaches on microsatellite marker data.

#### Results

Each of the algorithms detected four genetic clusters with different cluster compositions at different areas and identified highest degrees of differentiation between populations on the right and left bank of the Rhine river, between Pfalz and Eifel/Hunsrück and to a lesser degree between Westerwald and Taunus and between Eifel and Hunsrück.However, there was a high genetic connectivity within large regions.

#### **Discussion and Conclusion**

Thus genetic evidence suggests barriers of different strength that might be helpful in a setup of complex and expensive measures in the fight against the spread of animal diseases such as the ASF. The described methodical approach could also provide valuable information for other threatened regions to support the fight against ASF.

Viral diseases

#### ASSESSMENT OF THE PREVALENCE OF SWINE INFLUENZA VIRUS SUBTYPES IN SPAIN DURING 2014-2020

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#### **Background and Objectives**

Swine Influenza Virus (SIV) is characterised by subtypes by the most important 2 surface glycoproteins, Haemagglutinin (H) and Neuraminidase (N), involved in infection, pathogenicity and immune response. Prevalence of SIV subtypes may change over time. In order to set suitable control measures in farms, it is important to identified SIV subtype/s. The aim of this study was to describe the most prevalent subtypes of SIV in Spain.

#### **Material and Methods**

The study was carried out from Nov'14 until Nov'20 in farms located in Spain. A total of 569 samples were taken from pigs suffered SIV-like clinical signs. 376 nasal swabs (NS), 103 oral fluids (OF), 83 lungs (L) and 7 bronchoalveolar lavages (BAL) samples were collected and RT-PCR was performed to assess and subtype SIV.

#### Results

A total of 375 out of 569 samples (65.91%) were SIV positive by RT-PCR and on 346 of them (92.27%) subtyping was performed. Positive results by type of sample were: NS: 243/64,63%; OF: 67/65.05%; L:62/74.70; BAL: 3/42.86 In decreasing order number positive samples and subtype prevalence SIV were: subtype H1huN2 (74, 21.39%), subtype H1avN2 (73, 21.10%), subtype H1avN1 (60, 17.34%), subtype HxNx (38, 10.98%), subtype HxNI (23, 6.65%), subtype H1panN1 (12, 3.47%), subtype H3N2 (12, 3.47%), subtype H1huN1 (11, 3.18%), subtype H3N1av (6, 1.73%), subtype H1panN2 (5, 1.45%), subtype H1huNx (5, 1.45%), subtype H1avNx (2, 0.58%), subtype H3Nx (1, 0.29%) and subtype HxN2pan (1, 0.29%).

#### **Discussion and Conclusion**

The most prevalent subtypes were H1huN2, H1avN2 and H1avN1, being involved in 60% of SIV studied cases in Spain during 2014-2020. Moreover, pandemic strains are also circulating. Comparing with other studies, these SIV prevalence results show an evolution of SIV subtypes in Spain over time

Viral diseases

## A CROSS-SECTIONAL STUDY: THE ROLE OF GILTS IN IAV TRANSMISSION AND EVALUATION OF IAV GILT VACCINATION IN DANISH SOW HERDS.

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#### **Background and Objectives**

Swine influenza A virus (swIAV) is widespread in the pig production. Previous studies have shown that "new gilts" play a major role in introductions and maintenance of swIAV in herds. There are several ways to introduce new gilts into herds including quarantine and different vaccination strategies. The objective was to clarify the role of gilts in the transmission of swIAV in Danish sow herds and evaluate the effect of quarantine measures and vaccination.

#### **Material and Methods**

The study was conducted through observational cross-sectional studies performed in five vaccinated and five un-vaccinated Danish sow herds. Blood- and nasal swab samples of gilts, 1st parity sows, and piglets were collected in different sections of the production system and analysed for the presence of swIAV and antibodies. An association between the seroprevalence, detection of swIAV, quarantine measures and vaccination strategies were investigated to identify possible risk factors for swIAV introductions and persistence within the herds.

#### Results

Preliminary results revealed that all ten herds had antibodies against swIAV and 9/10 were positive for swIAV in nasal swabs. Viral shedding was identified in the end of the quarantine period in 6/10 herds primarily in vaccinated herds. Furthermore, viral shedding in piglets was observed in 6/10 herds with no differences between vaccinated and un-vaccinated herds.

#### **Discussion and Conclusion**

The prevalence of swIAV in piglets seemed to be associated with the herd having swIAV positive gilts in the end of the quarantine period. Vaccinated herds had a higher seroprevalence compared to non-vaccinated herds, and an increase in seropositive gilts was observed between the entry and end of the quarantine in these herds. This suggested that proper gilt immunization resulted in reduction in the number of naïve gilts being introduced into the sow herd. However, the management of the quarantine is as important and greatly influence the effect of vaccination.

Reproduction

#### BACKFAT THICKNESS HAS AN EFFECT ON WITHDRAWAL TO ESTRUS INTERVAL IN GILTS SYNCHRONIZED BY ALTRENOGEST

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#### **Background and Objectives**

The aim of this study is to investigate the relationships between blood progesterone levels (P4), backfat depth and the withdrawal hormone to estrus interval (WHEI) in gilts synchronised using altrenogest (Regumate<sup>®</sup>).

#### **Material and Methods**

30 Landrace x Yorkshire crossbred gilts were accommodated in the same condition and health program. All gilts were fed 20 mg/d altrenogest for 18 days. All gilts showed estrus within 7 days after hormone withdrawal. Blood collection from external jugular vein and backfat depth measurement (BF) were performed in all sows at Day -3, 0, 3, 18 of feeding period and the day of estrus or Day 7 after hormone withdrawal. Serum was obtained from whole blood and a monoclonal antibody ELISA kit was used to evaluate P4. Serum obtained from Day 18 was assessed for serum altrenogest level using LC-MS. The P4 and BF were assessed for correlation with WHEI. Sows were divided into 3 groups according to BF (low: <13.5 mm, moderate: 14-16.5mm and high: 17 mm). All groups classified were used as data comparisons which were analyzed using ANOVA except for the farrowing rate which was tested by Chi-square test.

#### Results

The results show that P4 (r=0.3, P=0.04) and BF (r=0.5, P<0.0001) have strongly a positive correlation with WHEI and have a negative correlation (r=-0.2, P=0.03) with the number of piglet born alive (BA). The sows with high BF have a longer WHEI (P=0.04), higher serum altrenogest at D 18 (P=0.02) and a lower BA (P=0.01) than the sow with low and moderate BF. The present study shows that the farrowing rate of the sows with moderate BF is better than the sow with low and high BF (P=0.01).

#### **Discussion and Conclusion**

BF plays an important role on WHEI in gilts synchronized using altrenogest and that moderate levels (14-16.5mm) will produce the most ideal results.

Immunology and vaccinology

#### A CONTROLLED, RANDOMIZED COMPARISON OF LUNG HEALTH FOLLOWING VACCINATION WITH A PORCINE CIRCOVIRUS TYPE 2 - MYCOPLASMA HYOPNEUMONIAE COMBO-VACCINE OR AN INDIVIDUAL PORCINE CIRCOVIRUS TYPE 2 VACCINE AND A MHYO VACCINE

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#### **Background and Objectives**

Both Porcine Circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhp) are worldwide, potentially severely loss-causing pathogens. Prophylactic solutions exist as both PCV2-Mhp-combo vaccines and separate vaccines on the individual pathogens. The objective of this randomized, controlled study in a PCV2-Mhp-combo and individual PCV2 plus Mhp vaccination was to assess and compare the lungs lesion scores (LLS) and average daily weight gain (ADWG).

#### **Material and Methods**

In a Danish organic specific pathogen free finisher farm, positive to Mhp and Actinobacillus pleuropneumoniae type 2, 4 batches of 300 7-week-old piglets were ear tagged and randomized into 3 groups, Vac-A: Circovac 0.5ml and Hyogen 2.0ml, Vac-B: Combined PCV Mhyo 2.0ml plus, Control: non-vaccinated, at the time of weaning (D0) and vaccinate according to groups. 10 randomly chosen pigs out of each groups were serum sampled D0, D49, D70 and D91. Mhp-ELISA and PCV2-qPCR were performed at the DTU Vet-institute. ADWG was evaluated in all three groups from the time of first positive Mhp-serology in the non-vaccinated group. In total 448 pigs included.LLS assessed via the Ceva Lung Program (CLP) performed by the Danish SEGES Laboratorium on randomly selected lungs of each group in the two batches positive. Statistics for weighted LLS were performed in Kruskal-Wallis test with Stata 15.

#### Results

No PCV2-qPCR positives in any groups. Mhp-seropositive controls: 20-30% at D70 and 50-80% at D91.Mhp-like LLS: Control 6.61, N=132; Vac-B 4.02, N=122 (vs control p=0.0004); Vac-A 1.40, N=120 (vs Control p=0.0000; vs Vac-B p=0.0096).ADWG, standardised 13-115 kg: Control 884 g/day, Vac-B 890 g/day and Vac-A 898 g/day.

#### **Discussion and Conclusion**

Both vaccine groups reduced statistical significantly the Mhp-like LLS. On top of that, the Mhp protective capacity of Vac-A was significantly better than the Vac-B. Both vaccine groups were providing numerically better ADWG than the non-vaccinated.

Immunology and vaccinology

## PRRSV-1 UPREGULATES IN VIVO INHIBITORY PD-L1 AND TIM-3 IMMUNE CHECKPOINTS IN BRONCHOALVEOLAR LAVAGE CELLS IN A STRAIN DEPENDENT FASHION

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#### **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is characterised by high antigenic and genetic variability, with the appearance of virulent strains in the recent years. PRRSV has the ability to alter the host's immune response, highlighting the interest on deciphering specific signaling pathways involved in the immune regulation. Immune checkpoints are regulatory molecules that trigger immunosuppressive signals associated to the "exhaustion" of T cells, which leads to the reduction of cell proliferation, cytokine production and cytotoxic activity. However, studies on the role of immune checkpoints during PRRSV infection are scarce. The aim of this study was to determine changes in the expression of immune checkpoints in bronchoalveolar cells from piglets infected with PRRSV strains of different virulence by using data obtained by RNASeq.

#### **Material and Methods**

Seventy four week-old piglets were randomly distributed in 3 experimental groups: (i) control, mockinoculated; (ii) 3249 (low virulent strain) and (iii) Lena (virulent strain) (10<sup>5</sup> TCID<sub>50</sub> intranasal). Clinical signs were daily recorded, and animals were sequentially euthanised from day 1 to day 13 post-inoculation (dpi). Pulmonary viral load was determined by qPCR. After bronchoalveolar lavage (BAL), RNA was extracted from BAL cells using TRIzol and cDNA was sequenced via 2 x 50 paired-end sequencing on llumina HiSeq 2000.

#### Results

PD-1, PD-L1, CTLA4, LAG3 and TIM3 were differentially expressed from 6 or 8 dpi onwards in both infected groups. Lena infected pigs showed an earlier (6 dpi) and higher expression of PD-L1 and TIM3 transcripts when compared with 3249-infected pigs. No marked changes were observed among infected groups for PD-1, CTLA-4 and LAG-3 transcripts.

#### **Discussion and Conclusion**

Our results highlight the upregulation of immune checkpoints along PRRSV infection. The higher and earlier upregulation of PD-L1 and TIM3 immune checkpoints by Lena when compared with 3249 strain might be associated with the higher virulence of the former.

Herd health management and economy

#### MANAGEMENT MATTERS; ASSOCIATIONS BETWEEN APP ANTIBODY RESPONSE AND ANIMAL FLOW MEASURES

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#### **Background and Objectives**

The aim of this study was to evaluate the association between the management regarding mixing of pigs and seroprevalence to Actinobacillus pleuropneumoniae (App) in 22-weeks old pigs.

#### **Material and Methods**

Mixing of pigs within and between different age groups on farms was audited with a newly developed semiquantitative model: "mix-index". The mix-index of 24 App positive multipliers in the Netherlands was subsequently categorised based on rank (Cat. 1 – 3). ApxIV antibody (ApxIV-Ab) was measured with ApxIV-ELISA and seroprevalence estimated, by sampling five 22 wk old gilts thrice annually between 2018-2020. A Chi-squared test was used to test the differences between the proportion of ApxIV-Ab+ animals between samplings and between farms with different mix-index categories. Mixed effect logistic regression modelling was used to determine risk estimates for an ApxIV-Ab+ result given the mix index category and to determine the effect of seasonality.

#### Results

The proportion of ApxIV-Ab+ animals is significantly higher in Cat 3 farms (0.82) than in Cat 1 farms (0.32) (p<0,05). The mixed model resulted in OR = 31.8 (95% CI 4.4 – 294.0) for a positive test result in 22-weeks old gilts for Cat 3 farms compared to farms with a mix-index score in Cat 1 and OR=7.4 for a positive test result when comparing Cat 2 farms to Cat 1 farms. Moreover, a significant difference in test results between the sampling moments was found. The mixed-effect model including sampling moment as a fixed effect, fitted the data better, suggesting a possible seasonal influence.

#### **Discussion and Conclusion**

The model outcomes indicate that the mixing of pigs is associated with an increases of the App prevalence in gilts in App positive multiplier herds. Implication: reducing frequency of direct animal contact may not only be important but also feasible for lowering App prevalence in swine herds.

Herd health management and economy

#### A RELEVANT DIAGNOSIS ASSOCIATED WITH A TAYLOR-MADE ACTION PLAN TO REDUCE ANTIMICROBIALS' USE

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#### **Background and Objectives**

By considering the farm specific situation and the context, the objective of this clinical case is to show how a relevant diagnosis associated with a taylor-made action plan can reduce the antimicrobial use in a farm.

#### **Material and Methods**

A PRRSV free farrow-to-finish farm of 450 sows started the implementation of an autogenous vaccine on sows in 2018 for streptococcial management. Nevertheless, six-to-eight weeks-old piglets showed in 2019 arthritis, neurological symptoms or loss of weight. As the farmer treated them systematically with antimicrobials (trimethoprim-sulfonamide) from the onset of clinical signs, the mortality rate was low (1.7%). To investigate this case, the antimicrobial treatment was stopped and four untreated piglets with nervous signs were taken for diagnostic investigation (necropsy, bacteriology, PCRs). For the differential diagnosis, rectal and floor samples were added for Edema Disease. Moreover, nasal and blood samples were taken on weaners with hyperthermia to search for Swine Influenza Virus.

#### Results

All the SIV samples were negative, only Escherichia coli 0139 K82 Stx2e and FI8 has been found on two necropsied piglets and in the rectal samples (no septicemic bacteria). To confirm this result, we proposed to vaccinate half of the four weeks old piglets in the following batch. As an outbreak with 5% mortality and nervous signs started, the farmer decided to vaccinate all the batch with a commercial vaccine. After vaccination the mortality rate dropped to 1% and clinical signs were not observed anymore. Technical performances were significantly improved and the return on invest positive.

#### **Discussion and Conclusion**

By stopping the systematic antimicrobial use, we have observed the emergence of an Edema Disease outbreak. This case highlights the importance to convince a farmer to stop systematic antimicrobials use to propose a clinical investigation based on a diagnosis in unbiased conditions and to propose a taylor-made action plan to solve the problem and reduce the use.

Herd health management and economy

#### BRIX REFRACTOMETRY TO MEASURE IMMUNOGLOBULINS ON-FARM IN NEONATAL PIGLETS

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#### **Background and Objectives**

Sufficient colostrum intake (CI) and immunoglobulin absorption are essential for an appropriate passive transfer of immunity via the colostrum. This study investigated 1) digital Brix refractometry to measure immunoglobulins in serum of neonatal piglets, 2) the agreements between Brix refractometry and a) optical refractometer, b) electrophoresis, and c) ELISA, and 3) threshold values for failure of passive transfer (FPT) for different immunoglobulin concentrations.

#### **Material and Methods**

Forty-five sows and 269 piglets from three different farms were enrolled in the study. Piglets were weighed at birth and 24 hours later to calculate CI. Serum was collected at 24 hours after birth and analysed for serum total protein (STP; optical refractometer),  $\gamma$ -globulins (electrophoresis), % Brix (Brix refractometry) and immunoglobulin G (IgG; ELISA).

#### Results

The median (interquartile range, IQR) CI was 412 (196) g per piglet. Median (IQR) STP,  $\gamma$ -globulin and % Brix concentrations in piglet serum were 60 (11) g/L, 35 (10) g/L and 8 (2) %, respectively. Average (± SD) IgG concentration was 49 ± 23 g/L. Passing-Bablok regression revealed a strong concordance between % Brix and STP (Kendall's tau: 0.620, P < 0.001) and % Brix and  $\gamma$ -globulin concentration (Kendall's tau: 0.575, P < 0.001, n = 267). The agreement between the Brix refractometer and IgG concentration was poor (Kendall's tau: 0.267, P < 0.001). Receiver operating characteristic curves were performed to evaluate test characteristics of Brix refractometry for three  $\gamma$ -globulin cut-off values, i.e. 10, 20 and 30 g/L. The % Brix cut-off values resulting in the optimal combination of sensitivity and specificity were 5.4 (100 and 98.5%), 7.0 (100 and 89.3%) and 7.9 (90.1 and 80.6%), respectively.

#### **Discussion and Conclusion**

Digital Brix refractometry is a sufficiently fast and practical method to assess serum immunoglobulin concentrations on-farm in neonatal piglets. However, additional studies are needed to validate the thresholds for FPT found in this study.

Herd health management and economy

## FIELD EVALUATION OF HAEMOGLOBIN (HB) LEVEL AND FACTORS INFLUENCING HB STATUS IN PIGLETS AT WEANING ON POLAND FARMS

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#### **Background and Objectives**

Iron deficiency anaemia (IDA) is a serious health problem in piglets and it is controlled by routine application of iron by injection. The aim of the study was to evaluate the haemoglobin level (Hb) at weaning in the commercial farms in Poland and to assess the possible influence of size of the piglet, the sow Hb level and type of iron form.

#### **Material and Methods**

Sixteen randomly selected farms from different geographical areas in Poland, using different iron based products supplementation were included. Within each farm, ten randomly selected litters from different parity sows have been assessed (30 piglets/farm, 491 piglets in total). Small, medium and large piglet per litter were sampled as well as sows from corresponding litters (n=164), Hb levels were measured immediately on farm test (HemoCue  $\mathbb{R}$ ). Piglets were classified as follows: Hb levels < 9 g/dL anemic, Hb levels ≥ 9 g/dL and < 11 g/dL are suboptimal and Hb levels ≥ 11 g/dL are optimal.

#### Results

Overall, 15 percent of the sampled piglets were anaemic, 59% were sub-optimal and 26% optimal. The type of iron treatment has significant effect (P= 0.0368) on Hb level at weaning. There was a weak correlation between Hb level of sow and Hb levels of large size piglet (P= 0.0413). Significant differences were found between the piglets of different size (P=0.0002). The best performing were small piglets (mean Hb= 10.46 g/dL, 15 % anaemic and 32% optimal). The large piglets were especially at risk (mean Hb= 9,91 g/dL, 17 % anaemic and 18% optimal).

#### **Discussion and Conclusion**

Clear effect of size of the piglet was demonstrated, large piglets were at risk of development of IDA, as well as the effect of sows' Hb on Hb level on this category of piglets. Forty-eight sows (29,2%) were found anaemic at weaning.

**Bacterial diseases** 

### ASSOCIATIONS BETWEEN MICROBIOME, CLINICAL, PATHOLOGICAL AND CLASSICAL MICROBIOLOGICAL FINDINGS IN PIGS WITH RESPIRATORY DISEASES

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#### **Background and Objectives**

Diseases of the respiratory tract are of great importance for pig production worldwide. The large number of different interacting pathogens and their strain-specific characteristics with regard to resistance and virulence factors pose a major challenge for diagnostics. It has long been known that, in addition to the pathogens that are primarily regarded as causative, a variety of other pathogens are causative, modifying or indicative of the overall occurrence of respiratory diseases. The aim of the present study was therefore to characterize the entire microbiome of the lung using bronchoalveolar lavage (BAL).

#### **Material and Methods**

Forty pigs from 29 herds with Respiratory Diseases were studied by clinical examination, examination of lung function by Impulse Oscillometry (IOS), BAL, gross pathology and histopathology, molecular diagnosis of pathogens' nucleotides and microbiological methods. The complete microbiomes of the lungs were analysed by whole genome sequencing of the BAL fluids.

#### Results

Twenty different bacterial genera were found in the BAL fluid samples. Alterations in clinical findings, IOS, pathology and pathological histology were significantly associated with the quantitative identification of bacteria of the genera Actinobacillus, Pasteurella and Mycoplasma. However, most strains were not Actinobacillus pleuropneumoniae or Pasteurella multocida. Additionally, some bacteria species could serve as markers for healthy lungs, as they never occurred together with any clinical or pathological findings.

#### **Discussion and Conclusion**

Next step in the ongoing study is to characterize the identity of the Actinobacillus and Pasteurella species. Based on this study, we hope to achieve a more comprehensive and rapid characterization of microbial findings related to respiratory diseases, including the integration of sequence data on virulence and resistance factors of the participating germs in one step.

**Bacterial diseases** 

#### EUROPEAN SURVEY ON LUNG LESIONS IN SLAUGHTER PIGS

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#### **Background and Objectives**

Lung lesions scoring at slaughterhouses provide valuable 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 the problems with enzootic pneumonia end pleuropneumonia. The aim of this survey was to collect the results of lung scoring performed in most of swine producing European countries in 2019.

#### **Material and Methods**

Ceva Lung Program scoring methodology was implemented to score the lesions at the slaughterhouse. The results were collected from 20 European countries in the 12 months period from December 2018 till end of November 2019. The mean values and quartiles 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). For the two latter indicators the results from France were not included, because there they were not scored routinely.

#### Results

The total number of scored lungs was 425058 from 3566 reports with the average of 119 lungs per batch. The median value of %BP was 40,37% with the Q1=19,83% and Q3 63,10%. The median of affected parenchyma was 5,51% with the Q1=2,86% and Q3=8,83%. For % DP the median, Q1 and Q3 were 9,88%; 3,48% and 23,55% respectively and for APPI the corresponding values were 0,42; 0,18 and 0,87 respectively.

#### **Discussion and Conclusion**

The results of this survey conducted in 20 European countries in 2019 demonstrated very similar distribution of the values as the previous year 2018. With the almost 4% more lungs scored in 2019, those results confirm the value of CLP as a repeatable scoring methodology. The values of A.p. like lesions are worse than in 2018, which may be related to the continuous decrease of the use of antimicrobials in European swine herds.



# POSTERS



#### REDUCTION OF BOAR TAINT CARCASS RISK IN ENTIRE MALE PRODUCTION USING ENTERISOL® ILEITIS

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#### **Background and Objectives**

The whole pork industry is looking for solutions to mitigate the risk of boar taint on entire male carcasses. Three substances are responsible for carcass odor, including skatole which is produced by bacteria in the digestive tract. Change in microbiome is assumed to minimize the risk of boar taint. The aim of this study was to evaluate the effect of Enterisol<sup>®</sup> lleitis on boar taint occurrence and backfat skatole level in herds with high prevalence of boar taint on entire males.

#### Material and Methods

Two herds, members of Cooperl, producing Nucleus Px(LWxLD) crossbred pigs and with a history of high level of taint carcasses were included in the study. The pigs were slaughtered in a single slaughterhouse performing human nose detection of taint carcasses routinely. In both herds, Lawsonia intracellularis infection was confirmed by serology and Enterisol<sup>®</sup> lleitis vaccination was implemented at 7 and 12 weeks of age respectively. Boar taint detection, using a scoring system from 1 to 5 (carcasses with scores  $\geq$  3 were considered "taint"), was performed at slaughter on all entire males. The percentages of taint carcasses, before and after vaccination, were compared with a Chi-square test. Backfat was sampled on taint carcasses to analyze skatole and androstenone concentrations.

#### Results

In total, 4820 male carcasses were included in the evaluation. The percentage of taint carcasses decreased significantly from 3,45% to 2,31% (p=0,02). On 141 taint carcasses, 67 backfat samples were analyzed for skatole and androstenone. The mean concentration of skatole decreased significantly from 221 ng/g to 154 ng/g (p=0,04), while androstenone concentration remained stable.

#### **Discussion and Conclusion**

This study confirms the benefit of the Enterisol<sup>®</sup> lleitis vaccination on the reduction of boar taint risk. The decrease of skatole level in the vaccinated pigs confirms the hypothesis of the positive effect of Enterisol<sup>®</sup> lleitis on gut health.

NURSERY MORTALITY MONITORED WITH STATISTICAL PROCESS CONTROL OVER MORE THAN 2 YEARS IN A FARM, WHICH FACED AN ACUTE PRRS OUTBREAK AND IMPLEMENTED CHANGES IN THE VACCINATION PROGRAM

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#### **Background and Objectives**

Infection with PRRS virus may lead to significant losses in productivity of breeding and growing pigs. PRRS control may utilize several measures including vaccination. Statistical process control (SPC) is a straightforward option to monitor and compare performance over time.

#### Material and Methods

The investigation was conducted on a one-site, farrow-to-finish farm with 2000 sows in Croatia. The farm is PRRSV positive since 2014, however, pigs were only vaccinated against PCV2 and Mycoplasma hyopneumoniae (Mhyo). A new, severe PRRS outbreak affected performance from week 48/2018. Along with management measures in the breeding unit, whole herd vaccination was implemented from week 8/2019. All suckling piglets, 14 days and older, were vaccinated with Ingelvac PRRSFLEX® EU, followed by routine vaccination at three weeks of age, concurrently with PCV2/Mhyo. From week 39/2019, PCV2/Mhyo vaccines were changed to FLEXcombo®. No other major changes influencing performance were implemented during the observation. Weekly data of nursery mortality was compared for four periods by SPC: PI: PCV2/Mhyo vaccinated; P2: during PRRS outbreak; P3: PRRSFLEX & PCV2/Mhyo vaccinated; P4: PRRSFLEX & FLEXcombo® vaccinated. Transition periods were excluded.

#### Results

Mean of nursery mortality increased significantly (P1: 1.8 vs. P2: 12.1) during the acute PRRS outbreak. With PRRS vaccination mortality dropped significantly (P3: 3.3), but did not reach pre-outbreak level. After additional change to FLEXcombo, nursery mortality dropped again significantly (P4: 1.9) to pre-outbreak level. Differences were considered significant with p<0.01 (Bonferroni correction; Control charts displayed in poster).

#### **Discussion and Conclusion**

PRRSV, PCV2 and Mhyo, do influence each other and may all be controlled, but not eliminated by vaccination. Changes in the vaccination program and/or individual vaccines within the program may change the disease situation and associated performance. SPC is a valuable tool to analyse large continues data sets, taking into consideration mean and variation of the data associated with process changes.

#### MECHANICAL CHARACTERISTICS OF CLAW HORN AND THEIR ASSOCIATIONS WITH CLAW LENGTH AND LESION SCORE IN CULLED SOWS OF 3 GREEK HERDS

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#### **Background and Objectives**

Claw health in sows is important in terms of welfare and productivity. However, data on biomechanical properties of sow claw horn is scarce. Hence, the objective was to investigate the mechanical indices of claw horn and their association with claw length and lesion score.

#### Material and Methods

The feet of 185 culled sows from three Greek herds (A: 64 sows; B: 64 sows; C: 57 sows) were collected at slaughter. Sows had different genotypes (A: Topigs; B: Danbred; C: PIC), but were reared on similar conditions and fed rations with identical levels of chelated Zn, Mn and Cu. All hooves were macroscopically examined for lesions and scored using a severity scale ranging from 0 to 2 at five anatomical sites. The dorsal, diagonal, and heel-sole lengths of claws and the dew claw lengths were measured with a digital caliper. A slice from the dorsal wall of each claw was used to assess its mechanical characteristics with three-point bending test. Young's modulus, yield stress and maximum stress were determined.

#### Results

Sows from herd A differed (P<0.05) from those of herd C; Young's modulus values for each of the eight claws were higher and yield stress and maximum stress were higher for four and six claws, respectively. In sows of herd B Young's modulus values were lower for the five claws compared to sows of herd A while only in one claw yield stress was higher. All claw lengths and mean lesion score for each claw were significantly lower in sows of herd A compared to those of the other two herds. The stiffness of claw horn was negatively correlated with claw length and lesion score. A positive correlation was observed between length and lesion score.

#### Discussion and Conclusion

Overgrown claws are susceptible to lesions and have inferior mechanical properties.

#### URINARY TRACT INFECTIONS IN CULLED SOWS FROM GREEK HERDS: PREVALENCE AND ASSOCIATIONS BETWEEN FINDINGS OF HISTOPATHOLOGY, BACTERIOLOGY AND URINALYSIS

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#### **Background and Objectives**

Urinary tract infections (UTI) of sows which include cystitis, which may progress to ureteritis and pyelonephritis affect their productivity, longevity and welfare. We determined the prevalence of UTI by histopathology and bacteriology and investigated possible associations between histologically confirmed cystitis and the results of urinalysis and urine cultures in sows from 3 Greek farrow-to-finish herds.

#### Material and Methods

Routinely culled sows were included in the study. Their urinary bladders were collected from abattoirs and examined histopathologically. Furthermore, urinalysis and urine cultures were performed on urine samples aseptically collected from the bladders.

#### Results

Histologically confirmed cystitis was evident in 85/185 (45.94%) culled sows. Among those, 44 (51.76%) suffered from acute and 41 (48.24%) from chronic inflammation. The majority of the positive urine cultures were due to colonization of the urinary tract with E.coli (55.81% of the total cases), followed by Staphylococcus spp. (18.60%). Sows with bacteriuria were 2.30 (p=0.03, 1.10-4.83) times more likely to have histologically confirmed cystitis compared to sows with negative urine cultures. Bacteriuria was associated with proteinuria (p<0.01), urine pH (p<0.01) and presence of sediment (p<0.01) in urine. Sows with proteinuria had 9.72 (2.63-35.88) times higher odds of bacteriuria than those without. Histologically defined cystitis was associated with proteinuria (p<0.01) and urine pH (p<0.01). Sows with proteinuria were 5.18 times (2.03-13.2) more likely to have histological lesions consistent with cystitis, than those without.

#### **Discussion and Conclusion**

In the studied herds, UTI affected almost one out of two culled sows. Bacteriuria, which was more common among sows with UTI than those without, was mainly ascribed to members of the intestinal and environmental microbiota. Proteinuria and the existence of urine sediment which were associated with UTI may be proposed as likely on-farm predictors of UTI in live sows.

#### PREVALENCE AND IMPACT OF PORCINE EAR NECROSIS ON THE AVERAGE DAILY WEIGHT GAIN IN NURSERY PIGLETS

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#### **Background and Objectives**

Porcine ear necrosis (PEN) is characterized by an uni- or bilateral blue to black discoloration of the ear tip or margin followed by necrosis and mainly nursery piglets are affected. Different risk factors have been described such as infections, ear biting, high stocking density, poor ventilation, mycotoxins in the feed, and insufficient enrichment of the environment. The aims of this study were to evaluate the prevalence of PEN and the impact of PEN on the piglets' growth.

#### Material and Methods

Six consecutive batches of weaned piglets (ranging from 560 to 750) were included. During the 7 week nursery period, the presence and severity of PEN were recorded weekly in each weaning batch, and by the same person. Individual weighing included 401 animals, and allowed to calculate the average daily weight gain (ADWG) for affected and non-affected pigs. Severity scoring included mild, moderate, severe and very severe lesions.

#### Results

At the end of the nursery period, 23% of all piglets were affected. The prevalence increased with weeks postweaning, especially from week 5 post-weaning onwards, reaching from 11 to 32% between groups at the end of the nursery. Only one batch showed a 2% decrease during the last week. The mean ADWG ( $\pm$ SD) for pigs with (n=158) and without (n=243) lesions was 394g ( $\pm$ 65g) and 391g ( $\pm$ 71g), respectively (P = 0.469). Mild, moderate, severe and very severe lesions represented, respectively 85.0%, 13.9%, 1% and 0.1% of all lesions.

#### **Discussion and Conclusion**

The prevalence of PEN increased with time spent in the nursery and the overall prevalence was variable between batches. As most of the affected piglets had mild lesions (crusts), it could explain the non-significant difference in ADWG between affected and non-affected animals. Further research is warranted to assess the impact of more severe lesions.

#### IMPACT OF MULTIPLE COUGHING OUTBREAKS DUE TO INFLUENZA ON DAILY GROWTH PERFORMANCE IN A FINISHING FARM MONITORED BY SOUNDTALKS

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#### **Background and Objectives**

Cough is a common symptom of finishers of which the concurrent impact on performance is poorly understood. The objective of this study was to compare and quantify the impact in daily growth (ADGW) of a multiple disease-coughing outbreak in a finishing batch of pigs under commercial conditions.

#### **Material and Methods**

The study was performed in an open finishing pen provided with an optiSORT sorting gate with automatic body weight recording (Hoelscher & Leuschner GmbH & Co. KG). Cough monitors (SOMO, SoundTalks NV, Belgium) were also installed and an algorithm-based respiratory distress index (RDI) was continuously generated and aggregated daily from the farm-recorded sound. A mathematical model based on daily growth of previous batches (reference growth curves) was proposed to accommodate the linear relation between cough-free daily growth curves and time through linear mixed models. 95% confidence limits over time were provided as graphical tools to evaluate whether the potential cough-affected growth curves (RDI greater than 1) was found within the expected bounds constructed over reference cough-free growth curves. To quantify this difference, a regular linear model was considered and performed in R.

#### Results

Individual ADGW data of a total of 350 pigs and the average daily RDI was recorded for 129 days. Two coughing outbreaks were detected and diagnosed as influenza by PCR, lasting 14 and 7 days respectively, with a mean/max RDI of 2.50/4.99, and 2.50/3.87, respectively. Overall, there were no statistical differences in the curves' growth within the periods of influenza. Despite abnormal lower growth detected during the first outbreak that severely impacted ADGW, a compensatory growth, observed during the second outbreak, mitigated the overall impact.

#### **Discussion and Conclusion**

Results of this study highlight that even mild clinically cough episodes could potentially impact performance parameter such as ADGW. However, more growth curves would be needed to more accurate predict impact and to account for potential confounders.

#### MONITORING TOE LENGTH AND ANISODACTYLIA AND INVESTIGATING ASSOCIATION WITH FOOT TEMPERATURE OF CROSSBRED FI GILTS FROM 3 GREEK HERDS

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#### **Background and Objectives**

Common claw abnormalities observed in sow breeding herds are overgrown claws. Gilts may become lame and culled even at first parity. Infrared thermography (IRT) has been used to detect signs of inflammation in lower limbs. The objective was twofold: i) to investigate toe growth and anisodactylia (toe dissimilarity) in FI gilts, from weaning up to their first farrowing and ii) to investigate possible association with foot temperature.

#### Material and Methods

A total of 139 crossbred FI gilts of 3 commercial herds in Greece (Herd A Topigs:27; Herd B Danbred:70; Herd C PIC:42), were used. Gilts were fed similar rations with identical levels of chelated Zn, Mn and Cu. Toe measurements included dorsal, diagonal, heel-sole, dew claw length. Anisodactylia, was assessed by the dorsal length difference between claws. Foot temperature was measured at first farrowing with IRT in the areas of carpus/tarsus (TI), upper metacarpus/metatarsus (T2), lower metacarpus/metatarsus (T3) and phalanges (T4). Locomotion soundness and backfat loss of all sows were scored/measured at farrowing.

#### Results

At weaning, toe lengths differed between herds and were lowest in Herd B (P<0.001). Anisodactylia was lowest in Herd C compared to others (P<0.001). At first farrowing, toe lengths and anisodactylia were lower in Herd A compared to B and C (P<0.001). TI, T2, T3 in the rear right foot were lowest in Herd C (P<0.05). Toe lengths of front feet were positively correlated with TI, T2, T3, and T4. Backfat thickness at service and farrowing differed (P<0.001) among herds and were highest in Herd B.

#### **Discussion and Conclusion**

Toe length differences and anisodactylia were present in gilts from different genotypes even at the time of weaning and at their first farrowing. Increased toe lengths of front feet of gilts at first parity were associated with increased foot temperature.
# ASSOCIATION OF TOE LENGTH AND ANISODACTYLIA WITH FOOT TEMPERATURE OF PUREBRED SOWS FROM 3 GREEK HERDS

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# **Background and Objectives**

Claw overgrowth may cause abnormal mechanical stresses in feet resulting in discomfort, tissue damage and localized increase in temperature suggesting inflammatory response. Infrared thermography (IRT) was used to measure feet temperature in purebred sows (GP) of three genetic lines. Association between toe length abnormalities and feet temperature was assessed.

# Material and Methods

A total of 86 GP sows of 3 genetic lines (Line A: Topigs, n=45; Line B: Danbred, n=19; Line C: PIC, n=22) were used. Sows were reared throughout their productive life under similar conditions and were fed rations that had identical levels of chelated Zn, Mn and Cu. Toe measurement included dorsal, diagonal, heel-sole, and dew claw length. Anisodactylia (toe dissimilarity) was assessed by the dorsal length difference between medial and lateral claws of each foot. Skin temperature was measured with IRT in the areas of carpus/tarsus (TI), upper metacarpus/metatarsus (T2), lower metacarpus/metatarsus (T3) and phalanges (T4). Locomotion soundness was scored at weaning while backfat loss was measured at farrowing and at weaning.

# Results

Toe lengths of all feet and anisodactylia mainly in the rear feet differed between lines (P<0.05), being lower in sows of Line A. In the rear feet, TI, T2, T3, T4 were significantly (P<0.05) lower in sows of Line A than the other two lines. In all lines, TI, T2, T3, T4 were positively correlated with diagonal claw length and rear feet anisodactylia, and negatively correlated with front feet anisodactylia of sows. Backfat loss and mean locomotion score were lower in sows of Line A compared to those of Lines B or C.

# **Discussion and Conclusion**

Claw length and anisodactylia were associated with the genetic line, while backfat loss was lower in those with reduced anisodactylia. Increased rear feet anisodactylia and diagonal claw length may predispose to an increased foot temperature.

# EVALUATION OF THE ECONOMIC IMPACT OF PRRS VIRUS ASSOCIATED TO PRRS STATUS IN DUTCH SOW FARMS

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# **Background and Objectives**

Netherlands is one of the main European pig producing countries where PRRS is an endemic disease causing significant productive and economic losses. The aim of this study was to establish a systematic monitoring programme for PRRS in order to classify farms and evaluate the productive and economic impact of PRRSV infection under Dutch field conditions.

#### **Material and Methods**

Between January and June 2020, 8 breeding herds with 5,200 sows all together located in the Netherlands voluntarily enrolled in a half-year PRRSV monitoring programme following the classification proposed by Holkman et al. All the farms adopted a diagnostic monitoring protocol which consisted of monthly individual blood tests of 30 pre-weaned piglets by RT-PCR (5 pools of 6 each one). Based on the diagnostic results, farms were classified as: Negative (N), Provisional negative (PN), Positive Unstable (PU) and Positive Stable (PS).

#### Results

A total of 169 weekly data were analysed: 141 weeks were classified as PU and 28 weeks as PS. Statistical significant differences were observed in piglet born alive ratio (-1.84% PU vs. PS), stillbirth piglet per litter (+0.34% PU vs. PS), pre-weaning mortality (+0.22 PU vs. PS) and weaned piglets per litter (-0.47 PU vs PS). We could estimate a reduction of 1.13 weaned piglet/sow/year due to instability (0.47 weaned piglet/-litter x 2.4 litter/sow/year = 1.13 weaned piglet/sow/year). Considering that the average size was 656 sows/farm, the annual economic impact of PRRS virus in a PU farm was 22,238 € ([656x1.13]x30) (average weaned piglet price in the Netherlands was 30€).

# **Discussion and Conclusion**

These results provide better understanding of the productive and economic impact of PRRSV circulation in breeding herds, reinforcing the efforts to stabilize and maintain stable the PRRS-positive farms.

IMPACT OF CYSTOISOPORA SUIS ON GROWTH PERFORMANCE AND DIGESTIVE HEALTH OF PIGLETS - A CONTEMPORARY STUDY IN FRANCE

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# **Background and Objectives**

Porcine neonatal coccidiosis caused by Cystoisospora suis is one of major causes of diarrhea in suckling piglets worldwide. Coccidiosis is controlled by routine application of toltrazuril. The objective is to evaluate a combinatorial injectable product (toltrazuril/gleptoferron) recently registered.

# **Material and Methods**

A commercial farm located in West part of France, without control program based on toltrazuril application was selected. C. suis was confirmed based on flotation before starting the study, as well during the trial in control group. Two groups of piglets (G1 and G2, n = 182 and 191 piglets) from one batch (29 sows) were randomly allocated based on parity of the sows in parallel design and treated either with a fixed dose 1.5 ml of 45 mg toltrazuril + 200 mg gleptoferron IM per piglet (Forceris®) on the 2<sup>nd</sup> day of life (DOL) (G1) or 200 mg of gleptoferron/piglet on 2<sup>nd</sup> DOL (G2). Animals were observed daily for general health and faecal litter score was assessed. Mortality, number of treatments and weight gain were recorded. Statistical analysis was done by T-student test.

# Results

Mortality was the same in both groups (4.5%). The mean weight was 460g higher in G1 vs. G2 (7.110 kg and 6.650 kg; p=0.004) at weaning (28 DOL). The average daily gain (ADG) was 226g vs. 205g in G1 and G2 respectively (p= 0.0001). More litters from G2 reported diarrhea with longer average duration (2.5 days/ G1 and 5 days/ G2). Ten litters (G2) with the longest duration diarrhea (more than 6 days) were selected and C. suis presence was confirmed, 6/8 were positive at weaning. No positive litter was observed in G1.

# **Discussion and Conclusion**

Clear negative effect of clinical coccidiosis on production results was demonstrated. Targeted treatment according to described protocol provided good efficacy against clinical signs of coccidiosis and improved significantly ADG and weaning weight.

# MONITORING OF HEPATIC LESIONS AT SLAUGHTER IN HEAVY PIGS AND THEIR CORRELATION WITH ANTHELMINTIC PLANS ON FARM

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# **Background and Objectives**

Abattoir post-mortem inspections offer a useful tool for animal-health monitoring and a source of data for epidemiological investigation. Aim of the present work was to provide feedback on prevalence and severity of lesions of the liver in batches of Italian heavy-pigs (165 kg live weight; 9 months of age). Moreover, results were compared to the type of anthelmintic treatment administered at the farm.

#### **Material and Methods**

In total, 205 batches of heavy-pigs from 75 intensive commercial farms located in Northern-Italy were monitored throughout a 6-months period. Within each batch, an average number of 100 livers was individually scored, assigning a value of 1-3. Anthelmintic treatments recorded among farms were: levamisole (x1, x2, x3 administrations), fenbendazole, ivermectin, flubendazole.

#### Results

Statistical analysis showed a strong farm effect (44 and 36% of variation for mean liver score and severe lesions respectively). Mean liver score was  $1.3\pm0.2$  and frequency of severe lesions was  $9.5\pm8.2\%$ , with no differences between farms that used anthelmintic (n=169) and farms that did not use it (n=36). However, among farms applying an anthelmintic plan, the lowest level of lesions was showed by farms administering levamisole x3, compared to the highest showed by farms administering levamisole x2 (mean score=1.21 vs 1.55; severe lesions=4.9 vs 16.6%; P<0.05).

#### **Discussion and Conclusion**

The frequency of severe lesions observed seems to be higher than other reports in Europe, even if it is impossible to directly compare different studies. This hypothesis in heavy-pigs may reflect evidence of a within-herd parasite reinfection at a later stage due to the prolonged finishing period. This indicates a lack of/inadequacy in adequate parasite-control plans in many farms, as suggested by the different treatments analysis. Probably, the implementation of an anthelmintic plan by adding a third treatment in case of levamisole in heavy-pigs is guarantee of a longer protection against later reinfections.

# POSITIVE EFFECT OF ENTERISOL® ILEITIS ON BOAR TAINT RISK IN CARCASSES: PROOF OF CONCEPT IN A FRENCH FARROW TO FINISH HERD

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# **Background and Objectives**

The rearing of entire male pigs may lead to the production of meat with an unpleasant odor. Previous studies suggest a possible correlation between ileitis and the occurrence of boar taint in carcasses. The assumption that preventing ileitis may reduce the production of skatole and the risk of boar taint in carcasses was made. The objective of this study was to assess the effect of the Enterisol<sup>®</sup> lleitis vaccination, in a herd positive for Lawsonia intracellularis (Li), on the rate of boar taint.

# Material and Methods

This study was conducted in a 380-sows Farrow to Finish herd raising entire males on 3 fattening sites. The Li positive status was confirmed by PCR on fecal samples and by serology. From mid-October 2018, the Enterisol® lleitis vaccination was implemented on 7-week of age pigs using dosing pump. Boar taint was systematically evaluated at slaughterhouse using the human noses method. The rate of boar taint between the period before vaccination and the period after vaccination was compared using a Fisher test. The analysis was also performed by carcass weight classes.

#### Results

In total, carcasses from 7889 entire males were assessed. The rate of boar taint carcasses significantly decreased, from 2,41% in non-vaccinated pigs to 1,60% in vaccinated pigs (p=0,013). A fattening site effect was observed with a greater reduction in sites with higher initial boar taint rates. The reduction of the boar taint rate was not correlated to the weight at slaughter.

# **Discussion and Conclusion**

As far as we know, this study is the first one assessing the effect of Enterisol® lleitis on boar taint occurrence. This proof of concept needs additional investigations to confirm the benefit of the oral vaccination against ileitis on boar taint considering several co-variables such as weight, age at slaughter or fat thickness, and including skatole concentration evaluation on fat.

# IMPACT OF SOW HERD VACCINATION AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS TYPE 1 (PRRSV-1) ON REPRODUCTIVE PERFORMANCE ON A ONE-SITE FARM IN SERBIA

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# **Background and Objectives**

PRRSV can cause significant impact on reproductive performance in endemically infected herds. This study aimed to investigate sow performance before and after implementation of a sow herd vaccination against PRRSV on a farm in Serbia.

# Material and Methods

The study was conducted on a farrow-to-finish farm with 2300 sows producing with weekly batch farrowing. The producer was dissatisfied with low farrowing rate with high variation (mean±SD=76.3%±4.5). Diagnostics confirmed the presence of PRRSV. No PRRS vaccination was implemented prior to this study. Vaccination started in October 2018 with double mass vaccination (four weeks apart) of the breeding herd, including gilts over 150 days of age, with ReproCyc<sup>®</sup> PRRS EU. Vaccination of the sow herd was repeated every 3 months. Performance data of sows inseminated from October 2017 onwards was collected over a period of 12 months before (last insemination September 2018, last farrowing January 2019) and compared with 19 months after the start of the vaccination programme. No other changes in vaccination programmes or major changes in management, feeding or housing were implemented during the observation period.

# Results

PRRS vaccinated sows (vac) had a significantly higher pregnancy rate checked on day 25-30 (mean<sub>vac</sub>=90.5% vs. mean=85.7%, p=0.005) and farrowing rate (mean<sub>vac</sub>=82.3% vs. mean=76.3%, p=0.001) and significantly lower regular returns to oestrus (mean<sub>vac</sub>=5.6% vs. mean=10.2%, p<0.001). Live-born piglets per litter did not differ significantly before and after the implementation of PRRS vaccination (mean<sub>vac</sub>=10.3 vs. mean=10.1, p=0.252).

# **Discussion and Conclusion**

The results show a positive impact of sow herd vaccination against PRRSV on the performance of the sows. Based on the pregnancy rate, farrowing rate and the number of live-born piglets the benefit-cost ratio of sow herd vaccination against PRRSV is 2:1.

# EVALUATION OF THE ECONOMIC IMPACT OF PRRS VIRUS IN THE NURSERY PHASE

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# **Background and Objectives**

Veterinarians and producers often ask themselves what the real impact of the PRRS virus in the growing phase is. Although there are some studies that estimate the impact of the disease in the breeding herd, knowledge in the nursery phase in Europe is limited. The aim of this study was to estimate the economic impact of PRRS in the nursery phase depending on the PRRS status at the source farm.

# Material and Methods

Between October 2018 and December 2019, 11 breeding herds with a multi-site system in the north of Italy were enrolled in a PRRSV monitoring programme following the classification proposed by Holkman et al. The farms adopted a protocol which consisted of monthly individual due-to-wean blood testing of 30 piglets by RT-PCR (5 pools of 6 each one). Based on the diagnostic results at the breeding herd, the PRRS status of each batch of piglets produced was classified as: Negative (N) or Positive (P).

# Results

A total of 161 batches of piglets from the 11 breeding herds that represents 460,000 piglets were analysed: 55 batches were classified as N and 106 as P and monitored during the nursery phase. Statistically significant differences were observed in average daily gain (AD: 402 g. in N vs. 378 g. in P, p-value: 0.03). Mortality (5.17% in N vs. 5.64% in P, p-value: 0.061) and medication cost (3.06€/animals in N vs. 3.57€/animal in P, p-value: 0.102) did not show statistically significant differences among groups.

# **Discussion and Conclusion**

The economic impact of PRRS in the nursery phase in terms of FCR, mortality and medication cost was €3.67 per 31kg pig. These results provide a better understanding of the economic impact of PRRSV in the growing phase, reinforcing the efforts to stabilize the breeding herds in order to produce a negative flow of piglets.

EFFICACY OF AN INJECTABLE TOLTRAZURIL-GLEPTOFERRON TO CONTROL IRON DEFICIENCY ANAEMIA AND COCCIDIOSIS (CYSTOISOSPORA SUIS) ON AVERAGE DAILY GAIN IN AN OUTDOOR HERD IN THE UK

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# **Background and Objectives**

Coccidiosis caused by Cystoisospora suis is a frequent cause of neonatal diarrhoea in piglets, causing reduced growth. Information about prevalence and impact of coccidiosis in outdoor farms is limited. Requirements for iron supplementation in outdoor pig units is often dismissed despite studies showing increased weaning weights following supplementation. The aim of this comparative study is to determine the impact of supplementing outdoor piglets with Forceris® on weaning weight and Average Daily Gain (ADG) under field conditions where Cystoisospora suis was confirmed by histopathology prior to the study.

#### Material and Methods

367 piglets from 34 litters were included and randomised based on litter and parity. 183 piglets were treated with a combined injectable gleptoferron-toltrazuril. 184 piglets were left untreated with access to iron via soil, as per the normal farm protocol (control group). All piglets were individually weighed and ear tagged at initial treatment (day 1, 2 or 3 of age) and re-weighed at weaning.

#### Results

There was no significant difference between initial weights of the treatment and control group (2.01kg and 1.98kg respectively, p=0.5026). There was a significant difference between weaning weights of the treatment and control group (8.42kg and 7.75kg respectively, p=0.0008). ADG was significant between both groups, with treated and control piglets growing on average 279g/d and 250g/d respectively (p=0.0001). Over the trial period this equated to an average of 667g extra growth per pig following treatment.

When compared to the control group, piglets in the treatment group had more uniform weights at weaning (CV% 17.9vs27.5) and a greater proportion of pigs >8kg (62.4% vs 43%).

### **Discussion and Conclusion**

The results of this study clearly demonstrate that treatment with Forceris® resulted in higher ADG in pigs during the trial period, with more uniform weaning weights recorded.

# THE IMPACT OF INJECTABLE TOLTRAZURIL ON WEANING WEIGH - A COMPARATIVE FIELD TRIAL.

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# **Background and Objectives**

Neonatal coccidiosis (caused by Cystoisospora suis) is considered to be a relevant cause of weaning weight impairment. Recently a combination of gleptoferron and injectable toltrazuril (Forceris®) was shown to improve the control over the agent and the piglets' weaning weight. The purpose of this field trial was to assess the effect of this injectable toltrazuril in the piglets' weaning weight in commercial farms in comparison with untreated piglets or piglets treated with oral toltrazuril.

# Material and Methods

In three farrow-to-finish farms (previously confirmed as C. suis positive), several litters were selected (farm A - 25 litters; farm B - 34 litters; farm C - 30 litters) and randomly distributed into two groups (IM and control group). Both groups were balanced regarding sows' parity and litter size. All piglets were ear tagged and weighed at 24 hours of life and at the day prior to weaning. In farm A, the control group remained untreated, whilst in farms B and C the control groups were treated with oral toltrazuril (20mg/kg) according to the standard procedure of both farms. The IM groups in all farms were treated with 1,5mL of Forceris™ at 24 hours of life.

# Results

In farm A the IM piglets were 0,148kg heavier at 24 hours of life (1,611kg vs 1,463kg), and at 22 days of age were 0,398kg heavier (6,475kg vs 6,076kg; p<0,05). In farm B the IM piglets were 0,144kg lighter at 24 hours of life (1,485kg vs 1,629kg), but 0,203kg heavier at 25 days of age (6,362kg vs 6,159kg; p>0,05). In farm C the IM piglets were 0,082kg heavier at 24 hours of life (1,821kg vs 1,739kg), and 0,356kg heavier at 21 days of age (6,153kg vs 5,797kg; p<0,05).

# Discussion and Conclusion

In this trial the piglets treated with injectable toltrazuril were heavier at weaning, even when compared with orally treated piglets.

# COMPARISON OF AN INJECTABLE TOLTRAZURIL-GLEPTOFERRON AND AN ORAL TOLTRAZUTIL+ GLEPTOFERRON FOR THE CONTROL OF ANAEMIA AND COCCIDIOSIS UNDER FIELD CONDITIONS

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# **Background and Objectives**

Iron-deficiency anaemia and Cystoisospora suis infection are considered as re-emerging problems in suckling piglets. Both conditions are controlled by application of different iron and toltrazuril-based oral products. Recently a combinatorial product (toltrazuril/gleptoferron) has been developed for parenteral application.

# Material and Methods

A commercial farrowing farm located in Brittany, with history of coccidiosis was selected to run the study. Two groups of piglets (from 42 sows = 1 batch) were randomly established based on litters and parity of the sows in parallel design and treated either with a fixed dose 1.5 ml of 45 mg toltrazuril + 200 mg gleptoferron IM per piglet (Forceris®) on the 2<sup>nd</sup> day of life (DOL) (GI) or 20 mg toltrazuril/kg body weight as an oral suspension (Baycox®) on 4<sup>th</sup> DOL (G2). Group (G2) received 200 mg of gleptoferron/piglet on 2<sup>nd</sup> DOL. Animals were observed daily for general health and feacal litter score was assessed. Mortality, number of additional treatments, weight gain, hemoglobin level (Hb) by Hemocue® were examined.

# Results

Due to farm management and early weaning we collected individual datas on G1(n=170) and G2(n=211) at weaning (28 DOL), the mean weight was 120g higher in G1 than G2 (8.22 kg and 8.10 kg; p=0.55) with more homogenous distribution. The average daily gain (ADG) was 258g vs. 250g in G1 and G2 respectively. Lower percentage of small piglets (< 7kg) (21% vs 28%) and more piglets with ADG >250g (57% vs 49%) was recorded in G1. More litters from G2 reported diarrhea signs with longer average duration (3 days/G1 and 3.6 days/G2). Hemoglobin levels were the same in both groups.

# **Discussion and Conclusion**

Forceris prevented for clinical signs of coccidiosis and provided a positive impact on the homogeneity of piglets. Both gleptoferron based products enabled to reach optimal level of Hb at weaning.

# EFFICACY OF AUTOGENOUS SOW-VACCINES AND IODINE SPRAY ON MORTALITY AND UMBILICAL OUTPOUCHINGS IN PIGLETS FROM TWO DANISH HERDS

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# **Background and Objectives**

The aim of this study was to evaluate if autogenous vaccines based on bacteria, isolated from piglet umbilici and/or topical iodine application of the umbilicus at birth, would affect prevalence of mortality and umbilical outpouchings (UO).

# Material and Methods

In two Danish herds 30 one to seven day-old piglets with clinical signs of umbilical infection, but never treated with antimicrobials were necropsied. Aseptically incision of the umbilical protrusion (UP) was performed and the underlying tissue, joint and meninges were cultivated. Eschericia coli and Streptococcus dysgalactiae isolated from UP tissue and also identified from either joint or meninges, were selected for autogenous vaccines for both herds. Correspondingly, an adjuvant placebo was produced at Vaxxinova GmbH. A two-way factorial study design was carried out as a randomized, blinded trial where sows were vaccinated or placebo injected twice prefarrowing, and after shortening of the umbilical cord half of their piglets in each group were sprayed with iodine 7% on the umbilicus.

# Results

A total of 5,852 piglets were included. Preliminary results for pigs aged 12 weeks for herd A and B respectively, showed a total prevalence of UO of 9.9% and 8.3% and a total mortality of 15.2% and 20.1%. Mixed models with sow as random effect showed a significantly lower mortality among pigs born from vaccinated sows in Herd A (12.9% vs. 16.8%, p=0.02). In Herd B iodine treatment of the umbilicus showed fewer cases of UO in the placebo group (7.1% vs. 9.8%, p = 0,046). However, this effect was not observed in piglets from vaccinated sows. Autogenous vaccination did not affect the prevalence of UO.

# **Discussion and Conclusion**

In this trial mortality was reduced in one herd in offspring from sows vaccinated with an autogenous vaccine. The prevalence of UO was reduced by iodine treatment in the placebo group of one herd only.

# MANAGING SHIGA TOXIN-PRODUCING E. COLI USING QUALITY CONTROL CHARTS FOR ROUTINE HEALTH AND PRODUCTION MONITORING IN PIG FARMING

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# **Background and Objectives**

Intestinal infection with Shiga-toxin-producing Escherichia coli leads to a widespread swine disease known as edema-disease (ED), mostly after weaning. To reduce antimicrobial treatments, preventive strategies should be applied. Beside the common control approach of the herd, the efficacy of a prevention plan (ED-PrevPlan) might be monitored using Quality-Control-Charts (QCC), a statistical tool to sustain health stability by routinely monitoring productive parameters. Aim of QCC analysis is to recognize when a parameter goes out-of-control, to allow the producer to apply corrective intervention.

# Material and Methods

The establishment of QCC in monitoring the ED-PrevPlan in an infected weaning site (1800 weaners/batch, slatted floor, dry ad-libitum feeding) has been reported over a retrospective 5-years period analysis (n=66 consecutive batches). ED-PrevPlan was applied in batches 1-12 and 64-66: piglets' vaccination at 5-days of age (Ecoporc-Shiga, IDT-Chemifarma); low-energy diet; ad-libitum fiber; all-in-all-out management. For QCC establishment, total mortality and feed-conversion-rate (FCR) for each batch were recorded; the statistics and the intervention limits (IL=mean ±3-fold sd) for both parameters were based on data from the first 12 batches to allow detection of out-of-control batches (values out of IL) from batch 13 ongoing.

# Results

Mortality evaluation using QCC revealed a production system under-control (mean=3.44%; upper-IL=10.1%; lower-IL=0.0%; sd=2.23%) during the application of the ED-PrevPlan, and 3 out-of-control batches (n.13=17.0%; n.18=20.1%; n.39=17.3%) when no ED-PrevPlan was applied. FCR did not show any out-of-control batch (mean=2.14; upper-IL=2.58; lower-IL=1.83; sd=0.02) during the entire period of analysis.

# **Discussion and Conclusion**

QCC revealed that mortality in an infected herd went out-of-control several times without an ED-PrevPlan, differently from FCR probably due to the intervention on feed energy. With minimal efforts, QCC seems to be a promising tool to evaluate health stability. Further studies are needed to investigate the efficacy of the vaccination alone in an ED-PrevPlan in order to maintain feed-energy.

# PRRS TYPE 1 MLV MASS VACCINATION IN COMMERCIAL SOW HERDS IS SAFE

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# **Background and Objectives**

Different PRRS vaccination protocols for sow herds in the Netherlands are used. Main arguments against mass vaccination protocols are suspected productivity effects due to vaccination during critical phases of the sows' reproductive cycle. In this study we compare production results in sow herds after PRRS type 1 MLV mass vaccination to the baseline production.

# Material and Methods

Retrospectively production data were obtained from the farm management systems. In total, 8 farms and 70 mass vaccination events using PRRS type 1 MLV vaccines were analyzed for different production results. Baseline production was defined as the production results in the period up to 6 weeks prior to mass vaccination. Post-vaccination period was defined as the period up to 6 weeks following mass vaccination including the week of mass vaccination. Both periods were analyzed using Statistical Process Control to identify weekly changes of more than 2 Standard Deviations (>2SD) on a herd level. The incidences of >2SD changes over the herds were expressed as average incidences per week.

# Results

Incidence rates of negative production results per week of >2SD changes comparing Baseline production to Post-vaccination production: Pregnancy rate at day 35 (representing return to estrus) 0.044 versus 0.052, Farrowing rate (representing total pregnancy losses including abortions) 0.065 versus 0.030, Still born per litter (representing piglet quality) 0.048 versus 0.032

# **Discussion and Conclusion**

The incidence rate of >2SD changes per week was scattered evenly over the Baseline and Post vaccination periods. Whole herd vaccination advantages are a larger homogeneity of immunity at population scale, better herd immunity, less administration, lower number of missed vaccinations and reduced labor, and is seen as a 'stupid proof' vaccination scheme. We conclude that the PRRS type 1 MLV mass vaccination is safe and recommended for the control of PRRSV in a sow herd.

COMPARING BRONCHOPNEUMONIC LESIONS AND ENZOOTIC PNEUMONIA INDEX (EP INDEX) BETWEEN M.HYO UNVACCINATED PIGS, PIGS VACCINATED WITH OTHER M.HYO VACCINES AND HYOGEN® VACCINATED PIGS

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### **Background and Objectives**

Mycoplasma hyopneumoniae (M.hyo) is the primary cause of enzootic pneumonia and is an important pathogen in the Porcine Respiratory Disease Complex (PRDC), which has a huge impact on the production cost of fatteners. Lung scores are performed in the slaughterhouse where bronchopneumonic lesions are indicative for M.hyo infection.

# Material and Methods

Lung scores are performed using the Ceva Lung Program (CLP) as described previously. A CLP is performed at the request of the herd veterinarian for one of the following reasons: evaluate the implemented vaccination protocol, respiratory symptoms during fattening or general health check of the fatteners. The data used for this analysis were collected in Belgium from October 2019 until September 2020. 8714 lungs from 56 different batches were examined, distributed as follows: 719 lungs from 6 M.hyo unvaccinated batches of pigs, 4324 lungs from 30 batches of pigs vaccinated with another M.hyo vaccine and 3671 lungs from 20 batches vaccinated with Hyogen® (HG).

#### Results

The unvaccinated pigs and the pigs vaccinated with another vaccine had 22.95% and 25.35% of bronchopneumonic lesions respectively, compared to 14.19% of bronchopneumonic lesions for the HG vaccinated pigs. The difference in bronchopneumonic lesions between unvaccinated pigs, pigs vaccinated with another vaccine and HG vaccinated pigs was significant (Chi-Square test: P<0.05). The unvaccinated pigs and the pigs vaccinated with another vaccine had an EP index of 0.990 and 0.949 respectively, compared to 0.411 for the HG vaccinated pigs.

#### **Discussion and Conclusion**

HG vaccinated pigs have significantly less bronchopneumonic lesions and the severity of the lesions is lower compared to M.hyo unvaccinated pigs and pigs vaccinated with another M.hyo vaccine. Some confounding factors such as the fact that unvaccinated and vaccinated animals might originate from herds with a different health status might not have been taken into account in this study. Therefore, more research is needed.

REPRODUCTIVE PERFORMANCE MONITORED WITH STATISTICAL PROCESS CONTROL OVER MORE THAN 2 YEARS IN A FARM, WHICH FACED AN ACUTE PRRS OUTBREAK AND IMPLEMENTED PRRS CONTROL MEASURES INCLUDING VACCINATION

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# **Background and Objectives**

Infection with PRRS virus may lead to significant losses in productivity of breeding and growing pigs. PRRS control may utilize several measures including vaccination. Statistical process control (SPC) is a straightforward option to monitor and compare performance over time.

#### **Material and Methods**

The investigation was conducted on a one-site, farrow-to-finish farm with 2000 sows in Croatia. The farm is PRRSV positive since 2014, however, did not see the need for specific PRRS control measures prior to a new and severe PRRS outbreak in late 2018 (week 44/2018). Control measures included loading and closing the herd for six months, limiting cross-fostering, no use of foster sows (until Sep/2019), strict hygiene at processing and whole herd vaccination (from week 8/2019). The breeding herd, including all gilts, was vaccinated twice four weeks apart with ReproCyc® PRRS EU, followed by quarterly mass vaccination. Four key parameters were analysed via SPC starting from week 20/2018 until week 40/2020. Weekly data was compared before (period 1), during the acute PRRS outbreak (period 2) and after PRRS control started (period 3). Spermicide substances in semen tubes temporarily influenced performance negatively. This data and a transition period were excluded from the analysis. No other major changes or events occurred during the observational period.

# Results

Means in period 1, 2 and 3 respectively were: Pregnancy rate:  $93.4^{\circ}$ ,  $92.9^{\circ}$ ,  $93.3^{\circ}$ ; Farrowing rate:  $91.9^{\circ}$ ,  $85.0^{\circ}$ ,  $90.2^{\circ}$ ; Live-born piglets:  $14.6^{\circ}$ ,  $13.5^{\circ}$ ,  $14.3^{\circ}$ ; Pre-weaning mortality:  $10.7^{\circ}$ ,  $19.2^{\circ}$ ,  $11.0^{\circ}$  ( $^{ab.c}$ : different superscripts indicate significant differences of the mean with p<0.017 (Bonferroni correction), control charts displayed in poster).

### **Discussion and Conclusion**

Reproductive performance stabilized after implementation of the PRRS control program and returned close to pre-outbreak values and variation. SPC offers a straightforward way to analyse large continuous data sets, taking into consideration mean and variation of the data associated with process changes.

EVOLUTION OF HEALTH EXPENSES AND TECHNICAL-ECONOMICAL EVALUATION AFTER IMPLEMENTATION OF LEPTOSPIROSIS VACCINATION AT THE LEVEL OF A GROUP OF FARMS

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# **Background and Objectives**

Leptospirosis is present in swine, with rare acute forms, but frequent reproduction disorders. Its control relied in France on biosecurity and antibiotics use, but since 2018 a vaccine with a leptospirosis indication is available, Porcilis<sup>®</sup> Ery+Parvo+Lepto. The aim of this study was to assess the technical, health and economic interest of this new vaccination in a group of farms.

#### Material and Methods

Evolutions in expenses per slaughtered pig for antibiotic treatments against leptospirosis, and evolutions in reproductive performances were observed before and after vaccination against leptospirosis in farms without other contemporary change, respectively in 15 farms for periods of one year, and in 10 farms among them (2940 sows) for periods of 6 months in same seasons.

#### Results

After vaccination against leptospirosis, expenses for antibiotic treatments against leptospirosis (tetracycline, penicilline and streptomycine) were reduced in all 15 farms by 0,12 to 0,92 euros per slaughtered pig (mean value 0,46). In the 10 farms with data available, fertility is maintained without treatment (mean value from 90,44 to 90,99%), and number of weaned piglets increased (average gain of 0,4 weaned piglet/litter and 1,3/productive sow/year) mainly due to an increase in prolificacy (average total born from 15,76 to 16,20 and born-alive from 14,60 to 14,95 per litter), and ability to reach weaning.

#### **Discussion and Conclusion**

In this study the vaccination against leptospirosis has a real clinical and economic interest in a context of proven disease. Altogether, extra cost of the vaccine, reduced expenses for antibiotics treatments, increase in number of weaned piglets lead to a gain of 2,85 euros per slaughtered pig per year, based on references on the last 5 years of the technical and economic data management system of the pig company to which those farms belong, and to a gain of 3,31 euros for year 2019 of same references.

# DO WE VACCINATE OUR GILTS TOO LATE IN HUNGARY AGAINST PARVO 1? - A FIELD STUDY

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# **Background and Objectives**

Porcine Parvovirus 1 is still one of the most important infectious disease in the swine sector, causing mainly disorders of the reproduction system. Sometimes the losses are not so apparent, like in case of SMEDI, or abortion; but it can cause severe production losses such as decrease in the number of live born piglets, sows returning to heat, sows' lower pregnancy and/or farrowing rate, and non-homogeneous litters. The aim of this study was to highlight the importance of timing of vaccination of the gilts against PPV-1.

# Material and Methods

We surveyed 27 Hungarian swine farms in 2019, and 2020. We always used the same protocol for blood sampling: 3-6-9-12-15-18-21-24 weeks old pigs were sampled, and 10 samples were taken in every age group. We used the PPV Hemagglutination Inhibition (HI) test for determining the antibody levels in sera.

# Results

The average time of first seronegativity, after maternal immunity loss was the following on the 27 Hungarian farms: at 3 weeks of age 7.4% of farms, at 7 weeks 3.7%, at 9 weeks 29.6%, at 10 weeks 29.6%, at 11 weeks 7.4%, at 12 weeks 14.8%, at 13 weeks 3.7%, and at 19 weeks 3.7% respectively. In the older groups (>15 weeks) we found antibodies from PPV-1 infection.

# **Discussion and Conclusion**

The first vaccinations of gilts are done after PPV-1 infection on many Hungarian farms, so the antibodies in the blood can interfere with the vaccine antigens. We advise to measure the antibody levels of the offspring on the farms and rethink the time of Parvo vaccination of gilts. Specially if there is suspect of Parvo reproductive problems. Vaccination should be done when maternally-derived antibody levels are low and antibodies from PPV-1 infections are not yet present.

# SALIVA BIOMARKERS FOR ANTI-MICROBIAL NEEDS VERIFICATION IN PIGS.

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# **Background and Objectives**

Reduction of the need for anti-microbial usage in sustainable pig production is widely discussed and highly promote in Europe and worldwide as a public health measure to reduce anti-microbial resistance. Inflammatory and infection biomarkers are increased during infection in both serum and saliva samples of pigs. The present pilot study shows an analytical tool for the assessment of inflammatory-infection condition prior the use of anti-microbials in pigs.

# Material and Methods

Two groups of 5 pigs were monitored during anti-microbial therapy in field conditions.

Group 1: pigs with respiratory symptoms (fatigue, dyspnea and prostration) due to Actinobacillus pleuropneumoniae infection based on necropsy.

Group 2: pigs with severe diarrhea probably caused by the high mycotoxin content detected in the diet since common infections (Salmonella spp., Brachyspira hyodisenteriae and Porcine epidemic diarrhea virus) were discarded by microbiological and PCR analysis.

C-reactive protein (CRP) and adenosine deaminase (ADA) were quantified in saliva samples. A repeated nonparametric multiple comparisons test was used for statistical comparisons.

# Results

In group 1, median CRP (22.58 ng/mL) and ADA (691 U/L) levels before treatment (day 0) were statistically higher than those observed at the end of the anti-microbial therapy (day 4) (4.12 ng/mL and 285 U/L for CRP and ADA respectively). However, no variation in any biomarker was observed in pigs with diarrhea during treatment (overall levels around 12.9 ng/mL and 346 U/L for CRP and ADA respectively).

# **Discussion and Conclusion**

The quantification of inflammatory-infection biomarkers in porcine saliva seems to be useful to distinguish between animals that require anti-microbial therapy due to an infection condition, to those subjected to non-infectious condition that would required other type of intervention. Moreover, the proposed analytical tool could be also used to verify the efficacy of the anti-microbial therapy by monitoring the levels of biomarkers until the normal range values will be reached.

# INVESTIGATION OF PORCINE RESPIRATORY DISEASE COMPLEX: ASSESSMENT OF 2 YEARS USING A FARM ANALYSIS KIT.

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# **Background and Objectives**

This study investigated the infection dynamics of Porcine Respiratory disease complex (PRDC) caused by Mycoplasma hyopneumoniae (Mhp), PRRS virus (PRRSv), influenza virus (IAV), porcine circovirus type 2 (PCV2) using oral fluid (OF) sampling kits on 49 affected farms.

# Material and Methods

In each herd, 3 ropes were placed in 3 pens of 3 different batches : T0 (batch whith acute coughing ), T-1 (batch 3-5w before), and T+1 (batch 3-5w after). OF samples were analyzed by qualitative multiplex PCR for PRRSv, IAV, PCV2 and Mhp (IVD-GmbH, Germany).

# Results

The age at T0 ranged from 8 to 25 weeks (average 17.4) and the cough index from 0.65 to 18 coughs/min/100 animals (average 3.7). The Mhp seroprevalence ranged 25% to 100% (average 88%). PRRSv was present in 39% to 35% of the farms before and during the cough, decreasing to 23% after the clinical episode. The same trend was observed for IAV: 43% before, 41% during the cough episode and 34% after. Regardless of the time of detection, PCV2 incidence varied little (37%-40%). The frequency of Mhp increases from 27% to 85% at T+1 and when age at T0 increased. At T0 Mhp was more often found in association with at least one of the 3 pathogens tested (51%) than alone (18%) and its incidence was lower than other pathogens at T-1. When coughing occurs, it was mostly between 15 and 22 weeks of age (30/49 farms).

# **Discussion and Conclusion**

Infection dynamics of PRDC is specific of each herd .The detection of pathogens by PCR does not necessarily mean clinical disease. However, for these 49 herds, results highlighted the role of PRRSv and IAV as primary PRDC agents, whereas it was rarely the case for Mhp. PRRSv was usually detected 1 month after animal regrouping (weaning, fattening). Does it promote PRRSv contamination?

OPTIMIZING LABOR TIME FOR PREVENTION OF COCCIDIOSIS AND ANAEMIA IN SUCKING PIGLETS USING AN INJECTABLE COMBINATION OF TOLTRAZURIL AND IRON.

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# **Background and Objectives**

Different management procedures on piglets are performed in their first week of life. One of the most important practices is the use of injectable iron to prevent iron-deficiency anaemia and the oral administration of toltrazuril for prevention of coccidiosis caused by Cystoisospora suis. Recently, an injectable combination of toltrazuril and gleptoferron (Forceris™, Ceva) has been developed to simplify such piglet's management for farmers. Thus, the aim of this study was to compare the effect of combined toltrazuril+gleptoferron and an injectable iron/oral Toltrazuril on the time of piglet's management procedure.

#### Material and Methods

This trial was performed in a farrow-to-finish farm with history of coccidiosis. A farrowing batch was randomly selected and its litters at 2 days of life (doa) were divided into two treatment groups. Group A was treated with 1.5mL of the injectable toltrazuril+gleptoferron and group B followed the usual farm procedure (oral toltrazuril and injectable iron). Ear-tagging and tail docking was performed in both groups during piglet's management. Time of management per litter was recorded, considering the number of piglets/litter. Time of management per piglet (seconds±SD) was calculated and compared between treatments by Student's t-test. Significant differences were considered when p ≤0.01.

#### Results

A total of 735 piglets from 55 sows were included: 34 litters in group A (485 piglets) and 21 litters in group B (250 piglets). Time of management per piglet in each litter was significantly reduced (p<0.01) in group A (23.41±3.49) compared to B (28.52±3.75).

#### **Discussion and Conclusion**

Time for piglet's management was significantly reduced using Forceris™, representing a labour-saving and valuable strategy that reduced stressful procedures in piglets in their first week of life.

# EFFICACY OF NEEDLE-FREE IRON DELIVERY, AN ON FARM DOUBLE BLIND RANDOMIZED CONTROLLED CLINICAL TRIAL

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# **Background and Objectives**

Piglets will develop a hypochromic microcytic anaemia if they are depleted of iron at young age. Needle-free injection devices are more often used for routine administration of iron to piglets. However, the efficacy and safety are not yet well studied. This study aims to study the efficacy and safety of needle free delivery of gleptoferron-iron on D3 for the prevention of iron deficiency anaemia in 4 week old pigs.

# Material and Methods

A double blind randomized clinical trial was conducted with 72 pigs from 9 litters. From each litter, 3 piglets were randomly allocated to the needle group, 3 to the needle-free group and 2 pigs to a non-treated control group. Pigs were weighed and blood (EDTA + Serum) was collected at D3, D14, D26 and D70. Blood was tested for haematological parameters (Hb, Ht, MCV, MCH, MCHC, CHr) as well as serum iron, iron binding capacity and iron saturation. A linear mixed effects model with random litter effect was used to compare the effect of needle-free delivery with needle injection on serum haemoglobin and haematocrit respectively on D26.

# Results

Although variation of Hb on D26 was increased in the needle-free group compared to the needle group, no significant differences between needle-free and regular injection of gleptoferron iron on Hb and Ht at D26 were found. In the control group, however, Hb and Ht at d26 was significantly lower. Interestingly, weight of the pigs between groups did not differ at any time point. Other haematological results showed almost equal results for both needle-free and needle injection group. No adverse signs were noted in all groups.

# **Discussion and Conclusion**

Needle-free iron delivery is as effective and seems as safe as injection per needle to prevent anaemia at weaning.

# Funding

This study was funded by MS Schippers, Bladel, The Netherlands

# PIG HEALTH INFO SYSTEM: DEVELOPMENT OF AN APP FOR HEALTH DATA RECORDING

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# **Background and Objectives**

Data regarding the current health situation in Swiss pig husbandry is hardly available. The veterinarians' documentation is very heterogeneous and findings are exclusively stored in their local software. Neither in quality nor in quantity does this data suit for a nationwide monitoring. However, such an overall monitoring of animal health data would be of high value in relation to health surveillance and early recognition of infectious disease outbreaks. To supply this need, in this project, a system is developed which allows digital recording and storage of pig health data. Additionally, a procedure will be implemented for data analysis and visualisation of information.

# Material and Methods

The veterinarians are provided with an application running on their smartphones, which is used to record findings while examining a farm. This application is especially developed to fulfil the veterinarians' requirements and to enable them to document their examination digitally and well structured. The application does not only cover symptoms and diagnoses but also observations on surroundings and management. The veterinarians decide case-related about the extent of their documentation. Having completed the recording of a new data set, a report is generated and automatically sent to the farmer and the veterinarian.

# Results

Starting later in this project, newly recorded data will be analysed for abnormal accumulations of specific findings to identify potential disease outbreaks. Hence, if required, veterinarians and farmers in the affected area can be warned and requested to be attentive. Moreover, necessary arrangements to stop spreading of a transmissible disease could be initiated.

# **Discussion and Conclusion**

In addition to the improvement in the veterinarians' documentation of findings, the project is expected to have a positive impact on animal health and animal welfare. As potential health risks can be detected at an earlier stage, measures can be taken in good time and thus the number of affected animals can be limited.

# A COMPARISON OF TWO PRRSV SURVEILLANCE TECHNIQUES IN A GERMAN SWINE HERD.

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# **Background and Objectives**

PRRSV surveillance is of utmost importance in both negative and vaccinated swine herds, to reduce economic losses. Sampling piglets at weaning time using serum blood samples is a very common way to monitor PRRSV (Holtkamp et al. 2011). However, processing fluids are a sensitive, practical and time-effective tool to monitor PRRSV in sow herds (Vilalta et al. 2018). The aim of this study is to compare both techniques in an assumably stable breeding herd.

# Material and Methods

A 3.600 head sow farm with a weekly production cycle and whom are vaccinated consistently 3 times a year, was examined for PRRSV stability. Two to five day old piglets were examined by processing fluids (PF) at day of processing (ear tags, tail docking, castration) and at intervals of four weeks. The same groups were sampled by 30 blood samples at the time of weaning. Processing fluid samples and blood samples were taken in accordance to sow parities. Each parity group (1, 2-4, 5+) was represented by 10 BS and 2 PF samples, which were tested for PRRSV by RT-PCR.

# Results

There was no PRRSV detection in processing fluids. One pooled blood sample at third (BS3, parity 1) and two pooled blood samples at fourth point of time (BS4, parity 2-4) were PRRSV positiv. Sequencing of ORF5 revealed a 99.5% homology of used vaccine strain.

# **Discussion and Conclusion**

Due to the good sensitivity of processing fluids in detecting PRRSV, the investigated sow population can be declared as stable. On the other hand, because of the recurrent PCR positive results in some of the blood samples, the whole herd cannot be defined as stable (Holtkamp et al. 2011). Therefore blood samples could provide good information about possible lacks of the internal biosecurity measures, if processing fluids are delivering negative results.

### GASTRIC ULCERS IN SOWS AT HERD LEVEL

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# **Background and Objectives**

For 3 years, SEGES Danish Pig Research Centre has performed an extensive screening for gastric ulcers in all Danish sow herds holding over 200 sows, as an agreement between SEGES, The Danish Veterinary Association and The Danish Veterinary and Food Administration, as a part of the Danish Pig Welfare Action Plan. The objective was to monitor the occurrence of gastric ulcers in Danish sow herds on herd level and to initiate action plans in herds with high prevalence of gastric ulcers.

# Material and Methods

From each herd, 20 stomachs were collected at 5 slaughterhouses in Denmark. The stomachs were evaluated at the Laboratory for Pig Diseases (SEGES) and scored on a scale from 0-10, where 0 denoted no ulcer and 10 the most severe ulcer. Scores 7-10 were defined as problematic. A herd problem was defined as the herd having more than 50% of the 20 stomachs with score 7-10. In this case, the owner was to complete an action plan for reducing gastric ulcers, controlled by Baltic Control as part of the DANISH Product Standard Certification.

# Results

By November 2019, 999 herds have been included and 34 action plan demands have been issued, i.e. 3.4% of the herds had gastric ulcer problems. Baltic Control have inspected 28 of the 34 herds and all, but one, presented an action plan. The effect of the action plans has not been further investigated.

# **Discussion and Conclusion**

When the screening started in 2017, the expected prevalence of issued actions plans was 25% of investigated herds. The much lower prevalence can be explained by extensive focus on preventing gastric ulcers in Danish sows for the last 5-7 years. Therefore, the prevalence of herds with actions plans is low - 3.4%, which also indicates the Danish farmers' interest in reducing sows' gastric ulcers.

# REPRODUCTIVE DISORDERS FOLLOWING LEPTOSPIRA POMONA INFECTION IN A SOW FARM, RESULTS FOLLOWING TO ANTIBIOTIC TREATMENT AND VACCINATION WITH PORCILIS® ERY+PARVO+LEPTO

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# **Background and Objectives**

Leptospira Pomona is known as cause of reproductive failures in sows. Antibiotic treatment was the usual way to handle Leptospirosis in the past. The vaccine availability of Porcilis® Ery+Parvo+Lepto gives us the opportunity to reduce clinical signs and / or infection of nine relevant Leptospira serovars.

# Material and Methods

In a 500 sow farm with good reproductive performance (over 30 piglets nursed/sow/year over years; vaccination Parvovirus, Erysipelas and PRRS EU) abortions increased dramatically. After detection of Leptospira Pomona (by PCR in abortion material) sows were treated with trimethoprim/ sulfonamide. Despite of improved hygiene precautions and change of antibiotic treatment to doxycycline problems were diminished but did not disappear completely during the next months. With the availability of Porcilis® Ery+Parvo+Lepto vaccination was introduced.

# Results

Herd data (time delayed analysis to farrowing) in the acute phase (Jan-Jun 2018): abortions 6.7 %, farrowing rate 80.7 %, dead born piglets 9.3%, mummies 0.86%; results from sows with 2fold doxycycline treatment (Jul-Dez 2018): abortions 3.6 %, farrowing rate 86.9 %, dead born piglets 9.3%, mummies 0.54%; results (Jan-Jun 2019) from sows with 2fold vaccination with Ery+Parvo+Lepto: abortions 0.2 %, farrowing rate 85.8 %, dead born piglets 7.6%, mummies 0.47%. Data from the vaccinated period compared to acute period showed 97 % reduction of abortions, an increase of farrowing rate by 6.3 %, 18.2 % less dead born piglets, 45.3% decrease of mummies and 6.23 % more live born piglets/sow/year (40.1 piglets/sow/year to 42.6).

# **Discussion and Conclusion**

Antibiotic treatment can reduce clinical symptoms caused by Leptospira Pomona but does not solve the problem on a sustained basis. Vaccination with the Ery+Parvo+Lepto vaccine aids in the protection of sows from Leptospira Pomona related reproductive disorders and helps to ensure high health and performance.

# LUNG LESION SURVEY IN SLAUGHTER PIGS USING CEVA LUNG PROGRAM IN THE NETHERLANDS; A RETROSPECTIVE COMPARISON

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# **Background and Objectives**

The aim of the study was to evaluate the prevalence and severity of lung lesions in slaughter pigs at different slaughterhouses in the Netherlands and to compare these with the previous period.

# **Material and Methods**

In the period of November 2018 and November 2019 (P1) a total number of 58 batches which included 8.253 lungs were scored according to the Ceva Lung Program method (Cvjetković 2018). The results were evaluated and compared with the previous period November 2016 till October 2018 (P2) in which a total number of 54 batches with 6.121 lungs were scored. All data are expressed as median.

#### Results

The % of bronchopneumonic lungs which is suggestive for EP caused by M. hyopneumoniae (M. hyo), was 19,06% versus 18,33% for P1 and P2. The % of affected surface of the bronchopneumonic lungs was 4,32% and 4,73% respectively. The % of scarring was 11,04% and 7,32% respectively. The % of cranio-ventral pleurisy was 2,13% and 3,97% respectively. The % of lungs with dorso-caudal pleurisy which is suggestive for previous Actinobacillus pleuropneumoniae (A.p.) infections, was 10,18% and 19,73% and the APP index was 0,28 and 0,52 respectively.

# **Discussion and Conclusion**

The results of the lung scoring of PI indicated a high rate of EP-like lesions. Compared to P2 it remains similar with almost no differences except for an increase in % of scars and a decrease in the % of cranio-ventral pleurisy. Although, compared to the overall European results described before, the EP-like lesions are still less prevalent. On the other hand, there is a notable decrease of A.p.-like lesions and APP index in PI compared to P2. The results of PI are comparable with the overall European results. The control of M. hyo and A.p. infections remains a major challenge and farm-specific control programs should be evaluated regularly.

# THE STANDARD AND THE BETTER ANSWER TO E. COLI MANAGEMENT IN SOWS

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# **Background and Objectives**

In piglets, like humans, E. coli bacteria are part of the natural, healthy intestinal microbiome. However, E. coli are also well described as pathogens leading to diarrhoea and even death. A farm in Italy with 150 sows managed, experienced a severe outbreak of E. coli in piglets between weaning and 15 kilos. Mortality and heterogeneity were at a level that endangered the continued economic viability of the farm.

# Material and Methods

The conventional control strategy (TI) of frequent treatments with colistin/doxycycline in feed or colistin in drinking water were applied for 8 months. Parenteral treatments with enrofloxacin were administered as needed. The situation was not improved in terms of mortality or recovering performance. Following this approach the farm decided to try a more preventative approach (T2). Parenteral treatments were continued as needed, but addition of antibiotics in feed and water were suspended. The piglet feed was reformulated to contain an intestinal acidifier (encapsulated) at 4kg/t and beta-(1,3)-glucan of algal origin to help support the developing immune system at 0.2 kg/t. Additionally drinking water pH was adjusted to of 3.6 with organic acids.

# Results

T2 reduced antibiotic use to 8.17mg per kg BWG from 26.96mg/kg BWG while T1 was applied. Mortality in T2 was at 6.69%, compared to 11.4% in T1; average daily gain improved to 610g/d in T2 compared to T1 with 520g/d.

# **Discussion and Conclusion**

When a severe E. coli outbreak occurred antibiotics were not able to alleviate symptoms or to decrease mortality. Even in the surviving piglets' performances were affected, impacting body weight gain and uniformity. Microflora modulation and immune support, combined with the shrewd use of antibiotics, succeeded to reduce mortality rates and to restore body weight gain. The crucial outcome for the farm was that the switch to prevention allowed them to remain in business.

# COMPARISON OF THE EFFICACY AGAINST M.HYO AND PCV2 OF CIRCOVAC® AND HYOGEN® VS. A PCV2/M.HYO COMBINED VACCINE

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# **Background and Objectives**

The aim of this study was to evaluate the efficacy of simultaneously administered 1-shot vaccines Circovac® and Hyogen® compared to a PCV2/M.hyo combined vaccine. The latter is preferred by some farmers for convenience reasons.

# Material and Methods

1207 pigs were vaccinated 3 days before weaning. 603 were simultaneously vaccinated with Circovac® (0,5 ml, I.M.) and Hyogen® (2ml, I.M.) (group A). 604 were vaccinated with a PCV2/M.hyo combined vaccine (2ml, I.M.) (group B). Per sow, half of the female and male piglets were allocated to group A, the other half was allocated to group B. The M.hyo efficacy was evaluated by lung scoring using the Ceva Lung Program and 10 bronchopneumonic lungs per group were analyzed with a RT-qPCR M.hyo. The efficacy against PCV2 was evaluated by calculating the average daily gain (ADG) and mortality.

# Results

In group A, 552 lungs and in group B, 535 lungs were scored. The enzootic pneumonia (EP) index was 0,33 and 0,70; % of bronchopneumonic lungs was 8% and 18% (p<0,05); % of scars was 1% and 12% (p<0,05); % of cranial pleurisy was 0% and 3% (p<0,05) in group A and B respectively. The median number of M.hyo DNA copies was 3,26x10<sup>6</sup> in group A (QI = 9,48x10<sup>5</sup>; Q3 = 7,56x10<sup>6</sup>) and 8,72x10<sup>6</sup> in group B (QI = 2,47x10<sup>5</sup>; Q3 = 2,48x10<sup>7</sup>) (n.s.). The ADG from vaccination until slaughter was 674 g/day and 672 g/day (n.s.) and mortality in fattening was 1,7% and 1,9% (n.s.) in group A and B respectively.

# **Discussion and Conclusion**

Comparable results in ADG and mortality were observed in both groups. Group A showed significant better lung health compared to group B and numerically lower M.hyo DNA load. In this study, Hyogen<sup>®</sup> showed its superiority in reduction of lung lesions over the PCV2/M.hyo combined vaccine.

# COMPARISON OF THE PHARMACEUTICAL PROPERTIES OF TWO INJECTABLE TOLTRAZURIL/GLEPTOFERRON BASED PRODUCTS

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# **Background and Objectives**

Formulation of pharmaceutical products and their pharmaceutical properties are important parameters with significant influence on the drug absorption, concentrations in the target tissues and consequently the therapeutic effect. Recently toltrazuril/gleptoferron based combination products were launched in EU for the concomitant control of coccidiosis and iron deficiency anaemia (IDA). The goal of this study was to investigate the pharmaceutical properties of two different injectable products.

# Material and Methods

Two different injectable products Forceris® (30 mg/ml toltrazuril + 133 mg/ml iron as gleptoferron suspension) (product A) and product B (36.4 mg/ml toltrazuril and 182 mg/ml iron as gleptoferron) were compared for viscosity, foaming property and syringeability based on standardize procedure. For the evaluation of foaming and general aspects of products the test-tube with 7 g of product and manual agitation during 45 s was used. Foam heights at different times were recorded. Viscosity measurements were performed on viscosimeter (Thermo Scientific HAAKE RotoVisco 1) at 20 °C. Syringeability was evaluated as the time needed for injecting 10 ml volume under the constant pressure of 10N in six replicates (two needle sizes 1.2 mm, 0.8 mm were tested). The obtained mean values were compared.

# Results

Foam formation remained present in product B more than 10 minutes with tendency to flocculate and sediment more rapidly. Product A foam formation was very limited and disappeared in less than 1 minute. Product A was 3x less viscous (8 mPas vs. 24 mPas) than product B. For both needle sizes product A had better syringeability than product B with lower variability (CV (%)).

# **Discussion and Conclusion**

Results of this study demonstrate that for the pharmaceutical properties of Forceris ® were better when compared to other product with potential favorable effect on easiness of application and bioavailability. Especially foaming can influence negatively the dosage and consequently the therapeutic effect.

# FIELD EVALUATION OF HEMOGLOBIN (HB) LEVEL IN PIGLETS AT WEANING ON BELGIAN FARMS

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# **Background and Objectives**

Iron deficiency anemia (IDA) is a serious problem in neonatal piglets. The aim of this study was to evaluate the hemoglobin (Hb) level of piglets at weaning on Belgian farms and to assess the iron status at weaning and the possible influence of the piglet size.

# Material and Methods

21 randomly selected farms using different injectable iron supplements were included in the study. Ten randomly selected litters per farm from different parity sows have been assessed. One large, one medium and one small piglet at weaning age (average 20,24 days) were sampled. In total 630 piglets. The most common parameter to indicate IDA is Hb concentration. The Hb concentration was measured on the farm with the Hemocue<sup>®</sup> Hb 201+ system. Piglets were classified as follows: Hb levels <90 g/l are considered to be anemic, Hb levels ≥90 g/l and <10 g/l are suboptimal and Hb levels ≥10 g/l are optimal.

#### Results

The average Hb level of piglets at weaning was 97.25 g/l with a standard deviation of 14.68 g/l. 55% of piglets had Hb levels  $\geq$ 90 g/l and <110 g/l. 19% of the piglets had Hb levels  $\geq$ 110 g/l and 27% of piglets showed Hb levels <90 g/l. The average Hb level of large, medium and small piglets was respectively 96.36 g/l, 97.59 g/l and 97.82 g/l (n.s). The group of large piglets had numerically more anemic piglets (n=61) compared to the group of medium (n= 50) and small piglets (n=56).

# **Discussion and Conclusion**

Anemia is highly prevalent in piglets at weaning. Routine examinations of hemoglobin levels and re-think of iron-supplementation practices is recommended. Large piglets are more at risk for IDA.

# EARLY APPLICATION OF PARENTERAL TOLTRAZURIL-IRON COMBINATION (FORCERIS®) IS COMPARABLE TO LATER TREATMENT IN THE CONTROL OF EXPERIMENTAL CYSTOISOSPOROSIS IN SUCKLING PIGLETS

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# **Background and Objectives**

Cystoisosporosis (coccidiosis) is a leading cause of diarrhea in suckling piglets and is controlled by metaphylactic toltrazuril application. Recently, a single dose combination product (Forceris®) for intramuscular application has been developed for the control of piglet cystoisosporosis and prevention of iron deficiency anaemia. In previous experimental studies, it was shown that treatment with Forceris® on the 2<sup>nd</sup> day of life (dol) followed by experimental infection with Cystoisospora suis on the 3<sup>rd</sup> dol supressed completely the oocyst shedding and significantly reduced diarrhoea and consequently improves body weight gain and health of treated piglet compared to infected untreated control.

# **Material and Methods**

A subsequent study with experimental infection conducted on the 1<sup>st</sup> dol and treatment on the 2<sup>nd</sup> dol was conducted to determine the efficacy of Forceris® when applied after the onset of neonatal infections. Piglets were randomly assigned to the Forceris® group (n=13; 45 mg toltrazuril + 200 mg iron/piglet), and to the Control group (n=12; 200 mg iron/piglet). General animal health was recorded daily and body weight was determined weekly during the study (1<sup>st</sup> – 29<sup>th</sup> dol). Individual faecal samples were collected from the 5<sup>th</sup> – 18<sup>th</sup> dol and examined for faecal consistency and the presence of occysts.

# Results

In the Control group all piglets shed countable oocysts, while the Forceris® group remained negative (p <0.0001). Diarrhoea was seen in all animals in the Control group and in one animal in the Forceris® group (p <0.001). The body weight gain was significantly depressed in the Control group compared to the Forceris® group during the first two weeks post-challenge (p=<0.0001).

# **Discussion and Conclusion**

Forceris® was safe to use and effective in a single application against experimental infections with C. suis on the 1<sup>st</sup> dol and can be recommended for treatment of porcine coccidiosis in neonatal piglets.

AN UPDATE ON THE SITUATION OF THE PORCINE ENZOOTIC PNEUMONIA AND PORCINE PLEUROPNEUMONIA IN PORTUGAL USING SLAUGHTERHOUSE LUNG EVALUATION DURING 2018 AND 2019.

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# **Background and Objectives**

Ceva Lung Program (CLP) is running in Portugal since December 2015 and evaluates the presence of bronchopneumonic lesions and dorsocaudal pleurisy at slaughter. Although these lesions are not pathognomonic, their occurrence is usually associated with two agents: Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae. For this reason, this lung lesion scoring system is useful tools to evaluate farms' health status and to validate the results of enzootic pneumonia and swine pleuropneumonia control programs. The purpose of this study was to update the Portuguese CLP results for the years 2018 and 2019 in comparison with previous published results (Costa and Nunes, 2019).

#### Material and Methods

Between November 2018 and October 2019, 222 batches of pigs from Portuguese farms were scored at slaughter using the CLP methodology (which includes Modified Madec System and Modified SPES as described by Cvjetković et al, 2018).

#### Results

In average, each batch had 25,3% of bronchopneumonic lungs (vs 27,1% in 2017/18) resulting in a 1,07 Madec Index (vs 1,16 in 2017/2018). The average prevalence of cranial pleurisy was 4,8% (vs 5,8% in 2017/2018) and the average prevalence of scarring was 2,2% (2,9% in 2017/2018). Regarding dorsocaudal pleurisy, the average prevalence was 13,8% (vs 18,2% in 2017/2018), resulting in a 0,37 APP Index (vs 0,49 in 2017/2018).

#### **Discussion and Conclusion**

These results show a reduction in all types of monitored lesions in comparison with the previously published results, which might indicate a positive evolution of the respiratory health control programs. Still, the results also suggest that pathogenic agents like M. hyopneumoniae and A. pleuropneumoniae may remain with an important presence in the Portuguese swine herds and that controlling the Porcine Respiratory Disease Complex remains a challenge. In the future, it will be important to maintain the CLP program in the long term to better evaluate the evolution of the respiratory health status of the Portuguese farms.

# SEASONAL VARIATION OF LUNG LESIONS AT SLAUGHTER: A STATISTICAL ANALYSIS OF THE CEVA LUNG PROGRAM RESULTS IN PORTUGAL.

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# **Background and Objectives**

Bronchopneumonic lesions (BP) and dorsocaudal pleurisy (DCP) remain common findings at slaughter in Portugal (Costa and Nunes, 2019). Some authors have found relevant differences of lung lesions' prevalence along the year in France (Maynard et al, 2019) and Spain (del Carmen et al, 2018). The aim of this study was to assess the variation of these lesions' prevalence and severity along the year in Portugal.

# Material and Methods

Between December 2015 and October 2019, 715 batches of pigs were scored at slaughter using the CEVA Lung Program. The batches were divided into four groups: spring (monitored between March 22<sup>nd</sup> and June 21<sup>st</sup> – 172 batches), summer (between June 22<sup>nd</sup> and September 21<sup>st</sup> – 234 batches), fall (between September 22<sup>nd</sup> and December 21<sup>st</sup> – 161 batches) and winter (between December 22<sup>nd</sup> and March 21<sup>st</sup> – 148 batches). Four parameters were studied: percentage of BP, average Madec Scoring (MS), percentage of lungs with DCP and average APP index (APPI). The distribution of the results was tested for normality (Shapiro-Wilks test) and analyzed with Wilcoxon rank-sum test for statistical significance.

# Results

The results show some variation between seasons. The best results were obtained in summer (DCP of 14,3% and APPI of 0,39) and fall (BP of 26,5% and MS of 1,14), with the worst results being found in winter monitoring (BP of 29,4%, MS of 1,32, DCP of 20,2% and APPI of 0,54). Nonetheless, no differences with statistical significance were found.

# **Discussion and Conclusion**

These results suggest that, regardless of some numeric variation, lung lesions occurred with no significative difference among seasons in Portuguese slaughter pigs. The constant presence of lesions at slaughter along the year (also found by Maynard et al, 2019 and del Carmen et al, 2018) shows the importance of maintaining respiratory health programs that cover the farm all year long.

# SOCIALIZING PIGLETS IN LACTATION: EFFECTS ON PERFORMANCE AND ON SWIAV INFECTION DYNAMIC AFTER WEANING

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### **Background and Objectives**

Housing systems allowing piglets to familiarize with non-litter mates pre-weaning can reduce stress and aggression after weaning, leading to performance improvement. Pre-weaning mixing makes also possible precocious pathogens transmission and can change infection dynamic at herd level. In a closed herd that recently dealed with recurrent flu on weaners, socializing piglets in lactation was partially done to follow performance and swine influenza type A (swIAV) infection dynamic differences.

#### **Material and Methods**

For experimental group S, the solid barriers between 14 farrowing pens were removed on day 7 after farrowing. In control group C, the 14 litters were kept under conventional conditions during all lactation period. At weaning (21 days), piglets from the two groups were put in separated pens in the same room. The individually identified piglets (42 piglets/group) were weighted at 5, 21 and 77 days. Weight and Daily Weight Gain (DWG) at different periods of time were compared between groups (Student tests performed). Blood and nasal swabs samplings were done at 21 and 28 days respectively.

#### Results

Piglets from group S have a lower weight at inclusion (2.23kg versus 2.43kg) and an inferior 5-to-21 days DWG (25g/day less). The weaning-to-77days DWG was however superior for group S compared to group C (44.2g/day more) leading to a 77-days weight numerically superior for group S piglets. At weaning all piglets had maternally derived antibodies (MDA) against H1N1pdm swIAV. No swIAV was isolated on nasal swabs and no flu-like syndrom occurred.

#### **Discussion and Conclusion**

A better DWG of the socialized piglets after weaning is observed in this farm and this result is consistent with other experiments made by same authors in several farms. The MDA protection provided by the vaccinated sows (with Respiporc FLUpan® H1N1) can explain the H1N1pdm infection fade-out on 28-days-old piglets whatever the group.

# INFECTION DYNAMICS OF MYCOPLASMA HYOPNEUMONIAE, SWINE INFLUENZA A VIRUS AND PCV2 IN SELF REPLACEMENT GILTS: A LONGITUDINAL STUDY FROM WEANING TO FIRST FARROWING

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# **Background and Objectives**

Gilt population management is a pivotal issue to control the spread of infections within the breeding herd. Knowledge on the infection dynamics in this population contributes to define preventive and control measures. The study aimed at assessing the infection dynamics of Mycoplasma hyopneumoniae (Mhp), swine influenza A virus (swIAV) and porcine circovirus type 2 (PCV2) in self-replacement gilts in a farrow-tofinish herd (2,000 sows) vaccinated against those pathogens.

# **Material and Methods**

A sample of 21 gilts was selected at weaning (24 days old) and followed until first litter's weaning (401 days old). Blood and tracheo-bronchiolar mucus (TBM) samples were taken every 4 weeks. Cough index was assessed twice a week. Antibodies against swIAV were tested by ELISA in sera. Mhp and PCV2 genomes were detected by PCR in TBM and sera, respectively.

# Results

Coughing outbreaks were recorded during the quarantine (172 to 201 days old) and after exposure to the sow herd in service area (204 to 248 days old) and gestation room (262 to 307 days old). Seroconversions to swIAV were detected during the nursery phase, at the end of the finishing phase and at the first month of gestation. Mhp was detected at a low level until 240 days old. The frequency of Mhp PCR-positive pigs noticeably increased from 240 to 297 days old following the clinical outbreak in the sow herd and massive seroconversion to swIAV. Some gilts were Mhp positive at farrowing. A low frequency of PCV2-viremic gilts was found from 24 days old to the first month of gestation.

# **Discussion and Conclusion**

Infection dynamics differed according to the pathogen. Infection pressure increased during the first month of gestation when the gilts were exposed to the sow herd. Measures to reduce infection pressure in the sow herd may contribute to a more gradual exposure of the gilts to respiratory pathogens.

# THE EFFECTS OF PRRS ON HUNGARIAN PIG FARMS' PROFITABILITY, BIG DATA ANALYSIS OF PUBLIC DATABASE

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# **Background and Objectives**

The digitalization of the agricultural processes facilitates the data analyses. Our big data analysis presents the economic impact of porcine reproductive and respiratory syndrome (PRRS) on the Hungarian pig herds in 2017 and 2018.

# **Material and Methods**

We collected and analyzed the pig breed, farrowing, mortality, sow culling, hog sales and PRRS categorization data of 22 swine farms by using the public database of the National Food Chain Safety Office and the information of the local veterinarians. The production data were categorized by the different stages of PRRS infection: (1) acute infection, (2) mass vaccination, (3) controlled chronic PRRS infection and (4) free status. Data from three genetic lines were compared. We used Structured Query Language Microsoft SQL, R programming language, IBM SPSS Statistics to analyze the raw data, and the Kruskal-Wallis test in the statistical analysis.

# Results

Our results show that PRRS causes high volatility in the production parameters of breeding sows, and their culling rate increases by 8-10% which mainly affects the multiparous animals, furthermore, there is a significant increase (p<0.001) in the prevalence of repeat breeders. For the finishers the fattening period extends by 10% which adversely affects the live animal rotation on the farms. The mortality of the sows, piglets and finishers increases. The PRRS outbreak has huge detrimental impact on the production in the consecutive half year. Nevertheless, special symptoms can be detected months before the outbreak, indicating the appearance of a possible infection.

# **Discussion and Conclusion**

The PRRS causes both significant direct production losses during the first 5 months after the outbreak and indirect production losses which are associated with the change in the live animals' rotation due to varying sow population and longer fattening periods. Big data analyses using the databases of the public administration would help to take control measures in time and would support the long-term planning.
# THE CHALLENGE OF AGRICULTURE 4.0 IN ANIMAL HUSBANDRY, OR HOW CAN WE GROW THE PROFITABILITY USING DIGITAL RAW DATA?

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# **Background and Objectives**

Farms with a medium-level production and using digital data collection provide a large amount of raw data on quality, quantity and profitability of daily livestock production processes. These are suitable for carrying out professional analyses using them for growing their profitability

#### Material and Methods

We collected digital data of Hungarian pig farms (2000-40000 fattening pigs in a year) between 2015 – 2018. There were analyzed housing, feeding, animal health, environmental parameters, animal movements, etc. data from different data source within the farms too, also information from public data stores. We created rotations of fattening groups and we compared KPI's (key performance indicators) and other effects on the production by rotation. We used Structured Query Language (Microsoft SQL Server Express 2017), R programming language (R version 3.4.3) and IBM SPSS Statistics (version 25) to find the unexplored, hidden information in these raw data.

#### Results

Based on our analysis, the results of each rotation showed significant differences of 50-80%. Reducing such discrepancies in production increases average production results, improves financial efficiency and makes production more predictable. We found use of "hidden" good practices, and offered elimination of "hidden" bad practices, we can increase the efficacy of production up to 10% during the first year.

#### **Discussion and Conclusion**

When we analyse fattening pig production in their monthly income and expenses (because of 3.5 to 4 months long fattening periods and weekly start of new groups) we usually see the mixed results of approximately 12-15 batches. The mix of these data does not explain properly whether one group increased or decreased the performance. Comparing the indicators of effectiveness, and the effects on the given group show which factors has effects on the profitability of the given farm.

# PIG PRODUCTION COMPANY IS SIGNIFICANTLY ASSOCIATED WITH THE PRESENCE OF NEW PRRSV STRAINS AT FARM LEVEL

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#### **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRSV) is one of the most important viral diseases affecting pig production. The introduction of new PRRSV strains and/or the appearance of new outbreaks due to endemic PRRSV strains are key points to control this important disease under field conditions. The aim of this research work was to find out whether the presence of new PRRSV strains in sow herds could be associated with the pig production company providing services to these farms.

#### **Material and Methods**

377 sow herd farms were monitored from 2016 to 2019. These farms belonged to 11 pig production companies. A PRRSV outbreak was diagnosed based on a combination of performance records, clinical observation, pathological examination and laboratory testing using samples of piglets to detect the virus through qRT-PCR. Moreover, all PRRSV positive samples were sequenced using Sanger technology for ORF5 and similarity analysis between strains was carried out using CLC Genomics Workbench 11.0®. Two strains were classified as different with a similarity lower than 97% in ORF5 sequence.

#### Results

different between pig production companies ranging from 1 to 3. Moreover, in four companies, the incidence of new PRRSV strains (75%) was significantly higher than the incidence due to strains detected previously in the same company (25%) whereas the contrary was observed for the other seven companies (44 and 56% for new and previously detected PRRSV strains, respectively).

#### **Discussion and Conclusion**

Molecular epidemiology of PRRSV is able to monitor the evolution of PRRSV strains in relation with pig production companies. It must be carried out additional studies to decipher the factors involved to avoid the presence of new PRRSV strains through a pig production company.

#### MULTIFACTORIAL DIETARY STRATEGIES CAN REPLACE USE OF HIGH LEVELS OF ZINC OXIDE

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# **Background and Objectives**

Nutritional alternatives are being developed intended to work without ZnO inclusion. However, no consistent replacements have been found. This study analyses different dietary strategies, with optimal feed additive (FA) packages, to support growth of piglets without using ZnO.

# Material and Methods

192 weaned piglets were randomly allocated to 4 dietary treatments with 3 animals per pen: negative control (NC), 105 ppm zinc, 10 pens; positive control (PC), 2500 ppm ZnO phase 1, 10 pens; VITAL, 105 ppm zinc, FA as fungal fermented rye, including water acidification (0.2%), 22 pens; BENCHmark diet, 105 ppm zinc, including water acidification (0.2%), 22 pens; BENCHmark diet, 105 ppm zinc, including water acidification (0.2%), 22 pens; BENCHmark diet, 105 ppm zinc, including water acidification (0.2%), 22 pens. Piglets were fed ad libitum. Animals were monitored for performance and health. Statistical analysis of performance was done using ANOVA with treatment and pen as fixed factors and weaning weight as covariate, followed by a Tukey post-hoc analysis. Health parameters were subject to a chi<sup>2</sup>-analysis.

#### Results

Weaning weight was similar between groups. Daily weight gain was numerically lowest in NC during all phases which resulted in 0.6kg lower body weight at d42 in NC (P=0.29). There were no differences between weight and weight gain of the PC, VITAL and BENCH. Feed intake did not differ between groups. Overall feed conversion rate was numerically higher in NC compared to other groups. Incidence of diarrhoea and morbidity was numerically higher in PC compared to other treatments (P>0.05). Incidence of treatments was similar between groups. Piglet mortality was numerically lower for VITAL, 1.5% vs 7% in other groups (P=0.12). With a piglet value of €25, a profit per pig was calculated: NC=€11.1, PC=€11.3, VITAL=€12.6, BENCH=€11.0.

#### **Discussion and Conclusion**

Data shows, compared to high levels of ZnO, innovative dietary strategies including water acidification and FA can reach at least similar zootechnical and economic performance while maintaining post-weaning piglet health.

#### ECONOMIC ASPECTS OF ANTIBIOTIC USE ON LARGE-SCALE HUNGARIAN PIG FARMS

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# **Background and Objectives**

Use of antibiotics (AB) is an actual topic and one of the most important issues in the European, one-healthdriven pork production. Sound, life science-based analyses, novel approaches and interpretation of the daily AB use would be essential to sustain a safe and healthy pork production. These are also important issues when we evaluate the real efficacy and economy of the applied antimicrobial protocols.

#### Material and Methods

In our survey, between 2018 and 2019, we systematically collected and analysed data about the daily practice of AB use, pathogens' identification, and their minimal inhibition concentration (MIC) tests' results in 15 selected large-scale swine herds. Special (VacSafe) colorizing technic was also used to visualize pigs' individual AB intake.

#### Results

According to the summary of product characteristics (SPC), out of 15 farms, 11 applied contraindicated drugs at the same time which resulted in 30-85% extra drug cost. The results of the colorizing method showed that only 50-80% of animals received the sufficient amount of ABs on 5 farms, because water pump dispensers (WPD) were not able to serve the needed concentration into the water in the dedicated timeframe. Some farms where more different Actinobacillus serotypes and MICs were founded 20-80% ineffective use of AB was observed. Some identified pathogens have intracellular (IC) lifecycle. Lack of IC MIC tests and knowledge on pharmacokinetic and pharmacodynamic caused approximately 100% extra cost.

### **Discussion and Conclusion**

Our findings show that specific audits on AB use can help swine farms modify their antimicrobial treatment and metaphylactic protocols, so that they can improve the prudent use of ABs and the profitableness of their production.

# COMPARATIVE STUDY ON THE PRODUCTION PARAMETERS, ANIMAL HEALTH STATUS AND DRUG USAGE OF HUNGARIAN SWINE FARMS

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# **Background and Objectives**

Antibiotics play a critical role in improving the health of animals and humans. The responsibility of the correct use leads us to reach the one health concept. Antibiotic-free pork production is a good way to create an added-value product. We, swine veterinarians have the responsibility to manage well the prevention or treatment strategies on farms.

#### Material and Methods

In our study we examined the relationships of the general animal health status and the management of the surveyed Hungarian farms with special regard to the antibiotic usage (AU). The data were collected from 13 large scale farms 2017-2019. By using a questionnaire, we collected the production parameters, the animal health status, (pathogens, the severity of lesions) and AU. The data were checked by farm visits and were analysed under different farm conditions.

## Results

According to the results the most common pathogens were Mycoplasma hyopneumoniae (100%), Lawsonia intracellularis (93%), swine influenza (85%), Actinobacillus pleuropneumoniae (53%) and among the secondary pathogens Streptococcus suis (77%) and haemolytic Escherichia coli (92%). Only 5 farms had well managed biosecurity system. All farms had a sort of prevention strategies, but only 2 farms controlled their effectiveness. None measured the effectiveness of disinfectants. 31.8% (15.8%-48.1%) of the total veterinary cost was accounted for the AU. Three farms had not any antibiotic resistance test results. The most commonly used antibiotics (doxycycline, amoxycillin, enrofloxacin), which were used for the treatment of Porcine Respiratory Disease Complex gave 60% of the total drug cost. Three farms used injectable antibiotics at newborn piglets for preventive purposes.

#### **Discussion and Conclusion**

In our study we revealed that detailed information and knowledge about animal health management and the prevailing pathogens on the farm have primary importance in order to manage correctly the problems and reduce the high level of antibiotic use.

# TIME TO STABILITY MONITORED AFTER LOAD CLOSE HOMOGENIZE USED TO ELIMINATE PRRS TYPE 2 IN DANISH SOWHERDS

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#### **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome (PRRS) is a devastating disease causing high mortality and economic loss to pig producers all over the world. Over the last decade the Load Close Homogenize (LCH) model including 2 times mass vaccination of a sowherd has become a preferred and generally accepted way of eliminating PRRS. When an infected sowherd wants to eliminate PRRS it is of high importance that an estimated Time To Stability (TTS) can be predicted. TTS is defined as the time from intervention until the 1<sup>st</sup> PRRSv negative bloodtests on newly weaned pig. This was examined in 3 cases.

#### **Material and Methods**

3 sowherds was infected with PRRS Type 2 simultaneously from the same source of gilts. Within 4 weeks from verification of the infection all 3 farms was loaded with gilts for 28 weeks according to LCH protocol. At the same time management was changed to strict "10 Golden Rules". Massvaccination of all animals of more than 4 weeks of age was performed with Ingelvac PRRS MLV. The massvaccination was repeated after 4 weeks. After 12 weeks from 1<sup>st</sup> massvaccination diagnostics of PRRSv by PCR of processing fluids and blood from newly weaned pigs was conducted. The diagnostics was ongoing weekly, until all samples tested negative for PRRSv in order to establish TTS.

#### Results

TTS on the 3 farms was 27, 29 and 44 weeks respectively after 1<sup>st</sup> massvaccination. All farms tested negative on processing fluids sooner than the TTS.

#### **Discussion and Conclusion**

This casereport confirms the predicted TTS after LCH from previous studies. When advising farmers it is important to be able to estimate TTS in order to calculate a ROI. Furthermore this study concludes that use of processing fluids in combination with blood from newly weaned pigs is an accurate and helpful tool to establish TTS.

# THE DISTRIBUTION OF OPINIONS REGARDING CHANGES IN PRODUCTION PERFORMANCE AND ECONOMIC IMPACT OF PRRSV IN SWINE FINISHING OPERATIONS

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#### **Background and Objectives**

The impact of swine diseases is typically measured by changes in feed efficiency (FE), average daily gain (ADG), and mortality (MORT%). Economic analysis is used to estimate financial impacts of these changes. Limitations of these analyses include idiosyncratic characteristics of production settings and challenge level. Results are often static due to contemporaneous seasonal effects, costs and prices. An alternative method is eliciting the opinions of experts from their experience across many different farms, diseases, seasons, costs and prices.

#### Material and Methods

We anonymously surveyed 39 EU veterinarians and industry professionals, eliciting their experience with changes in ADG, FE and MORT% from a PRRSv infection in finishing. The participants gave the mean, minimum, and maximum changes in these metrics. Participants also provided opinions regarding correlations between elicited variables. Individual triangular distributions were combined into a group distribution for each metric. Monte Carlo simulation of a bio-economic finishing model was then undertaken to estimate changes in net income per head. Distributions of recent EU costs and hog prices were used.

#### Results

Based on elicited opinions, the weighted average change in ADG was from healthy mean 826g (StDev 102g) to PRRSv mean 733g (STDEV 97g). For FE, healthy mean 2.61 (StDev 0.21) to PRRSv mean 2.84 (StDev 0.34) and MORT%, healthy mean 3.0% (StDev 2%) to PRRSv mean 6% (StDev 4%). Total head finished and sold from 1,000 started was healthy mean 969 (StDev 20) to PRRSv mean 944 (StDev 35). The resulting mean cost of PRRS in the finishing phase was estimated to be €8.87 per head.

#### **Discussion and Conclusion**

Opinions regarding the impact of PRRSv in finishing reveal it is believed to be substantial. These opinions are directly related to the level of investment in prevention and biosecurity and are therefore critical to reducing the economic and societal losses, as well as animal welfare impacts of disease.

#### LUNG LESION SURVEY USING CEVA LUNG PROGRAM IN RUSSIA: COMPARISON OF PERIODS 2018 AND 2019

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## **Background and Objectives**

Slaughterhouse lung scoring is the effective way for assessment of respiratory health and efficiency of control programs based on vaccination on swine farms. Ceva Lung Program (CLP) is standardized tool allowing for rapid scoring and it was successfully used for evaluation of real prevalence of EP and A.p like lesions on farm at national level with the possibility to assess the economic impact on production results. The aim of our study is to provide the regular update on the prevalence and severity of lesions caused by EP and A.p in the Russia (RU) and compare it with the status in 2018.

#### Material and Methods

During the period of the year 2019 a total number of 290 batches (median: 100 lungs scored per batch) and total of 29216 lungs were scored from different farms in different regions of Russia. Broncho-pneumonic lesions (EP like lesions) and percent of Dorsocaudal Pleurisy (A.p-like lesions) were evaluated to assess the level of EP and A.p infections. Results were compared with the previous period (2018).

#### Results

The significant decrease % of affected lungs by EP like lesions was observed on Russian farms from 21.00 % to 14.75 % with 3.38 % affected average surface of pneumonic lungs. Slight decreasing trend was observed as well for A.p like lesions prevalence from 7.00 % to 6.00%. All values expressed as median.

#### **Discussion and Conclusion**

For both EP-like and A.p-like lesions a decreasing tendency was observed and this trend continues to be present already from 2017. The explanation is rather complex and several factors influence it, such as implementation and improvement of management strategies for these pathogens including effective vaccination and biosecurity. Other factor is – the introduction of new M. hyo negative status farms in Russia.

# FIELD EVALUATION OF HEMOGLOBIN (HB) LEVEL IN PIGLETS AT WEANING ON EUROPEAN FARMS

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## **Background and Objectives**

Iron deficiency anaemia (IDA) is the most commonly recognized clinical condition of fast growing piglets and is considered as an emerging problem. The most common parameter to indicate IDA is hemoglobin concentration (Hb). The aim of this study was to assessed IDA prevalence in selected EU countries and to identify risk factors.

#### **Material and Methods**

Eight countries were included in the survey and 2349 piglets were assessed (large, medium and small) (Austria n= 90, Belgium n= 300, Czech n= 510, Denmark n= 300, France n=360, Germany n= 210, Holland n= 291, Portugal n= 288). Litters from different parity sows were randomly selected (10 litters/30 piglets per farm). The Hb was measured with the Hemocue<sup>®</sup> Hb 201+. Piglets were classified as follows: Hb levels < 90 g/l are anemic, Hb levels  $\ge$  90 g/l and < 110 g/l are suboptimal and Hb levels  $\ge$  110 g/l are optimal. The effect of the size of piglets, type of iron and parity of sows were evaluated.

# Results

In total 14,7% (317) piglets were anemic in this survey with significant differences among included countries (P < 0,0001). The highest prevalence of IDA was observed in Belgium and France (34% and 18,1%) where oral form of iron was frequently used. Significant differences in % of anemic piglets were established for different forms of iron: dextran x gleptoferron x oral form (16,2%; 7,9%; 34,3%) (P < 0,0001). The effect of the size of the piglet and parity was not significant (P= 0,07999; P= 0,34662) with more anemic piglets from young parities.

#### **Discussion and Conclusion**

In our survey differences in prevalence of IDA were observed among different countries in EU with 14,7 % anemic piglets in total. Oral form of iron administration, low parity of sows (1-2) and large size of piglet seems to be risk factors for IDA.

# BATCH EFFECT ON DEVELOPMENT OF HEMATOLOGIC VARIABLES DURING THE COURSE OF REPRODUCTIVE CYCLE OF SOWS

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# **Background and Objectives**

Some hematologic variables like hemoglobin concentration (Hb), erythrocyte count (RBC) and hematocrit (HCT) are traditionally used to assess anemia in sows. Automated hematology analyzers generate extended variables like mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and red blood cell distribution width (RDW), which could be more informative. The main objective of this study was to describe the trends in development of these hematologic variables during the reproductive cycle and across batches.

# Material and Methods

Fifty multiparous sows belonging to two consecutive weekly batches (25 sows in each) were included in the study. These sows were selected from a large group of sows based on their low Hb. The sows were injected twice with normal saline intramuscularly (12.5 mL) at gestation day (GD) 56-59 and GD 70-84 because they served as negative control group in another trial. Blood samplings were done at time points S1 (GD 49-62), S2 (GD 97-112) and S3 (Post-farrowing day 19-37). The sows were snare restrained and blood was withdrawn into EDTA tubes from jugular vein. Complete hematology testing was done using the Advia 2120i Hematology system.

## Results

The development of traditional variables in the two batches of sows differed. In batch 1, Hb, RBC and HCT increased at S2 with a drop at S3. However in batch 2, Hb, RBC and HCT decreased at S2 with a further drop at S3 except Hb which rose slightly. Non-traditional variables showed similar trends in both the batches. MCV and RDW rose at S2 and then decreased at S3. MCH and MCHC decreased at S2 and rose at S3.

# **Discussion and Conclusion**

The hematologic variables varied between batches in the group of sows selected based on low Hb. However, no groups with normal/high Hb were studied. This should be taken into account, when interpreting hematologic variables for diagnostic purposes.

#### THE ROLE OF THE FARM ENVIRONMENT IN THE PERSISTENCE OF PCV2 INFECTION IN VACCINATED SWINE HERDS

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# **Background and Objectives**

Vaccination against PCV2 has proved to be effective for controlling viremia and clinical signs. However, its use has not managed to eradicate the infection in farms, which suggests that other elements may be involved in the virus persistence. Therefore we performed this work to identify the farm areas where PCV2 can accumulate and persist.

#### **Material and Methods**

We used swabs to take environmental samples from four farrow-to-weaning farms which had been vaccinating against PCV2 for years. These samples, which included surfaces of different farm areas, staff and utensils, were analysed by qPCR.

#### Results

PCV2 DNA was detected in the environment of all these farms. Overall, the offices seem to be the most contaminated areas (mean of 1.72x10<sup>6</sup> PCV2 copies/swab), although viral DNA was also detected in samples from the warehouses of two farms (mean of 2,02x10<sup>4</sup> PCV2 copies/swab). Likewise, staff clothes or working footwear were contaminated by PCV2 in all the farms. Samples from the perimeter were also positive in two of the farms (main entrance of the farm and loading docks, with a mean of 4.91x10<sup>2</sup> and 8.77x10<sup>2</sup> PCV2 copies/swab respectively). In contrast, the areas that host pigs were contaminated in just one farm. These positive samples were taken in the floor of the gestation pens (2.63x10<sup>3</sup> PCV2 copies/swab) and in the corridors of the gestation and farrowing areas (1.74x10<sup>5</sup> and 2.45x10<sup>2</sup> PCV2 copies/swab respectively). All the samples from the weaning area were negative

# **Discussion and Conclusion**

These results suggest that the presence of the virus in elements of the warehouses and offices may be one of the reasons why the virus has not been eradicated in farms. Moreover, the detection of PCV2 in working clothes and samples from different areas suggests that environmental sampling could be used for locating infected subpopulations and areas through which the infection could be reintroduced.

# IMPACT OF ORAL VACCINATION AGAINST LAWSONIA INTRACELLULARIS ON WEIGHT VARIABILITY AT THE END OF FATTENING IN IBERIAN PIG BREED

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# **Background and Objectives**

Porcine Proliferative Enteropathy caused by Lawsonia intracellularis (Li.) is an enteric disease of pigs affecting most of the Spanish farms subclinically with a relevant economic impact. The aim of this study was to evaluate the impact of oral vaccination against ileitis on variability of the weight in Iberian breed pigs reared in an intensive (indoor) production system.

# Material and Methods

This study was conducted in a multi-site farm with 2,500 lberian sows located in Spain with subclinical lleitis, confirmed by ELISA (IgG). 2,880 fattening pigs were included in the study (1,440 orally vaccinated via drinking water 3 weeks after weaning (V) and 1,440 non-vaccinated (NV) with Enterisol<sup>o</sup> lleitis ). In order to minimize the seasonal impact on results, 8 vaccinated batches and 8 non-vaccinated batches were filled alternatively. All pigs were individually weighed in the fattening unit at placement and 37, 73, 109, 142 and 177 days after. Average daily gain (ADG, kg/d) and antibiotics costs ( $\in$ ) were also assessed.

#### Results

The weight at placement in the fattening unit was the same, The differences between vaccinated and non-vaccinated animals at 37, 73, 109, 142 and 177 days after replacement were + 1.48, +2.57, +4.64, + 3.2 and + 6 respectively in the vaccinated animals (p<0,05). The SD of the weight at placement was the same. The differences between vaccinated and non-vaccinated animals at 37, 73, 109, 142 and 177 days were -8,26%, - 8,76%, -8,7%, -7,96% and -8,7% respectively in the vaccinated animals (p<0,05). The ADG, was 657 (V) vs 639 (NV) and the reduction of the use of antibiotics in the vaccinated group represented the 74.6% compared to the non-vaccinated one.

#### **Discussion and Conclusion**

In this field experience, in which pigs were suffering from subclinical ileitis, vaccination with Enterisol<sup>o</sup> lleitis increased the weight of pigs and decreased the variability (SD) within the vaccinated animals.

### FACTORS THAT RELATE TO LOW ANTIBIOTIC USE IN WEANED PIGLETS IN THE NETHERLANDS

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# **Background and Objectives**

From 2009 to 2018 Dutch pig husbandry reduced antibiotic use by 58%. As the highest Daily Defined Dosage Annually (DDDA) is in piglets from weaning until 10 weeks of age, it is useful to focus on factors that may affect DDDA in this production phase. The goal of this study is to describe risk factors for low antibiotic use in weaned piglets, housed on the same location as the sows, in the Netherlands.

# Material and Methods

A list of 18 farm specific variables was used to audit a random selection of 59 individual Dutch sow herds. The results were analyzed using Excel by comparing them to DDDA in the weaned piglets. 'DDDA high' and 'DDDA low' were defined using the dataset median as cut off value (DDDA 11.8). All other numerical variables were categorized the same way: above or below median. Chi<sup>2</sup> testing was performed to find the significantly related variables.

#### Results

Significant associations with low DDDA: Conservative farmers versus Entrepreneurs, Sow herds smaller than 500 heads, Less than 340 piglets per batch, Not using any of the following piglet vaccinations: PCV2, M.hyopneumoniae and PRRSV, The use of 2 or less different vaccinations in piglets

#### **Discussion and Conclusion**

As the farmers character may be aligned with herd size we checked and found these variables independent of each other. Farm size and DDDA was related and this looks multi-variable. DDDA and piglet vaccinations were related. We explain this by the piglets' health: to control poor health more antibiotics are used and often combined with preventive interventions like vaccination. Poor piglet health causes either economic losses that may motivate the Entrepreneurs or welfare issues that motivate Caretakers. Taking the character of individual farmers into account give new opportunities to further decrease weaned piglets DDDA in The Netherlands.

PREVALENCE AND SEVERITY OF THE ENZOOTIC PNEUMONIA AND PLEUROPNEUMONIA IN BRAZILIAN PORK FARMS BASED ON THE LUNG LESIONS SCORE FROM 2016 TO 2019

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#### **Background and Objectives**

Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae remain important respiratory pathogens in the swine industry worldwide. As a common practice, inspections of lung lesions at slaughter are used for monitoring and a clear correlation between lung lesions, the economic impact of the disease and the efficiency of vaccination has been reported<sup>2</sup>. The aim of this study is to evaluate the level of EP and A.p-like lesions on Brazilian pig farms in the period of 2016-2019, using the Ceva Lung Program tool (CLP).

#### **Material and Methods**

In the period from 2016 to 2019 a total number of 1,360 batches and 129,539 lungs were scored. The animals came from farms located in the main swine producing states of Brazil, including the South, Southeast and Midwest. EP-like lesions and dorsocaudal pleurisy scores (A.p-like lesions) were evaluated using the CEVA Lung Program methodology and compared between years.

#### Results

Lung lesions typical for EP ranged from 51.72% to 66.67%. The affected surface area of the pulmonary parenchyma in the pneumonic lungs varied between 4.92% and 5.57%. Cranioventral pleurisy presented variation between the years analyzed between 2.00% and 6.90%. Regarding pleuropneumonia, a variation between 4.00% and 6.00% of the lungs were affected by A.p-like lesions with a variation of the APPI index between 0.13 and 0.21 during the years analyzed. All values are expressed as median

#### **Discussion and Conclusion**

These results show a significant prevalence of Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae infections, which can also be shown in other countries. The year 2016 presented larger EP like lesions, when compared to the following years. Monitoring tools such as slaughter lung inspections are extremely relevant for estimating the prevalence and severity of lesions. And the periodicity of these inspections should contribute to generating a consistent database and analyzing the success of the control programs of these important pathogens.

#### ONLINE MONITOR OF PIG HEALTH IN THE NETHERLANDS, RESULTS AND TRENDS

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## **Background and Objectives**

Since 2016 the Dutch pig health monitoring system is extended with an obligatory clinical signs surveillance by practitioners, the Online Monitoring.

# Material and Methods

Using an online application, veterinary practitioners register during monthly farm visits whether clinical signs are present. If so, they record the age group involved, 'syndrome', main clinical signs and most likely diagnosis. Practitioners can review the information of their own practice in relation to the whole of the Netherlands or to their region, using an online dashboard.

#### Results

Veterinary practitioners recorded information from about 3,200 farm visits every month in 2019. On many farms several age groups are present, so the average monthly number of records is 7,850. In 33% of these records a health issue is mentioned. These issues involve respiration (31.8%), digestion (18.8%), systemic disease (16.6%), locomotion (10.8%) central nerve system (9.7%), behaviour (6.4%), skin (3.1%) and reproduction (2.8%). A most likely diagnosis is mentioned in 71.6% of the recordings. In most cases this is an infectious disease (60%). The following pathogens are reported as most likely causes of health problems: Streptococcus spp. (17.8%), Actinobacillus pleuropneumoniae (7.7%), Lawsonia intracellularis (6.4%), Influenza virus (6.3%), Escherichia coli (6.2%), PRRS virus (3.0%), Mycoplasma hyopneumoniae (2.2%), Clostridium spp. (1.5%), Haemophilus parasuis (1.4%) and Pasteurella spp. (1.1%). If a non-infectious cause is assumed, it mostly includes aspects of housing (5.1%) or feeding (4.0%). Over the last three years certain diagnoses show distinct seasonal fluctuations, like for instance infections with Lawsonia intracellularis (increase in summer), PED (rarely in summer) or Influenza virus (decrease in summer). Incidentally, some health problems show a remarkable trend. For instance, there were significantly more reports of a high mortality rate in sows in the (hot) summer of 2019.

# **Discussion and Conclusion**

The Dutch Online Monitor provides very useful health data for veterinary practitioners and the pig industry.

# FIELD APPLICATION OF AN ECONOMIC SIMULATION TOOL IN 21 GERMAN PIG HERDS TO ESTIMATE THE COST OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME AT FARM-LEVEL

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#### **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) continues to be a major economic threat to modern pig production worldwide. Whereas (financial) damage for farms is obvious in acute PRRS outbreaks, it is less so with endemic infections, since clinical impact and disease severity can differ greatly between PRRSV endemically infected farms. This makes the quantification of financial losses challenging. Therefore, aim of this study was to apply a PRRS cost simulation tool to systematically assess and quantify the economic effect of PRRSV at farm level in endemically infected German pig herds.

#### **Material and Methods**

From a total of 21 sow herds with documented endemic PRRSV infection, data on health and production performance, farm management and environment to be fed into the calculator were collected during a farm visit. Blood samples were taken to substantiate the PRRSV status of each herd.

#### Results

The median of calculated farm budgets for these 21 endemically PRRSV infected farms was - 31 € per sow and year, whereas according to the simulator it would have been 260 €, had these farms been PRRSV negative. The median total loss attributable to PRRSV across all farms was 77,000 € per farm per year, and 250 € per sow and year. The impact of PRRS on farm profits was -19.1% on average and -41% in the worst case.

#### **Discussion and Conclusion**

Figures from this simulation of losses can be considered to give a good idea of the economic damage to be expected in endemically PRRSV infected herds. Even if the herd is endemically PRRSV infected, a non-negligible loss due to PRRS can be expected, and the farm will benefit economically from a concerted PRRS control. The calculator has proven itself in the field to render a plausible estimation of the loss due to PRRS in endemically infected farms.

# OREGANO OIL IS ABLE TO SUPPORT SOW PERFORMANCE AND PROGENY HEALTH WITH A REDUCED NEED FOR MEDICATION

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#### **Background and Objectives**

With increasing pressure on the global pig industry to reduce the use of antimicrobials whilst maintaining animal performance and welfare, there is a growing interest in sustainable natural alternatives. Oregano (Origanum vulgare) was supplemented to sows and piglets to assess the effect on early life health and long-term performance.

#### Material and Methods

Sixty-two multiparous sows across two weekly batches were randomly allocated to control (CON) or oregano essential oil supplementation (OS) at an equivalent rate of 500g/t. Treatments were top dressed to sow diets daily from seven days prior to farrow until weaning (~26 days). All piglets were ear-tagged at birth and any cross-fostering was carried out within treatment. At two weeks of age, piglets from all treatment groups were offered creep feed containing OS at 1kg/t. Piglets were re-weighed approximately 10 weeks after weaning to understand any long-term benefits of oregano essential oil supplementation. All data were evaluated using SAS (SAS Institute inc. 2013).

#### Results

Piglets from the OS treatment had significantly improved average daily weight gain from birth to weaning (p=0.016), and significant higher body weight at one week of age with  $2.23\pm0.03$  kg compared to  $2.10\pm0.029$  kg for CON (p=0.006). Health records showed that piglets in OS litters had significantly reduced incidence of health problems such as lameness or swollen joints resulting in a 4.2% reduction in therapeutic treatment (p=0.072) and reduced mortality from 14.3% to 10.9%. Post weaning average daily gain was numerically increased in OS group compared to control (p>0.05).

#### **Discussion and Conclusion**

Piglets from sows receiving OS had significantly improved growth rates pre-weaning with a reduced incidence of morbidity and mortality and lower need for medication. This study supports the approach of using Oregano essential oil products such as Orego-Stim<sup>®</sup> as a natural tool to improve sow and progeny health and performance economically.

# QUANTIFICATION OF POSTPARTUM DYSGALACTIA SYNDROME: A CLINICAL CASE IN A FRENCH FARROW-TO-FINISH HERD

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# **Background and Objectives**

Colostrum intake is a key factor for piglet health during lactating and post weaning periods. It is dependent on both the sow production and the piglets' capability to ingest. In a 450 sows farrow-to-finish herd exhibiting health problems (arthritis, hyperthermia) on weaners we suspected an insufficient transfer of maternal immunity. We used the recently developed practical protocol for evaluation of colostrum intake in commercial farms (Leneveu et al., ESPHM 2019).

# **Material and Methods**

Piglets from 6 litters were individually tagged and weighed immediately at birth and 24h later to calculate the 24h weight gain (WG24). For each piglet: the rank, the hour of birth and the weights were recorded. 24 hours after birth, piglets were blood sampled to determine serum total IgG level by RID method. Results were compared with reference data obtained from 10 French commercial farms in 2018. Farmers' practices and farrowing environment were also evaluated.

## Results

Individual data were obtained for 90 piglets from 6 litters. WG24 was very low (48.9 g on average when the mean value in the reference population is 88,4 g) whatever the birth weight, suggesting that the main issue was an insufficient production of colostrum by sows. Moreover, 30.9% of piglets exhibited a low concentration of IgG in serum (<20g/L). All the colostrum deficient piglets were from 2 litters (a gilt and a parity 3 sow) with average WG24 values of +23.2g and -2.3g respectively.

# **Discussion and Conclusion**

Our investigations showed that (i) this herd exhibited a postpartum dysgalactia syndrome (PDS) (ii) two out of the six sows were more affected. This protocol proved to be an efficient tool for raising farmer awareness on this syndrome by quantifying its impact. An investigation on PDS aetiology was initiated subsequently.

### BIOSECURITY NETWORK ANALYSIS FOR BETTER ASF PREVENTION

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# **Background and Objectives**

African Swine Fever (ASF) is one of the most important infectious diseases of the pig production. An ASF biosecurity (BS) program should represent a hierarchy of conceptual, structural, and procedural components (bioexclusion and biocontainment) aiming at preventing disease transmission within and across farms. The primary focus of bioexclusion is to limit the level of exposure to pathogenic agents. This requires a systematic approach to prevent pathogen movements across the farms' biosecurity network (BN). The HPBA established a scientific committee to develop a special audit tool for BN analysis (BNA).

#### **Material and Methods**

The BNA will be applied for each member of HPBA. Pilot study was started in September 2019. A questionnaire consisting of 100 questions was compiled to measure the standard BS on the surveyed farms. On a 1-4 scale (1 is the worst, 4 is the best practice) each BS parameter was evaluated. Out of the 100 parameters 10 special question were selected to demonstrate the vulnerability of the surveyed farms within the network in case of an ASF outbreak. The given points were multiplied to highlight the main problematic farms within the network.

#### Results

The results of the pilot study show that this multilevel measurement approach can help the pig farmers' association to put more focus on members with higher risk so that all the network members can reduce the risk of contagious diseases, especially ASF. After the first experiences of the pilot study slaughterhouses, feed distributors, live pig transportation companies and other swine farm suppliers joined the BNA.

#### **Discussion and Conclusion**

The rapid enlargement of the network facilitates the members to better focus on the most problematic farms by using the BS network analysis tool; reducing the risk of an ASF outbreak in commercial pig production.

#### INGELVAC PRRSFLEX INCREASES AVERAGE DAILY WEIGHT GAIN IN DUTCH FINISHING PIGS

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# **Background and Objectives**

In a farrow to finish farm with a suboptimal historical growth of 783 grams/day, Circo-vaccination was implemented. A group of pigs was vaccinated against PRRS to evaluate its effect on ADG.

# Material and Methods

Pigs were vaccinated with Ingelvac CircoFLEX<sup>®</sup> (CF) at 3 weeks of age. In total 339 pigs were additional vaccinated with Ingelvac PRRSFLEX EU<sup>®</sup> 2 weeks after weaning (CPF). Grower pigs were weighed at allocation (18 pigs/pen) and again after 15 weeks of finishing. Each pen was scored weekly for the presence of conjunctivitis, sneezing, and coughing (0-4; 0=no signs; 4=severe). Sixteen pens were selected for fixed spatial sampling of oral fluids (OF) at 4, 8 and 12 weeks of finishing. OF was tests by PCR for PRRS, Mhyo, SIV and PCV2. At 15 weeks of finishing, 1 blood sample per pen was tested for the presence of antibodies against PRRS, Mhyo, SIV and PCV2. At 0 and PCV2. ADG was tested using Generalized linear model using treatment and sex (SAS).

#### Results

OF indicated active SIV and PRRS infections during the starter and grower period. CPF had higher PRRS SPratios when compared to CF (1.87 vs 1.80; P<001). ADG of CPF was 43 gram/day higher when compared to CF (937 vs. 894 gram/day; p<0.05). CPF had a significant lower eye score during the first 6 weeks (P<0.001). Only minor symptoms like black eye lids (score 1) were observed which faded away during finishing.

#### **Discussion and Conclusion**

With a gross profit of €30.51 per pig (Dutch 2018 average) €30.51/894=€0.034 extra benefit per gram growth resulting in an economic benefit of €1.46 per pig. Assuming that FCR will improve when ADG is improved, the probable result will even be (far) larger. Ingelvac PRRSFLEX increased ADG with 43 grams resulting in an improved economical benefit, after deduction of vaccine costs.

# USAGE OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) IN SOWS ON 690 PIG FARMS IN SWITZERLAND

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# **Background and Objectives**

Along with vaccinations, antimicrobials and antiparasitic drugs, NSAIDs are among the most frequently used preparations in sows in modern pig production. Main indications are the alleviation of fever and pain caused by infectious diseases or injuries. In Switzerland, on farms participating at the Suissano Health Program, all treatments with NSAIDs are recorded. The aim of the present study was to analyse the use of NSAIDs in gestating and lactating sows with regard to treatment frequency and main indications.

#### **Material and Methods**

In 690 study farms, housing 14,000 lactating and 43,000 gestating sows, all treatments with NSAIDs between September 2018 and August 2019 were recorded using electronic treatment journals. For every study farm, the number of treatments and the number of treatments per animal in this period were calculated and the main indications for treatments with NSAIDs were determined.

#### Results

The number of treatments was 32211 in lactating sows and 12572 in gestating sows. Lactating sows were significant more frequently treated with NSAIDs than sows (p < 0.001), with a median of the number of treatments per sow per year of 0.84 in lactating sows (maximum 17.6) and 0.13 in gestating sows (maximum 4.8). The main indication for NSAID treatments in gestating sows was lameness with a proportion of 87% of all treatments. In lactating sows, the main indications were related to the Post Partum Dysgalactia Syndrome (74%) and lameness (10%).

# Discussion and Conclusion

Treatments of sows with NSAIDs occurred regularly in the study farms and with varying frequency. For the future, it should be discussed whether the frequency of treatments with NSAIDs may be suitable for the use as an indicator for systems assessing animal health.

# ASSESSING COLOSTRUM INTAKE BY MEASURING ERYSIPELOTHRIX RHUSIOPATHIAE SERUM IMMUNOGLOBULIN LEVELS OF SOWS AND PIGLETS

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# **Background and Objectives**

Colostrum consumption is essential for piglet survival and development. Piglets depend on colostrum intake for immune transfer as it provides a source of maternal antibodies measurable on their blood, so a practical way of evaluating intake would be by measuring serum antibody levels in young piglets. The aim of this field study is to indirectly estimate the level of colostrum intake by measuring and comparing Erysipelothrix rhusiopathiae serum immunoglobulin levels on seropositive sows and their offspring, as most herds are routinely vaccinated against it.

# Material and Methods

The field study was carried out in 3 commercial farms vaccinating sows against swine erysipelas (SE) using a bivalent commercial vaccine SE + porcine parvovirus on day 14 of lactation. Blood was collected from 10 sows of multiple parities and 3 piglets per litter (not cross-fostered) at 7 days of age, in each farm (total of 30 sows and 90 piglets). All serum samples were tested for SE specific antibodies using a commercial ELISA kit, and a piglet titer/sow titer % ratio was calculated. Higher % ratios indicate better colostrum intake (higher antibody levels): ≥80% positive; >60%- <80% doubtful; ≤60% negative.

# Results

2 sows (6,7%) were seronegative to SE and removed from the trial. Insufficient colostrum intake was observed in 6% of piglets (negative ratios); 10,7% of piglets had doubtful ratios; 83,3% had sufficient colostrum intake with positive ratios.

#### **Discussion and Conclusion**

These results showed that only 83,3% of sampled piglets had enough SE serum antibodies; thus, ensuring optimal colostrum intake in the first 24 hours is still a critical point in farms. Furthermore, seronegative sub-populations of sows were found in this study, probably affecting passive immunity transfer to piglets. This technique is a simple, unexpensive and useful field tool to determine the success of colostrum intake, although it can not establish a quantitative measurement of passive immunity in each piglet.

#### ANALYSING PIG HEALTH - THE SMART ANIMAL HEALTH PROJECT IN SWITZERLAND

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# **Background and Objectives**

The Swiss Federal Food Safety and Veterinary Office together with the Federal Office for Agriculture is pursuing the goal of focusing more closely on animal health and welfare in the livestock sector. To this end, an animal-assisted method for the objective and systematic recording and assessment of animal health and welfare of the most important Swiss livestock species should be established.

#### Material and Methods

At the beginning, the project strategy was coordinated between the farm animal species i.e. cattle, calves, small ruminants, poultry and pigs. In a first step all available information and databases such as animal traffic data, anti-microbial usage data, slaughter analyses etc. were collected and tested for their suitability for the assessment of animal health and welfare. In order to record and assess animal health and welfare as validly as possible, a wide-ranging literature search was carried out and all relevant stakeholders of the pig industry were brought together, so that their opinions and experiences on individual parameters of animal health and welfare and welfare were collected. Measurands were defined for categories such as performance parameters, biosafety, animal environment, animal behaviour and animal health. This measurands are now being tested on 30 farms as part of a pilot programme and the results will be checked using the already established Welfare Quality® protocol.

#### Results

The data will be combined as a score for the different categories as well as an overall score for swine health and welfare on farm. Due to this a benefit for farmers, veterinarians and politicians in monitoring the farm health should be generated.

# **Discussion and Conclusion**

The aim of this project is to develop a valid, accurate, reproducible, safe and fraud-proof methodology for monitoring animal health and welfare in Swiss livestock that combines available data on farms.

# COMPARATIVE STUDY ON THE IMMUNE AND PRODUCTIVE RESPONSE OF SUVAXYN® CIRCO (ZOETISTM) AGAINST 2 DIFFERENT PCV2 MONOVALENT VACCINES IN A PORTUGUESE PIG FARM

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## **Background and Objectives**

Evaluate the fattening performance and economic benefit of piglet groups vaccinated with 2ml of Suvaxyn® CIRCO (A), as compared to piglets vaccinated with 2 different PCV2 monovalent vaccines (B and C) in a Portuguese farm.

#### Material and Methods

At 3 WoA piglets were weaned, divided into 3 groups of 250 piglets (same number of piglets per litter in each group), individually identified by ear tag, vaccinated (vaccine A, B or C), weighed and mixed housed in weaning rooms. At around 10 WoA, 220 animals from each group were moved to a fattening farm, individually weighed at arrival and divided in 3 different barns. 30 blood samples per group were taken at 10, 13, 18 and 24 WoA and tested for qPCR PCV2 and RT-PCR PRRS EU/USA. Feed consumption per group was registered during fattening. At slaughter, group live weights and individual carcass weights were registered. Individual live weight at slaughter and individual Average Daily Gain during fattening (ADG) was estimated from carcass weight and mean carcass yield. Average profitability per pig was estimated using an economic simulator (SIP Consultors). Means between groups were tested with ANOVA and Tukey's post-hoc test.

#### Results

PCV2 virus was detected in 3/24 pools in group A, in 9/24 in group B and in 4/24 in group C. Animals in group A had a mean estimated ADG 52 grams higher than animals in group B and 33 grams higher group C (p<0.001). The Economic FC during fattening was 2,34 Kg/Kg in group A, 2,47 in group B and 2,43 in group C. The estimated mean profitability per pig was  $4.78 \in (B)$  and  $3.27 \in (C)$  higher in the Suvaxyn® CIRCO group.

#### **Discussion and Conclusion**

In this study, vaccination with Suvaxyn® Circo effectively reduced viremia and allowed better productive and economic results compared with the other two vaccines.

# TRUST MEASUREMENT BETWEEN FARMERS AND VETERINARIAN IN PIG VETERINARY MEDICINE: A FIRST STEP TO IMPROVE FARMER'S COMPLIANCE

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# **Background and Objectives**

Co-design and co-development of innovative strategies with animal health professionals to foster more prudent use of antimicrobials in animal production is essential. To implement such strategies, it seems important to take into account interpersonal trust between veterinarians and farmers. The objective of this study is to develop a scale measuring farmers' trust in veterinarians.

#### **Material and Methods**

The construction of the scale relied on the Churchill method. A first version of the Trust in Veterinarian scale (TiVS) was based on a literature review in the field of clinical trust and patient-doctor interaction scales. Then, we conducted four focus-groups with pig veterinarians and 26 qualitative open-ended interviews with farmers to explore veterinarians' and farmers' perceptions and views on trust. A qualitative evaluation of the resulting scale by professionals involved in pig industry (veterinarians, engineers, scientists, farmers) helped avoid redundancies and clarify each item. Finally, a pilot study was carried out with five pig farmer-veterinarian couples to test the relevance of the scale in field conditions and validate a final scale.

#### Results

From the literature, a list of 41 items, based on a multidimensional theoretical framework, explored seven dimensions of trust (Competence, Integrity, Honesty, Confidentiality, Fidelity, Caring, Overall trust). After the qualitative analysis, the adapted-scale version VI comprised 52 items divided into seven dimensions with a new (Availability) and a compiled dimension (Honesty/Integrity). After the qualitative evaluation by 25 pig professionals, 30 items were selected for version V2. This scale has been tested and validated on field via the pilot study to obtain the final version.

#### **Discussion and Conclusion**

TiVS is the first scale that has been validated in pig veterinary medicine to assess farmers' trust in their veterinarians. It will be used in a future cohort study with 30 farmer-veterinarian couples to explore the relationship between trust and pig farmers' compliance to veterinary advice.

#### EXPLORING ORAL FLUID MICROBIOME IN PIGS

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# **Background and Objectives**

Microbiome diversity is associated with health outcome. As oro-nasal mucosa is the first barrier against many pathogens and oral fluid (OF) collection is easy and non-invasive, OF microbiome appears an interesting target for diagnostic purposes. To gain knowledge on the OF microbiome in pigs we analysed differences between sampling matrices, variation between animals and associations with age.

### **Material and Methods**

In 2 farms, paired OF samples were collected using Salivette® (S) and Puritan Swabs® (PS) from 2 clinically healthy sows per farm, and 5 clinically healthy piglets per sow at 5 different timepoints (t1-t5; 4, 9, 12 and 18 weeks of age). Microbiome analysis was performed using 16S rRNA gene sequencing. Repeatability of the analyses was assessed by comparing two time points 1 day apart (t3-t4). 🛛 diversity was measured using Shannon index. Correlation between samples at different levels was determined by Cramer's V.

#### Results

In total, 4645 and 5498 different species were identified using S and PS, respectively. Streptococcus suis and Haemophilus parasuis were found among the 20 most commonly present species. Increased from t1 (below 4.5) to t3 (5.4), and then became stable. With S, Increased per litter and unexpectedly, it was the lowest in the oldest pigs (t5). A higher correlation was seen specifically between piglet samples originated from the same farm, sow and timepoint (Cramer's V 0.53 – S- and 0.65 – PS-). When specifically looking at the correlation between timepoints, t3 and t4 showed the highest correlation (Cramer's V 0.57 – S- and 0.6 – PS).

#### **Discussion and Conclusion**

Results show the complexity of microbiome analysis, highlight the importance of the sampling procedure and provides insights on sampling guidelines for future studies. OF microbiome analysis can have great potential as a research tool.

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# PROPHYLACTIC USE OF MELOXICAM AND PARACETAMOL IN A FARM WITH A HISTORY OF POSTPARTUM DYSGALACTIA SYNDROME

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#### **Background and Objectives**

Postpartum dysgalactia syndrome (PPDS) is a major economic problem in modern sow farms. Non-steroidal anti-inflammatory drugs are used as part of treatment. The current study investigated if meloxicam or paracetamol, when administered prophylactically and orally to sows prior to parturition, improved sow and piglet health as well as performance in a farm with PPDS problems in sows.

#### **Material and Methods**

Sixty sows and 978 piglets were enrolled in the study. Sows were randomly divided into three groups: a nontreated control group (CG), a meloxicam-treated group (MG) and a paracetamol-treated group (PG). Treatment was administered orally for 7 days from gestation day 113 onwards. Performance and health parameters investigated in sows were gestation length, farrowing duration, litter characteristics, colostrum yield and quality, litter weight gain, weaning-to-oestrus interval, pregnancy rate, rectal temperature, acute phase proteins and inflammatory markers, backfat, constipation and feed refusal. Investigated parameters in piglets were birthweight, average daily weight gain, colostrum intake and mortality.

#### Results

The PG had a significantly (P=0.04) lower rectal temperature (mean±SD:  $38.09 \pm 0.18^{\circ}$ C) than the MG ( $38.24 \pm 0.18^{\circ}$ C), but not than the CG ( $38.22 \pm 0.18^{\circ}$ C). Sows of the PG had a significantly (P=0.001) longer gestation length (116.3 ± 0.9 days) than sows of the CG (115.3±0.6 days), but not than the MG (115.9 ± 0.9 days). All the remaining parameters showed no significant differences between groups. Retrospective evaluation of the health parameters showed that 9/20, 7/20, and 5/20 of the sows in the CG, MG, and PG, respectively, were defined as clinically PPDS-affected.

#### **Discussion and Conclusion**

Preventive oral treatment with meloxicam or paracetamol did not ameliorate the PPDS situation of the sows in the farm. The insufficient presence of clinical signs complicated the evaluation of a therapeutic effect.

This study was funded by the "ECPHM research grant 2019".

# CO-INFECTION WITH MYCOPLASMA HYORHINIS AND GLAESSERELLA PARASUIS CAUSING RESPIRATORY DISTRESS IN WEANED PIGLETS? - A CASE REPORT

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#### **Background and Objectives**

In routine diagnostics, Mycoplasma (M.) hyopneumoniae-like lesions can often be observed macroscopically and histologically in the absence of detection of M. hyopneumoniae. Nevertheless, in some of these cases, other pathogens like M. hyorhinis or Glaesserella (G.) parasuis can be detected instead. In literature, the relevance of these agents as primary cause of these lesions is discussed controversially.

#### Material and Methods

In a farrow-to-finish farm in Austria, respiratory distress was observed in piglets two weeks after weaning. Three untreated, clinically affected piglets were sent to the Vetmeduni Vienna for further diagnostics. Moreover, a farm visit and lung evaluation at slaughterhouse were conducted after the incidence of clinical symptoms had decreased in the nursery but increased in younger fattening pigs.

#### Results

All three investigated animals showed a catarrhal-purulent bronchopneumonia. Additional histological findings were described as peribronchial and perivascular interstitial infiltration with non-purulent inflammatory cells. PRRSV, PCV2, Influenza A virus and M. hyopneumoniae were not detectable by PCR. In culture, high levels of G. parasuis (serotype 4) and M. hyorhinis were identified. Furthermore, PCR results for Influenza A virus from nasal swabs collected during the farm visit were negative and serum samples were PRRSV PCR-positive (97% homology with a vaccine strain). At the slaughterhouse, 70% of the lungs showed M. hyopneumoniae-like lesions. As a consequence, the herd-attending veterinarian decided to implement a two-shot vaccination against M. hyopneumoniae and clinical symptoms declined in the subsequent groups.

# **Discussion and Conclusion**

Since other pathogens were not detectable and only three piglets were necropsied, the impression could arise that G. parasuis and M. hyorhinis were responsible for the respiratory distress. Later diagnostic approaches suggested that in this case other pathogens, which were not detected at first, might have acted as a precursor. Altogether, this case demonstrates that one-time necropsy of clinically-affected animals might not be sufficient to fully evaluate the situation at farm level.

#### FRIENDLY SAMPLING ALTERNATIVE FOR PRRSV MONITORING

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## **Background and Objectives**

Intensive porcine respiratory and reproductive virus (PRRSV) monitoring in boars producing semen for artificial insemination (AI) is important, but requires repeated sampling of the same animals. To avoid negative effect of repeated sampling, animal friendly sampling methods by using one drop of blood collected in an E-swab or FTA-card offers an interesting alternative to conventional blood sampling. In this study the relative sensitivity of the (blood) E-swab and (blood) FTA-card was estimated in comparison to serum (from the same sample).

#### **Material and Methods**

Blood samples from 30 animals per farm, from 6 farms with PRRSV suspicion were collected using serum tubes, E-swabs and FTA-cards (180 samples per matrix in total) and PRRSV PCR was performed.

#### Results

Relative sensitivity of E-swab was 82% and of FTA card 68% over 111 positive serum samples. The average Ct value of E-swab and FTA-card samples was 2 and 3 Ct higher than sera, respectively. All serum samples with Ct values below 32 (72/111) were also detected from E-swabs samples. Serum samples with Ct values above 38 (2/111) were not detected from E-swabs samples. Fifty seven per cent of the serum samples with Ct values between 32 and 38 (37/111) had also a positive result in E-swab samples.

#### **Discussion and Conclusion**

The higher Ct values are probably due to the dilution factor applied during the collection and RNA extraction process (blood on the E-swab was diluted in 1 mL buffer and in 0.3 mL on the FTA-card, versus direct extraction on serum). Despite the moderate sensitivity of E-swab, it offers an alternative to serum for PRRSV monitoring in AI stations when increasing sample size and/or sampling frequency (this is possible due to the low invasive way of the sampling method) and assuming low Ct values (below 32) in case of a PRRSV introduction in a naïve herd (e.g. AI stations).

#### H3N2 SUBTYPE OF HUMAN ORIGIN INFLUENZA VIRUS OUTBREAK IN AN ENZOOTICALLY H1N1 INFECTED PIG FARM

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# **Background and Objectives**

Swine influenza virus (SIV) infection in pigs is clinically characterized by respiratory signs, fever and reproductive disorders. The most prevalent subtypes are H1N1, H1N2 and H3N2. The aim of this report was to describe the impact of an H3N2 SIV epizootic outbreak in a swine farm already enzootic for the H1N1 subtype.

#### **Material and Methods**

Sows from an 850-sow farm experienced respiratory signs, fever and anorexia. Respiratory problems affected different farm modules and reproductive losses appeared in breeding and gestation areas. In the farrowing area, stillbirths increased and number of weaned piglets decreased. Respiratory signs and mild increased mortality were observed in the nursery. Replacement gilts were not affected during the outbreak. Initially, the veterinarian took 20 and 14 blood samples from sows within an 8 day-interval, to check against PRRSV by RT-PCR and ELISA, and SIV by ELISA.

#### Results

Sera were negative for PRRSV by RT-PCR and ELISA. Influenza test was positive in the first sampling and ELISA values were two-fold increased in the second serum group. Later on, paired sow serum samples (n=15) were analyzed to assess the subtypes involved by hemagglutination inhibition assay. Sows were all positive against avHINI. Fourteen samples were negative (titer less than 1/20) against the huH3N2 SIV subtype, but 3 weeks later 15/15 sows had an increased titer (1/160-1/1260). Respiporc FLU3 (CEVA), a vaccine containing the three SIV subtypes, was applied. No further SIV outbreaks were recorded and gilt vaccination was implemented into the general vaccination scheme of the farm.

## **Discussion and Conclusion**

The present report describes the epizootic presentation of a SIV outbreak caused by a huH3N2 subtype in a farm which was already enzootically infected by a H1N1 subtype. The control of the infection in the incoming replacement gilts was key to prevent further episodes of the respiratory and reproductive presentations due to SIV.

# DETECTION OF MYCOPLASMA HYOPNEUMONIAE IN PROCESSING FLUIDS IN THE EVENT OF A CLINICAL RESPIRATORY DISEASE OUTBREAK

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#### **Background and Objectives**

Diagnosis of early infection with Mycoplasma hyopneumoniae (M. hyopneumoniae) remains challenging. Recently, M. hyopneumoniae has been detected in processing fluids (PF), which consist of serosanguineous exudates from tissues obtained after tail docking and castration of newborn piglet. This study investigated the putative use of PF to detect M. hyopneumoniae in the event of a clinical respiratory disease outbreak in a previously M. hyopneumoniae negative sow farm.

#### **Material and Methods**

The study was performed in a 5,450 sow-breeding farm deemed as negative for M. hyopneumoniae and clinically stable for porcine reproductive and respiratory syndrome virus (PRRSV). To monitor for PRRSV, the farm routinely tested three pooled PF of 15 litters each on a weekly basis. Additionally, forty-five individual litter samples were stored once monthly and tested if needed. The first week of August 2018, an outbreak of respiratory disease was detected and diagnostic laboratory tests confirmed the coexistence of M. hyopneumoniae along with other bacteria and porcine circovirus type 2 (PCV2). Once confirmed a porcine respiratory disease complex outbreak, a retrospective testing of PF for M. hyopneumoniae by real-time PCR was performed. Ninety PF stored between March 19 and October 8, 2018, were tested.

#### Results

All pooled PF tested negative for M. hyopneumoniae by real-time PCR, except 3 suspects collected on August 13, 20 and 27, which showed Ct values >37. These three PF were collected while the clinical respiratory disease outbreak was taking place. The positive PF from August 27 was composed of PF from 12 litters that were individually tested. All but one litter PF with a Ct value of 32.21 were real-time PCR negative.

#### **Discussion and Conclusion**

Our results provide new insights into the value that testing PF may have to detect M. hyopneumoniae in breeding herds. Further investigation is needed to better understand the meaning of detecting M. hyopneumoniae in this sample type.

# EVIDENCE OF THE PORCINE CIRCOVIRUS 3 (PCV-3) TARGETING TISSUES OF FETUSES AND STILLBORN PIGLETS FROM REPRODUCTIVE FAILURE CASES IN SPANISH PIG FARMS

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## **Background and Objectives**

PCV-3 has been detected in pigs displaying different clinical signs as well as in healthy animals; lately it has been most frequently associated to reproductive failure cases. Thus, this study aimed to assess the frequency of PCV-3 detection and potentially associated lesions in tissues of fetuses and stillborn piglets sampled during outbreaks of reproductive failure.

#### Material and Methods

Fetuses and stillborn piglets from 53 cases of reproductive failure from Spanish pig farms were collected and analyzed by PCV-3 qPCR. The presence of PRRSV, PCV-2 and PPV nucleic acid was also assessed. Samples with a high PCV-3 load were tested by PCV-3 in situ hybridization (ISH), sequenced and phylogenetically analyzed.

#### Results

PCV-3, PCV-2, and PRRSV were detected by qPCR in 18/53 (33.4%), 5/53 (9.4%) and 4/53 (7.5%) cases, respectively, while PPV was not detected. Mild arteritis and periarteritis were present in multiple tissues in the PCV-3 ISH-positive cases. Three out of the 6 PCV-3 qPCR-positive cases tested by ISH were also positive. The main labelled cells were smooth muscle cells of arterioles from different tissues such as heart, kidney and spleen; macrophage-like cells in lung and kidney were also labelled.

#### **Discussion and Conclusion**

The present work pointed out a potential role of PCV-3 in reproductive failure cases in pig breeding herds in Spain. Since the simple viral detection of an endemic virus does not imply the causality of the clinical condition, detection of PCV-3 within lesions provides a stronger evidence of putative association between the presence of the virus and the clinical outcome.

# ASSOCIATION BETWEEN THE PRESENCE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN SEMEN, SERUM AND TESTES AT DIFFERENT TIMES DURING AN ACUTE INFECTION IN BOARS

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#### **Background and Objectives**

In July 2019, a PRRSV-free boar station was infected with PRRSV-1, and subsequently more than 40 herds were infected with the same variant of PRRSV. Transmission of PRRSV through semen has been documented in several reports. However, there is only limited knowledge of the relationship between the presence of PRRSV in serum, testis and semen, in naturally infected boars. The aim of the present study was to compare the presence of PRRSV in serum, semen and testicular tissues from boars euthanized shortly after the outbreak.

#### **Material and Methods**

In total, 35 boars were included in the study. 19 had been infected for weeks, 16 only within the last week. Semen was collected the day before euthanasia. Immediately after euthanasia blood and testis including epididymis were collected. All serum, semen and testis samples were tested for PRRSV by RT-qPCR.

## Results

None of the 35 boars showed any clinical signs during the infection. Of the boars that had been infected for weeks, four boars were positive in serum but negative in semen while there were three boars that were PRRSV-positive in semen but negative in serum. Among boars that were infected within the last week, seven boars were PRRSV-positive in serum but negative in semen, and only one boar was positive in semen and negative in serum. Samples from testicles and epididymis were more often PRRSV-positive than semen and more samples from boars that were infected within the last week were positive.

#### **Discussion and Conclusion**

Material collected from natural PRRSV infected boars seem to reveal that serum is the most sensitive sampling material for the detection of PRRSV. However, a negative test of serum is not equivalent to that other tissues are PRRSV negative. The results should be taken into consideration when control programs for boar stations are designed.

# ASSESSMENT OF THE IMPACT OF THE RECOMBINANT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS HORSENS STRAIN ON THE REPRODUCTIVE PERFORMANCE IN PREGNANT SOWS

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### **Background and Objectives**

In July 2019 PRRSV-1 was detected in semen from a boar station in Denmark. Severe disease, with clinical signs similar or exceeding those typical of PRRSV-1 infection, was reported by several sow farms previously negative. A new recombinant strain (Horsens) between Suvaxyn PRRS MLV and Unistrain PRRS was found. This study evaluated the impact of the recombinant strain and a reproductively pathogenic field PRRSV-1 isolate on reproductive performance in seronegative sows infected in the last third of gestation.

#### **Material and Methods**

PRRSV naïve sows were intranasally inoculated with: NTX (4) non-infected; T01 (5) PRRSV Olot/91 strain; and T02 (6) Horsens strain. All sows farrowed naturally. Blood was periodically collected for to measure PRRSV viremia (RT-qPCR). Reproductive performance was evaluated. Viral loads were measured in blood from piglets at birth and at weaning, in lungs from stillborn pigs and in bronchoalveolar lavages (BAL). Lung macroscopic lesions at weaning were evaluated.

#### Results

Sows farrowed for NTX/T01/T02: 89.7%/36.0%/49.9% live piglets; 78.0%/27.1%/41.7% healthy piglets; 8.8%/0.9%/2.9% low viable piglets; and 10.3%/64.0%/50.1% stillborn pigs; weaned piglets: 89.9%/49.0%/53.2%. 100% T01 and 50.0% T02 sows were viremic.

At birth 98.9% T01 and 65.0% T02 piglets were viremic . At weaning 100% T01 and 50.0% T02 piglets were viremic. In BAL, 100% T01 and 28.6% T02 piglets were PRRSV positive at weaning.

In stillborn lungs, 84.4% T01 and 67.4% T02 piglets were PRRSV positive. At necropsy, 68.8% T01 and 38.1% T02 piglets had macroscopic lung lesions.

#### **Discussion and Conclusion**

Horsens recombinant strain is apparently less virulent than Olot/91 under laboratory conditions; its impact on reproductive performance was numerically lower than the other PRRSV-1 subtype 1 strain.

# IDENTIFICATION OF LINEAR CONSERVED EPITOPES ON THE ECTODOMAINS OF ENVELOPE PROTEINS OF PRRSV-1 USING ELITE AND POOR NEUTRALIZING SERA

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#### **Background and Objectives**

The high antigenic variability of porcine reproductive and respiratory syndrome virus (PRRSV) results in the generation of neutralizing antibodies (NA) with poor cross-reactivity. However, some individuals are capable of neutralizing viruses of different origins, indicating the existence of conserved neutralizing epitopes (NE). The objective of this study was to identify the linear epitopes of PRRSV-1 envelope protein ectodomains and their potential role in cross-neutralization.

#### Material and Methods

Twenty-two hyperimmune monospecific sera and four PRRSV-1 isolates previously confronted with these sera in seroneutralization assays were used. The nucleotide sequences of the ORFs 2-6 of these viruses were used to predict the amino acid sequences of the ectodomains of GP2 to GP5 and M proteins of the corresponding viruses. These amino acid sequences were used to design overlapping dodecapeptides later used in a Pepscan assay.

#### Results

The peptides used in this study were, in most cases, only recognized by a small proportion of sera, including the NE previously described in the literature. However, they were relatively conserved between isolates, with the only exception of the NE located in the ectodomain of GP4, which is highly variable and only recognized by homologous sera. When the recognition pattern was compared between elite and poor neutralizing sera, no clear differences could be identified.

#### **Discussion and Conclusion**

The peptides used in this study, including previously described NE, can be considered poorly immunogenic as they are recognized by a limited number of sera. This finding, along with the absence of differential peptide recognition patterns between elite and poor neutralizing sera, seems to indicate that the linear immunogenic epitopes identified in PRRSV-1 envelope proteins are not involved or, at least, are not the sole responsible, for the elite neutralization phenotype. Thus, it can be hypothesized that elite neutralizers might recognize either conformational unidentified epitopes or an undetermined combination of linear epitopes.

# VACCINATION OF NEONATAL PIGLETS AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) SIGNIFICANTLY REDUCES VIREMIA AND VIRAL SHEDDING

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## **Background and Objectives**

PRRSV is still one of the most important viruses in the global swine industry and is often controlled by the use of modified live virus (MLV) vaccines. The aim of the present study was to evaluate the ability of Suvaxyn® PRRS MLV to reduce viremia and viral shedding following experimental infection with a recent, virulent PRRSV-1 field isolate (PRRSV AUTI5-33 or Acro PRRSV).

#### Material and Methods

On the first day of life, 21 piglets were intramuscularly vaccinated and 20 piglets were sham-treated, followed by an intranasal infection with PRRSV AUTI5-33 at 28 days of age. Piglets were followed for two weeks after challenge before necropsy was performed. Serum, nasal and oral swabs were collected every 2-3 days after challenge and analyzed by a PRRSV AUTI5-33 specific RTqPCR. Cut-off values for considering animals as virus positive were defined as >2500 genome copies/mL serum, >50000 and >5000 genome copies/mL for nasal and oral swabs, respectively.

#### Results

Significantly lower PRRSV RNA loads were measured in serum and nasal swabs of vaccinated piglets on most sampling days. All of the non-vaccinated piglets were viremic and shed virus in nasal swabs, whereas only 71.4% of the vaccinated animals were viremic and less than half of the vaccinated animals shed virus (38.1%). In addition, the percentage of days with viremia in the vaccination group was 19.6% compared to 55.1% in the sham-treated group. In the vaccination group, the percentage of days with nasal shedding was 1.9% and with oral shedding was 0.1%, while in the sham-treated group it was 26.6% and 2.9%, respectively.

#### **Discussion and Conclusion**

A single dose of Suvaxyn® PRRS MLV administered to one-day-old piglets was able to significantly reduce the amount of viremia and viral shedding after experimental infection with a virulent PRRSV-1 field isolate in comparison to non-vaccinated challenged pigs.
DIVERSITY OF SWINE INFLUENZA VIRUS SUBTYPES DETECTED FROM RESPIRATORY CASES OVER THE PAST 3 YEARS IN SPAIN

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## **Background and Objectives**

Swine Influenza Virus (SIV) is one of the most important respiratory pathogens in pigs. Genetic diversity of this virus is expanding over the years, which is manifested by changes in the predominant subtypes. The objective of this study was to assess the annual diversity of the different subtypes detected during 2018-2020.

## **Material and Methods**

A total of 150 cases that showed clinical signs compatible with SIV infection were used for the present study. In all of them, SIV had been detected and successfully subtyped by RT-PCR. The sample matrix from which the virus was detected was variable depending on the farm. In 98 cases, the sample taken was nasal swab; in 32 cases lung tissues were used and, finally, 20 of the samplings were carried out by collecting oral fluids. The variety of subtypes obtained were compared within each year.

## Results

The distribution of the subtypes detected in 2018 was as follows: HlavN2 (34.9%), HlhuN2 (28.6%), HlavNI (20.6%), HlhuNI (6.3%), HlpanNI (4.8%), H3N2 (3.2%), H3Nlav (1.6%) and HlpanN2 (0%). In 2019, the prevalence of each subtype was: HlhuN2 (36.1%), HlavN2 (19.4%), HlavNI (13.9%), HlhuNI (8.3%), HlpanNI (8.3%), H3Nlav (8.3%), H3N2 (5.6%) and HlpanN2 (0%). Finally, the subtype diversity found in 2020 was: HlavN2 (37.7%), HlhuN2 (30.4%), HlavNI (13.0%), HlpanNI (5.8%), HlpanN2 (5.8%), HlhuNI (4.3%), H3N2 (2.9%) and H3Nlav (0%).

## **Discussion and Conclusion**

In each of the three years evaluated, HlavN2 and HlhuN2 subtypes were found with the highest frequency. In addition, the same pattern was also observed in each year with respect to HlavN1, being the third most detected subtype. On the other hand, a slight increase in the detection of pandemic lineages (HlpanN1+HlpanN2) was perceived from 2018 to 2020.

# TWO NOVEL PORCINE TESCHOVIRUS STRAINS AS THE CAUSATIVE AGENTS OF ENCEPHALOMYELITIS IN THE NETHERLANDS

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## **Background and Objectives**

Porcine teschovirus (PTV) circulates among wild and domesticated pig populations without causing clinical disease, however neuroinvasive strains have caused high morbidity and mortality in the past. In recent years, several reports appeared with viral agents as a cause for neurologic signs in weanling and growing pigs among which PTV and new strains of PTV were described.

## **Material and Methods**

Case presentation

#### Results

On two unrelated pig farms in the Netherlands the weanling pig population showed a staggering gate, which developed progressively to paresis or paralysis of the hind legs with a morbidity up to 5%. After necropsy we diagnosed a non-suppurative encephalomyelitis on both farms, which was most consistent with a viral infection. PTV was detected within the central nervous system by qPCR. From both farms PTV full-length genomes were sequenced, which clustered closely with PTV-3 (98%) or PTV-11 (85%). Other common swine viruses were excluded by qPCR and sequencing of the virus.

## **Discussion and Conclusion**

Our results show that new neuroinvasive PTV strains still emerge in pigs in the Netherlands. Further research is needed to investigate the impact of PTV and other viral agents causing encephalomyelitis within wild and domestic pig populations supported by the awareness of veterinarians.

## PORCINE CIRCOVIRUS TYPE 2 (PCV2) GENOTYPING IN AUSTRIAN PIGS IN THE YEARS 2002 TO 2020

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## **Background and Objectives**

PCV2 is one of the most destructive viral diseases with high economic impact. Since the mid-2000s, two global genotype shifts from PCV2a to PCV2b and from PCV2b to PCV2d have been observed. In 2018, eight genotypes, PCV2a-PCV2h were proposed by Franzo and Segalés (PLoSONE 13(12):e0208585). In the present longitudinal study, the occurrence of these eight genotypes and the PCV2 strain variability were investigated in Austrian pigs.

## **Material and Methods**

147 FFPE tissue samples from pigs with histories of PCV2-SD or PDNS submitted from 2002-2020 for routine diagnostics were sequenced on the capsid protein gene. Sequences were analyzed by the Neighbour Joining method. Additionally, haplotype and nucleotide diversity were determined.

#### Results

Sequences of PCV2a (7.5%), PCV2b (73.5%), PCV2d (18.4%), and PCV2g (0.6%) were found. PCV2c, PCV2e, PCV2f, and PCV2h were not obtained. PCV2a was present only occasionally from 2002-2011. PCV2b was detected in every study year except 2020. In four study years, all samples were of this genotype. PCV2d was detected in 2004 for the first time. After a 7-years-break, it was found in 2012 and from 2014 on in every study year with increasing prevalence. PCV2g was detected only in one sample from 2009. Additionally, the variants of the nucleotide sequences were evaluated and 72 different haplotypes were detected. PCV2a showed the highest haplotype diversity followed by PCV2b and PCV2d. The nucleotide diversity was generally low.

## **Discussion and Conclusion**

In 2002, PCV2-SD was reported in Austrian pigs for the first time. Already by that time, only a few PCV2a strains with high haplotype diversity could be detected, which indicated the decreasing importance of this genotype. The evolution and predomination of PCV2b and PCV2d and the varying extent of haplotype diversity over the years may be a product of different factors including natural evolution, natural selection and vaccination pressure.

## CHALLENGES IN INFLUENZA A DIAGNOSTICS IN A GILT PRODUCING FARM - A CASE REPORT

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## **Background and Objectives**

Influenza A virus (IAV) is an economically important respiratory disease in swine worldwide. Diagnostic tests are available for direct or indirect detection of active influenza A infection or previous exposure, respectively. While viral shedding is of short duration and direct detection is dependent on sampling time point, measuring antibody responses by quantitative Hemagglutination-inhibition (HI) assay is a reliable alternative method and mostly replaces direct detection. The HI panel mostly includes characteristic classical HlavNI and pandemic HINI, HlhuN2 and H3N2 strains. This case report emphasises possible problems in HI test result interpretation.

## Material and Methods

In a closed gilt producing herd gilts for own replacement showed fever and respiratory distress shortly after reintroduction into the sow herd. Nasal swabs were taken from three representative gilts (day 0) and HlavNI was detected by PCR in all three samples. However, no HlavNI seroconversion using HI assays could be detected between day 0 and 15. Single serum samples of gilts between 12 and 22 weeks of age (n=25) showed either no or very low (1:20) titers against the HlavNI used in the routine HI-test. Older sows showed titers between 1:20 and 1:160 against all strains included in the HI test except for H3huN2.

## Results

When expanding the HI-panel by seven further HIavNI strains, a clear seroconversion could be seen at two HIavNI strains used in all three PCR positive tested gilts; high titers were also present in the majority of the other sows tested. Subsequent vaccination against the classical IAV strains improved respiratory health.

#### **Discussion and Conclusion**

The present case report highlights that for successful diagnosis of influenza infections and further prevention strategies it is crucial to combine both direct and indirect detection methods. Additionally, it is advisable to continuously adopt panels of IAV strains used in HI-assays and include current strains circulating in the respective area.

## EXPLORATORY STUDY ON DETECTION OF PORCINE CIRCOVIRUS 3 IN PORK MEAT

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## **Background and Objectives**

Porcine circovirus 3 (PCV-3) is a single-stranded circular DNA virus infecting swine. Other viruses like Torque teno viruses (TTVs) have been found in consumable pork meat in the past. The elevated consumption of pork meat and the fact that PCV-3 is ubiquitous in swine farms may suggest a similar situation for this virus. Therefore, the objective of the present study was to assess whether consumable pork meat contains PCV-3 DNA.

## Material and Methods

A total of 30 pork meat commercial samples including processed (bacon [n=8], cured sausage [n=5], ham [n=8] and Majorcan sausage [n=1]) and non-processed (loin [n=4] and sausages [n=4]) ones were included in this study. DNA was extracted from the supernatant of tissue homogenised and tested by PCV-3 PCR and qPCR when positive.

## Results

Up to 67% (20/30) of samples were PCR positive for PCV-3 DNA. All non-processed samples (loin and sausages) were positive for PCV-3 and contained the highest viral load (mean Ct of 33.38 and 29.97, respectively). Among the processed samples. Majorcan sausage had the highest viral load with a Ct of 32.02, followed by cured sausage (4 out of 5 [80%], mean Ct of 34.24) and bacon (6 out of 8 (75%), mean Ct of 35.42). Ham samples had the lowest PCV-3 load, with only 1 out of 8 samples (12.5%) being PCR positive (non-quantifiable viral load).

## **Discussion and Conclusion**

Present results confirmed the detection of PCV-3 DNA in processed and fresh pork meat. Since the percentage of samples from non-processed meat was higher than processed one, it is likely that curation procedures may exert an effect on PCV-3 genome integrity and, therefore, jeopardize its detection by PCR when present. Overall, these data likely reflect the ubiquity of PCV-3 in the swine industry.

COMPARISON OF SWINE INFLUENZA TYPING RESULTS IN GERMANY FROM JANUARY TO SEPTEMBER IN 2018, 2019 AND 2020

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## **Background and Objectives**

Influenza A virus (IAV) has a key importance in the porcine respiratory disease complex (PRDC). However, besides the co-infecting agents, the cause of disease is influenced either by the pigs age, health/immune status or the influenza strain involved. The aim of this study is to give an overview on passive Influenza surveillance in Germany over the past three years.

## **Material and Methods**

This report summarizes Influenza subtyping results from January to September in the years 2018, 2019 and 2020 in Germany. Nasal swabs (pooled per 5), oral fluids, bronchoalveolar lavage or lung tissue were either taken in farms with acute clinical signs linked to influenza or in farms with unclear, persistent respiratory or reproductive symptoms. Samples were analyzed for IAV by PCR. Subtyping of samples with a Ct-value ≤30 was done by multiplex real-time PCR.

## Results

In total 1119 samples of 614 farms were analysed. In the investigated periods most farms were positive for H1avN1 (2018: 57,1%; 2019: 39,2%; 2020: 44,4%) followed by H1huN2 (2018: 17,3%; 2019 19,3%; 2020: 15,5%). In 2018 10% of the farms were positive for pandemic IAV strains (panH1N1 and panH1N2), this increased to 20,3% in 2020. H3N2 was detected in 7,3% of the farms in 2018 and decreased to 3,9% in 2019. More than one subtype was found in 11,1% (2018), 4,8% (2019) and 11,1% (2020) of farms.

## **Discussion and Conclusion**

The detection rate of pandemic subtypes increased between 2018 and 2020. It is possible that several subtypes can be present on a farm at the same time. To ensure the full picture is gathered, the sample size plays an important role. This data shows that it is essential to know the exact strains circulating on swine farms so that the correct vaccination strategy can be implemented.

# DETECTION OF PORCINE PARVOVIRUS (PPV) 27A-LIKE STRAIN IN A FRENCH HERD: A CASE REPORT.

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## **Background and Objectives**

More than 70% of reproductive failure in sows due to an infectious agent are caused by either PRRS virus, PCV2 or PPV. PPV vaccination of sows is widely used to prevent SMEDI (Stillbirth, mummification, embryonic death and infertility) syndrome. The objective of the case report is to describe a PPV outbreak due to an emerging strain in a French farm.

## Material and Methods

In Summer 2020, a significant increase of mummies was observed in a farrow to finish farm (>500 sows) located in Brittany. The sows were routinely mass vaccinated against PRRS virus and PCV2, every 12 weeks. The reproductive herd was regularly monitored for PRRS and was considered as stable according to the AASV guideline. The sows were also vaccinated against parvovirosis, leptospirosis and Erysipelas one week after farrowing. Diagnostic work included PCV2, PPV and PRRS PCR on fetuses' samples collected from 12 litters (Labofarm, France). Two PPV positive samples were submitted for Nanopore sequencing (PathoSense, Belgium).

## Results

PPV PCRs on pooled samples from all the 12 litters were positive with low Ct (<12) whereas PRRS and PCV2 PCRs were negative. Using Nanopore sequencing, PPV was detected at a high viral load on both samples, PCV3 was detected at low load in one sample only indicating that PPV is most important in the clinical picture. The genetic analysis for the PPV gene encoding VP2 showed that the 2 investigated strains were genetically closely related to 27a-like strain.

#### **Discussion and Conclusion**

As far as we know, it is the first time that a PPV 27a-like strain (Cluster D) was characterized in France. This finding is consistent with previous reports showing the emergence of cluster D strains in Northern Europe. According to the literature, these strains seem to be more virulent than historical strains thus a close surveillance is necessary.

# ANALYSIS OF SAMPLES SUBMITTED FOR INFLUENZA DETECTION BY MULTIPLEX PCR FROM DUTCH PIG FARMS IN THE PERIOD JANUARY 1ST TO OCTOBER 1ST, 2020

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## **Background and Objectives**

Swine Influenza A viruses are highly prevalent in swine in The Netherlands. Both 'classical' and pandemic SIVstrains circulate in the Dutch swine herds. Identification of circulating strains was performed, important for the right choice of vaccine to apply in a herd, future vaccine development and monitoring of the zoonotic potential.

## **Material and Methods**

Nasal swabs, lungs, oral fluids or BALF were collected by Dutch veterinarians from pigs suspected of suffering from flu. Samples were sent to IVD in Hannover, Germany for diagnostic by PCR (VetMAX-Gold Influenza SIV-PCR swine). Results were expressed as negative (Ct-value >40), weakly positive (38-40) or positive (<38). Subtyping was done by PCR (SIV Influenza Subtypisierung Multiplex-PCR, Henritzi et al., 2016).

#### Results

A total of 103 sample-submissions from 89 herds were received, consisting of a total of 318 samples/ pooled samples: 98 submissions of nasal swabs (N=294), 4 submissions (N=12) of oral fluids, 2 submissions of udder/nose wipes (N=8) and one only medium without swab (N=4). Two submissions had combined sample types. Of these 318 samples, 204 were positive of which 8 had a Ct-value >38 (weakly positive). 75 farms (84%) tested positive at least once. 79 submissions (77%) had at least one positive sample. From 117 samples a subtype of SIV could be determined: HlavNlall: 46, HlpdmN2: 30, HlhuN2: 25, HnegN2: 4, HlavN2: 3, HlhuNlall: 3, HlavNneg: 3, HlhuNneg: 1, HlpdmNlall: 1, HnegNlall: 1. In seven farms two different SIV-types were found. Spatial distribution (will be shown at the conference) shows that all SIV-types are distributed over all regions with pig farms.

#### **Discussion and Conclusion**

Nasal swabs are a good tool to diagnose SIV infection. Although HlavNlall is still the most important type in swine in the Netherlands, HlpdmN2 is becoming more prevalent. Additionally 3 different reassortants were found. Interestingly, no H3N2 was found in this period in contrast to previous years.

# COMPARISON OF SWINE INFLUENZA TYPING RESULTS IN DUTCH SWINE FARMS JANUARY TILL SEPTEMBER 2018, THE SAME PERIOD IN 2019 AND 2020

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## **Background and Objectives**

Influenza A virus (IAV) has a key importance in the porcine respiratory disease complex (PRDC). Since 2009 H1N1pdm strains have been detected in swine herds next to the classical strains and they have changed the infection dynamics on farms. Identification of circulating strains was performed, to give an overview Influenza during the past three years.

## Material and Methods

Nasal swabs, lungs, oral fluids or BALF were collected by Dutch veterinarians from pigs suspected of suffering from flu. Samples were sent to IVD in Hannover, Germany for diagnostic by PCR (VetMAX-Gold Influenza SIV-PCR swine). Subtyping of samples with a Ct-value <30 was done by multiplex Real-Time PCR (Henritzi et al, 2016).

## Results

In total 834 samples of 165 different farms were analyzed. In the investigated periods most farms were positive for HlavNI (2018: 53,8%; 2019 38,5% and 2020: 39,3%) followed by HlhuN2 (2018: 38,5%; 2019 20,5% and 2020: 21,4%). In 2018 14,2% of the farms were positive for pandemic IAV strains (panHlNI and panHlN2), this increased to 17,9% in 2019 and to 26,4% in 2020. H3N2 was detected in 7,7% of the farms in 2019, none in 2018 and 2020. HlavN2 was found in 7,7% in 2018, the same 7,7% in 2019 and this decreased to 2,6% in 2020. More than one subtype per farm have been found in 7,7% (2018), and 8,9% (2020). None in 2019. Changes in geographic distribution of the different types of SIV will be shown at the conference.

## **Discussion and Conclusion**

Nasal swabs are a good tool to detect SIV infection. The detection rate of pandemic subtypes increased from 2018 to 2020. It is possible that several subtypes can be present on a farm at the same time. These data show that it is essential to know the circulating strains on swine farms exactly so that the right vaccination strategy can be implemented.

## PRRS PIGLET VACCINATION AT 1 DAY OF AGE EFFECTS ON NURSERY MORTALITY

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## **Background and Objectives**

The objectives of this study were to evaluate the nursery PRRS stability and performance/economic benefit in PRRS vaccinated batches, as compared to previous non vaccinated batches after an outbreak.

#### **Material and Methods**

The study was conducted in an iberian farm, in a dense pig area, producing 440-weaned piglets per week. A PRRS outbreak occurred in 2019 with reproductive disorders in sow herd, and respiratory clinical signs in nursery. The study compares non vaccinated batches weaned between week 1 to 17 of the year (Group A), and vaccinated batches between week 18 to 45 (Group B). Piglet vaccination started on week 15, when viremic newborn stopped, thanks to improve the management and internal/external biosecurity rules in sow herd. Piglets received an intramuscular injection with 2 ml of a PRRS MLV vaccine at first week of age.

#### Results

Wild type PRRS strain viremia was detected in nursery until week 23 of the year by qPCR (Bio-T kit® PRRSV DIVA RXN), and vaccine type strain (96V198) was detected during the vaccination period. Group A had a nursery mortality mean of 10,06% and decreased to 2,81% in Group B, decreasing from 44 to 12 dead piglets/week. The statistical significance by Poisson regression model is based on the coefficient between both groups with an estimated value of -0,012 with a standard error of 0,10857. The production cost was  $49,42 \in$  in group A and  $46,56 \in$  in group B. The cost of the intervention was calculated by the vaccine dose price and vaccination labor ( $1,2 \in$ /pig). During this study, the market piglet price was  $61,8 \in$  (Iberian pig market) and the return of investment (ROI) of 4,5.

## **Discussion and Conclusion**

Under the conditions of this study, Suvaxyn PRRS MLV vaccination of non-viremic piglets at first week of age stabilized and improved the mortality rate associated with PRRSv in nursery.

LUNG LESIONS AND VIRAL LOAD IN LUNG TISSUE IN VACCINATED AND NON-VACCINATED PIGLETS AFTER EXPERIMENTAL INFECTION WITH PRRSV AUTI5-33 (\*ACRO" PRRSV)

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## **Background and Objectives**

The aim of the study was to provoke lung lesions in weaned piglets after experimental infection with PRRSV-1 isolate AUT15-33. Another objective was to test the efficacy of Ingelvac PRRSFLEX®EU, a commercial modified live virus vaccine, in order to decrease lung lesions and viral load in lung tissue after infection with AUT15-33.

## Material and Methods

Vaccinated and non-vaccinated piglets (4groups,n=16/group) were intranasally infected with low dose (1x10^3 TCID50) or high dose (1x10^5 TCID50) of PRRSV AUTI5-33 28 days post vaccination (D28). Half of the animals of each group were euthanized and necropsied 14 days post infection (D42), remaining animals four weeks later (D70). Gross lung lesions were macroscopically classified according to severity, location and percentage of the affected lung lobe. The total histo-score was calculated from the sum of lesion severity and extension of each of the histologically examined parameters (pneumocytic hypertrophy and hyperplasia, septal infiltration with mononuclear cells, intra-alveolar necrotic debris, intra-alveolar inflammatory cell accumulation, perivascular inflammatory cell accumulation) in all lung lobes. Tissue samples were collected to assess viral load in lung using the AUTI5-33 specific qRT-PCR.

#### Results

A significant difference (p<0.05) in gross lung lesions between vaccinated, high dose infected and non-vaccinated, high dose infected pigs was detected while there was a numerical difference (p<0.1) between vaccinated, low dose infected and non-vaccinated, low dose infected animals during first necropsy (D42). The total histo-score was significantly higher (p<0.05) in non-vaccinated compared to vaccinated animals during first necropsy. Viral load in lung tissue differed significantly (p<0.05) between vaccinated and non-vaccinated infected animals during first necropsy. During second necropsy (D70), no significant differences in lung lesions and viral load in lung tissue were present between treatment groups.

#### **Discussion and Conclusion**

Vaccination was able to reduce severity of lung lesions and viral load in lung tissue under experimental conditions.

# SWINE INFLUENZA VIRUS STRAINS (SIV) FOUND IN SAMPLES FROM SWINE IN THE UNITED KINGDOM FROM JANUARY TO OCTOBER 2020

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## **Background and Objectives**

Swine influenza A virus (SIV) can have a large economic impact on the pig industry, and the zoonotic threat also makes it a concern for public health. A study has shown that 56% of UK farms were positive for influenza, based on 146 samples from nine farms. Both 'classical' and pandemic SIV-strains circulate in the UK swine herd. Diagnosis of strains is important both for the right choice of vaccine to apply in a herd, for future vaccine development and monitoring of the zoonotic potential.

## **Material and Methods**

Nasal swabs, were collected by UK veterinarians from pigs suspected of suffering from flu. Samples were sent to IVD (Innovative Veterinary Diagnostics laboratory) GmbH in Seelze, Germany for diagnostic by PCR (VetMAX-Gold SIV RNA Detection Kit, Life Technologies). Results were expressed as negative, weakly positive (Ct-value 38 - 40) or positive (Ct-value <38). Subtyping was done by PCR (SIV subtyping multiplex real-time PCR) if Ct-value was less than 30.

## Results

A total of 58 submissions out of 98 submissions analysed by IVD had 1 or more positive results (59%). In most cases nasal swabs were submitted pooled by 4 or 5 swabs in one pool. At least one strain was found in 33 submissions which included a total of 751 samples, all of which were nasal swabs, with 40 strains recorded in total: 24 H1huN2 (60%), 2 H1avN2 (5%), 2 H1huN1pdm (5%), 4 H1pdmN2pdm (10%) and 8 H1pdmN1pdm (20%). There were 5 submissions with two or more strains (15.15%).

## **Discussion and Conclusion**

The sampling technique used by UK veterinarians to detect SIV is often successful (59%). Based on the results, H1huN2 is the predominant strain found in the UK. Pandemic strains are also commonly detected including H1pdmN2pdm and H1pdmN1pdm, and to a lesser extent pandemic strain reassortments such as H1huN1pdm. Infections with multiple stains are not uncommon.

## CONTROLLING PRRSV AND PREVENTING RECOMBINANTS; A CASE REPORT.

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## **Background and Objectives**

Mutation and recombination are common processes inherent to PRRSv nature to the point most existing strains are considered as mosaics. Literature reports recombination cases involving MLV and wild virus or PRRSv1 and PRRSv2. Certain parts of the industry are afraid of creating new strains with enhanced virulence – but there is no evidence that recombinant strains are more pathogenic than their parental strains.

## Material and Methods

PRRSv positive 250 sows (three-week batch) farrow-to-finish farm in Northern-Ireland.

Vaccination protocols included MLV for sows (4 blankets/year) and 4 week-old piglets (different products, same strain).

Clinical respiratory problems occurred on 12-16 weeks-old pigs causing mortality (>5% each batch) and losses involving a recombinant PRRSv stain (MLV + wild-virus detected by ORF2 to ORF7 sequencing).

MLV vaccines were replaced due to concerns on piglets/growers vaccination efficacy; no cooldown period was possible. PRRSv status was monitored during the next year.

## Results

Viremia was found in 4 and 16 weeks-old pigs on repeated occasions. We identified different events and accidents undermining vaccination efficacy including wrong fridge temperature set-up, frozen vaccines, vaccines kept out of the fridge, and biosecurity breaches. The monitoring (PCR+sequencing) program detected a new wild-virus circulating but fine-tuning vaccination protocols prevented PRRSv disease. The recombined MLV/wild-virus was never found again, and stabilization was eventually achieved (PRRSv negative weaners and no viremia in growers/fatteners).

#### **Discussion and Conclusion**

In this case, the recombinant MLV strain was involved causing disease, but both disease and virus stopped being detected once the farm was stabilized. It is unknown the virulence of the parental wild strain but the recombinant MLV was not more virulent than other PRRSv strains found in the region.

Ongoing increases in PRRS genome sequencing practices allow the identification of more recombinant strains causing viremia or clinical disease. Good vaccination practices are paramount to control PRRSv and preventing recombinations.

# HISTOLOGICAL EXAMINATION OF TESTES AND EPIDIDYMIDES FROM BOARS NATURALLY INFECTED WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV)

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## **Background and Objectives**

Since the summer of 2019, more than 40 Danish herds experienced infection with a new PRRSV-1 variant originating from an outbreak on a PRRSV-free boar station. The aim of the present study was to disclose potential histological lesions in testes and epididymides of boars naturally infected with PRRSV together with immunohistochemical identification of PRRSV.

# Material and Methods

Tissue section (n=1) from the right testis and the right epididymis (n=3; caput, corpus and cauda) of 35 naturally infected boars and 10 controls were examined histologically by staining with Haematoxylin and eosin, and immunohistochemically for PRRSV-1. A rabbit anti-PRRSV1 nuclear protein antiserum (Alpha Diagnostic Intl. Inc.) was used for immunostaining.

## Results

In the testes, focal, interstitial mononuclear cell infiltration, hyperemia, focal, tubular degeneration (atrophy), and areas of necrotic tubuli seminiferi were observed in both infected and controls. Five of the infected testicles had areas of denucleated Leydig cells, and in one case, multinucleated giant cell formation in tubuli seminiferi was observed. In the epididymides, interstitial mononuclear cell infiltration and vacuolization of the epithelium were occasionally observed in both groups. In the epididymis of 10 of the infected boars, mononuclear cell infiltrations were observed in the lumen of the ductus epididymidis. Additionally, four of these presented multinucleated giant cell formation.

Immunohistochemistry revealed few, focally distributed, infected macrophages in the interstitium of one and three of the infected testes and epididymides, respectively.

## **Discussion and Conclusion**

The histological examination revealed only few, localized lesions in the tissues, and little differences were observed between the groups. In addition, only four of the 38 infected animals presented PRRSV-1 positive cells. The present study emphasizes, that PRRSV is the cause of only minor histomorphological lesions in the testes and epididymides, and that the viral antigen can only be sporadically identified within the tissues.

# ANALYSIS OF FACTORS INFLUENCING GROWING PERFORMANCES IN A CLUSTER OF FARMS UNDER DIFFERENT PRRS CONTROL PROTOCOLS IN SPAIN

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## **Background and Objectives**

PRRS vaccines and biosecurity protocols can play a relevant role on productive parameters in PRRS endemic farms. Therefore, the purpose of this trial was assess the impact of changes in their PRRS control program.

## **Material and Methods**

This trial involved 173 fattening barns, 72 of which, issued from 4 sources, following a 3 phases approach (TRAD) and the remaining 101 coming from 3 sources under a wean-to-finish system (WTF). The 7 source farms were under a PRRS control program with a modified live vaccine (protocol A) in sows and were shifted to the 5 step process BI approach, involving vaccination of sows (protocol B) or both sows and piglets (protocol C). PRRS vaccines used in this new approach were Ingelvac Reprocyc<sup>®</sup> PRRS EU for sows (2ml IM) and Ingelvac PRRSFLEX<sup>®</sup> EU for piglets (1ml IM) (Boehringer Ingelheim Vetmedica, GmbH). For statistical analysis, GLM models or mixed models were applied according to the variables under study, and their distribution.

## Results

As there was an association between productive system and productive indexes, each production system was analysed independently. While, for ADWG, statistical differences were only related with slaughter weight in both systems, FCR was associated with both slaughter weight and PRRS protocol, with a significant improvement after vaccination of piglets in both systems (A vs C, p<0.05). In addition, mortality rate was related to source farm, and significantly reduced with protocol C in WTF farms (A vs. C, p>0.05). In fact, in TRAD farms, there was a positive impact of change of protocol, but the differences observed were not significant

## **Discussion and Conclusion**

In spite of the multivariate character of productive performance indexes, the implementation of the BI 5 phases approach yielded an improvement of the standardized FCR and the mortality, that reached statistical significance in case of vaccination of the piglets.

DETECTION OF DIFFERENT SUBTYPES OF INFLUENZA A VIRUS ON BELGIAN FARMS SUFFERING ACUTE RESPIRATORY DISEASE

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## **Background and Objectives**

Influenza A viruses (IAV) are divided into different subtypes. In Europe, the most prevalent subtypes are HlavNl, HlhuN2, H3N2, HlpdmNl and HlpdmN2. The aim of this study is to investigate the diversity of IAV in Belgian pig herds suffering clinical outbreaks.

## Material and Methods

57 Belgian swine herds with an average of 408 sows, suffering acute respiratory symptoms, were sampled from January 2020 until October 2020. In 62% of the herds nursery piglets were sampled, in 20% of the herds sows were sampled and in 18% of the herds fattening pigs were sampled. In total 118 PCRs were performed (50 oral fluid, 47 nasal swabs, 10 TBS, 9 lungs and 2 blood sera).

#### Results

48 (=41%) of these PCR's were positive for IAV and 70 (=59%) PCR's were negative. 32/57 (=56%) of the farms were positive for IAV. Positive samples were subtyped. 6 farms had a non typable strain. 10 farms had a pandemic strain: 5 farms were only positive for H1pan and the neuraminidase could not be typed (=H1panNx), H1panN1 was found in 3 farms, H1panN1pan in 1 farm and H1panN2 also in 1 farm. A classical subtype was found on 16 farms: H1avN1 was found on 14 farms, H1avN2 on 1 farm and H1huN2 also on 1 farm.

## **Discussion and Conclusion**

The results show that IAV is commonly found on Belgian farms. We can conclude that HlavNI is the most dominant subtype. The detection of pandemic strains is rising. Pandemic subtypes were found on 30% of the IAV positive farms indicating a rapid spread within the pig population. It is therefore important to protect swine herds against both classical and pandemic strains to obtain complete protection against circulating strains.

# COMPARISON OF THE REPRODUCTIVE PERFORMANCE OF SOWS BEFORE AND AFTER VACCINATION WITH A KILLED PRRSV VACCINE AS A BOOSTER TO A MODIFIED-LIVE VACCINE, IN A GREEK FARM

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## **Background and Objectives**

PRRSV is one of the most important pathogens in pigs worldwide, which can severely affect the reproductive efficiency of a farm. Different vaccination protocols with killed and/or modified live vaccines have been used in sows to control the disease, with various results. The present study aimed to compare the reproductive performance of sows before and after vaccination with a killed PRRSV vaccine as a booster to a modified-live vaccine, in a Greek farm.

## Material and Methods

Reproductive data from a Greek farrow-to-finish farm with 700 sows, for years 2017 and 2018, were studied retrospectively. The number of liveborn and weaned piglets and the number of mummies and stillbirths per litter per week were compared before (2017) and after (2018) starting vaccination with the killed virus vaccine Progressis®, at the day 90 of gestation, as a booster to a modified-live vaccine for PRRSV. Also, abortion and return to estrus rate was recorded and compared.

#### Results

The mean number of live born and weaned piglets was 14.51 ( $\pm$ 0.89) and 12.73 ( $\pm$ 0.55), respectively, in 2017 and 14.99 ( $\pm$ 0.57) and 13.06 ( $\pm$ 0.46), respectively, in 2018 (P<0.05, for all comparisons). The mean number of stillborn and mummified piglets was significantly lower in 2018 compared to 2017, with 1.29 ( $\pm$ 0.43) versus 1.90 ( $\pm$ 0.57) stillborn piglets (P<0.05) and 0.23 ( $\pm$ 0.16) versus 0.33 ( $\pm$ 0.23) mummies (P<0.05). The frequency of abortions differed significantly (P<0.05) before and after boosting with the killed vaccine, with 3.12% and 1.52% abortions, respectively. Return to estrus rate was 8.5% in 2017 and 7.5% in 2018 (P>0.05).

#### **Discussion and Conclusion**

The results of the present historical control trial show that vaccination with the killed virus vaccine Progressis® as a booster to a modified-live vaccine can improve sows' reproductive performance, under the specific farm conditions.

## DETECTION OF AN INFLUENZA A VIRUS, PANHIN2, IN A SWINE HERD IN BELGIUM

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## **Background and Objectives**

Different influenza A virus (IAV) subtypes circulate in swine herds in Belgium, both classical and pandemic subtypes. Shortly after the emergence of the human pandemic H1N1 2009 IAV (panH1N1(2009)), variants of this strain were detected in swine populations worldwide. The first detection of this strain in a swine herd in Belgium happened in 2018. The strain panH1N1 was found on a farm where piglets showed mild respiratory symptoms (coughing and sneezing) shortly after weaning. Reassortants of IAV are often found, among them also reassortants between pandemic and classical lineages. Here we describe the detection of a panH1N2 in a swine herd in Belgium.

#### Material and Methods

Piglets of a 300 sow farm in a 4 week batch system had a dry cough at the end of the farrowing period and in the first weeks of the nursery. Piglets didn't respond to an antimicrobial treatment. Sows showed no reproductive symptoms. Eleven tracheo bronchial swabs (TBS) were taken from 5 weeks old, coughing pigs and analysed with Pathosense (full diagnostic platform based on 3rd generation sequencing system, Laboratory of Virology of the Faculty of Veterinary Medicine UGent) and a multiplex PCR of IVD (IVD GmbH, Innovative Veterinary Diagnostics, Seelze, Germany).

#### Results

TBS were positive for IAV and the subtype panH1N2 was determined. To our knowledge, it is the second detection of panH1N2 in Belgian pigs. After the start of vaccination, the clinical situation improved.

## **Discussion and Conclusion**

Nowadays, IAV disease has a more chronic character and can stay on a farm for a long period of time. Since IAV can act as a door opener for secondary bacterial infections and create a huge economic impact, it is important to rapidly control IAV infections. This result is also a confirmation of the rapid spread of pandemic IAV subtypes in Belgian swine herds.

# IMMUNE CHECKPOINTS ARE UPREGULATED IN THE THYMUS OF PIGLETS INFECTED WITH PRRSV-1 STRAINS OF DIFFERENT VIRULENCE

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## **Background and Objectives**

Porcine respiratory and reproductive syndrome virus (PRRSV) produces a dysregulation on the host immune response. Immune checkpoints are positive or negative regulatory receptors expressed on immune cells. The present study aims to evaluate the expression of inhibitory immune checkpoints in the thymus of piglets infected with PRRSV-1 strains of different virulence.

## Material and Methods

Sixty-five 4 week-old piglets were distributed in three groups: control, 3249-infected group (low virulent strain) and Lena-infected group (high virulent strain). Animals were euthanized at 1, 3, 6, 8 and 13 days post-infection (dpi) and thymi were collected to analyze PRRSV viral load, immunohistochemistry and relative quantification of immune checkpoints (PD-L1, PD-1, TIM-3, CTLA-4, LAG-3, CD200R and IDO-1).

## Results

Viral load was higher and earlier in Lena-infected pigs. PD-L1, TIM-3, CTLA-4, LAG-3 and IDO-1 were upregulated in the thymus of both infected groups from 6 dpi, but with a higher expression in Lena group. All of them showed a progressive decrease until the end of the study but TIM-3 which steadily increased until the end of the study. The immunohistochemical study allowed determining the distribution of positive cells, whereas CD200R<sup>+</sup> cells were observed in the cortex and medulla, PD-L1<sup>+</sup> cells were mainly observed in the thymic medulla of infected animals.

## **Discussion and Conclusion**

Considering the role of both the thymus in the maturation of T cells as well as of inhibitory immune checkpoints leading to the exhaustion of T cells, among other functions, deciphering the work of these molecules in the thymus along PRRSV infection may represent a cornerstone to understand PRRSV immunobiology. PD-L1, TIM-3, CTLA-4, LAG-3 and IDO-1 could play an important role in the immunosuppression observed in PRRSV-infected pigs, especially those infected with the virulent PRRSV-1 strain Lena.

# IMPACT OF AN EARLY PIGLET VACCINATION ON THE CLINICAL EXPRESSION OF PRRSV INFECTION, A FRENCH FIELD CASE

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## **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome (PRRS) is the most damaging viral disease in the swine industry. In Brittany, PRRSV is considered enzootic. So far, only PRRSV-1 strains have been identified. We aim at describing a field case where early vaccination helped controlling mild clinical expression of post-weaning PRRSV circulation.

## **Material and Methods**

In a 320-sow farrow-to-finishing farm in Brittany, in the spring of 2019, numerous cases of ear necrosis appeared 2-3 weeks into post-weaning, and performance dramatically decreased at the start of the finishing period, without any respiratory signs. This occurred in successive batches. In this farm, all piglets are routinely weighed upon transfer to the finishing unit. The farm, located in a high-density pig production region, routinely vaccinated sows against PRRS with a modified-live vaccine (MLV), on a quarterly basis (mass vaccination). Early vaccination was implemented in piglets at 5-8 days of age. The MLV previously used in sows for mass vaccination was changed to the same vaccine in piglets in order to avoid using two different MLV vaccines in the farm.

#### Results

The impact on reduction in ear necrosis became visible as soon as the first batch of vaccinated piglets entered the finishing unit and their average individual weight was 3.0 kg higher, as compared to the three previous batches. Average individual weight in the 11 following batches vaccinated was improved by 3.6 kg.

#### **Discussion and Conclusion**

Ear necrosis is a multifactorial condition with a genetic and an infectious component. PRRSV, as PCV2, have been associated with its occurrence. In this case, the only change was in the PRRS vaccination protocol. Results suggest that the addition of PRRS vaccine in piglets at early age might have had a positive impact against PRRSV circulating in the nursery. This field report provides some evidence of the importance of PRRS control in piglets at early age.

# CONTROL OF THE HORSENS HIGHLY-PATHOGENIC RECOMBINANT DUAL-MODIFIED-LIVE PORCINE REPRODUCTION AND RESPIRATORY SYNDROME VIRUS VACCINE-STRAIN IN A DANISH SWINE FARM

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## **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major cause of losses in swine farms worldwide. The occurrence of potentially high-virulent mosaic recombinants between two or more strains are well-described, between field strains, field strains and modified live virus (MLV) vaccine strains and MLV strains alone. The Horsens is a highly-virulent recombinant of two MLV PRRSV-1 strains. Here we report a case of viral farm control of the Horsens-PRRSV-1 with an inactivated PRRSV-1 vaccine (INV-1).

## **Material and Methods**

A former PRRSV free Danish sow-to-30kg farm, with continuous-flow farrowing facilities, was infected by the Horsens strain via seminal doses of an infected boar-stud. A severe clinical outbreak commenced Q3'2019 with high losses and extensive viremia demonstrated by PCR in both sows and nurseries. An initial double mass vaccination of all breeding-stock with INV-1 (Progressis®, Ceva, France) November and December 2019, was followed by vaccinating young gilts once with PRRSV MLV in quarantine, boosted 4 and 8 weeks later by INV-1. Out of quarantine, pregnant females were boosted 3 weeks prior to each farrowing. Farm quarterly production data are reported and serum was investigated by PRRSV-PCR and antibodies (PRRSV-Ab) in sows and PRRSV-Ab in weaners.

## Results

Quarterly production data from Q3'2019 to Q3'2020: Pre-wean mortality: 34.0%, 14.4%, 11.3%, 14.0%, 15.5%. Wean-30kg mortality: 4.6%, 17.1%, 4.6%, 3.8%, 4.2%. Live-born/litter: 16.7, 13.2, 17.2, 17.6, 18.4. Still-born: 14.1%, 25.2%, 14.2%, 14.2%, 13.7%.Serum sampling at 02.03.2020 & 27.08.2020 of 15 female breeding-stock and last-week weaners each timepoint respectively, revealed negative PRRSV-PCR and stable uniform PRRSV-Ab in the sows. The last-week weaners were very low level PRRSV-Ab positive in the first sampling and all negative in the second sampling.

## **Discussion and Conclusion**

The applied PRRSV-INV-1 protocol appears to restore full productivity and fully control the circulation of the virulent recombinant Horsens-PRRSV-1.

# PRELIMINARY DETECTION OF PRRSV, PCV2 AND HEPATITIS E VIRUS IN ORAL FLUID, FAECES AND POOLED SERA SAMPLES STATUS AT SIX SLOVENIAN PIG FARMS

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## **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Porcine Circovirus Type 2 (PCV2) and Hepatitis E virus (HEV) are important viral disease causative agents detected in pig oral fluid (OF), faeces and serum during infection. The objective of our study was to evaluate which sample provides the most adequate result.

## Material and Methods

Group samples of OF, faeces and 10 individual sera were obtained from 6 pig farms from different pig categories: 5 week-old (w/o), 7 w/o, 9 w/o, 11 w/o, 13 w/o weaners, fatteners and breeding sows. Nucleic acid was isolated with QIAamp Viral Mini kit (Qiagen, Leipzig). 10 L of OF and faeces eluents from each category were pooled to determine presence of the viruses on tested farms; when positive, then faeces, OF were tested separately for each pig category. If both OF and faeces tested negative, two pools of 5 individual sera were tested for all three pathogens to confirm the initial result. Samples were compared using Fisher's exact test, p-values less than 0.05 were considered statistically significant.

## Results

PRRSV and HEV were found on one farm and PCV2 was found on three. For PCV2 detection, Fisher's exact test has proven OF samples are more appropriate for detection than faeces (p<0.05). It happened twice, that PCV2 was not found in OF or faeces, but was later confirmed in the pooled sera samples, whereas for PRRS and HEV the initial negative result in OF and faeces was negative in sera pools as well.

## **Discussion and Conclusion**

In 3 different samples, positive results were more often obtained from OF than from faeces or serum pools of the same pig category; statistically significant difference was only confirmed for PCV2, where OF was considered to be more appropriate sample for detection than faeces. We confirmed OF holds strong diagnostic value for detection of these viruses.

# MATERNAL ANTIBODIES OF OFFSPRING BORN TO GILTS THAT WERE VACCINATED WITH INGELVAC PROVENZA® AS NEONATAL PIGS

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## **Background and Objectives**

Maternal antibodies (MAB) to Influenza A virus (IAV-S) inhibit response to killed IAV-S vaccine but strong MAB do not reduce mortality of pigs when infected with IAV-S. Therefore piglets must form their own IAV-S immune response. This can be achieved using a live attenuated influenza vaccine (LAIV) at an early age such as Ingelvac Provenza® (Boehringer Ingelheim Animal Health USA Inc., St. Joseph, Missouri). The objective of this evaluation was to determine the MAB status of piglets born to gilts which were vaccinated with INGELVAC PROVENZA as suckling pigs compared to offspring of older sows on the same farm where dams receive semiannual mass vaccination with a custom made IAV-S vaccine (CMV; Newport Laboratories Inc., Worthington, Minnesota).

## Material and Methods

Beginning February 2018, all pigs at a sow farm were vaccinated intranasally with 1ml of INGELVAC PROVENZA at 4-7 days old. A CMV was administered to all females in March 2019. In April and August 2019, serum samples were collected from 60 piglets at 10-15 days old; half born to dams that were vaccinated with INGELVAC PROVENZA as neonates and half born to older parity sows. Samples were tested using IDEXX Swine Influenza Virus Ab Test (IDEXX Inc., Westbrook, Maine) at ISU-VDL.

#### Results

ELISA showed all samples collected 6 weeks post-CMV were positive with strong MAB (average S/N=0.129 (positive S/N<0.600)) regardless of dam LAIV status. Conversely, samples collected 20 weeks post-CMV from offspring of gilts that received LAIV were 63.4% positive (average S/N=0.483), while piglets born to non-LAIV females were 93.4% positive (average S/N=0.263). The odds ratio that offspring born to an older sow would have positive MAB was 8.11.

#### **Discussion and Conclusion**

This study provides preliminary evidence that LAIV in replacement gilts and suckling pigs, the key subpopulations promoting IAV-S transmission at the sow herd level, helps to stimulate piglet immunity.

# EVALUATION OF THE DIAGNOSTIC PERFORMANCE OF REALPCR\* CSFV RNA TEST, A NEW REVERSE TRANSCRIPTASE REAL-TIME PCR FOR DETECTION OF CLASSICAL SWINE FEVER VIRUS (CSFV) RNA

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## **Background and Objectives**

Classical swine fever (CSF) is a highly contagious, OIE-listed disease of pigs that is endemic in parts of Eastern Europe, Asia and Central and South America. Prompt laboratory confirmation in CSF-suspected cases is a key part of control and elimination strategies in CSF-free regions.

This study evaluates the performance of a new RT-PCR for the detection of CSFV RNA.

## **Material and Methods**

Fifty CSFV isolates (genotypes 1.1, 1.2, 1.3, 1.4, 2.1, 2.2, 2.3 and 3.4) were used to test the inclusivity of the RealPCR CSFV RNA Test, PCR-1 [IDEXX Laboratories, Inc., Westbrook ME, USA]. Sixty CSFV-positive blood, serum and tissue (kidney, lymph-node, spleen and tonsil) samples and 80 samples (blood, serum, fecal swabs, oral swabs and tissues) from experimental infections were used to compare the performance of PCR-1 and an accredited PCR (PCR-2). The limit of detection (LOD) of PCR-1 was analyzed using log10 dilutions of three CSFV-positive sera and compared to PCR-2 and a commercial PCR kit.

This study was performed at the Institute of Virology, University of Veterinary Medicine Hannover, Foundation, Germany.

## Results

The inclusivity study showed that PCR-1 detected all the strains and genotypes tested, representing the genetic variability of CSFV. Compared to the accredited PCR, PCR-1 showed comparable or improved sensitivity, with lower average Ct values in all sample types, particularly in tissues. PCR-1 correctly identified 80 samples (100%) from experimentally infected pigs whereas PCR-2 failed to detect one blood and two fecal swab samples containing low genome loads. At the highest PCR-positive dilution, only PCR-1 was able to detect CSFV RNA, showing a LOD of 1 log10 better than the other PCRs tested.

## **Discussion and Conclusion**

This study shows that the RealPCR CSFV RNA Test is highly inclusive, with better diagnostic sensitivity and LOD than the other PCR tests evaluated.

## PREVALENCE OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) IN LIVE BORN CRUSHED SUCKLING PIGLETS

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## **Background and Objectives**

Porcine circovirus type 2 (PCV2) is an important pathogen in the pig population and causes major economic losses to the pig industry worldwide. While horizontal transmission is common, vertical transmission can occur during gestation, throughout birth or by contact to the mother or the farrowing environment. The present study aimed to examine the occurrence of PCV2-infections in live born crushed suckling piglets.

## **Material and Methods**

Tissue pools consisting of lung, heart, spleen, inguinal lymph node and thymus from 211 piglets from 13 German and 3 Austrian piglet producing farms were examined for the presence of PCV2-genome by PCR. Moreover, single tissue examination was conducted to evaluate the distribution of PCV2-genome in different organs. The age of the piglets ranged from 1 to 25 days.

#### Results

In 87.5% of the examined farms, 57.8% of the examined litters and 53.1% of the examined piglets PCV2-genome was detected. The prevalence of PCV2-DNA positive piglets in the 16 farms ranged from 0% to 84.6%. The thymus displayed the most frequent PCV2-DNA positive tissue (55.6% positive samples), followed by the inguinal lymph node (54%), spleen (52.4%), lung (50.8%) and heart tissue (44.4%).

## **Discussion and Conclusion**

These results show a frequent occurrence of early PCV2-infection of suckling piglets in German and Austrian piglet producing farms. However, this study cannot provide insight into the exact time of infection with PCV2, as this study was conducted on live born piglets and it is possible that PCV2-infection happened intrauterine during gestation or early after birth. Nevertheless, it can be assumed that the frequent detection of early PCV2 infected piglets is of major importance for the horizontal distribution of this pathogen within a farm. The examination of crushed piglets instead of euthanized individuals is an important issue considering animal welfare and the 3R (reduction, refinement, replacement) in animal testing.

# INTERDEPENDENCY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS AND SWINE INFLUENZA A VIRUS IN UK PIG FARMS.

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## **Background and Objectives**

Porcine Reproductive and Respiratory Syndrome virus (PRRS) and Swine Influenza A virus (SIA) are increasingly reported as concurrent infections and can be highly impactful on productivity in growers. The objective of this study was to investigate the interdependency of PRRS and SIA in UK pig farms.

## Material and Methods

Blood or oral fluids from growing pigs was submitted to SciTech Laboratories, UK, from clinical cases or general health control. They were investigated by either ELISA in blood against PRRS or SIA or by PCR on oral fluids for the presence of PRRS or SIA. Samples were from the veterinary practitioner's routine diagnostic samples and no fixed protocol was used. Each submission was reviewed and only the 86 farms where it was possible to make a clear farm diagnosis of the presence of both pathogens were included. Farms were allocated in 4 groups: PRRS+/SIA+, PRRS+/SIA-, PRRS-/SIA+ and PRRS-/SIA-. A Chi-square test and a Relative Risk (RR) was performed.

## Results

The Chi-square value was 18.1 and highly significant (p=0.00002). The RR for having SIA if the farm was also PRRS+ was 4.1 (p=0.0026) and the RR for having PRRS if the farm was also SIA+ was 2.0 (p=0.0003).

#### **Discussion and Conclusion**

The study concludes that the risk of being infected with PRRS or SIA increase if the other pathogen is present. This highlights the importance of concurrent infections and the necessity to investigate for both pathogens in clinical cases that are not clearly single factorial. It is not possible to reach a single conclusion for the reason of this interdependency. Obvious suggestions could be that the immunosuppression caused by one opens the door for the other, poor internal or external biosecurity, exposure from neighboring farms in pig dense areas or poor control of the health of purchased pigs

## TECHNICAL-ECONOMIC IMPACT OF PRRSV CIRCULATION IN A WEAN-TO-FINISH PRODUCTION FARM

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## **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) is one of the pig diseases with the highest economic impact; however, French farmers still ask for proof of the return on the investment in piglet vaccination.

## Material and Methods

In this field study, vaccination was implemented on a wean-to-finish farm in Western France that receives PRRSV-free piglets from a single source. That wean-to-finish farm broke with PRRSV at the beginning of 2018. The farm's veterinarian visited the farm in August 2018, after being called because of deterioration of growth performances and losses rate over the first half-year, in association with coughing from 12 weeks of age onwards. Serological profiles identified positive animals at 16 weeks of age; vaccination was implemented on piglets at 35 days of age, with Suvaxyn® PRRS MLV (starting in September 2018), together with a review of ventilation and internal biosecurity measures.

#### Results

The farmer noticed a major improvement in the clinical condition of finishers from the first batches of PRRS vaccinated pigs. When comparing the performance of the batches produced over the first half-year of 2018 to those produced over the same period of 2019, a decrease in mortality rate (-2.2%), and in the age at departure (-6 days) were observed, together with an increase in the wean-to-finish ADWG (+32 g/d) and in the live-weight at departure (+4 kg).

## **Discussion and Conclusion**

This field trial confirms that piglet vaccination in the face of active PRRS virus circulation is a key factor in lowering the clinical impact of respiratory disease complex. Further cost calculation showed a return on investment of systematic piglet PRRS vaccination of  $3.2 \notin$ /pig, including the cost of the vaccine. This result allows the veterinarian to justify the changes in the farmer's daily routine, which led to improved animal health, animal welfare and farmer income.

# IMMUNE RESPONSE AND PROTECTIVE EFFICACY OF TYPE 1 PRRSV MLV WHEN ADMINISTRATED INTRAMUSCULARLY (IM) OR INTRADERMALLY (ID) AGAINST SINGLE HP-PRRSV OR CO-CHALLENGE WITH TYPE 1 PRRSV

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## **Background and Objectives**

Co-existence of both genotypes of PRRSV had been endemic in several Asian countries, including Korea, Vietnam, and Thailand. The co-infection of both genotypes complicates successful control program. Therefore, the objective of this study was to evaluate efficacy of Type 1 PRRSV MLV (UNISTRAIN PRRS) when vaccinated via IM or ID against HP-PRRSV or co-challenge with Type 1.

## **Material and Methods**

Forty-two, PRRSV-free piglets were randomly allocated into 7 groups (n=6). GI and G4 were IM groups (2 ml/pig). Meanwhile, G2 and G5 were ID groups (0.2 ml/pig). G3, G6, and G7 served as control. At 35 days post-vaccination (DPV), GI-G3 were challenged with HP-PRRSV and G4-G6 were co-challenged with Type I and HP-PRRSV intranasally. G7 was left as non-vaccinated/non-challenged group. Blood samples were collected at 0, 7, 14, 21, 28, 35 DPV, 7, 14, and 35 days post-challenge (DPC). Sera were analyzed for PRRSV-specific antibody using ELISA and SN assay, and PRRSV RNA was quantified using RT-qPCR. PBMC were isolated and used for in vitro stimulation to determine IFN- $\boxtimes$  using ELISPOT assay and quantify porcine IL-10 using ELISA. Pigs were necropsied at 7 and 35 DPC and lung lesion was determined.

# Results

Following vaccination, ID-groups had shorter viremia and lower PRRSV RNA compared to that of IM-groups. ID-groups had significantly lower IL-10 level than IM-groups, but IFN- $\boxtimes$ -PCs were significantly higher. Following challenge, viremia and lung score at 7 DPC were significantly lower in ID-groups compared to IM-groups.

## Discussion and Conclusion

In conclusion, the results demonstrated that Type 1 PRRSV MLV administered, either by IM or ID, provided protection against challenge with HP-PRRSV, either alone or co-challenge with Type 1 PRRSV as demonstrated by the reduction of lung lesion and viremia. Moreover, ID route might represent an alternative to improve vaccine efficacy as it provided lower IL-10 and higher IFN- $\square$ -PCs.

## PORCINE EPIDEMIC DIARRHEA: 6 YEARS OF SEROLOGICAL SCREENING IN BELGIUM

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## **Background and Objectives**

Porcine Epidemic Diarrhea (PED) is caused by the PED virus which induces diarrhea in pigs of all ages. Since 2000, the swine populations in many European countries, including Belgium, became seronegative. Since 2013 in the USA, cases of highly virulent strains were reported. In 2014, PED was again observed in European countries. First new reports of PED in Belgium date from 2015. Clinical signs were mild. An annual serological screening has been performed since 2014 to follow up the PEDV-status of the Belgian pig population.

## **Material and Methods**

In Belgium, all pig herds are obliged to analyse at least yearly a number of serum samples for the mandatory Aujeszky monitoring. Since 2014, an ad random selection of the available samples from the Aujeszky screening in sows was made for a serological screening for PEDV antibodies. Maximum 5 serum samples per selected herd were analysed. Yearly at least 334 sera samples of minimum 68 herds were analysed with IPMA to detected PEDV-specific IgG antibodies.

## Results

In the serological screening in 2014, all samples were negative. In the serological screenings of 2015 and 2016/2017 antibodies against PEDV were detected in 57% and 25% of the herds and 10% and 2% of the sows, respectively. A herd is considered as positive if at least one sow has antibodies against PEDV. Since 2018, antibodies were no longer detected.

## **Discussion and Conclusion**

In 2014, the Belgian pig population was serologically negative for IgG antibodies against PED. As a consequence of the detection of PED in Belgium in 2015, antibodies were detected in half of the investigated Belgian pig herds. These percentages decreased clearly in 2016/2017 and antibodies against PEDV were no longer detected in 2018 and 2019. Therefore, we concluded that there is currently no or only very low circulation of PEDV in Belgium.

# PRRS STABILIZATION IN A FARROW-TO-FINISH FARM USING MASS VACCINATION OF PIGS FROM 1 DAY OF AGE AND LONGITUDINAL MONITORING OF THE COMPLIANCE WITH BIOSECURITY

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## **Background and Objectives**

The aim of the study was to evaluate the results of a protocol aiming to control viral circulation using a MLV vaccine licensed from 1 day of age.

## Material and Methods

The study was conducted in a 250 sow-farrow-to-finish farm with one off-site fattening unit, and contaminated by PRRSv in November 2017. A mass vaccination with SUVAXYN® PRRS MLV of all the animals in both sites was conducted in December 2017 and one month later. Piglets were then batch-to-batch vaccinated twice at around 4 days of age and 3 weeks later. Strict biosecurity measures were implemented. Their observance was monitored: first using a calculating tool to check purchase of needles and vaccines, which were compared with the number of animals to be vaccinated; and second, using a geofencing system, named "Move & Improve", able to track people's movements to check consistency of comings and goings.Virological and serological monitorings were implemented at the end of the protocol in both sites. In 4 successive batches at 3 weeks apart, litters were RT-PCR tested at weaning using both blood (154 in total) and saliva samples (112 in total). In each finishing site, blood from 15 ear tagged pigs was taken every 3 weeks and tested by ELISA from the end of post-weaning to slaughter.

## Results

Consumption of needles and vaccines was quite consistent with the number of animals vaccinated throughout the protocol. Thanks to the Move & Improve tool, a biosecurity mistake was quickly detected and corrected in the off- site unit. PRRSv could not be detected in any sample in due-to-wean piglets and in growers.

## Discussion and Conclusion

All the analytical results confirmed the success of the stabilization program. We have now to improve external biosecurity measures in order to stop vaccination of the sows and regain a PRRS-free status.

# SWINE INFLUENZA STRAINS (IAV) FOUND IN SAMPLES FROM SWINE IN THE NETHERLANDS IN Q1 - Q3 OF 2019

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## **Background and Objectives**

Swine Influenza A viruses are highly prevalent in swine in The Netherlands. Both 'classical' and pandemic SIVstrains circulate in the Dutch swine herds. Identification of circulating strains was performed, important for the right choice of vaccine to apply in a herd, future vaccine development and monitoring of the zoonotic potential.

## **Material and Methods**

Nasal swabs, lungs, oral fluids or BALF were collected by Dutch veterinarians from pigs suspected of suffering from flu. Samples were sent to IVD in Hannover, Germany for diagnostic by PCR (VetMAX-Gold Influenza SIV-PCR swine). Results were expressed as negative (Ct-value >40), weakly positive (38-40) or positive (<38). Subtyping was done by PCR (SIV Influenza Subtypisierung Multiplex-PCR, Henritzi et al., 2016).

#### Results

A total of 42 sample-submissions from 38 herds were received, consisting of a total of 209 samples / pooled samples: 35 submissions nasal swabs (N=184), 2 submissions (N=7) lungs, 3 submissions (N=6) oral fluids, 1 (N=4) BALF and 1 of 7 nasal swabs and 1 BALF. In most cases nasal swabs were submitted pooled by 4 or 5 swabs in one pool. In 6 submissions pools were made by IVD. 33 Submissions had 1 or more positive results (78.5%). 29 Out of 36 submissions of nasal swabs were positive, 1/2 lungs, 3/3 oral fluids and 1/2 BALF were positive. A subtype was found in 39 samples out of a total of 23 submissions: 15 HlavN1, 8 HlhuN2, 3 H3N2, 7 Hlpdm strains, 3 reassortants HlavN2 and 1 HlhuN2 & H3swN2. In 2 samples only a Nlall or N2 could be found.

#### **Discussion and Conclusion**

The sampling technique used by Dutch veterinarians to detect SIV is often positive (78.5%). Both 'classical' H1N1, H1N2 and H3N2 and pandemic (H1pdmN2) strains were found, but also a reassortant H1avN2. Mixed infections are rare.

# VACCINATION OF SOWS AGAINST PORCINE CIRCOVIRUS TYPE 2 IN SUBCLINICALLY INFECTED HERD DOES NOT IMPROVE REPRODUCTIVE PERFORMANCE.

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## **Background and Objectives**

Porcine circovirus type 2 (PCV2) plays an important role in the development of several disease syndromes, collectively described as porcine circovirus-associated disease (PCVAD). Previous reports proved that even subclinical infections with PCV2 may negatively affect herd productivity. The objective of this study was to assess an impact of sows vaccination against PCV2 on reproductive parameters in high-performing sow farm.

## Material and Methods

The study was conducted in a PCV2 subclinically infected farm of high-health status. Standard PCV2 vaccination protocol in the farm included vaccination of piglets at 4 weeks of age (w.o.a), replacement gilts (31 w.o.a.) and sows (after weaning). (n=538) were randomly divided into 3 groups, according to PCV2 vaccination protocol applied in the next reproduction cycle. Sows were vaccinated intramuscularly with 1ml of Ingelvac Circoflex at 1<sup>st</sup> (n=183) or 28<sup>th</sup> (n=180) day after weaning. The last group (n=175) remained unvaccinated. Sows were artificially inseminated after natural return to heat. The number of liveborn, stillborn, mummified and weak piglets were compared (Kruskal-Wallis one-way ANOVA).

## Results

In summary, 538 parities were analysed (10483 total born, 392 mummified, 1342 weak piglets). No statistically significant results of observed parameters between groups were observed, regardless of the time of vaccination or the sows parity.

## **Discussion and Conclusion**

Previous studies have shown that vaccination of sows against PCV2 improved the reproductive performance of the subclinically infected sow herd. In the present study no impact of sows vaccination on the reproductive performance could be observed. Nevertheless, vaccination of replacement gilts, especially originating from high health status farms, against PCV2 may be beneficial to prevent PCV2-associated reproductive disease in their first litters.

MONITORING TIME TO STABILITY OF AN ACUTE PRRS-INFECTED SOW FARM COMPARING 3 DIFFERENT DIAGNOSTIC TESTS AFTER IMPLEMENTATION OF LCH-MODEL

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## **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) is a devastating disease causing high mortality and economic loss. Over the last decade the LCH-model has become a preferred and generally accepted way of eliminating PRRS.The aim of this study was I) to monitor Time to Stability of a PRRS-infected sow herd using the LCH-method including 2 x mass vaccination with Ingelvac PRRS MLV and 2) to compare 3 different diagnostic methods: a) Processing Fluids (PF), b) Placenta Umbilical Cord serum samples (PUCS) and c) blood PCR on due to wean piglets (DTW).

## Material and Methods

A 750 sow farm was infected with PRRS-type 2: LCH and mass-vaccination were initiated 1 month after introduction of PRRSv. After initial mass-vaccination the farm was vaccinated with MLV every 3 months because of PRRS positive farms nearby. Every second week for 40 weeks the herd was tested for PRRS by PCR on:Processing fluids on testicles and tale-cuts from all pigs born in the week of sampling PUCS on 25 placentas. Blood sampling of 60 DTW piglets

## Results

The number of weeks from first mass vaccination until all PCR-pools were negative was 14 weeks for PF, 30 weeks for PUCS and 24 weeks for DTW piglets.

#### **Discussion and Conclusion**

Implementation of the LCH-model was effective in eliminating PRRS from this herd. In this study we found the Time to Stability on DTW-piglets to be 24 weeks, which correlates with findings by other authors. PF seems to be a less sensitive method as it was already PRRSv-negative in week 14 despite that the herd at this time was still positive on PUCS. This can be due to a large sample-size as we included up to 500 piglets in the PF-sample. When determining time to negative, both sensitivity of the test and sample size are important parameters, as the PRRS-prevalence will be extremely low towards negativity.

## PCV2 INFECTION DYNAMICS IN GILTS, SOWS AND PIGLETS ON BELGIAN FARMS

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## **Background and Objectives**

The aim of this study was to monitor the PCV2 infection dynamics in gilts, sows and piglets and if necessary to optimize the PCV2 vaccination schedule.

## **Material and Methods**

Blood samples were collected on 9 farms. In each farm, 5 sexually mature gilts at entry in the quarantine, 5 gilts aged 3-4 weeks older, 10 sows of different parities, 10 piglets at an avg. age of 3,5 weeks (PCV2 vaccination age) and 10 piglets 3-4 weeks older were sampled. A PCV2 qPCR test was performed on pooled samples of gilts and piglets. A PCV2 antibody ELISA (SERELISA® PCV2 Ab Mono Blocking, Synbiotics) was performed on samples of sows and piglets at vaccination age. Titers > 350 are positive and the maximum titer equals 2484.

#### Results

Viremic gilts were found on 2/9 farms and 3/17 pooled samples were qPCR positive. Antibody titers of all sows were positive and the median titer was 2484, indicating that 50% of the sows had a maximum antibody titer. Antibody titers of piglets at vaccination age, representing the maternal antibody status, show a median titer of 1319 (min = 150; max = 2484; QI = 610; Q3 = 2484). In total 3/36 pooled samples of piglets were qPCR positive, indicating an early PCV2 infection. These pools were all from one farm.

## **Discussion and Conclusion**

Viremic gilts in the quarantine require a check if they were properly vaccinated as piglet and if biosecurity measures are respected. Farm differences were observed in piglet antibody titers. Farms vaccinating the sows showed the highest piglet antibody titers. Research is needed to better understand the relationship between maternal antibodies at vaccination and PCV2 vaccine efficacy. It is also important to check early PCV2 infection, since vaccinated piglets need to develop protective immunity before PCV2 infection.

# AIR SAMPLE AS A WELFARE FRIENDLY SUBSTITUTE FOR NASAL SWAB TO DETECT SWINE INFLUENZA A VIRUS IN PIG POPULATION?

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## **Background and Objectives**

This field study aimed at assessing the ability to detect swine influenza A virus (swIAV) in bioaerosols collected at the pig's breathing zone compared to samples taken from live animals.

## Material and Methods

The study was carried out in five batches of pigs with influenza-like syndrome (rectal temperature  $\ge$  40.5°C, coughs, sneezes). In each batch, nasal swabs were taken from a sample of six pigs with hyperthermia. Then, three groups of two pigs were formed. Bioaerosol samples were collected at the breathing zone (5 to 100 cm from the pig) of the two animals using a portable, silent and self-contained air sampler based on electrostatic precipitation (BIODOSI®, CEA-Tech). Dust particles were collected on a stainless steel ring placed in the sampler. The ring was then removed and placed into a transport medium. Three sampling times (5, 10 and 15 min) and two volumes (2 and 12.5 ml) of minimum essential medium (MEM) for transport were tested. All samples were analysed by M-gene real time RT-PCR.

#### Results

In the three batches where swIAV genome was detected in nasal swab supernatants, 4–6 pigs tested positive with Ct values ranging from 17.2 to 38.1. In those batches, at least one air sample also tested positive, with the 5min\*12.5ml MEM sampling conditions having the lowest detection rate (5/9 positive samples) and the 15min sampling time the highest (9/9 positive samples with 2 or 12.5 ml MEM). Ct values of air samples ranged from 31.4 to 39.5.

## **Discussion and Conclusion**

This field study showed that swIAV may be detected in the air from infected pigs using handheld electrostatic precipitator device. Although the low apparent genomic loads in these air samples (Ct>30) did not permit to investigate the virus infectivity, air sampling at the pig's breathing could be a promising way to conduct non-invasive viral surveillance among swine farms.

## EMERGING AND IMPACT OF PORCINE CIRCOVIRUS TYPE 2D IN DENMARK

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## **Background and Objectives**

Porcine circovirus type 2 is a small DNA-virus, which can be divided into genotypes based on genetic differences. During recent years, a new genotype (genotype d) has evolved to be the most prevalent genotype in Asia and North America. Furthermore, some studies have indicated that this genotype induces more severe clinical signs and has been related to vaccine failures in the field. The objective of this study was to investigate the genotypes presently circulating in Denmark and to relate the genotype to clinical signs and/or decreased efficacy of PCV2 vaccines.

## **Material and Methods**

Archived samples from herds collected between 2014 and 2018 were included. These samples had previously been tested positive for a moderate to high level of PCV2 by qPCR. In addition, samples received in 2017-2018 from case herds with PCV2 associated clinical signs (wasting, un-thriftiness, poor performance) or typical pathological signs (enlarged lymph nodes, wasting) were included. PCV2 was genotyped by full genome SANGER sequencing of PCR products followed by phylogenetic analysis including representative virus sequences retrieved from GeneBank.

## Results

The sequencing revealed that PCV2b was the only genotype identified in 2014-2016, whereas both PCV2b and PCV2d genotypes were detected in 2017-2018. In two herds, both PCV2b and PCV2d were identified in the same sampling. Interviews with owners of the case herds revealed in all cases that the PCV2 related problems had been controlled either by change in management or by vaccination.

## **Discussion and Conclusion**

This study revealed that PCV2d is evolving also in Denmark, but did not sustain that PCV2d has been associated with more severe disease in Danish pig herds. Furthermore, the available vaccines seem to be effective, also towards the PCV2d genotype.
# THE PATTERNS OF DETECTION AND GENETIC DIVERSITY OF PORCINE CIROVIRUS TYPE 3 (PCV3) IN EIGHT POLISH PIG FARMS

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## **Background and Objectives**

Porcine circovirus type 3 (PCV3) is spread globally, but the biology of infection and its role for swine health remains unknown. The objective of this study was to investigate the prevalence of PCV3 in different clinical materials and to assess the genetic diversity of the virus in Poland.

# Material and Methods

Samples of serum, feces and oral fluid were collected from 6-10 pigs, at 3-20 weeks of age, from 8 farms. Samples were pooled by 4-6 before DNA extraction and tested with in house quantitative real time PCR (Ct≤37 were considered positive). Overlapping PCV3 genomic fragments were amplified from selected virus positive samples and sequenced in order to obtain complete genome sequences of Polish strains.

#### Results

PCV3 was detected in all farms in at least one sample type. On average, 50.8% of oral fluid samples were positive for PCV3 and viral loads ranged from 2.5 to 6.6 log<sub>10</sub> copies/mL. Overall, 23.5% of fecal pools reacted positive with viral loads from 2.5 to 5.5 log<sub>10</sub> copies/mL. PCV3 viremia was detected in 31.4% of serum pools. The level of PCV3 viremia was from 2.8 to 5.5 log<sub>10</sub> copies/mL. Eleven complete genome sequences were obtained. Phylogenetic analysis indicated that Polish sequences show genetic diversity similar to previously described in other countries.

## **Discussion and Conclusion**

Our results indicate that PCV3 is likely to be widely spread in Polish pig farms, which differ in the virus detection patterns. Generally, the viral load are similar to those observed in subclinical infections with porcine circovirus type 2 (PCV2). Interestingly, PCV3 can be shed with feces even by non-viremic pigs.

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# THE IMPACT OF VACCINATION AGAINST PORCINE CIRCOVIRUS TYPE 2 (PCV2) ON THE VIRUS DETECTION IN DIFFERENT CLINICAL MATERIALS AND GENETIC DIVERSITY

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# **Background and Objectives**

Vaccination against porcine circovirus type 2 (PCV2) is highly efficacious in controlling clinical PCV2 diseases, but the virus remains highly prevalent on farms. The aim of the study was to compare PCV2 detection rates and quantity in clinical materials from farms with different strategies of vaccination against PCV2.

# Material and Methods

Serum, feces and oral fluids from 11 farms with piglet vaccination (VACI) and 11 farms with vaccination of sows and their progeny (VAC2) were obtained. Samples were pooled by 4-6 before DNA extraction and tested with qPCR. Fisher and Mann-Whitney tests were used for statistical comparison. Additionally, nucleotide sequences of PCV2 ORF2 were determined from selected samples.

## Results

In five farms, no viremia or shedding with feces was detected. The virus was the most common in oral fluids (52.7% and 38.8% positive samples from VAC1 and VAC2, respectively). PCV2 was less frequent in feces pools and it was found in 39.9% pools from VAC1 and 23.7% from VAC2. 28.0% and 5.6% of serum pools from VAC1 and VAC2 reacted PCV2-positive, respectively. Significant reduction (p<0.05) of PCV2 detection rates and viral loads was observed in VAC2 in comparison with VAC1 farms. Three genotypes, PCV2a, PCV2b and PCV2d, were detected.

# **Discussion and Conclusion**

PCV2 remains highly prevalent in Polish pig farms, but the lack of viremia or shedding with feces from five farms indicates that the infection can be successfully controlled. No link between a specific PCV2 genotype and viral load was observed. Combined vaccination of sows and their progeny seems to be more efficacious in eliminating PCV2 viremia than vaccination of only piglets. However, poor biosecurity and hygiene, as well as incorrect vaccine handling and administrations, may hamper vaccination efficacy.

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## RESILIENT EFFECTS OF SGKI AND TAPI DNA MARKERS DURING PRRSV OUTBREAKS IN REPRODUCTIVE SOWS

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# **Background and Objectives**

The genetics of pig resilience is a key issue to overcome stress. In pigs, a major stressor is the porcine reproductive and respiratory syndrome virus (PRRSV), which causes serious health problems and productivity drops in farms. Based on reported regions in chromosome 1 and 7 influencing reproductive performance and immunity response during PRRSV outbreaks, we selected SGK1 and TAP1 as candidates for resilience.

# Material and Methods

Reproductive data from a PRRSV-positive stable sow farm was collected for almost 3 years until a PRRSV epidemic outbreak occurred in 2017. First, we screened genetic variability of SGK1 and TAP1 loci by Sanger sequencing. Next, we genotyped DNA from 315 Landrace x Large White sows for three mutations by high resolution melting including a 3'UTR variant of SGK1 gene, SGK1\_e15 (rs338508371, C>A); and two synonymous variants in TAP1 gene, TAP1\_e1 (rs1109026889, C>T) and TAP1\_e5 (rs80928141, C>T). The effect of the different markers on reproductive traits has been evaluated by mixed effects model.

#### Results

Our study reports that TAP1\_e1 affected the number of total born (P<0.05), stillborn (P<0.05) and total losses (P<0.05). However, there is no relation with the epidemic status of the farm. On the other hand, SGK1\_e15 and TAP\_e5 showed evidence of resilience during an outbreak affecting the number of born alive (P<0.05) and the number of mummies, respectively (P<0.001). Thus, in the PRRSV outbreak, SGK1\_e15 AA sows had around 2 less piglets born alive per parity (P<0.01) as compared to AC sows and TAP\_e5 TT sows around 1 mummy more than CC sows (P<0.001).

# **Discussion and Conclusion**

Our results indicate that SGK1 and TAP1 are potential markers for resiliency in PRRSV-infected sows as long as they are associated with the number of born alive and the number of piglets lost during a PRRSV outbreak.

## CALCULATION OF TIME TO PRRSV-STABILITY AND PRODUCTION LOSSES IN A FRENCH BREEDING UNIT

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# **Background and Objectives**

The aim of the study was to calculate the time to PRRSv-stability (TTS), the time-to-baseline-production (TTBP) and total losses in a European context.

## Material and Methods

The study was conducted in a breeding 1000-sow herd, conducted in 10 batches (farrowing each 2 weeks, weaning age at 21 days). After the detection of the contamination by a PRRSv-1 strain in January 2018, a mass vaccination protocol, using ReproCyc® PRRS EU, associated with strict internal biosecurity measures was implemented aiming to achieve PRRS stabilization. The success of the vaccination protocol was determined as the failure to detect PPRSv RNA in serum of pre-weaning pigs by RT-PCR, tested in 4 different batches.TTS is defined as the time in weeks taken to produce PRRSv negative piglets. Day 1 was the day of the 1<sup>st</sup> mass vaccination.TTBP is defined using statistical process control methods to represent time to recover the number of pigs weaned per batch that the herd had before PRRSv contamination. The data from the 20 batches prior to the outbreak were used as baseline data. A kappa (weight) of 0.400 and 3 sigma were used to define the control limits (according to Linhares 2017).Total losses was measured by the number of pigs not weaned per 1000 sows between the time of PRRSv detection until TTBP was reached.Statistical analysis were performed using SAS 9.2 software.

#### Results

TTS was 22 weeks, TTBP was 7.66 weeks and total losses were 2421.68 piglets.

## **Discussion and Conclusion**

This study is the first one, in our knowledge, aiming to calculate TTS, TTBP and total losses in our European context. Face to a PRRSv-1 contamination in a naïve herd, TTS and TTBP were below those previously described in the US after a PRRSv-2 outbreak but total losses calculation was similar.

## PRICIPLES OF PRRSV ERADICATION IN HUNGARIAN SWINE HERDS

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# **Background and Objectives**

In EU with traditionally significant pig industries, the prevalence of PRRSV infections is high. Four countries PRRS-free: Norway, Sweden, Finland, and Switzerland. Therefore, the Pig Strategy of Hungary and the Foodchain Safety Strategy 2013–2022 prioritise the need to eradicate PRRSV. Uniquely, National PRRS Eradication Programme was introduced for the implementation of these strategies. The aim of the programme is to achieve long-term PRRSV-free status of all pigs on the territory of the country in order to reach a more efficient, economical and competitive international market position. Hungarian swine production is divided into two groups: backyard farms (<100 animals) and economic entities (>100 animals), mostly (85%) farrow-to-finish farms.

# Material and Methods

At the beginning, 34 761 sows were kept in backyards, and 118 178 sows on large-scale pig farms in Hungary. PRRSV prevalence in backyard farms was 3.7%, while on industrial farms it reached 38%. The legal basis of the programme is the PRRSV Eradication decree of the Minister, which was approved by the relevant EU veterinary committee and came into force after consultation with the key representatives of the pig industry, breeders, integrators, the veterinary authority and scientific experts. Method used for PRRS eradication in backyard farms was test and removal, and in industrial farms mainly depop-repop, or immunisation, monitoring and changes in farm management practices.

## Results

By the end of 2018, as a result of the programme, 10 out of the 19 counties and 120 out of 174 districts in Hungary became free from PRRS.

# **Discussion and Conclusion**

Principle of the National PRRSV Eradication Programme is the specification of a mandatory herd qualification regime for all pig farmers irrespective of the number of animals kept. Eradication is based on territorial principles, and results in a time-limited eradicating programme. Regular laboratory monitoring of swine herds plays a key role in the Hungarian PRRSV eradication process.

# APPLICATION OF NETWORK ANALYSIS TOOLS IN THE HUNGARIAN PRRSV ERADICATION PROGRAMME

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# **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major pathogen of swine, causing severe economic losses worldwide. In the past decade, multiple systems biology and network analysis tools have become available and applied in microbiology and epidemiology successfully.

## **Material and Methods**

Clinical samples were tested for the presence of PRRSV with the virotype PRRSV RT-PCR Kit. Complete ORF5 was amplified according to published protocols. Sequencing was performed using the Sanger method. 206 Hungarian sequences were generated, and together with 108 reference PRRS sequences, they were subjected to classical and network based phylogenetic analyses. Classical phylogenetic analysis was performed by the MEGA 6.0 software using the maximum likelihood algorithm. To generate network from ORF5 sequences, alignment scores were calculated by Needleman–Wunsch and Smith–Waterman algorithms for global and local pairwise sequence alignments. To generate a network where each viral sequence is a member, and their connections are represented with high alignment scores, we applied Prim's algorithm to identify a minimal spanning tree. As in these spanning trees the number of connections is limited, we enriched each node with high alignment score edges, if they were applicable.

# Results

The network, generated from 314 Hungarian and reference PRRSV ORF5 sequences derived from biological samples from swine holdings infected with PRRSV showed scale-free characteristics. High agreement observed between network modules and the standard clade (12) or lineage (16) based classification of individual sequences. By applying network analysis methods, we identified novel ways of infections between individual pig holdings and previously unknown epidemiological connections between infected herds. With the network analysis tool, we visualized the virus spreading in time, and observed important characteristics of vaccine strains.

#### **Discussion and Conclusion**

The network-based visualization of ORF5 sequence similarities are easy to use by field scientist, veterinary authorities in the national PRRS erradication program, and complementary to the classical phylogenic-tree based representation.

# GENOTYPING OF PORCINE CIRCOVIRUS 2 (PCV-2) IN VACCINATED FARMS DIAGNOSED WITH PCV-2-SYSTEMIC DISEASE

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# **Background and Objectives**

Vaccination against Porcine circovirus 2 (PCV-2) is a common practice all over the world. Vaccines prevent clinical disease but not infection. However, PCV-2-systemic disease (PCV-2-SD) is occasionally diagnosed in vaccinated farms. The present work aimed to assess the viral genotypes associated to disease cases in vaccinated farms in Spain (period 2009-2018).

# Material and Methods

Thirty-five cases diagnosed as PCV-2-SD at the Servei de Diagnòstic de Patologia Veterinària (UAB, Spain) were selected. They corresponded to pigs from PCV-2 vaccinated farms collected between 2009 and 2018, which displayed clinical signs compatible with the disease, lymphocyte depletion and granulomatous inflammation of lymphoid tissues and moderate to high amount of PCV-2 antigen detected by means of immunohistochemistry. The age of the animals varied between 8 to 16 weeks. DNA was extracted from the lymphoid tissues included in paraffin blocks, and the whole PCV-2 Cap gene was amplified by PCR. PCR products were purified, sequenced and genotyped.

# Results

PCV-2 Cap gene from all cases was successfully sequenced and genotyped. Two different genotypes were retrieved from these cases: PCV-2b (n=18) and PCV-2d (n=17). Only PCV-2b sequences were obtained between years 2009 to 2012 (14/18), and the remaining 4 sequences were found in the period 2015-17. In contrast, all PCV-2d sequences were found between 2014 and 2018.

# **Discussion and Conclusion**

The present study evidenced the dominance of PCV-2b and PCV-2d genotypes associated with cases of PCV-SD in vaccinated farms. The higher prevalence of PCV-2b associated disease cases before 2014 and the dominance of PCV-2d afterwards probably reflect the general prevalence of these genotypes over time. Although it has been suggested that PCV-2d could be more frequently involved in cases of PCV-2-SD in vaccinated farms, current results do not support such hypothesis.

## USE OF BIOPORTAL IN A PRRSV OUTBREAK IN A SPANISH FARM

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# **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the leading swine pathogens. The availability of sequence data from PRRSV from widespread geographic territories has enabled a better understanding of the fight against PRRS. New introduction of isolates and the evolving dynamics of the virus can be monitored by comparing sequences to previous viral isolates. Thus, the aim of this work was to monitor the genetic changes at a farm level by using Bioportal.

# **Material and Methods**

The study was conducted in a 1200 sows farrow-to-wean farm. Since August 2015, the farm was under a PRRS 5 step process control, including the implementation of three sow mass vaccinations (ReproCyc PRRS EU®) per year. Piglets receive a dose of Ingelvac PRRSFLEX EU® before weaning. During 2016 and 2017, we detected a resident strain (strain A), and since July 2019 we were able to detect a new strain (strain FAST). In order to evaluate the impact of the introduction of a new strain into the system several KPI (e.g. %fertility, %abortion) were analyzed. Two periods of time were analyzed, before and after the introduction of the new strain FAST.

## Results

We found out that strain FAST was highly prevalent in that area since 2016, specifically it has been detected in 72 different farms of 18 different companies. When comparing the productive results before and after the detection of the strain FAST, no differences were detected. The KPI were similar or even better after the detection of the new strain.

# **Discussion and Conclusion**

Results obtained by Bioportal give evidence that there was a new introduction of the FAST strain into the herd. Thus, Bioportal and improved control of external biosecurity were crucial to control the introduction of new sequences in a farm. Productive results revealed that vaccination prevented the herd from negative effects associated with PRRSV infection.

## STUDYING TYPE I PRRS SEASONALITY IN SPAIN

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# **Background and Objectives**

Some studies have identified a correlation between a new PRRSV infection and the season in North America. The intensive PRRS PCR diagnosis and the sequencing of these positive samples done by Boehringeringelheim Spain, since 2015, has generated a huge database to work with. Thus, the aim of this study is to report the evolution of the % of PRRS detection by PCR and the % of sequences done, during different periods (temperatures) of the year.

# Material and Methods

This study is a compilation of 4 years PRRS diagnoses. The periods have been divided in thirds (T): TI (December to March), T2 (April to July), T3 (August to November).We calculated Spanish mean temperatures, using weather AEMET stations. A total of 2818 samplings from 12 companies were included. The sequencing was provided whenever the veterinarians report clinical signs or changes in the PRRS status. To calculate the % of sequences by T, we used BI Spanish database (2430 sequences) coming from 102 different companies. Regression analysis, chi-2 test and comparison of means was conducted and p<0.05 was used as set up.

# Results

The proportion of positive samplings by T were 43% for TI and 39% for T2 and T3. No statistical differences were found in terms of % of diagnosis but a tendency (p=0.08) was observed for TI. The percentage of sequences done in every T was 42% for TI, 33% for T2 and 25% for T3, finding no statistical differences but T3 tended (p=0.07) to have the lowest percentage. A negative correlation between the mean monthly temperature and the percentage of sequences obtained by month were found (p=0.02; R2=19.12).

# **Discussion and Conclusion**

The percentages of positive diagnosis and sequences by third, tended to have higher values in TI. There was a negative correlation between mean monthly temperature and number of sequences found.

# PORCINE CIRCOVIRUS 3 DETECTION IN SERUM OF WILDLIFE SPECIES IN SPAIN

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# **Background and Objectives**

Porcine circovirus 3 (PCV-3) is an emerging member of the Circoviridae family. PCV-3 DNA is highly distributed among pigs and wild boars worldwide. Recently, its presence has been confirmed in Italian wild ruminants and related hematophagous ectoparasites. The present study aimed to assess the prevalence of PCV-3 in free-ranging wild ruminants and Lagomorpha species in Spain based on retrospective large-scale molecular survey and to perform a PCV-3 genetic characterization in the positive samples.

# **Material and Methods**

In total, 801 serum samples from hunted red deer (Cervus elaphus CE; n=108), roe deer (Capreolus capreolus CC; n=87), Pyrenean chamois (Rupicapra pyrenaica RP; n=133), Iberian ibex (Capra pyrenaica CP; n=92), mouflon (Ovis orientalis musimon OA; n=91), fallow deer (Dama dama DD; n =104), European rabbit (Oryctolagus cuniculus OC; n=101), and European hare (Lepus europaeus LE; n=85) were analyzed. PCV-3 DNA presence was investigated using a PCR method. Sequencing of Rep and Cap gene from positive samples was attempted and, when possible, aligned with available sequences in Genbank.

# Results

Three out of 801 (0.4%) serum samples were positive by PCV-3 PCR, corresponding to one red deer (0.9%), one mouflon (1.1%), and one fallow deer (0.96%). All samples collected from Lagomorpha species were negative for the presence of PCV-3 DNA. The partial Rep gene sequences were obtained from all three positive wild ruminants, while the complete Cap gene sequence was retrieved from the positive fallow deer. The obtained sequences showed a high nucleotide identity with a PCV-3 sequence from a Spanish wild boar but a low similarity with the virus detected in Italian chamois and ticks.

# **Discussion and Conclusion**

The present study indicated that wild ruminants and lagomorphs do not play a significant role in the epidemiology of PCV-3 in Spain, suggesting that these infections may be caused by eventual spill-over events.

# EVALUATION OF PCV2 MATERNALLY DERIVED ANTIBODY LEVELS AND PCV2 AND PRRSV GENOME DETECTION IN SERUM SAMPLES FROM 3-WEEK-OLD PIGLETS

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# **Background and Objectives**

Porcine Circovirus 2 (PCV2) and Porcine reproductive and respiratory syndrome virus (PRRSV) are two of the most important viruses in the swine industry worldwide. Sampling of piglets at weaning provides very useful information to adapt vaccine protocols to the farm conditions. The objective of this study was to assess PCV2 maternally derived antibody (MDA) levels as well as the presence of PCV2 and PRRSV genomes in 3-week-old piglets in Spanish farms.

## **Material and Methods**

This study was carried out in 35 farms located in different geographical areas of Spain. In these farms, no PCV2 sow vaccination programs were being applied. At 3 weeks of age, 10–15 piglets per farm born from different parity sows were randomly selected. They were bled and serum samples (n=367) were processed by PCV2 ELISA and PRRSV PCR (5 samples/pool). In 32 out of the 35 farms presence of PCV2 viremia was assessed by qPCR (5 samples/pool).

#### Results

The average PCV2 MDA-level detected in the tested farms was 0.71±0.37 S/P values. Nine out of the 35 farms (26%) showed very heterogeneous PCV2 antibody levels at weaning (>50% coefficient of variation [CV]). Sixteen out of the 35 farms (46%) presented at least one PCV2 seronegative animal at 3 weeks of age. High mean MDA levels (>1 S/P) were detected in 11% of farms and low mean levels (<0,35 S/P) in 9% of farms. PCV2 PCR-positive piglets were only found in one farm whilst in 37% of the tested farms, the presence of PRRSV was confirmed in 3-week-old piglets by PCR.

# **Discussion and Conclusion**

Instability of PCV2 and PRRSV in sow herds is demonstrated around the time of piglet vaccinations, posing a risk of reduced subsequent vaccine protective efficacy. This fact must be taken into account when establishing control measures against relevant diseases.

OPTIMIZED BIOSECURITY AND MANAGEMENT IN 40 FARMS/SITES RESULTED IN COMPLETE PRRSV CONTROL IN A HIGHLY PIG DENSE AREA OF DENMARK WITHIN 6 MONTH

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## **Background and Objectives**

Growing pigs represents the absolute majority of PRRSV infected pigs in an area. Growing pigs have a longer duration of viremia and shed PRRSV for a longer period than the adult breeding stock. Control of PRRSV circulation in growing pig populations is a combination of correct biosecurity, pigflow, management and immunization.

## **Material and Methods**

The study was conducted in 40 farms owned by 15 different producers and involved 10.035 sows from 8 farms, 53.230 nursery pigs placed in 14 different sites and 40.870 finishers placed 25 different sites. From the beginning of the study, all sow sites except one were stable positive by Ingelvac PRRS MLV vaccination or negative (following AASV definition). 3 nursery and 13 finishers sites were ELISA positive (exposed to PRRSV). A COMBAT (Comprehensive Online management Biosecurity Assessment Tool) survey was conducted in all herds to determine biosecurity level and to determine the need for improvement of management and pig flow. All sites received a follow up visit from a veterinarian to guide improvements.

#### Results

COMBAT revealed that PRRS positive sow sites (AASV classification 1 & 2vx) needed to improve handling of pigs (mixing age groups and weaned pigs in farrowing room), wash and disinfection procedures and introduction of semen from positive boar stud was risky. For finisher and nursery sites the flow of transportation vehicles, logistics for removal of dead pigs and movement of people between sites needed attention. Within 6 month, all nursery and finisher sites were PRRSV negative.

#### **Discussion and Conclusion**

Combination of improved biosecurity and pigflow management in a large number of herds in a very pig dense area, based on COMBAT risk evaluation, was able to eliminate PRRSV circulation in sow, nursery and finisher sites at the same time as immunization of breeding stock was systematically maintained. This is by far the largest successful PRRS control project in Denmark.

# DETECTION OF PORCINE CIRCOVIRUS TYPE 3 IN POLISH WILD BOAR POPULATION

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# **Background and Objectives**

Porcine circovirus type 3 (PCV3) is a newly described third member of the Circoviridae family able to infect swine. Viral DNA has been detected in pigs suffering a variety of clinical/pathological conditions, as well as in apparently healthy animals. The presence of PCV3 DNA was also reported in wild boar population in different countries. The aim of the present study was to assess the prevalence of PCV3 in Polish wild boar population and evaluate the frequency of PCV3 genetic material among wild boars of different age categories.

# **Material and Methods**

Serum samples from 284 wild boars culled during 2017/2018 and 2018/2019 hunting seasons were collected. Wild boars were numbered and categorized according to age (teeth method) and weight into three age groups: juveniles (up to 12 months; n = 20), subadults (12-24 months; n = 123) and adults (over 24 months; n = 141). The presence of PCV3 DNA was detected using real-time PCR method (source?).

## Results

In summary, 125 out of the 284 serum samples (44.01%; 95% CI, 38.36 - 49.83) were PCV3-positive. Animals infected with PCV3 were found in all age groups. However, the highest prevalence rate was detected in juveniles (60%;95% CI, 38.66 - 78.12); while in subadults and adults the prevalence was lower and reached 45.53% (95% CI, 37.00 - 54.33) and 40.43% (95% CI, 32.68 - 48.68), respectively.

# **Discussion and Conclusion**

The results of the present study clearly demonstrated that PCV3 is highly prevalent in Polish wild boar populations, especially in wild boars up to 12 months. Those observations support the role of wild boars as a possible natural reservoir for PCV3.

# AIR SAMPLING AS A USEFUL TOOL FOR DETECTING AND MONITORING PCV2 INFECTION

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# **Background and Objectives**

PCV2 is an environmentally resistant virus. This characteristic could be used to monitor infections by this virus using air samples. Therefore, we performed this work to try to establish a relationship between blood viremia and the viral load detected, by qPCR, in air samples.

# Material and Methods

For that, we monitored three consecutive fattening batches from a PCV2 unvaccinated farm which performed an AI-AO system. The first batch (n=360) was used as a control batch whereas the others were vaccinated with Suvaxyn PCV (Batch A, n=380) and Ingelvac CircoFLEX (Batch B, n=490). Each batch was monitored at 10, 12, 14, 16 and 18 weeks of age, taking blood samples from 60-65 pigs and six air samples

## Results

The first detection of viremia in the control batch occurred at 12 weeks of age, when it peaked with 4.71x10<sup>7</sup> PCV2 copies/blood ml. From this week onwards, viremia was still detected with prevalence rates between 24% and 100%. In vaccinated batches, viremia was first detected later: at 14 (Batch A) and 18 (Batch B) weeks respectively. The prevalence in these batches from the first detection of the virus to the end of the study was 1.7% and 1.6% respectively, with a maximum value of 9.55x10<sup>4</sup> PCV2 copies/blood ml.Whenever viremia was detected, viral load was also present in the air. Moreover, in the control batch and in Batch A, the virus was detected earlier in the air samples than in the blood ones. However, the highest viral load was found at the same time in both types of samples (4.26x10<sup>6</sup> PCV2 copies/1000L of air), subsequently decreasing and showing a strong correlation between them (R<sup>2</sup>=0.86; p=0.073).

# **Discussion and Conclusion**

The use of air samples enables the detection of PCV2 infections with a prevalence lower than 5%. Furthermore, this method can be used to monitor the infection in unvaccinated farms.

# STILLBIRTHS, ARTHROGRYPOSIS AND PREWEANED NERVOUS DISEASE: EVIDENCE FOR PORCINE CIRCOVIRUS 3 (PCV-3) INVOLVEMENT

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## **Background and Objectives**

Self-limiting disease involving multisystemic inflammation in stillborn and preweaned pigs with nervous disease was investigated in two herds.

# **Material and Methods**

Clinical and herd details were obtained and postmortem examinations with histopathology, in situ hybridization (ISH) to detect PCV-3 and virology (PCRs, sequencing, virus microarray) were performed.

#### Results

Widespread multisystemic inflammation, mainly lymphoplasmacytic, reflecting likely in utero viral infection was detected in tissues from seven stillborn piglets, four with arthrogryposis, and three 18-day-old piglets with postnatal tremor and paresis. One herd was affected in 2014, the other in 2018. Stillborn and arthrogrypotic piglets were in litters from sows of any parity, and in the 2018 incident, occasional piglets developed tremors and paresis post-natally. Virus microarray and/or next generation sequencing detected PCV-3 in all piglets with multisystemic inflammation. RT-PCR confirmed PCV-3 in all tissues with low Ct values and PCV-3 was demonstrated in association with histological lesions by ISH in two stillborn piglets with arthrogryposis from each of the two herds, and in two piglets with nervous disease. Tissues with low or no PCV-3 viral loads from pigs without multisystemic inflammation did not show PCV-3 genome by ISH. Atypical porcine pestivirus (APPV) was only detected by RT-PCR in piglets with nervous disease.

# **Discussion and Conclusion**

Detection of PCV-3 at high viral loads and by ISH in piglets with multisystemic inflammation provides good evidence of its likely causal association with disease on two breeding herds. APPV may also have been involved with PCV3 in the nervous disease occurring in some piglets. US researchers have demonstrated PCV-3 in foetuses and weak neonatal piglets with myocarditis by ISH and PCR. These cases are believed to be the first such report in Europe. Surveillance since 2011, by performing myocardial histopathology on foetuses and stillbirths, suggests these incidents are rare in GB pigs.

## SPLENIC INFARCTION IN PIGS WITH NON-NOTIFIABLE INFECTIOUS DISEASE

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# **Background and Objectives**

Splenic infarction is rarely seen in GB pigs and was found in individuals from five premises submitted for diagnostic investigation prompting consideration of porcine notifiable disease. Investigations resulted in endemic disease diagnoses.

# **Material and Methods**

Carcases were submitted to the Animal and Plant Health Agency with clinical and herd details and postmortem examinations were performed. Bacteriology, virology (PCRs, virus microarray, sequencing) and histopathology including immunohistochemistry (IHC) were performed.

## Results

Five groups of pigs were investigated, necropsies in each identified splenic infarction in one pig per group. One was an adult sow, one 5-weeks-old (preweaned) and three were postweaned (six to 10-weeks-old). Clinical signs included malaise, wasting, respiratory, diarrhoea and mortality. In three cases, findings including on-farm clinical information and absence of high fevers did not suggest swine fevers. Two cases (adult and preweaned) were reported to the Animal Health authorities and official investigations ruled out swine fevers. Porcine Reproductive and Respiratory Syndrome (PRRS) due to PRRSV-1 was confirmed in two. In the other three cases, histopathology revealed lymphoid lesions consistent with porcine circovirus-2 disease (PCVD) and botryoid intracytoplasmic viral inclusions and/or strongly positive PCV2 antigen labelling confirmed PCVD. Microarray in four cases did not detect further viruses. One PRRS case had systemic streptococcal infection. PRRS cases had necrotising interstitial pneumonia, vasculitis and lymphadenopathy. PCVD pathology included bronchointerstitial pneumonia, pulmonary oedema, excess pleural fluid, variablesized lymph nodes and/or hepatitis.

# **Discussion and Conclusion**

Splenic infarcts are rarely seen in GB pigs; PRRS or PCVD were diagnosed as causes in five cases. It remains important to consider notifiable diseases whenever splenic infarcts are seen as in two of these cases. Thorough clinical histories, measurement of rectal temperatures of sick pigs and examination of representative numbers of carcases in each case proved vital in assessing cases, together with comprehensive diagnostic testing.

## EPIDEMIOLOGY OF THE DANISH PRRS OUTBREAK 2019

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## **Background and Objectives**

PRRSV-1 was detected in samples taken as part of the routine PRRSV surveillance in a PRRSV negative boar station in July 2019, followed by subsequent transmission to herds and reports of reproductive failures and high piglet mortality. The aim of this study was to document transmission from the boar station and the production losses.

## Material and Methods

The routine surveillance at the boar station consisted of testing for antibodies against PRRSV every second week. Blood samples from the boar station taken prior to the outbreak were tested for PRRSV by RT-qPCR to assess the most likely day of PRRSV introduction. An intensive surveillance program was implemented for 3 weeks in the breeding- and multiplier herds. In the production herds, samples were taken if clinical signs of PRRS appeared. All herds infected with PRRSV were contacted and production data collected and analysed.

#### Results

Based on the results of the analysis of available samples, the most likely time of introduction of the virus into the boar station was predicted to be during the first week of July. Between July 1 and 26, 71 breeding- and multiplier herds and approximately 630 production herds received semen from the boar station. Since the virus isolated in the boar station was different from all other PRRSV strains characterized globally, it was possible to confirm – by sequencing – if a given herd was infected though semen. Until November 2019, the new strain of PRRSV has been confirmed from four multiplier herds and from 36 production herds. The analysis of the production data is still ongoing, but for the 3 herds analysed so far, the piglet mortality was increased by a factor 1,8–3,9 when comparing 2–3 month before and after the outbreak.

#### **Discussion and Conclusion**

The virus seems to cause sustained disease in infected herds. Further studies are in progress to document the production losses.

## DETECTION OF ATYPICAL PORCINE PESTIVIRUS IN PIGLETS WITH CONGENITAL TREMOR IN DANISH HERDS

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# **Background and Objectives**

Congenital tremor (CT) is a disease occurring mostly in few pigs/litters in the farrowing unit. In 2015, atypical porcine pestivirus (APPV) was first described in North America and later associated with CT symptoms in piglets. Since then, APPV have been detected in piglets with CT from 4 different continents, including North America, South America, Europe and Asia. In April 2018, the first detection of APPV was made in piglets associated with symptoms of CT from a Danish swine herd. Since CT is an occasional challenge in Danish herds, we initiated a study to clarify the relationship between CT and APPV in Danish swine herds. Furthermore, we validated blood as sampling material in detection of APPV.

# Material and Methods

A screening was performed in 10 herds with clinical symptoms of CT in a sporadic pattern. In each herd, blood samples were collected from 5 CT affected piglets in the farrowing unit. All blood samples were analyzed for APPV by RT-qPCR.

## Results

The results showed that, each blood sample from the piglets was positive for APPV with cycle threshold values ranging from 20.0-36.7. Only two herds out of ten experienced increased pre-weaning mortality in the farrowing unit due to CT. All the CT affected piglets came from gilts, although not all gilt litters in each herd were affected.

# **Discussion and Conclusion**

These preliminary results indicate a correlation between APPV infection and clinical symptoms of CT. However, the prevalence of APPV in Danish herds is unknown, and therefore blood samples from herds with no signs of CT will finally support the hypothesis, that APPV is involved in the pathogenesis of CT. Furthermore, the results show that blood can be used as sufficient sampling material for the analysis of APPV infection. Since the problem is only seen in some gilt litters, further research is needed to assess this issue.

# SWINE INFLUENZA VIRUSES ISOLATED DURING SWINE INFLUENZA'S OUTBREAKS IN ITALY

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# **Background and Objectives**

Pigs play an important role in influenza A virus (IAV) ecology. Swine IAVs of H1N1, H3N2 and H1N2 subtypes are enzootic in pigs globally, with genetic and antigenic differences between geographic regions. Aim of this study is to report updated information on IAVs isolated during swine influenza's outbreaks in Italy.

# **Material and Methods**

During the period 2017-2019, 4787 swine IAV outbreaks involving 1207 farms in Northern Italy have been confirmed by RT-PCR for M gene. Five hundred eighty-nine IAV positive samples were further characterized by multiplex RT-PCR subtyping. Two hundred ninety-one positive samples were genetically characterized by NGS full genome sequencing assigning each virus to its lineage and genotype.

# Results

The prevalence of the different subtypes was: 28% H1N1, 36% H1N2, 9.6% H3N2 and 2% H1N1pdm09. In 4 farms (0.7%), two strains circulating concurrently in the same outbreak were detected. The H1N1 subtype was avianlike origin (90%), H1N1pdm09 (6%) with a little percentage (4%) of reassortant strains. The H1N2 subtype was the most variable with the circulation of 12 different genotypes. The Italian H1N2 (human-like) was detected in 42% of the samples but in the 58% of the detected cases viruses were H1N2 of reassortant origin (pandemic and avian-like internal genes). The H3N2 was confirmed to be the most stable subtype.

# **Discussion and Conclusion**

In the last three years an increasing genetic variability was observed in the swine IAVs circulating in Italy. The HIN2 subtype is the most prevalent among viral subtypes and the most genetically variable and prone to reassortment. The importance of monitoring the genetic variability of IAV is enhanced by the economic importance of influenza in pigs and its role in PRDC but also because the pig's role as mixing vessel of viruses potentially zoonotic.

# IMMUNOPATHOGENESIS OF BRONCHOPNEUMONIA DURING THE EARLY PHASE OF INFECTION WITH PRRSV-1 STRAINS OF DIFFERENT VIRULENCE

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# **Background and Objectives**

Severe clinical signs and high mortality rate are hallmarks of highly virulent Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) strains. Virulent strains induce severe lung injuries, playing a role in bacterial secondary infection and the onset of bronchopneumonia. This study evaluates the role of different inflammatory mediators in the immunopathogenesis of the lung lesion in PRRSV-1-infected pigs.

# **Material and Methods**

Seventy-four-week-old piglets were randomly distributed in 3 groups and inoculated intranasally with 10<sup>5</sup> TCID<sub>50</sub> of PRRSV-1 3249 strain (low virulence) or Lena strain (high virulence); one group was mock-inoculated (control). Animals were necropsied from day 1 to 8 post-inoculation (dpi); lung lesions were recorded, and histopathological and immunohistochemical studies against PRRSV-N-protein, CD200R (immune inhibitory molecule), CD14 (specific LPS co-receptor) and iNOS (major mediator of inflammation) were conducted. Viral genome quantification assessed by RT-qPCR.

# Results

Lena-infected pigs showed the most severe gross and microscopic lesions, displaying suppurative bronchopneumonia from 6 dpi onwards. PRRSV load in the lung was always higher in Lena-infected pigs, peaking on 6 dpi (1,61E+07 eq CPD50/mL), coinciding with the highest number of PRRSV-N-protein<sup>+</sup> cells, whereas viral load of 3249-infected pigs reached the maximum at 8 dpi (1,94E+06 eq CPD50/mL). CD14 and CD200R were mostly expressed by intravascular and interstitial macrophages located inside or surrounding bronchopneumonia. The number of CD14<sup>+</sup> and CD200R<sup>+</sup> cells increased progressively in both groups Lena and 3249 from 3 dpi onwards, peaking on 8 dpi. The number of iNOS<sup>+</sup> cells was highest at 6 dpi and 8 dpi in 3249 and Lena groups, respectively.

# **Discussion and Conclusion**

The virulent Lena strain caused severe lung lesions associated to earlier and higher PRRSV replication. The higher frequencies of CD14<sup>+</sup>, CD200R<sup>+</sup> and iNOS<sup>+</sup> cells could be involved in regulating acute lung inflammation in PRRSV-1-infected pigs.

## PREVALENCE OF PRRS VIRUS IN NURSERY PIGS IN THE BENELUX IN 2019

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# **Background and Objectives**

PRRS is one of the most important pig diseases. Information about prevalence of PRRS in nursery piglets is currently limited, although these insights are important to decide the vaccination strategy. To gain insights into the prevalence of the virus in nursery pigs in the Benelux, PRRSv prevalence was evaluated in early and late stage of the nursery period in 122 farms.

# Material and Methods

Between March and May 2019, 122 farms were selected by veterinarians based on their interest to participate in the project. In each of the 122 farms, 4 pens of nursery piglets were sampled by oral fluids: 2 pens were sampled at the start (5-7w of age) and 2 pens were sampled at the end of nursery period (8-10w of age). Samples were analyzed by PRRS RT-PCR. In each farm, positive OF samples with low Ct-value were submitted for ORF-5 sequencing.

## Results

The presence of PRRSv in nursery piglets was confirmed in 71% of the farms. 62% of these positive farms were not vaccinating piglets. For the positive farms not vaccinating piglets, 57% were positive in the first part of the nursery period, while 43% were positive at the end. Since RNA quality of oral fluid is often bad, PRRS sequencing was only successful in few farms: 11/19 in NL (5 vaccine and 6 wild type virus) and 23/39 in BE (8 vaccine and 15 wild type virus).

# **Discussion and Conclusion**

The results of the PRRS prevalence project in 2019 in the Benelux demonstrated a high prevalence of PRRSv in the first part of the nursery period, comparable to the findings from 2016 to 2018.

# COMPLETE GENOME SEQUENCING OF INFLUENZA A VIRUSES (IAV) DIRECTLY FROM NONINVASIVE PIG FIELD SAMPLES

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# **Background and Objectives**

Influenza A viruses (IAV) cause respiratory disease in pigs and may contribute to other respiratory disease syndromes. Subtyping of IAV is most commonly performed based on hemagglutinin (HA) and neuraminidase (NA) coding genomic segments, either using subtype specific PCR or DNA sequencing. In order to monitor IAV evolution in pigs, the analysis of complete genomic sequences is necessary. The objective of this study was to assess likelihood to obtain complete genome sequences directly from clinical materials of conventional pigs.

# Material and Methods

Nasal swabs and oral fluid samples were obtained from 12 Polish conventional farms. The samples were tested with Virotype Influenza A RT-PCR Kit (Indical Bioscience, Germany). RNA from the samples with Ct values  $\leq$  30 was subjected to NexteraXT library preparation after cDNA synthesis, or after RT-PCR amplification of all genomic segments. Libraries were pooled and sequenced with 2 × 300-bp paired-end sequencing on a MiSeq system (Illumina, USA).

## Results

Sequencing directly from cDNA provided only fragmentary genomic information, irrespectively of Ct or sample type. On the other hand, segment amplification until present provided high quality complete genome sequences from two nasal swabs and one oral fluid sample (Ct  $\leq$  25). In addition, 26 good quality genome amplification products from both sample types (Ct  $\leq$  27), representing 11 farms, are currently being sequenced.

# **Discussion and Conclusion**

IAV complete genomic segment amplification allows sequencing of complete IAV directly from clinical samples. Despite mixed experience with DNA sequencing of RNA viruses from oral fluids (e.g. PRRSV), this material is suitable for sequencing of complete genomes of IAV. As oral fluid contains IAV RNA even in convalescent pigs, it can be considered a valuable sample for surveillance of IAV evolution in pigs.

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# COMPARISON OF DIFFERENT SAMPLING STRATEGIES FOR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) AFTER AN OUTBREAK IN A 3000-SOW HERD IN GERMANY

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# **Background and Objectives**

After outbreaks of PRRSV-infections in sow herds, the time-to-baseline production can take 18 to 55 weeks. Different sampling strategies (e.g., processing fluids [PF], serum, or oral fluids [OF]) and the influence of hygiene and pig flow management have been described to assess the PRRSV-status after an infection. Thus, we aimed to evaluate the PRRSV-status in pigs after an outbreak by using different sampling strategies.

## **Material and Methods**

In a 3000-sow herd located in Germany, a PRRSV EU outbreak occurred. Subsequently, sows (twice in a fourweek interval, ReproCyc® PRRS EU) and piglets (at three-weeks of age, Ingelvac® PRRSFLEX EU) were vaccinated. Five weeks after sow herd vaccination, every four-weeks PF (4x ten litters), serum (n=30), and OF (4 cotton ropes of 28 pigs each) specimens were collected from 3-days-old piglets, 3-weeks-old piglets, and 6- and 9-weeks-old pigs, respectively. PRRSV-detection was determined by using a PRRSV EU PCR and re-measured if positive by an in-house PCR to rule out PRRSV-vaccine strain 94881. Results from the different sampling strategies were used to evaluate the PRRSV-status.

#### Results

Both, PF and serum samples showed a correlation ( $\boxtimes$  0.63 [95% CI: 0.37 to 0.80]; p=0.0001); and significant decrease in PRRSV levels from week 1 (median: 4.9) to 35 (median: 0.0; p=0.0063), and week 1 (median: 5.3) to 35 (median: 0.0; p=0.0002), respectively, but not for the OF (p=0.6086). Results for PF and serum revealed an inconsistency in hygiene and pig flow management at the farm; however, an age-dependent pig flow procedure leads to zero PRRSV levels on any three consecutive measurements.

# **Discussion and Conclusion**

The 3000-sow herd had a stabilized production within 35 weeks after a PRRSV outbreak with the aid of vaccination, hygiene improvement, and age-dependent pig flow. Both PF and serum samples are equivalent sampling strategies to evaluate the PRRSV-status after an outbreak.

# GENETIC AND ANTIGENIC DIVERSITY OF DANISH PORCINE PARVOVIRUS STRAINS

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# **Background and Objectives**

Porcine parvovirus (PPV) is widespread in swineherds globally and causes reproductive failure manifested by the SMEDI-syndrome. DTU VET receives annually between 50-100 samples from aborted fetuses from Danish herds. During recent years, the average prevalence of positive submissions has increased from 4 % to 17 %. The aim of the study is to investigate the genetic and antigenic diversity of Danish PPV isolates. Furthermore, the cross reactivity of antibodies raised to commercial available PPV vaccines was tested

## **Material and Methods**

A total of 37 Danish field isolates of PPV were included in the analysis. These isolates originated from diagnostic submissions (fetuses) between 2006 and 2018. DNA was extracted from fetal tissue and the full VP1/VP2 gene sequenced. The sequences were compared to PPV sequences retrieved from GenBank, including available sequences of vaccine strains. Virus neutralization tests were performed to test the level of cross neutralization between antibodies raised to selected PPV vaccines and selected PPV strains.

#### Results

The phylogenetic analyses revealed that the sequences grouped into two defined clusters. All of the viruses collected in Denmark 2006–2009, clustered with older strains previously defined as genotype 1. Since 2009, the majority of the Danish field strains clustered together with recent German strains, including the genotype 2 reference strain 27a. Preliminary results of ongoing neutralization tests indicated that there was a marked difference in the level of cross reactivity between PPV isolates belonging to the different phylogenetic clusters.

# **Discussion and Conclusion**

A significant increase in number of foetuses positive for PPV coincided with a shift in genotype. Results of virus neutralization tests indicated that the genetic clustering also had effect to the level of cross-neutralization and by that indicate that genetic mix-match between PPV field and vaccine strains may influence the efficacy of commercial available vaccines.

## POINT-OF-CARE DIAGNOSTICS DEVICE FOR SWINE VIRAL DISEASES: THE SWINOSTICS H2020 PROJECT

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# **Background and Objectives**

The SWINOSTICS project aims to develop a novel field diagnostic device, based on advanced bio-sensing and photonics technologies to tackle selected emerging and endemic viral diseases of pigs.

## **Material and Methods**

The SWINOSTICS approach is based on the combination of photonic integrated circuit (PIC) and bio-sensing technology to detect the viruses. The targeted viruses in the project are: ASFV, CSFV, PPRSV, PPV, porcine circovirus 2, and SIV.In the present work the detection of PCV2 was aimed. Our biosensor PIC comprises three main building blocks: sensing ring resonators, light coupling block (grating couplers), and optical power distribution block. The ring resonators are used as sensor elements (using one resonant ring for each targeted virus), the light coupling section allows to introduce and extract the optical signals into/out of the PIC whilst the power distribution block is necessary to feed all the resonator rings of the PIC with a common laser source input. Several photonic sensor PICs were functionalized with the aim of detecting PCV2. On top of the surface, commercial rabbit polyclonal antibodies against PCV2 (Thermo Fisher Scientific #PA5-34969) were covalently immobilized in an oriented manner (by their carboxy terminal of the Fc part). PCV2b cell culture supernatant (qPCRCt:10) was used in serial PBS dilutions to test the functionalized PIC.

## Results

The functionalized rings showed remarkable shift in their resonance as compared to the non functionalized rings even up to 1/5000 dilutions of the original sample.

# **Discussion and Conclusion**

One of the fabricated PICs was used in a preliminary experiment for detection of PCV2 virus. After the functionalization of the rings with specific antibodies and attaching a microfluidic layer on the PIC, positive detection of several virus concentrations was achieved. Clinical samples and the detection other viruses are aimed as the next step of the project.

## IDENTIFICATION OF LATENT CONTACT PATHWAYS BETWEEN PIG PREMISES USING THE MENTAL MODEL APPROACH

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# **Background and Objectives**

Infectious disease spread among farms depends on type and frequency of transmission pathways. In addition to officially recorded animal transports, a range of unrecorded indirect contacts between premises occur. The role of these latent contacts in disease propagation is largely unknown within the Swiss pig industry. The aim of this study was to identify the most important contact pathways beyond pig transport.

# **Material and Methods**

We adapted the standard procedures from the Mental Model Approach (MMA), a commonly used method in risk research. First, seven experts in the field of pig production and health were interviewed. Based on their input, an initial disease pathway model was established. In a second step, a total of twenty farmers were selected for qualitative interviews, with the aim to validate the latent contacts identified by the experts and set them in relation to farm characteristics.

# Results

Preliminary results indicate that latent transmission pathways can be grouped into contacts via pig transport (e.g. lorry drivers entering the premises), professional and social contacts between farmers (e.g. sharing of farming tools), external collaborators accessing the barns (e.g. veterinarians) and living vectors (e.g. wild boar). All disease pathways identified by the expert pool, except boar sharing, were also mentioned by farmers.

# **Discussion and Conclusion**

These findings highlight the potentially high risk of indirect contacts for disease transmission between Swiss pig premises. The large overlap between disease pathways mentioned by experts and farmers shows that MMA is an appropriate tool to minimize social desirability bias when asking to reveal sensitive information. As a next step, a workshop with all experts will be conducted to find a final agreement on the importance and frequency of each indirect contact pathway. An infectious disease spread model based on both latent contact and official transport data will be built, in order to define recommendations for suitable disease surveillance strategies.

# SALMONELLA TYPHIMURIUM REDUCTION TO BELOW THE BACTERIOLOGICAL DETECTION LIMIT FROM A FARROW-TO-FINISH SWINE HERD WITH A HISTORY OF CLINICAL SALMONELLOSIS, USING SALMOPORC®, A LIVE ATTENUATED SALMONELLA TYPHIMURIUM VACCINE

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# **Background and Objectives**

Salmonella Typhimurium (ST) is an important zoonotic pathogen in swine, that can cause clinical disease. This case study describes a farrow-to-finish swine herd, producing its own replacement gilts, which experienced clinical outbreaks of salmonellosis caused by ST since 2002 in weaned piglets, growers, finishers and gilts, leading to high antimicrobial (Colistin) use. Aim of this study was to see whether it was possible to reduce ST to below the bacteriological detection level, using vaccination of sows and piglets according to the SPC with Salmoporc®, a live attenuated ST vaccine for swine.

# **Material and Methods**

Monitoring of Salmonella was done every month and later on every two months, using in total 20 pooled faecal, sock and dust samples per farm visit in the period from September 2016 to November 2019.

## Results

After starting in August 2016, within the first ten months the results showed a rapid decline of the clinical symptoms, the antimicrobial usage and the number of Salmonella-positive samples from 50% to 0%. During the winters of 2017/18 and 2018/19 the number of positive samples increased due to management factors. In July 2019, only two samples from a corridor were positive and in September and November of 2019 both samplings were completely negative. An additional sampling in October 2020 was also completely negative.

# **Discussion and Conclusion**

This case can be seen as a proof of principle that vaccination with Salmoporc® can be a tool to reduce ST contamination within a herd to below the bacteriological detection level. Also, clinical symptoms and antimicrobial use were no longer present or needed. Vaccination over an extended period of time can be a valuable additional tool, like in the poultry industry, to reduce the number of herds supplying S. Typhimurium infected swine to slaughterhouses and ultimately reduce the number of salmonellosis cases in humans.

# REDUCTION OF SALMONELLA SEROTITERS BY PROBIOTIC CLOSTRIDIUM BUTYRICUM IN COMMERCIAL CONDITIONS

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# **Background and Objectives**

A commercial fattening farm in Germany historically experienced high Salmonella titers at slaughter, despite using an all in – all out system with sanitation between batches. Hogs arrived at 30 kg (hybrids from a singular BHZP farm), with blood analysis on 60 random samples of an initial group prior to slaughter showing 43 positive results. The average Salmonella titer was 54.81. As a result the farm was classified as Salmonella category III, causing meat price deductions.

# Material and Methods

The next two consecutive batches of animals received dietary probiotic Clostridium butyricum (strain Miyairi 588, Miya-Gold<sup>®</sup>), for the first batch at a level of 500 g of product/ mton of feed ( $2.5 \times 10^{11}$  C. butyricum Miyairi 588 / mton of feed) for the first week. This dosage was then reduced to 300 g / mton of feed ( $1.5 \times 10^{11}$  C. butyricum Miyairi 588 / mton of feed) for the remainder of the fattening period. The second batch received again 500 g / mton of feed, but only in the growing stage (30 to 50 kg bodyweight). Before slaughter 60 blood samples were analysed of each batch, recorded as positive if their titer exceeded 40.

# Results

Blood analysis of the first supplemented batch showed 21 positive samples, compared to the initial group's 43. Average Salmonella titer was also lower, measuring 31.13 compared to the initial group's 54.81. This trend continued in the second supplemented batch, with only 16 positive samples. The average Salmonella titer was 26.92, effectively bringing the farm down to a Salmonella Category II.

# **Discussion and Conclusion**

Supplementation with probiotic Clostridium butyricum reduced Salmonella titers, confirming the potential of the probiotic to restrict Salmonella proliferation. As such, dietary probiotic supplementation deserves a place in on-farm food safety programs, reducing the risk of economic deductions at the slaughterhouse due to high Salmonella titers.

# THE PREVALENCE OF ANTIBODIES AGAINST HEPATITIS E VIRUS (HEV) BETWEEN 2018 AND 2019 AMONG DOMESTIC PIGS IN SLOVENIA

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# **Background and Objectives**

Hepatitis E virus (HEV) is important causative agent of acute viral hepatitis in humans, while infection in pigs is asymptomatic. The main route of human infection is through ingestion of contaminated pork liver and pork products. The aim of the study was to determine the prevalence of antibodies against HEV in Slovenian domestic pig population.

# Material and Methods

Study was conducted between September 2018 and October 2019. At the slaughterhouse, 5 individual sera samples were collected from every pig holding with more than 50 fatteners. The anti-HEV antibody detection was performed with ELISA PrioCHECK Porcine. Sampling and testing were carried out within the framework of an annual Order financed by the Veterinary administration of Republic of Slovenia.

# Results

A total of 1550 sera samples of fatteners from 311 pig farms were tested. Anti-HEV antibodies were detected in 36.9% of sera samples, 58.58% were free of specific antibodies and 4.51% of samples were interpreted as doubtful. Only 40.83% holdings were negative, while in 18.64% of positive pig holdings all 5 tested samples were positive. In 14.79% of holdings 4 out of 5 samples were positive, in 9.64% pig holdings 3 out of 5 samples were positive, in 8.36% 2 out of 5 samples and in 7.71% 1 of 5 tested samples were HEV antibody positive. The comparison of the results of herd prevalence between years 2018 and 2019 showed that significantly higher antibody prevalence of 80.6% was detected in 2019 compared to 51.55% in 2018.

# **Discussion and Conclusion**

The prevalence found is lower as in earlier studies in some EU countries. The most probable explanation for the incrise of herd prevalence is the import of growing pigs of unknown HEV status from different pig holdings originating from several EU countries and also an intensive animal trade between holdings. Importance of HEV infection as zoonosis is underestimated.

# POTENTIAL USE OF CLINICAL EXAMINATION AND SEROLOGICAL TESTING TO FORECAST THE 'POSITIVE FARMS' FOR LUNG LESIONS IN SLAUGHTERED PIGS

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# **Background and Objectives**

This study aimed to determine the potential use of clinical respiratory symptoms recorded on farm during fattening and serological testing for respiratory pathogens to predict the 'positive farms' for lung lesions in slaughtered pigs.

# **Material and Methods**

The study was conducted on 240 pigs originated from eight commercial farms. Two weeks before slaughter, pigs were clinically inspected on each farm for the presence of following symptoms: coughing, sneezing and laboured breathing. Serum samples were collected from pigs at slaughter and analysed for the presence of antibodies against Mycoplasma hyopneumoniae (M. hyopneumoniae), Actinobacillus pleuropneumoniae (APP), Swine Influenza Virus (SIV), Porcine Reproductive and Respiratory Syndrome virus (PRRSV), Porcine Circovirus type-2 (PCV2) and Porcine Respiratory Coronavirus (PRCV). The plucks of 30 slaughtered pigs from each farm were examined for pneumonia (Madec and Kobisch, 1982) and pleurisy (Dottori et al. 2007). Farms were classified as positive for pneumonia when the mean batch pneumonia score was higher than five. For pleurisy a farm was considered to be positive when at least one pig had a pleurisy score higher than two.

# Results

No relationship was found between clinical symptoms and serological values and lung lesions using Spearman correlation analysis (correlation coefficients ranged from 0.0078 to 0.2567; P>0.05). According to receiver operating characteristic curves and the area under the curves (AUC), 'positive farms' for lung lesions at postmortem inspection could not be accurately detected (P>0.05) by the clinical symptoms recorded on farm during fattening (pneumonia: AUC<sub>coughing</sub>=0.625; AUC<sub>sneezing</sub>=0.625; AUC<sub>labored</sub> breathing=0.688; pleurisy: AUC<sub>coughing</sub>=0.567; AUC<sub>sneezing</sub>=0.667; AUC<sub>labored</sub> breathing=0.500) and serological values (pneumonia: AUC<sub>Mhyopneumoniae</sub>=0.625; AUC<sub>APP</sub>=0.563; AUC<sub>SIV</sub>=0.656; AUC<sub>PRRSV</sub>=0.688; AUC<sub>PCV2</sub>=0.625; AUC<sub>PRCV</sub>=0.531; pleurisy: AUC<sub>Mhyopneumoniae</sub>=0.500; AUC<sub>APP</sub>=0.567; AUC<sub>SIV</sub>=0.667; AUC<sub>PRRSV</sub>=0.533; AUC<sub>PCV2</sub>=0.400; AUC<sub>PRCV</sub>=0.500).

# **Discussion and Conclusion**

The results of this study suggest that the recording of lung lesions at postmortem inspection is more reliable and feasible method for pig health and welfare monitoring than serological testing and recording of clinical symptoms on farm during fattening.

# LONGITUDINAL (2013-2018) OBSERVATION OF A GERMAN SOW FARM APPLYING VACCINATION AGAINST SALMONELLA TYPHIMURIUM AND A STRICT HYGIENE PROGRAM ON SALMONELLA SEROLOGY AND EXCRETION OF THEIR OFFSPRING

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# **Background and Objectives**

Salmonella Typhimurium (ST) continues to be a major cause of foodborne illness worldwide. Reducing ST prevalence on farm helps to minimise contamination at slaughter and therefore is an important step in improvement of food safety. Several studies have shown that vaccination with a Salmonella live vaccine is effective to reduce prevalence in sow herds and their offspring.

# Material and Methods

Here we show the effect of the use of an attenuated live vaccine (Salmoporc®, Ceva Animal Health) in a 2000 sow herd in Germany producing their own replacement gilts. In June 2013 a clinical outbreak of ST induced diarrhoea was observed in the nursery. Half a year later the fatteners decreased in their category in the serological Salmonella monitoring program. Since October 2013 a vaccination program including all sows (before farrowing) and gilts (twice orally as piglets and at 160 days of age) was implemented. Additionally an improved cleaning and disinfection action plan was rolled out. Regularly environmental samples were taken and the progress of the associated fattening farms was evaluated by the serological results of the Salmonella monitoring system.

# Results

Salmonella diarrhoea disappeared with the first vaccinated group of piglets in November 2013, leading to reduced antimicrobial use after weaning. All associated fattening units returned into the good category in 2015. With each sampling the amount of ST positive results declined, till in June 2016 all 20 socks and swabs taken in the nursery were negative. Since 2015 the farm delivers vaccinated gilts to a new built 3000 sow farm. On that farm until now no Salmonella was found in environmental swabs.

# **Discussion and Conclusion**

If vaccination and hygiene measurements are implemented and executed in a proper way, it is possible to reduce Salmonella prevalence on farm. Salmonella control never is a quick win and needs a long term contribution.

Veterinary public health

# HEPATITIS E VIRUS (HEV) IN SPANISH PIG HERDS; DETECTION AND CHARACTERIZATION

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# **Background and Objectives**

Hepatitis E is a feco-oral transmission zoonosis considered as a major problem for human health in developing countries and causes sporadic outbreaks in industrialized regions. The infection in pigs courses inaparent, nevertheless, human infection leads to self-limiting acute disease whose mortality rate may reach 4%.

The main objective was assesing the presence of HEV in Spanish pig herds and then conducting a preliminary characterization of the detected virus.

# Material and Methods

A qPCR assay was designed to recognize the gene coding the capside. Eighty samples coming from animals without hepatic damage were collected from Spanish farms during 2018. Three kinds of sample were included: liver(n=8), feces(n=37) and oral fluid(n=23). Positive samples were led to Sanger sequencing for partial ORF2 sequencing and phylogenetic study.

# Results

Every liver sample (8/8) resulted negative, nonetheless, 8 feces samples resulted positive (8/37, 21%; Cq values range: 23-35) and 4 oral fluid samples resulted positive (4/23, 17%; Cq range: 22-40). The 12 positive samples were attempted to sequence, however, only 5 (those whose Cq<32) succeded. All the samples belonged to subtype 3f. Moreover, very high homology (>95%) was found between sequences from our swine samples and those from human patients located in the South of France.

# **Discussion and Conclusion**

These results confirm that VHE is widely spread among Spanish pig herds and perhaps it has been undersestimated so far. We should consider our animals as a reservoir which causes sporadic outbreaks related with undercooked pork products and fecal contamination of water flows.

Other European countries reported that human and swine which live together in a high pression infection area also share the same subtypes of genotype 3 HEV. This, along with the fact that we also found very high homology between our swine HEV sequences and those coming from near located human infections highlights the zoonotic feature of this virus.

# TYPICAL ERRORS IN THE PRACTICAL ANTIBIOTIC USE ON LARGE-SCALE HUNGARIAN SWINE FARMS

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# **Background and Objectives**

Use of antibiotics (AB) is an actual topic and one of the most important issues in the European, one-healthdriven pork production. Sound, life science-based analyses, novel approaches and interpretation of the daily AB use would be essential to sustain a safe and healthy pork production. The aim of this pilot-study was to identify the typical errors, lack of knowledge and misunderstandings of AB use on the Hungarian swine farms to improve their prudent use.

# Material and Methods

In our survey, between 2018 and 2019, we systematically collected and analyzed data about the daily practice and approach of AB use, pathogens' identification, and their minimal inhibition concentration (MIC) tests' results in 15 selected large-scale swine herds. The use of PK/PD analysis was also checked.

# Results

Out of 15 farms 11 (73%) did not fulfil the provisions of summary of product characteristics (SPC) of the ABs on clinical particulars (interactions), and 9 drugs out of 15 (60%) were not soluble in the applied concentration. Misunderstanding of the actual concentration of the active substances were observed in 5/14 cases (36%). 5/15 (33%) water pump dispenser (WPD) systems were not able to provide the targeted concentration into the water in the dedicated timeframe. On some farms we found 5 different Actinobacillus serotypes in one herd with different MICs. We also identified the lack of special warning on the used AB nature on time and/or concentration dependence. Intracellular (IC) MIC were not measured (although IC pathogens were identified e.g. Lawsonia), and pharmacokinetic and pharmacodynamic analyses sometimes were not run and/or understood.

# **Discussion and Conclusion**

Our findings show that specific audits on AB use can help swine farms modify their antimicrobial treatment and metaphylactic protocols, so that they can improve the prudent use of ABs and the profitableness of their production.

# REDUCING PREVALENCE OF SALMONELLA BY WATER ACIDIFICATION IN A COMMERCIAL PIG HERD

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# Background and Objectives

Salmonella is a major concern globally; infection and contamination occurs at different levels of pig production [Regulation (EC) No. 2160/2003]. Reduction of Salmonella prevalence in the feed-to-food chain is necessary for preventing human Salmonellosis cases. Using organic acids in drinking water can improve water quality and because of their prolonged activity also contributes to the control of bacteria in the gastrointestinal tract of pigs. This study determines the effectivity of drinking water acidification on reducing Salmonella prevalence in commercial pigs.

# Material and Methods

On a commercial farm, 264 pigs (average body weight 22.95 kg) were randomly allocated to two treatments with 12 pens with 11 animals per treatment. Animals were fed a commercial diet (no antibiotics, no acidification). Treatments consisted of a control group and a group with 0.2% drinking water acidification (DWA) (water pH 3.6; Trouw Nutrition, the Netherlands) during grower and finisher phases. On day 106, blood samples from 40 animals (10 pens randomly selected per treatment) were taken for seroconversion analysis (optical density, OD) for Salmonella. Results were categorised as OD <10, 11-20, >20. Samples were considered positive when OD >40, according to IKB Dutch regulations. Data were analysed using PROC GLM procedure including dietary treatment as fixed effect for OD values, and PROC FREQ for Chi–Square test on Salmonella seroconversion from SAS.

# Results

Pigs provided DWA increased average water consumption the first four weeks compared to control (90 vs 68 liter/pig). Salmonella seroconversion analysis showed lower OD values for DWA compared to control (OD <10: 13 vs 6 samples, OD 11-20: 9 vs 7 samples, OD >20: 18 vs 27 samples). A significant decrease in OD >40 was reported in blood samples in DWA compared to control (20% vs 52%, P<0.001).

# **Discussion and Conclusion**

Reduction in Salmonella prevalence improved Salmonella category according to IKB Dutch regulations for the drinking water acidification group.

# A THREE YEARS SURVEY ON ANTIMICROBIAL USE IN A SAMPLE OF SWINE FARMS IN EMILIA ROMAGNA (ITALY): RESULTS OF THE APPLICATION OF REGIONAL PRUDENT USE GUIDELINES

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# **Background and Objectives**

The reduction of antimicrobial use (AMU) is crucial to contrast the development and spreading of antimicrobial resistance. From 2016 to 2018 a pilot Project founded by UE In Emilia Romagna (Italy) was developed in order to experiment the application of regional guidelines for antibiotic prudent use. Thirty pig farms (2 farrowing, 4 farrowing-weaning, 1 weaning, 20 fattening and 3 farrow-to-finish) were involved.

# Material and Methods

Guidelines on prudent AMU and farm biosecurity were distributed and discussed with the farmers and their veterinarians at the start of the project and during yearly meetings. AMU was calculated as number of DDDAit (Defined Daily Doses Animal for Italy) and reports were distributed and discussed. AMU was detailed by age group (sow, piglet, weaner or fattener), route of administration and antimicrobial classes.

# Results

During the three years of the study, overall AMU decreased in weaners (-42.4%), sows (-52.7%) and fatteners (-40.9%), while an increase was reported in piglets (+9.9%). Referring to HPCIAs (WHO's Highest Priority Critically Important Antimicrobials), a reduction was recorded in all the categories: fatteners (-92.9%), weaners (-92.2%), sows (-57.4%) and piglets (-22.8%) with a decline in colistin consumption that reached a -98.7 % in weaners. Additionally, macrolides administration decreased in both weaners (-85.3%) and fatteners (-96.6%) and 3<sup>rd</sup> generation cephalosporines consumption decreased in piglets (-50.4%). During the Project an overall reduction of premix use was recorded in all the categories, even though the oral route remained used the most in weaners, sows and fatteners.

# **Discussion and Conclusion**

This study highlights the relevance that education activities on antimicrobials administration and biosecurity may have on overall and HPCIAs AMU in Italian pig farms. Nevertheless, the ban of the use of colistin in association with other antimicrobials, intervened during the first year of the project, could had also an impact on AMU reduction.

## DATA COLLECTION ON USE OF ANTIMICROBIALS IN SWINE

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# **Background and Objectives**

Veterinary Research Institute Brno was granted to develop national record system for the data collection on use of antimicrobials in swine in the Czech Republic. No doubt the drug information shall disclose much; squeezing this lemon more could tell bigger story. The objective of the paper was to learn from and propose to the interested public solutions for reporting and sharing not only reported antimicrobials (medicinal products, MP) used in swine in accordance to EC regulation. The collected data from European region can show in broader scopes main branch weaknesses and strength and can enforce European position on the world pork market. The developed countries already running various systems, there is still the chance catch up and consolidate this European swine backyard for future reclaim. The main point remains collection of the data, and the structure should be given. The vital information for background structure of the database, are expected in January 2022, when the implementing act to regulation EC 2019/6 will be adopted.

## Material and Methods

Among the cornerstone items for further analysis belong individual animal or the group, the list of the authorized MPs and index diseases. Union product database of authorized MPs is vital for entering and sharing the same data. Index of diseases should be set as default, justifying administration of MPs.

## Results

We have created the list of basal 150 diseases, 40 symptoms and 20 interventions for completing records by veterinarians as well as breeders and skilled staff. Nevertheless, how much work should be done, boondoggle should be avoided.

# **Discussion and Conclusion**

Many of animal data are recorded yet, at the breeding and feeding systems, which should be brought into the play and included. The progress in the branch is under fast development; next steps, the system and European index of diseases shall be presented and discussed during the PHM symposia.
# SPATIAL AND TEMPORAL DETECTION OF MYCOPLASMA HYOPNEUMONIAE UNDER CONTROLLED AEROSOL EXPOSURE FOR GILT ACCLIMATIZATION

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# **Background and Objectives**

Mycoplasma hyopneumoniae (Mhp) is the causative agent of enzootic pneumonia in swine. Gilt acclimation methods for Mhp control, such as vaccination, a "seeder" model, or controlled exposure, are employed with the intent to minimize dam-to-piglet transmission and to establish Day 0 for elimination strategies. Controlled exposure via the aerosolization of Mhp herd-specific lung homogenate has been shown to induce infection under field conditions. However, limited information regarding the validation of such administration technique to obtain a reliable and uniform exposure is available. Therefore, the objective of this study was to evaluate the spatial and temporal detection of Mhp under controlled aerosol exposure conditions using various clinical and environmental samples.

# **Material and Methods**

Negative gilt developing units were selected for this study. At 0 days post-exposure (dpe), Mhp herd-specific lung homogenate was selected based on a diagnostic criterion and then aerosolized from a single location in the barn. Environmental and air particle samples were collected at different locations from the origin of exposure at 0, 7, and 14 dpe. Tracheal secretions were also collected from gilts (n=30) housed in pens throughout the barn to assess the spatial and temporal detection patterns for Mhp.

## Results

Prior to exposure, all samples were Mhp negative by PCR. At 0 and 7 dpe, a numerical increase in Mhp relative bacterial load was detected in environmental and air particle samples collected in the projectile path compared to those in the opposing direction. From the origin of exposure, Mhp was detected in the environment up to 4.6 meters. All gilts remained Mhp negative until approx. 14 dpe, in which 10% of pigs sampled and housed in pens located within 4.6 meters of the exposure origin became positive.

# **Discussion and Conclusion**

Overall, detection of Mhp appeared to be spatially associated, however, additional research is warranted to confirm findings.

# ANTIMICROBIAL RESISTANCE OF ALPHA- AND BETAHEMOLYTIC STREPTOCOCCUS SPP. ISOLATED FROM DISEASED PIGS - A THREE YEAR RETROSPECTIVE STUDY

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## **Background and Objectives**

While antimicrobial resistance rates of Streptococcus (S.) suis in pigs are frequently described, little is known about resistances of other streptococci in swine stocks. Therefore, the main objective was to evaluate antimicrobial resistances rates of other porcine Streptococcus spp.

# Material and Methods

Resistancy rates of 553 alpha- and beta hemolytic porcine Streptococcus spp. isolates against 13 antimicrobials were obtained by agar disk diffusion test according to CLSI and retrospectively analyzed. Isolates originated from different tissues of diseased pigs with known anamneses, but unknown pre-treatment. Diagnostic material was submitted by 39 different Austrian herd veterinarians.

## Results

67.8% of all tested isolates showed resistances against tetracycline, 67.6% to trimethoprim/sulfamethoxazole, 62.3% to clindamycin and 51.6% to erythromycin. 95% of all S. hyovaginalis isolates were resistant to those four antimicrobial substances. Isolates which were resistant to tetracycline were also more likely to be resistant to erythromycin and clindamycin. Resistances to beta-lactam antibiotics were only observed for S. alactolyticus and S.suis. Isolates from the joints had the highest resistance rates.

#### **Discussion and Conclusion**

Due to missing interpretative criteria for agar disk diffusion test of porcine streptococci, the interpretation of susceptibility testing can be challenging. Despite these difficulties, some differences in resistance profiles were determined. The assessment of antimicrobial resistances of discounted Streptococcus spp. is of importance to public authorities as it increases visibility of the general resistance situation.

# ANTIMICROBIAL SUSCEPTIBILITY OF STREPTOCOCCUS SUIS IN SWEDISH GROWER PIGS

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# **Background and Objectives**

Streptococcus suis is among the most important bacterial pathogens affecting pig production worldwide; however, until recently overt signs of the disease have rarely been noted in Sweden. The objective of this study was to investigate the antibiotic susceptibility of S. suis isolates from healthy Swedish grower pigs.

# **Material and Methods**

During 2018-2019 palatine tonsil swabs were taken from 200 healthy pigs aged 8-13 weeks from 20 Swedish commercial farms. Ten herds had repeatedly noted clinical signs of S. suis, whereas ten control herds had not. Swabs were cultured on colistin-oxolinic blood agar (COBA) at 37°C in 5% CO2 overnight. S. suis colonies were pure-cultured on horse blood agar at 37°C in 5% CO2 overnight and identified to the species level using MALDI-TOF MS with a cutoff score of 2.0. The antimicrobial susceptibility of 188 isolates was assessed by broth microdilution, using VetMIC GP-mo panels (SVA, Uppsala, Sweden). MIC values were interpreted using cutoffs according to Swedres-Svarm.

## Results

The prevalence of S. suis was 95% in both case and control herds. Antibiotic susceptibility testing revealed that 3.8% of the isolates, all from case herds, were resistant to penicillin, and 9.2% were classified as intermediate. The percentages of isolates not susceptible (resistant or intermediate) to tetracycline were similar in the two groups, at 100% and 98.9%, respectively.

## **Discussion and Conclusion**

This is the first report of Swedish S. suis isolates resistant to penicillin. Sweden has a very low level of antimicrobial usage in food-producing animals, and benzylpenicillin is the most commonly used substance in pigs. Compared to previous studies a higher percentage of isolates was found to be not susceptible to tetracycline. However, care should be taken when making comparisons between studies employing different methodologies and interpretive criteria.

# Reference:

Werinder et al. 2020, Acta Vet Scand. 62:36, pp. 1-9, https://doi.org/10.1186/s13028-020-00533-3

# SCREENING FOR LAWSONIA INTRACELLULARIS IN UK UNDER DIFFERENT CLINICAL SCENARIOS

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# **Background and Objectives**

Lawsonia intracellularis is the aetiological agent of lleitis, an enteric disease in pigs that may take different clinical forms. The aim of this study was to quantify the occurrence of L intracellularis infections in different clinical and subclinical scenarios in British pig herds.

# **Material and Methods**

In total, 22 British (9 outdoor; 13 indoor) herds with (Proliferative Hemorrhagic Enteritis, i.e acute & Porcine Intestinal Adenomatosis, i.e chronic) and without (i.e. subclinical) lleitis-like symptoms were selected. Cross-sectional sampling was performed at six sampling points (6/9/12/15/18/21 weeks of age). A minimum of 30 blood and 25 fresh faecal (directly from the floor) samples were collected from each herd. Faecal samples from the same herd and age group of animals were pooled in groups of five. Presence of L. intracellularis was determined by ELISA (antibodies) in blood and the bacterial shedding quantified by qPCR (bacterial load) in faeces. Full differential diagnosis was performed to confirm causality.

# Results

In total, 955 faecal and 562 blood samples were collected. Overall, 90.1% of the herds tested positive by both qPCR and serology, of which 86.4% presented a significant bacterial load (>3.5Log10 bact/mL). On average, 5.6%, 9.5%, 36.8%, 40.0%, 27.8% and 20.0% of the herds had a significant bacterial load at 6, 9, 12, 15, 18 and 21 weeks of age, respectively. From the positive herds, 15%, 55% and 30% presented an acute, chronic and subclinical form, respectively. All acute infected herds had a very high bacterial load (>4.5Log10 bacteria/mL). Whereas a very high/high bacterial load (>4.0Log10 bacteria/mL) was determined in chronic (63.7%) and subclinical (83.3%) infected herds.

# **Discussion and Conclusion**

L. intracellularis is highly prevalent in British herds, affecting many herds with significant infection levels. High infection levels were detected not only in herds with evident lleitis-like symptoms, but also in subclinical cases, even in the absence of clinical signs.

# EVALUATION OF AN AUTOGENOUS VACCINE AGAINST STREPTOCOCCUS SUIS SEROTYPES 1 AND 2 IN PIGLETS UNDER FIELD CONDITIONS.

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# **Background and Objectives**

Streptococcus suis is an economically important pathogen for the swine industry and for humans as a zoonotic agent. Since the pressure to reduce the use of antibiotics in Europe and no commercial S. suis vaccines are available, autogenous vaccines have become a useful strategy for its control. Therefore, the objective of this study was to assess the effect of an autogenous S. suis vaccine on mortality rate and antibiotic use during lactation period.

## **Material and Methods**

This trial was conducted in a 750-sow farrow-to-wean farm with history of meningitis associated to S. suis in suckling piglets. Autogenous vaccine was based on two S. suis serotypes (ST) isolated in the selected farm, STI and ST2. It was produced by Ceva Biovac, following the good manufacturing practices. Gilts and sows received two doses (2mL/dose) of autogenous vaccine at 6 and 3 weeks pre-farrowing (wpf) as primo-immunization. Sows were re-vaccinated at 3 wpf before each farrowing. Global and S. suis-associated mortality and antibiotic use (b-lactamics, mg/PCU) were monitored during lactation in two periods: non-vaccinated (NV) in May-October 2019 and vaccinated (V) in May-October 2020. Differences between periods were compared using Mann-Whitney U-test.

#### Results

Twelve piglet's batches (1.500 piglets/batch) coming from V sows and NV were monitored during lactation for 6 months. Global pre-weaning mortality in V scenario (7.67%) was lower than NV (10.50%). Mortality associated to S. suis due to meningitis was significantly reduced (p<0.05) in V scenario (0.97%) compared to NV (9.70%). The use of antibiotics against S. suis decreased significantly in piglets coming from V sows (0.22 mg/PCU) compared to NV period (3.93 mg/PCU).

# **Discussion and Conclusion**

Sow vaccination against S. suis prior farrowing reduced mortality due to meningitis and the use of antibiotics in piglets. Thus, it could represent an effective strategy for controlling S. suis in affected farms.

# PREVALENCE OF EDEMA DISEASE ESCHERICHIA COLI (EDEC) IN WEANED PIGLETS IN GERMANY

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# **Background and Objectives**

Escherichia coli bacteria encoding shigatoxin subtype Stx2e and F18-fimbriae are referred to as EDEC (edema disease E. coli). They are considered as the causative agent of edema disease in pigs, a systemic disorder associated with high mortality rates in piglets suffering from acute disease. The aim of this study was to – for the first time – determine the prevalence of EDEC at pen and farm level in weaned piglets in Germany.

# **Material and Methods**

In this cross-sectional study 99 pig farms of unknown infection status were visited. On each farm, five pens were selected for sampling (n = 481). Piglets in these pens were at an age of 1-2 weeks after weaning. In addition to boot swaps and chewing ropes, faecal samples (n = 2'405) were picked from the floor of each pen at five separate locations. All samples were subsequently analyzed for EDEC by bacterial culture and subsequent testing of E. coli isolates for genes stx2e and fedA by duplex-PCR. Boot swaps, oral fluids and chewing rope rinse samples were additionally analyzed by DNA extraction and stx2e/fedA-PCR.

# Results

Pens and farms were considered EDEC-positive if at least one sample from a pen or from a farm proved positive for EDEC and/or genes stx2e and fedA, respectively. Overall 24.9% (95% CI: 21 – 28.7%) of all sampled pens and 37.4% (95% CI: 28.3 – 46.5%) of all farms were classified as positive.

# **Discussion and Conclusion**

Based on this new sampling and laboratory testing protocol, more than one third of the pig producing farms in Germany are affected by E. coli strains that are considered capable to cause edema disease. Besides an evaluation of the relative sensitivity of the different sampling and testing protocols to detect stx2e and fedA genes, farm records collected through a questionnaire will be evaluated for possible risk factors for the occurrence of EDEC.

## SURVEILLANCE OF LEPTOSPIRA INFECTION IN UK SWINE

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# **Background and Objectives**

Scarce and old data is available on the prevalence of Swine Leptospirosis in UK. The aim of this survey was to estimate the extent of Leptospirosis and to identify the occurring serovars in British pig herds.

# **Material and Methods**

Laboratory data from blood samples collected throughout the UK from January 2019 to October 2020 was investigated. On average 10 sows, presenting recent reproductive disorders, were sampled per herd (n=48 herds). A total of 511 swine sera were tested by microscopic agglutination test (MAT) for antibodies against strains of eleven Leptospira serovars.

## Results

On average, 97.9% (47/48) of herds tested positive for Leptospiral infections. Average within-herd prevalence was 34.3%. Overall, 31.5% of sows tested positive for Leptospira. Of all positive samples, 60.2% reacted with only one Leptospira serovar and 39.8% reacted simultaneously with two or more serovars. The most frequently detected serovar was Bratislava, which was found in 41.6% of all positive sows, followed by Icterohaemorrhagiae (38.5%), Australis (26.1%), Autumnalis (25.5%), Copenhageni (17.4%), Tarassovi (4.3%), Grippothyphosa (3.7%), Canicola (1.2%), Saxkoebing (1.2%), Pomona (0.6%) and Hardjo in none samples (0%). On average, 3 different serovars were detected per herd. Of all positive herds, only 17.0% reacted with only one serovar, whereas 83.0% reacted simultaneously with two or more serovars. Most of the positive herds tested positive for serovar Bratislava (76.6%), followed by Icterohaemorrhagiae (57.4%), Autumnalis (53.2%), Australis (46.8%), Copenhageni (36.2%), Tarassovi (12.8%), Grippothyphosa (10.6%), Canicola (4.3%), Saxkoebing (2.1%), Pomona (2.1%) and Hardjo in none herd (0%).

# **Discussion and Conclusion**

A prevalence of about 31.5% for a leptospiral infection was determined for sows with recent reproductive problems in UK. Pigs in UK are probably a reservoir host for serovars Bratislava, Icterohaemorrhagiae, Australis, Autumnalis and Copenhageni. Incidental infections were detected for serovars Tarassovi, Grippothyphosa, Canicola, Saxkoebing and Pomona. Further investigations on risk factors is warranted.

## IMPACT OF ANAEROBIC DIGESTION OF PIG MANURE ON CAMPYLOBACTER SPP SURVIVAL IN BIOGAS PLANTS

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# **Background and Objectives**

In the context of developing renewable energies and recovering organic waste, on-farm anaerobic digestion represents an alternative way to handle biowaste with energy. In France, most of biogas plants (BP) fed with manure operate at mesophilic conditions converting organic matter to biogas and by-product degradation, i.e. digestate. This digestate, used as a fertilizer, is usually spread on agricultural land, either after storage or post-treatment.

## Material and Methods

The presence and numeration of thermotolerant Campylobacter spp. was investigated in pig manure and raw digestate of two mesophilic biogas plants (BPI and BP2) over one year. Three replicates of both manure and digestate were collected at each of the 8 visits. Isolates of Campylobacter were then typed by RFLP-PFGE using KpnI restriction.

#### Results

Campylobacter spp. was present in all manures (16) at an average concentration of 348 MPN/g ww and in only 9 out of 16 raw digestates at an average concentration of 3.5 MPN/g ww. Campylobacter species identified were in accordance with the pig manure in France, i.e. C. coli. A total of 82 Kpnl-profiles were generated from the 152 C. coli considered. After analysis with Bionumerics software, they clustered in 31 genotypes. Among them, 24 genotypes were identified for manure and only 22 for digestate. Genotypes were specific to the BP they originated from except for 1 common to both BPs. Moreover 5 and 9 genotypes were common between manure and digestate in BP1 and BP2, respectively.

## **Discussion and Conclusion**

Such treatment of pig manure can be effective in reducing the presence of this pathogen. Indeed, these results showed that thermotolerant C. coli and some genotypes were susceptible to mesophilic anaerobic digestion. It remains now to find out what allows some genotypes of C. coli strains to survive, or not, anaerobic digestion.

# INFLUENCE OF COLIPROTEC F4/F18 VACCINE ON ANTIMICROBIAL USE AND PERFORMANCES IN FRENCH FARROW-TO-FINISH FARMS

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# **Background and Objectives**

Post-weaning diarrhoea is an important animal health issue responsible for antimicrobial or Zinc Oxide use in a high proportion of affected herds. A live attenuated vaccine licenced a few years ago is a new perspective to prevent colibacillosis at weaning. A field study was performed to compare antimicrobial use and performances before and after implementation of vaccination.

# **Material and Methods**

Forty-five farrow-to-finish farms in which Coliprotec F4/F18 vaccine is administered to piglets to prevent colibacillosis at weaning were selected. Data on zootechnical performances and antimicrobial use were collected during a period of time including (i) 6 months before and (ii) 6 months after the vaccination onset. Indicators selected were Average Daily Gain (ADG), mortality rate and for antimicrobial use an indicator taking into account the exposure of weaners to antimicrobials used to treat digestive disorders (dig ALEA). Before/after comparisons were performed on (i) the 45 farms and (ii) two subsamples differing by the performances before the vaccination onset ("+ farms" and "- farms" respectively).

## Results

After vaccination, ADG from weaning to slaughter was higher (+7g/d on average) for the 45 farms and significantly higher (+25g/d) for "- farms" (p<0.0001). Mortality rate from weaning to slaughter significantly decreased in both cases (p=0.03 and p<0.001 for the 45 farms and the "- farms" respectively). AMU indicator significantly decreased by 68% for the 45 farms (p<0.0001).

# **Discussion and Conclusion**

Our study highlighted the positive effect of Coliprotec<sup>a</sup> F4/F18 vaccination especially in farms where digestive disorders at weaning were associated with low technical performances. When questioned about their perception of the impact of vaccination, farmers and vets involved the study stated it was beneficial to pig health and performances.

# ATYPICAL ESCHERICHIA COLI STRAINS PRODUCING SHIGA TOXIN 2 SUBTYPE E AND THEIR ABILITY TO CAUSE EDEMA DISEASE

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# **Background and Objectives**

Edema disease (ED) is considered a serious illness of weaned piglets and a reason for economic losses in pig holders. Typically, Escherichia coli strains harbouring the stx<sub>20</sub> gene, belonging to serogroups O138, O139, O141 or O147 and expressing adhesins F18, are described as the cause of this illness. On pig farms, stx<sub>20</sub> positive E. coli carrying different O-antigens and F18 negative are also detected. Therefore, this study aims to characterize stx<sub>20</sub> positive E. coli strains based on the determination of serogroups, adherence factors and haemolysis on blood agar. Furthermore, their ability to produce Shiga toxin 2 in vitro was assessed using a Vero cells assay and IC<sub>50</sub> was determined. The results were compared with strains typical for Edema disease.

## **Material and Methods**

For the study, 38 strains isolated from animals with clinical signs of ED were selected. In all strains, detection of Shiga toxin genes (stx), enteroxotin genes and F4, F18, F5, F6, F41 adhesins was performed. In vitro, cytotoxic effect using Vero cell assay was assessed.

## Results

In total, 20 strains associated with ED were determined as atypical. The serogroup was not specified, adhesins F18 were not detected, but in 5 strains (25%) high cytotoxic effect ( $IC_{50}$  >64000) was confirmed in vitro. Out of 18 strains detected as typical cause of Edema disease, most of them belonged to O139 and were equipped with fimbrial adhesins F18. In all these strains, the production of Shiga toxin was confirmed at least in the dilution of 1:1000.  $IC_{50}$  >64000 was detected in 4 strains (22%).

# **Discussion and Conclusion**

The results of our study confirmed that even atypical E. coli strains carrying the stx<sub>20</sub> gene may be involved in Edema disease. Therefore, the Shiga toxin confirmation is the crucial diagnostic step. All other characteristics are rather complementary.

## PREVALENCE OF STX2E POSITIVE ESCHERICHIA COLI ON SELECTED FARMS IN BELGIUM

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# **Background and Objectives**

Edema disease (ED) is an enterotoxemia that is caused by F18-positive Shiga toxin producing Escherichia coli (STEC), that affects weaned piglets, causing economical damage on farms worldwide. The aim of this study was to established the prevalence of STEC in selected farms in Belgium.

# Material and Methods

For this study, 10 farms were selected, that had previously described clinical symptoms, which might be characteristic for ED. Six pens/farm were randomly selected and in every pen, fresh faeces from at least five piglets was collected in order to prepare pool sample. Sixty swabs in total were transported for isolation of E. coli and identification of virulence factors by multiplex PCR (F4, F18, Stx2e, STa, STb and LT). A questionnaire was completed during every farm visit.

## Results

On six farms (6/10) isolated strains were positive for the Stx2e gene and one isolate was positive for both the adhesin F18 (FedF) and Stx2e genes. In total, eleven isolates were positive for Stx2e and seven isolates were positive for F18. Six farms were positive for different virotypes of enterotoxigenic E. coli (ETEC) linked to post-weaning diarrhoea. Three farms (3/6) had virotypes of ETEC in combination with E. coli having the Stx2e gene. Four farms were positive for E. coli isolates with Stx2e gene in combination with STa and STb or STb genes only.

# **Discussion and Conclusion**

Escherichia coli with possibility to produce Shiga toxin is a common finding on Belgian farms with historically reported clinical symptoms of ED. The extensive use of antibiotic treatment (all farms included), with routine medication on 4/10 farms, could be one of the possible reasons, why the clinical expression of ED is lower despite a high rate of Stx2e positive strains isolated.

DOSE SELECTION AND DOSE CONFIRMATION OF AIVLOSIN® 625 MG/G GRANULES FOR USE IN DRINKING WATER (TYLVALOSIN) FOR THE TREATMENT AND METAPHYLAXIS OF PIG ENZOOTIC PNEUMONIA ASSOCIATED WITH MYCOPLASMA HYOPNEUMONIAE (MHP) INFECTIONS

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# **Background and Objectives**

Following EMA/CVMP/627/2001-Rev.1 requirements, these studies aimed to select and confirm the optimal dose of a water soluble formulation of tylvalosin for the treatment and metaphylaxis of pig enzootic pneumonia associated with Mhp.

# **Material and Methods**

Treatments were given in water for 5 consecutive days. Efficacy was evaluated based on reduction of lung lesions and Mhp recovery vs. untreated controls. In a Mhp-only dose determination (DD) study (group-n=48), pigs were inoculated with Mhp and treated with 5, 7.5 or 10 mg tylvalosin/kg body weight (BW). In a Mhp-Pasteurella multocida (Pm) DD study (group-n=8), pigs were sequentially inoculated with Mhp and Pm and treated with 5 or 10 mg tylvalosin/kg BW. The selected 10 mg tylvalosin/kg BW dose was further evaluated in two dose confirmation (DC) studies using the same experimental models (Mhp-only study group-n=102; Mhp-Pm study group-n=44).

# Results

In the Mhp-only DD study, mean lung lesion scores (LLS) were 29.7, 26.3, 25.0 and 24.4 in the Control, 5 (p=0.19), 7.5 (p=0.07) and 10 (p=0.05) mg tylvalosin/kg BW groups, respectively. In the Mhp-Pm DD study, mean LLS were 14.1, 8.0 and 9.6 in the Control, 5 (p=0.11) and 10 (p=0.24) mg tylvalosin/kg pigs, and the lowest Mhp recovery from the lungs was at 10 mg tylvalosin/kg (p<0.01 vs. 5 mg/kg and Control). In the Mhp-only DC study, tylvalosin pigs had lower LLS than Control (6.52 vs. 14.97; p<0.001). In the Mhp-Pm DC study, tylvalosin pigs had lower LLS (3.32 vs. 8.37; p<0.01) and lower Mhp recovery from the lungs (p<0.01) than the Control.

# Discussion and Conclusion

These studies demonstrate that Aivlosin<sup>®</sup> 625mg/g granules (ECO Animal Health, UK) at 10 mg tylvalosin/kg for 5 days is efficacious against enzootic pneumonia caused by Mhp and has greater clinical and bacteriological efficacy than at the lower tested doses.

## HOW TO CONTROL MYCOPLASMA HYOPNEUMONIAE INFECTION IN BREEDING HERDS?

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## **Background and Objectives**

Mycoplasma hyopneumoniae (M.hyo) remains a threat to swine production although vaccination protocols are widely implemented. Literature highlights the relationship between sows and/or weaned piglets' M.hyo prevalence and lung lesions' severity. This study aims at evaluating the prevalence of M.hyo infection in breeding herds and in weaned piglets and at assessing the link between vaccination protocols and M.hyo excretion.

## Material and Methods

The study was carried out in 20 farms - located in the West of France - with different vaccination's protocol. Monodose vaccines from eight different specialties were used either on gilts (one farm), piglets (eight farms), or both (nine farms) and with different protocols for piglets (before, after or at weaning). Two farms did not vaccinate against M.hyo. To evaluate M.hyo excretion, laryngeal swabs were collected from 10 sows and for two piglets per sow just before weaning. In the post-weaning unit, six and nine-week-old piglets were individually swab-sampled. Oral fluids were collected with five ropes per age. In the fattening unit, only oral fluids were collected (four different ages). All samples were tested for M.hyo using qPCR.

#### Results

Only 18 piglets coming from three herds were shedding M.hyo at weaning (18/400) with a high variation of positive samples in each herd (from 5 to 75% shedding piglets per herd). Four herds had at least one PCR positive sow. The pair sow-piglet shedding was not systematic (only 11/18 positive piglets born from positive sows). Regarding the impact of vaccination, the results showed a positive trend of gilt vaccination to prevent the risk of having M.hyo positive piglets in post-weaning and during fattening.

## **Discussion and Conclusion**

The study highlights a low prevalence of sows and piglets shedding M.hyo at weaning and a variable prevalence at the herd level. The combined vaccination of gilts and piglets constitutes an efficient approach to control M.hyo infection across the entire farrow-to-finish process.

# ASSESSMENT OF THE EFFECT OF STEC-2E E.COLI POSITIVITY OF PIGLETS ON ZOOTECHNICAL PERFORMANCE ON A FARM WITH HISTORY OF EDEMA DISEASE

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# **Background and Objectives**

Edema disease (ED) is one of the major diseases in pigs during nursery. It is caused by Escherichia coli producing Shiga toxin 2e (STEC). The goal of the presented study was to investigate the possible effect of positivity and thus possible colonization of piglets by E. coli ( $stx_{2e}+/stx_{2e}-$ ) on production results during the nursery period, on a farm with a history of ED.

# **Material and Methods**

The study was carried out on a farm with known history of ED occurrence. In rectal swabs of 61 piglets, the presence of  $stx_{2e}$  using PCR was determined at 3 weeks after weaning and at the end of the nursery period. Piglets, positive at least once were assigned to group A ( $stx_{2e}$  +),  $stx_{2e}$  negative piglets were assigned to group B. They were weighted at weaning and at 69 days of age (DOA). Twelve piglets (12/61, 19.7%) were at least once positive for  $stx_{2e}$  gene and assigned into group A. The group B was composed of 49 piglets.

## Results

The isolated strain was confirmed as in-vitro Stx2e producer by Vero cell assay. There was no statistical difference between means  $(B - A) \pm SEM$  at weaning: 0.1335  $\pm$  0.3628 kg (p= 0.7142) and therefore both groups could be compared. The difference in mean weight gain at the end of the trial (69 DOA) was numerically better in group B: 16.76 kg vs. 14.68 kg (p= 0.08424).

# **Discussion and Conclusion**

Despite of low number of stx<sub>20</sub> positive animals detected in this study, a potential negative effect of possible colonization of piglets by STEC was observed. The numerical difference in weight gain between the groups was in the favor of Stx-2e negative piglets and was 2.08 kg, which was close to significance (p= 0.08424). Further studies are needed in order to confirm the possible, negative effect of colonisation.

# PROTECTION PROVIDED BY THREE DIFFERENT VACCINES AGAINST CHALLENGE INFECTION WITH ACTINOBACILLUS PLEUROPNEUMONIAEE SEROTYPE 4 AND 6 STRAINS

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# **Background and Objectives**

The distribution of Actinobacillus pleuropneumoniae (A.p.) serotypes, the causative agent of porcine pleuropneumonia differs substantially among countries. Serotype 4 (A.p.4) and 6 (A.p.6) are prevalent in Southern and Northern Europe, respectively. The aim of this study was to determine the efficacy of Coglapix<sup>®</sup> (Ceva), a toxoid+bacterin (serotype 1 and 2) vaccine, vaccine-C, and compare its efficacy to two widely used vaccines, a toxoid+OMP vaccine and another toxoid+bacterin (serotype 1, 2, 3, 4, 5, 7) vaccine. vaccines-A and -B, respectively.

# **Material and Methods**

Seven weeks old pigs vaccinated and then boosted ten weeks old, either with vaccine-A, vaccine-B, Vaccine-C and non-vaccinated positive controls were challenged with ~10<sup>6</sup> CFU/pig of the challenge bacterium suspended (A.p.4, isolated in Spain in 2017 and A.p.6, of Denmark, also from 2017) in PBS by the aerosol route, 13 weeks old. After one week of observation the surviving pigs were euthanized and their lung and pleura lesions scored (LLS) according to severity and extension, and group means were compared.

## Results

A.p.4 challenge: the mortality rates were 0, 5%, 5%, and 10% for vaccine-C, vaccine-A, vaccine-B, and for the positive control, respectively. The group mean LLSs were 0.66<sup>\$</sup>, 1.1<sup>α</sup>, 1.16<sup>α</sup>, and 1.44<sup>α</sup> for vaccine-C, vaccine-A, vaccine-B, and the positive control group, respectively. A.p.6 challenge: the mortality rates were 10%, 10%, 35%, and 30% for vaccine-C, vaccine-A, vaccine-B, and positive control group, respectively. The group mean LLSs were 0.92<sup>#</sup>, 0.96<sup>#</sup>, 1.66<sup>6</sup>, and 1.79<sup>6</sup> for vaccine-C, vaccine-A, vaccine-B, and the positive control group, respectively. Statistically significantly different groups (at 95% confidence level) indicated by differing symbols.

# **Discussion and Conclusion**

Vaccine-C confirmed its superior efficacy even against the heterologous serotype 4 and 6 A.p. strains, which represented the prevalent strains of their geographic origin, proving the broad protection range of this vaccine.

## SALMONELLA SPP. IN PIG FARMS - STILL AN ISSUE?

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# **Background and Objectives**

Salmonellosis is important zoonotic disease worldwide. Source attribution studies show pig meat as one of the common source of salmonella for humans in the European Union. Therefore, in many countries control strategies to reduce salmonella occurrence and various biosecurity measures are applied on farms. The project Biopigee from One Health European Joint Programme aims to identify the best biosecurity practices in pig farming to prevent the pork meat food chain from salmonella. This study, as a part of the project, was performed to obtain actual data on salmonella occurrence in pig farms in the Czech Republic.

# Material and Methods

Breeding (4), fattening (3) and farrow-to-finish (7) farms were sampled from October to November 2020. On each farm, 20 pooled samples of feces were taken in different pig categories based on the type of farm. Samples were processed according to ISO 6579:2017.

## Results

In total, 280 fecal samples were analysed. Salmonella spp. was confirmed in 3 breeding and 5 farrow-tofinish farms. All fattening farms were salmonella negative. The most frequently positive were gilts (37/124; 30%) followed by sows (5/26; 19%). Salmonella spp. in finishing pigs were found only on farrow-to-finish farms in almost 16% of samples (11/70).

# **Discussion and Conclusion**

These preliminary results confirm that salmonella is still an important issue in the Czech Republic, despite all farms reported applying of biosecurity measures and control strategies. Sampling and evaluation of the results on national level and their comparison on EU level will follow. This work was supported by the EU Horizon 2020 project EJP One Health No. 773830.

# A 3-YEAR RETROSPECTIVE INVESTIGATION ON THE PREVALENCE OF ALPHA- AND BETAHEMOLYTIC STREPTOCOCCUS SPP. IN DISEASED PIGS IN AUSTRIA

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# **Background and Objectives**

Even though Streptococcus (S.) suis is the most important streptococcal species for the world-wide swine industry, other Streptococcus species seem to represent an underestimated risk for porcine health. Objective of this study was to evaluate the tissue frequency of these species in diseased pigs with defined clinical anamnesis over a three year period.

# **Material and Methods**

553 alpha- and beta hemolytic porcine Streptococcus spp. isolates were included. They originated mainly from the respiratory tract, central nervous system, serosal tissue and joints of nursery and fattening pigs, but also from abortion material or samples of the genitourinary tract. After bacteriological examination isolates were further identified by MALDI-TOF.

# Results

Most isolates were identified as S. suis (54%), followed by S. dysgalactiae subsp. equisimilis (SDSE), S. hyovaginalis, S. thoraltensis, S. alactolyticus, S. porcinus and S. orisratti. All isolates from skin lesions belonged to the species SDSE, which were also significantly more often recovered from specimens of the genitourinary tract than S. suis. S. hyovaginalis was most frequently recovered from the upper respiratory tract of fattening pigs and S. thoraltensis from the genitourinary tract of sows.

# **Discussion and Conclusion**

Interpretation of findings of Streptococcus besides S. suis in tissues of diseased pigs is often difficult, especially if isolated of dead animals or without knowledge about premedication or the way the diagnostic sample was taken to exclude contamination. Nevertheless, some streptococcal species such as SDSE and S. thoraltensis might have higher impact on the clinical outcome than thought so far.

# SHIGA TOXIN 2E-PRODUCING E. COLI IN SWINE: PREVALENCE AND RISK FACTORS EVALUATION ON CZECH PIG FARMS

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# **Background and Objectives**

Edema disease (ED) in weaned piglets caused by Shiga toxin-producing Escherichia coli (STEC) is an important syndrome, causing losses due increased mortality and reduced weight gain in endemically affected farms. The aim of this presented study is to evaluate the prevalence of shiga toxin encoding E. coli (STEC-2e) and to identify possible risk factors.

# **Material and Methods**

A total of 15 farms were included. From each pen (4–5 pens sampled per farm), pooled sample from at least 3 randomly selected piglets/ pen ( $\pm$  3 weeks after weaning) was taken and sent for cultivation on bacteriological plates. From each plate, a mixture of presumptive E. coli positive colonies was picked and screened for Stx-2e + F18 genes using multiplex PCR assay. Data about farm management were always collected using a survey questionnaire. The logistic regressions model was used to assess the factors associated with the presence of Stx-2e/F18 genotype: farm size, early weaning, ZnO + acids use, restricted feed, wet feed. Covariates and interactions were tested at 10% (SAS® version 9.4).

# Results

Eight farms (53.3%) were positive for Stx-2e/F18 STEC strains. Additionally, 62.5% (5/8) of positive farms reported a history of clinical ED. 29 STEC strains (Stx-2e/F18) were isolated (168 samples in total – 17%). No significant risk factors associated with Stx-2e positivity were detected, only the trend related to the size of the farm (< 500 sows) was identified (p= 0.0774).

# **Discussion and Conclusion**

Overall, a high 53,3% rate of farm prevalence of shiga toxin encoding E. coli (STEC-2e/FI8) was found. Positive farms, with NO clinical ED history (3/8) had no clinical outbreaks within the near future after sampling. No specific risk factors were determined, however, for a more precise assessment, a higher number of farms should be evaluated.

# SEROPREVALENCE OF ANTIBODIES AGAINST LAWSONIA INTRACELLULARIS IN PIGS OF DIFFERENT AGES ON FARMS IN UKRAINE

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# Background and Objectives

# Background and Objectives

Porcine proliferative enteropathy (PPE) caused by Lawsonia intracellularis was first reported in Ukraine in 2008.

Serological studies in 2010/2011 demonstrated the seroprevalence of antibodies against Lawsonia intracellularis of

36.5%. Since then, no monitoring was done. This study investigate the current prevalence of Lintracellularis on large

swine farms in Ukraine.

# Material and Methods

Material and Methods

For serological testing, a commercial ELISA kit for specific L. intracellularis antibodies in porcine sera (SVANOVIR®

Lintracellularis/lleitis-Ab, Svanova) was used. We examined 619 blood serum samples from pigs of different ages,

originated from 13 farrow-to-finish large commercial farms (range from 520 to 13704 sows per farm) located in

different regions of Ukraine. Average number of samples per farm was 47 (range from 30 to 56 samples/farm). The

total number of samples from sows, gilts, 6-week old pigs, 9-week old pigs, 12-week old pig 15-week old pig, 18-week

old pig and 22-week old pig was 72, 54, 65, 100, 38,104, 86, and 100 respectively.

# Results

Results

Out of 619 tested samples 320 (51,7%) were positive for specific antibodies against L. intracellularis. We found Lintracellularis antibodies on all tested farms. The highest percentage of seropositive pigs was found among sows

(86,1%), finishers 22-weeks old (80,0%). Followed by gilts (79,6%),18-weeks old (76,7%), and 15-week old pigs (64,4%).

In growers the percentage of seropositive pigs was significantly lower (0.99% of all pigs of 6-12 weeks old).

# Discussion and Conclusion

Discussion and Conclusion

The data shows that Lintracellularis is widely spread on large swine farms in Ukraine. High percentage of seropositive

sows, gilts and finishers, indicate that these pigs' groups have the highest exposure to Lintracellularis. The investigation indicate, that the infection with Lintracellularis take place mostly at the age of about 12 weeks (early

finishing).

# FACTORS ASSOCIATED WITH STREPTOCOCCUS SUIS AUTOGENOUS VACCINE SUCCESS IN FRENCH PIG FARMS

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# **Background and Objectives**

Streptococcus suis (S. suis) is a commensal bacterium of the upper respiratory tract of pigs responsible for meningitis and septicaemia in piglets. Today with the need to reduce antibiotic consumption implemented by European laws, the administration of autogenous vaccine to sows appears an interesting medical option to prevent streptococcosis. However, the efficacy of these vaccines can vary considerably in the field. In the literature, only few studies investigating the efficacy's factors have been published with controversial results. The aim of this study was to assess the influence of herd-management factors on the efficacy of autogenous vaccine through the investigation of clinical cases.

# **Material and Methods**

Twelve farms whose sows were administered an oiled autogenous vaccine against Streptococcus suis were selected. Efficacy was assessed by comparing the frequency and severity of clinical signs and antibiotic treatments against streptococcosis before and after vaccine implementation. Data on farm management, preventive measures (biosecurity, hygiene, vaccinations), farm sanitary status and autogenous vaccine composition (S. suis serotypes and other bacterial species) were collected. Zootechnical performances and laboratory analyses were also recorded. A systematic visit of the farm was performed including the examination of all categories of pigs and the evaluation of housing conditions. A Multiple Correspondence Analysis was conducted to identify herd factors associated with failure or success.

# Results

Failure was significantly more frequent for farms with hyperprolific sows (P=0,005) and farms in which growing pigs exhibited digestive or respiratory disorders. Other herd-management practices like tooth grinding (P=0,02), insufficient hygiene and biosecurity were also associated with failure.

# **Discussion and Conclusion**

The levers highlighted in this study offer the opportunity for veterinarians to provide advices to farmers to improve efficacy of vaccination. The focus has to be placed on (i) colostrum intake, crucial for piglet immunity and jeopardised with hyperprolific sows, (ii) management of digestive and respiratory co-infections and (iii) compliance on hygiene and biosecurity.

# TRUEPERELLA ABORTISUIS - TEN YEARS IN GERMAN SOW FARMS

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# **Background and Objectives**

Arcanobacterium abortisuis was isolated first time from a placenta from a sow following abortion in Japan 11 years ago (Azuma et al., 2009). In 2011 the new genus Trueperella was proposed (Yassin et al., 2011). Even though isolation of Trueperella abortisuis (TA) was rare in those times it was clearly connected with abortion or endometritis (Metzner et al., 2013).

# Material and Methods

Cultivation of TA was carried out under microaerophilic conditions at 37°C on standard blood agar. Small colonies appear after 24 to 48 hours. Species identification was carried out by MALDI-TOF mass spectrometry.

## Results

More than 50 field isolates collected during last 10 years had been identified as TA. However an increase of isolations in the years 2018 to 2020 was observed in german samples. 109 vaginal swabs of sows with discharge have been collected in 2018 and 2019. Out of those TA was detected in a total of 26 samples. In almost all samples mixed infections with the following bacteria were detected: Streptococcus suis (35 positive samples), other Streptococcus species (63), Escherichia coli (25), Staphylococcus hyicus (17), Actinomyces hyovaginalis (15), Enterococcus species (9), Trueperella pyogenes (6), other Staphylococcus species (5), other bacteria (28). Virological or toxicological examinations were not carried out. Furthermore only four additional porcine isolates have been isolated from other organs.

#### **Discussion and Conclusion**

On the one hand the role of TA as a primary pathogen remains unclear. But on the other hand the new findings confirm the assumption that TA is specific for swine and has a strong affinity to the female reproductive organs. Since only a few publications of this emerging pathogen exist, it is likely to be underrepresented in routine diagnostics. And finally the present study shows that immune prophylaxis is possible by using autogenous vaccines.

# PREVALENCE OF BORDETELLA BRONCHISEPTICA IN HERDS WITH RESPIRATORY DISEASE. A SCREENING OF LIVE AND EUTHANIZED PIGS

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# **Background and Objectives**

Bordetella bronchiseptica is a frequent agent involved in respiratory disease on piglets and causes Non-Progressive Atrophic Rhinitis (NPAR) often seen with mucous secretion, deviated snout and bronchitis. B. bronchiseptica intensify other infections e.g. influenza and PRRS, and cause comorbidities such as pneumonia and septicaemia. Therefore B. bronchiseptica can, solemnly or together with other pathogens, cause decreased productivity.

## Material and Methods

During 2019 SEGES Laboratory of Pig diseases (Denmark) tested 65 euthanized pigs from several herds, showing rhinitis associated with B. bronchiseptica. The pigs where received for routine diagnostics and not all samples where tested for all agents. In 2020, a screening for B. bronchiseptica using Rhinicheck (PCR oral fluid samples on live animals) included 19 Danish sow herds was performed. The herds had a history of respiratory disease at the time of sampling. The sampling took place in 4 double-pens in each herd using one sampling rope per double-pen. The pigs where sampled at 1 week and 3 weeks after weaning, and all pens in each herd where sampled the same day.

#### Results

Out of 55 pigs with positive results in the routine testing, 26 were positive for B. bronchiseptica giving a prevalence of 47%. From the screening of live animals 17 herds where positive for B. bronchiseptica, hereof 4 with massive infections and 12 with moderate infections.

#### **Discussion and Conclusion**

bronchiseptica was the most frequent diagnosed respiratory disease agent on piglets with rhinitis send SEGES Laboratory of Pig diseases in Denmark for routine diagnostics and the prevalence of B. bronchiseptica among 19 herds with respiratory diseases where 89%. These results show that B. bronchiseptica is widespread agent in Danish farms, and due to the antibiotic reduction policies, other precautions, such as vaccination, should be considered to prevent B. bronchiseptica from creating problems in the stables.

# BRONCHOPNEUMONIC LESIONS AT SLAUGHTER IN ANIMALS VACCINATED WITH DIFFERENT MYCOPLASMA HYOPNEUMONIAE VACCINES IN GREEK SWINE FARMS

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# **Background and Objectives**

Vaccination of pigs is a common practice to control Mycoplasma hyopneumoniae (Mhyo) infection, which causes bronchopneumonia and predisposes to secondary infections of the respiratory tract. The objective of the present study was to compare the prevalence and severity of bronchopneumonic lesions among animals vaccinated with different Mhyo vaccines, in Greek swine farms.

# **Material and Methods**

From January 2016 to August 2019 examination of lungs at slaughter was performed for 55 different farms located in mainland Greece. Lungs were scored for bronchopneumonic lesions by using the Ceva Lung Program® Methodology and software tool. A questionnaire was used to collect all necessary information for the farms. The analytical unit was the lung.

#### Results

In total, 7908 lungs belonging to 113 different batches were examined. Although 8 different vaccines were recorded, 75% of the animals were vaccinated with one of the three vaccines: a monovalent double shot vaccine (A), a bivalent vaccine containing Mhyo and PCV2 antigens (B) and the monovalent single shot vaccine, Hyogen® (C). Animals vaccinated with vaccine C had 0.58- and 0.22-times lower likelihood (P=0.004 and P<0.001, respectively) to have bronchopneumonic lesions compared to vaccines A and B, respectively. Also, the percentage of consolidated tissue was by 0.78% and 3.6% lower (P=0.087 and P<0.001, respectively) for vaccine C compared to A and B, respectively. Results were adjusted for the random effect of the different farms and batches of animals.

# **Discussion and Conclusion**

According to the results of the present almost 4-year survey, the animals vaccinated with the monovalent single shot vaccine C were less likely to have bronchopneumonic lesions at slaughter compared to the animals vaccinated with the double shot and the bivalent vaccine, in the Greek farms. When lesions were present, the extent of affected tissue was lower for pigs which were vaccinated with vaccine C compared to the other vaccines.

# SEROLOGY - A NEW DIAGNOSTIC TOOL TO EVALUATE SEVERITY OF LAWSONIA INTRACELLULARIS INFECTION?

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# **Background and Objectives**

For many endemic diseases, increases in disease levels are associated with decreases in productivity. For Lawsonia intracellularis (LI), high levels of fecal shedding (qPCR) has been found related to reduced average daily gain (ADG). Due to infection dynamics, it is, however, impossible to know, if the sample is collected at or far away from the peak of infection. Serological tests do not suffer this disadvantage but have historically mainly been used to determine presence of infection rather than level. This study aims to evaluate if the magnitude of the serological response to LI is correlated to ADG.

# **Material and Methods**

In a farm with clinical disease caused by LI, 91 pigs from 25 different litters were weighed at 3 and 9½ weeks of age. Pigs were bled at 9½ weeks of age and serological response to LI was analyzed using ELISA-LAW Svanova test at MSD service laboratory in Boxmeer, NL. ADG and OD-values (% inhibition) were negatively correlated (r=-0.38, p<0.001).

# Results

After correction for weaning weight and room by linear regression, an estimated decrease in ADG of 1.3 g for every one unit increase in OD-value was found (R<sup>2</sup>=0.301). Applying this model estimate to the two most extreme OD-values in the data set (3.4 and 93.3 % inhibition), an ADG difference of 117 g for these two pigs is implied.

# **Discussion and Conclusion**

To our knowledge, this is the first time a correlation between the serological response and the ADG in the preceding period has been demonstrated. The magnitude of the serological response in a group of pigs might therefore be a useful diagnostic alternative to evaluate the economic impact of a LI infection.

# IDENTIFICATION OF ACTINOBACILLUS PLEUROPNEUMONIAE (APP) SEROTYPES IN SWINE PLEUROPNEUMONIA OUTBREAKS IN GERMAN SWINE HERDS

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# **Background and Objectives**

Pleuropneumonia remains an important challenge to swine production worldwide. A German serological APP-screening of ApxIV-positive farms (N=44), found APP-serogroup-ELISA herd prevalence of: 3/6/8/15 (81.8%), 2 (63.6%), 5 (63.6%), 12 (56.8%), 4/7 (38.6%), 10 (63.6%) and 1/9/11 (22.7%); 93.2% of the farms were serologically positive for 2-6 out of 7 ELISA-serogroups (Renken 2016). The objective of this study was, with highly reliable methodology on APP-isolates, to identify the clinically relevant serotypes, others than serotype 2, currently present in Germany, and in most resent isolates, to compare to present local lab methodology.

# **Material and Methods**

Sixty-four samples isolated from lung lesions typical for APP infection, were collected on as many German swine farms during clinical outbreaks of pleuropneumonia. All isolates, tested as non-serotype 2 App, were selected into two batches, one historical, 2002-19, and one recent, September 2019 to February 2020. APP isolation was performed at two local German labs, transferred to FTA-cards and shipped to Imperial College London. Serotyping, based on capsular loci, was carried out by multiplex-PCR (mPCR) as described by Bossé et al. (2018). The recent strains were also characterized by toxin gene PCR (Rayamajhi et al.2005) and agglutination with specific commercially available rabbit antisera (Porcs Reactif coagglutine, BioVac, Beaucouzé Cedex, France) at the local lab.

# Results

Historical batch 2002-19 (N=57): serotype 2 (17.5%), 5 (31.6%), 6 (8.8%), 9/11 (24.6%), 16 (12.3%), 9/11+16 (1.8%) and 9/11+18 (3.5%). Most recent batch (N=7) in mPCR versus traditional serotyping: 6 vs 8 (2), 7 & 18 vs non-typable (2), 9/11 vs 1, 9 & 9+11 (3).

# **Discussion and Conclusion**

The quality of the mPCR data in serotype identification is very high; at present state-of-the art, so the identification of APP serotypes 2, 5, 6, 7, 9/11, 16, and 18 as causes of acute clinical and pathological pleuropneumonia in Germany is highly reliable and more accurate compared to traditional methods.

# IDENTIFICATION OF ACTINOBACILLUS PLEUROPNEUMONIAE (APP) SEROTYPES IN SWINE PLEUROPNEUMONIA OUTBREAKS IN SPANISH SWINE HERDS

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# **Background and Objectives**

Pleuropneumonia remains an important challenge to swine production worldwide. Country APP serotype prevalence can change over time. In previous APP prevalence, coagglutination test studies in Spain: in 2009 Maldonado et al. only investigated the n=127 biovar-2 of N=502 isolates finding serovar 2 (1.2%), 4 (1.2%), 7 (17.3%), 11 (0.4%) and non-typeable (5.2%), in 1995 as Gutiérrez et al. investigated N=71 solely biovar-1 isolates and found serovar 4 (42.2%), 7 (22.5%) and 2 (12.8%) plus serovars 1, 3, 6, 8, 9, 12 and non-typable only in small numbers. The objective of this study was, in a coherent manner with highly reliable methodology, to identify the clinically relevant serotypes of APP currently present in Spain.

# Material and Methods

Fifty-seven samples of lung lesions typical of APP infection were collected during clinical outbreaks of swine pleuropneumonia between August and November 2019. APP isolation was performed at two local Spanish labs, transferred to FTA-cards and shipped to Imperial College London. All swine producing regions of Spain were represented in the 57 samples collected at as many finisher farms; pigs originating from 45 sow herds. Serotyping, based on capsular loci, was carried out by multiplex-PCR as described by Bossé et al. (2018).

#### Results

Eight serotypes were detected from the 57 APP isolates: Serotype 2 (11/57=19.3%), 4 (14/57=28.1%), 5 (2/57=3.5%), 6 (1/57=1.8%), 9 (8/57=14.0%), 13 (7/57=12.3%), 17 (13/57=22.8%), and Ap18 (1/57=1.8%).

# **Discussion and Conclusion**

Fifty-seven APP isolates, although representing all swine producing regions of Spain, cannot be considered truly representative; the rate of serotype identification cannot be translated into a country prevalence. However, the quality of data in the serotype identification is very high; at present state-of-the art, so the identification of APP serotypes 2, 4, 5, 6, 9, 13, 17, and 18 as causes of acute clinical and pathological pleuropneumonia in Spain is highly reliable.

# SEROTYPE ANALYSIS OF THE STREPTOCOCCUS SUIS STRAINS ISOLATED IN BELGIUM BETWEEN FEBRUARY 2018 UNTIL OCTOBER 2020

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# **Background and Objectives**

Streptococcus suis is an important swine pathogen that predominantly affects suckling and weaned piglets. Currently 35 different serotypes of Streptococcus suis are known. Septicaemia, meningitis and purulent arthritis are some of the lesions that occur. Streptococcus suis can be isolated from these lesions both as a primary or secondary pathogen. The study of the data shows which serotypes of Streptococcus suis are most commonly isolated in Belgium.

## Material and Methods

The strains are mainly isolated from weaned piglets and serotyped at the Belgian lab Diergezondheidszorg Vlaanderen (DGZ) and the isolates are subsequently transported to Ceva Biovac. At Ceva Biovac, the serotyping is repeated by co-agglutination (with antisera developed in-house) of the 12 most common serotypes to confirm the obtained result. The data used for this study originates from Ceva Biovac, where a database of all the strains used for their autogenous vaccines is kept. The focus of this study is limited to Streptococcus suis strains isolated in Belgium from February 2018 until October 2020 and the serotype from Ceva Biovac is used.

## Results

A total of 550 Streptococcus suis strains, submitted by 39 veterinarians from 126 Belgium sow farms and farrow-to-finish farms (representing 7.9% of farms), were analyzed. In descending order of serotype (ST) detection: ST9 (29%), ST7 (18.5%), non-typable (13.1%), ST2 (12.5%), ST4 (8.2%), ST8 (4.7%), ST1 (4%), ST3 (2.9%), ST1/2 (2.2%) and other serotypes (4.9%). Serotype 9 has remained the dominant serotype for the selected samples in Belgium during the period February 2018 until October 2020.

# **Discussion and Conclusion**

ST9, ST7 and ST2 remain the most important serotypes isolated in Belgium (excluding non-typable strains) for the period February 2018 until October 2020 which could be used in autogenous vaccines. There is no clear shift towards a different serotype yet, but there is a tendency towards more non-typable Streptococcus suis strains in 2019 and 2020.

## PREVALENCE OF CHLAMYDIA SUIS IN SWINE SAMPLES ANALYSED FROM 2018 TO 2020: AN EMERGING PATHOGEN?

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# **Background and Objectives**

Chlamydia suis infection in pigs has been associated with several clinical signs: conjunctivitis, pneumonia, enteritis and reproductive failure. Out of these signs, infection is often subclinical and C. suis detection is usually not performed in diagnostic routine. C. suis is frequently detected in mixed infections with other pathogens. The aim of the study was to evaluate the prevalence of C. suis in pig samples, alone or with other pathogens, and the correlation with clinical symptoms.

# Material and Methods

Eight hundred and thirty-seven samples (faeces, fetuses, sperm, nasal and bronchial swabs, vaginal and conjunctival swabs) were analyzed from 2018 to 2020. The pigs were sampled in Northern Italy farms. Samples were screened by Chlamydiaceae real-time PCR and positive samples were analyzed by C. suis-specific real-time PCR. The presence of co-infections and of clinical symptoms in pigs was evaluated.

## Results

Out of 837 samples, 101 resulted positive for C. suis (12%). Positive samples were faeces (28%), nasal and bronchial swabs (26%), fetuses (18%), sperm (10%), vaginal (10%) and conjunctival swabs (8%). A retrospective analysis showed that fetuses, nasal, bronchial and conjunctival positive swabs were collected from symptomatic pigs, while faeces, sperm and vaginal positive swabs mainly from asymptomatics.

Coinfections were detected in pigs with enteric and respiratory disorders: Salmonella spp. in faeces (10%), Mycoplasma hyopneumoniae (60%), Haemophilus parasuis (58%) Actinobacillus pleuropneumoniae (46%), PRRSV (23%), SIV (15%), Pasteurella multocida (15%) and Streptococcus spp. (8%) in nasal and bronchial swabs.

# **Discussion and Conclusion**

C. suis was mainly detected in nasal and bronchial swabs in association with other pathogens in symptomatic pigs. C. suis could act like a primary pathogen and infected pigs could be more susceptible to other infections, or like an opportunistic one. Moreover, some field isolates resulted positive for the resistance gene to tetracycline, which raises a public health concern since C. suis transmission to humans has been reported.

# ERADICATION OF MYCOPLASMA HYOPNEUMONIAE BY STRATEGIC MEDICATION WITH VETMULIN®

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## **Background and Objectives**

Despite double vaccination against M. hyopneumoniae, pigs originating from a birth to weaning herd of 700 non-vaccinated sows were still suffering from chronic coughing and poor growth. M. hyopneumoniae was diagnosed by multiple serology and PCR tests. The weaners were moved weekly to a compartmentalised nursery site and finally transferred to all-in all-out finishing stables, all at different locations. An eradication programme without farrowing stop and based on strategic medication with tiamulin (Vetmulin<sup>®</sup> 100g/ kg premix-Huvepharma<sup>®</sup>) was initiated.

## Material and Methods

A hygiene and disinfection protocol was installed. The sows and gilts were treated via feed at 10 mg tiamulin hydrogen fumarate/ kg bodyweight/ day for 2 weeks. From 1 week before till the end of this treatment period, the suckling piglets received a dose of tulathromycin (2.5 mg/kg intramusculary) every 5 days. Piglets born thereafter were considered to be free and were no longer vaccinated. Blood samples (n=195, ELISA) were taken at different ages until slaughter. PCR analysis was performed on 15 oral fluids and 3 pooled nasal swabs (sows, gilts, fatteners). The impact on the zootechnical performance was investigated.

## Results

Clinical signs of enzootic pneumonia were not observed anymore. All analyses were negative for M. hyopneumoniae. Comparing data the first 16 weeks after and 16 weeks before the treatment, a reduction of 1.3% pre-weaning mortality (12.3-13.6%) and an increase of 1.05 kg weight at weaning (7.26-6.21 kg) was registered. Exit weight in the nurseries increased by 4.56 kg (32.07-27.51 kg), daily weight gain improved by 76 g (477-401 g) and mortality rate was 1.76% lower (2.22-3.98%). An increase of 123 g daily weight gain (936-813 g) along with a decrease (-0.19) of the feed conversion rate (2.54-2.73) was noted in the fatteners.

## **Discussion and Conclusion**

M. hyopneumoniae can successfully be eradicated without farrowing stop by a strategic medication with tiamulin (Vetmulin<sup>®</sup>).

# INVESTIGATING AN EXTREME CASE OF EDEMA DISEASE, WITH 70% OF MORTALITY RATE

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# **Background and Objectives**

A sudden and extremely severe case of edema disease (ED) due to E coli producing Shigatoxine Stx2e (STEC), occurred in October 2018 in a 150-sow farrow-to-finish French farm, free of PRRSv and weaning 12.04 piglets per litter. Sows were vaccinated against PCV2, erysipelas, and E. Coli F4. Our aim was to report and describe this dramatic outbreak and to investigate retrospectively the potential factors associated to its occurrence.

## **Material and Methods**

Farm documents were scrutinized over a period of a year before and six months after the outbreak. Risk factors were investigated during a farm visit using semi structured interview of the farmer around the sequence and type of feeding program, possible changes in management or the environment or genetics. Environmental sampling was performed in rooms occupied by pigs and empty ones that had been cleaned and disinfected, for bacteriology and PCR to detect presence of STEC.

#### Results

It was the first occurrence on this farm. Two batches were particularly affected by cases of sudden deaths with a peak of mortality 8 days after weaning. The first, that was diagnosed based on necropsy and isolation of septicemic STEC (OI39K82, FI8, Stx2e), resulted in 20.4% mortality rate in nursery. Despite antibiotherapy and feed restriction, all sick pigs died. As mortality reached 70% for the second affected batch, implementation of vaccination with the anti Stx2e toxine vaccine Ecoporc® Shiga was decided for the 3<sup>rd</sup> batch and is still ongoing. During the 6 next months after, mortality rate returned below the reference of the year before the ED crisis started (2.1% vs 3.4%). Presence of risk factors such as high density, commingling litters and suboptimal cleaning/disinfection procedures were identified. Persistent environmental presence of STEC was demonstrated.

## **Discussion and Conclusion**

Reasons for such a high lethality ED crisis, rarely reported in literature, were probably multifactorial but remain unknown.

# PREVALENCE AND SEVERITY OF BRONCHOPNEUMONIC LESIONS SUGGESTIVE OF MYCOPLASMA HYOPNEUMONIAE INFECTION IN GREEK SWINE FARMS

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# **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is one of the two most important primary bacterial respiratory pathogens associated with lung lesions. Evaluation of lungs at slaughter has been used to quantify prevalence and severity of bronchopneumonic lesions suggesting Mhyo infection. The purpose of the present study was to assess the prevalence and severity of bronchopneumonic lesions in Greek swine farms.

# Material and Methods

From January 2016 to August 2019 examination of lungs at slaughter was performed for 55 different farms located in several geographical regions of Greece: Epirus, Central Greece, Peloponnese, Thessaly, Macedonia and Thrace. Lungs were scored for bronchopneumonic lesions by using the Ceva Lung Program® software tool based on the Madec and Kobisch scoring system. A questionnaire was used to collect all necessary information for the farms. The analytical unit was the lung.

# Results

In total, 7908 lungs belonging to 113 different batches were examined. The mean ( $\pm$ SD) of slaughter days for the examined animals was 167.8 ( $\pm$ 15.8). 98.8% (7813/7908) of the animals were vaccinated against Mhyo. The prevalence of lungs with Mhyo-like lesions was 45.7% (3613/7908). The affected parenchyma ranged from 0 to 66.5% of the lung with a median equal to 0% and a mean ( $\pm$ SD) equal to 3.3% ( $\pm$ 6.6). Fissures of collapsed alveoli were found in 7.2% of the lungs.

# **Discussion and Conclusion**

The results of the present survey show a high prevalence of bronchopneumonic lesions suggestive of Mhyo infection in Greek swine farms. However, the severity of the lesions was low which could be attributed to the high vaccination rate against Mhyo, which has been found to reduce lung lesions but cannot prevent transmission of the pathogen and colonization of the host. Therefore, except for vaccination, optimization of management practices and housing conditions are necessary to decrease the prevalence of Mhyo infection in Greek farms.

VACCINATION WITH AN E. COLI F4/F18 VACCINE FOR THE PREVENTION OF F4-ETEC POST-WEANING DIARRHEA RESULTED IN REDUCED POST-WEANING MORTALITY AND ANTIBIOTIC USE

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# **Background and Objectives**

Post-weaning Escherichia coli diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD, caused by enterotoxigenic E. coli (ETEC), typically provokes mild to severe watery diarrhea between 5-10 days after weaning, which may result in mortality. Recently, an oral live bivalent E. coli F4/F18 vaccine (Coliprotec<sup>®</sup> F4/F18; Elanco) was approved on the European market, which reduces the impact of PWD provoked by F4-ETEC and F18-ETEC. The objective was to evaluate mortality and antibiotic use following E. coli F4/F18 vaccination under field conditions.

## **Material and Methods**

A 160-sow farm (weaning at 26 days) with diagnosed problems of PWD due to F4-ETEC was selected. Control piglets received the standard treatment protocol with antimicrobials during the post-weaning phase. Vaccinated piglets were immunized at 21 days with an oral live bivalent E. coli F4/F18 vaccine. At weaning, no standard group medication (ZnO and antimicrobials) was applied for prevention of PWD. Several performance parameters were collected: treatment incidence ( $TI_{100}$ ), mortality and days in nursery. Vaccinated piglets (n = 3 groups) were compared to a historical Control piglets (n = 3 groups). Data were statistically analysed using JMP 14.0 – comparison of means.

#### Results

Oral E. coli F4/F18 vaccination significantly reduced  $TI_{100}$  (18.6 ± 6.3 days to 2.4 ± 1.9 days; P<0.05). Mortality rate significantly reduced (11.2 ± 2.6% in Control to 4.5 ± 1.5% in Vaccinated group; P<0.05) following vaccination. Days in nursery (48.5 ± 0.3 days) remained constant throughout the trial.

# **Discussion and Conclusion**

Live E. coli F4/F18 vaccination against PWD has a significant impact on mortality, in combination with a reduction in medication use. In conclusion, control of PWD through oral vaccination is a successful option in order to prevent piglets from the negative clinical outcomes of F4-ETEC infection during the post-weaning period.

ERYSIPELOTHRIX RHUSOPATHIAE SEROTYPE 15 ASSOCIATED WITH A PIG ERYSIPELAS OUTBREAK IN A COMMERCIAL FARROW-TO-FINISH HERD IN IRELAND

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# **Background and Objectives**

Pig erysipelas is a zoonotic disease caused by the bacterium Erysipelothrix rhusopathiae that affects adult and grow-finish pigs associated with sporadic cases or larger outbreaks of septicaemia with characteristic skin lesion or chronic polyarthritis. At least 28 Erysipelothrix serotypes have been recognised. Infection in clinically affected pigs is often associated to serotypes 1 and 2. Erysipelas control relies on sanitation and vaccination. Inactivated E rhusopathiae licenced vaccines based on serotype 2 or serotypes 1 and 2 are available in Ireland. This study summarises the findings associated with chronic E rhusopathiae infection in a commercial farrow-to-finish pig herd.

#### **Material and Methods**

A continuous flow farm with routine PCV2 vaccination (Circovac®, CEVA) once pre farrow used a commercial E rhusopathiae serotype 2-based bacterin (Porcilis Ery+Parvo, Intervet Ireland) which was administered to breeding pigs once pre weaning experienced clinical signs of erysipelas in finish pigs for several weeks from July 2020 characterised by sudden death, skin discolorations and lameness. Three 20 to 22-week-old dead pigs showing ear discoloration and loosing body condition were necropsied. Heart, lung, liver and joint swabs were collected for laboratory investigations.

## Results

The postmortem examinations showed pericarditis, valvular vegetative endocarditis with enlarged hearts and interstitial pneumonia. E rhusopathiae was isolated from pericardium, heart valves and joint swabs. Serotype 15 E rhusopathiae was identified. PRRS and swine influenza viruses lung PCR tests were negative. Vaccination programme of the home reared replacement gilts had been discontinued in October 2019. Birds were found inside pig buildings.

## **Discussion and Conclusion**

High-infectious pressure conditions in a continuos pig flow likely contributed to the outbreak. In addition the lack of PCV2 and Erysipelas vaccination in gilts and PCV2 piglet vaccination may have played a role. PCV2 may impair the immune system and contribute to bacterial infections. Compliance with hygiene, vaccination programmes and vaccine usage guidelines is essential.

# SCREENING FOR INTESTINAL PATHOGENS IN GROWING-FINISHING PIGS WITH ILEITIS-LIKE SYMPTOMS IN UK

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# **Background and Objectives**

Enteric disease in growing and finishing pigs has a long-term impact on production performance, health and antibiotic usage especially during late production stages. The aim of this study was to screen for pathogens causing enteric disease in British herds with lleitis-like symptoms. Scarce knowledge is available in the UK pig sector.

# Material and Methods

In total, 10 British herds were investigated. These herds were included only if the herd veterinarian noticed suspicious lleitis-like symptoms, ie. presence of enteric disease in growers or finishers, mainly characterized by scour, looseness, diarrhoea, growth retardation and/or mortality.Cross-sectional sampling was performed with focus on the period where clinical signs were present, including at least three sampling points. A minimum of 15 faecal samples were collected from each herd. Fresh faecal samples were collected directly from the floor. Samples from the same herd and same age group of animals were pooled in groups of five and singleplex-PCR testing was performed for the following bacteria: L. intracellularis, Salmonella spp (including S. Typhimurium) and both Brachyspira pilosicoli and Brachyspira hyodysenteriae. E. coli was excluded from the differential diagnosis, as no samples were collected on weaners/nursery piglets with clinical signs suggestive of Post-weaning diarrhoea.

## Results

In total, 165 faecal samples were collected. All herds (10/10) tested positive by PCR for L. intracellularis in most of sampling points. A low number of herds tested positive for Salmonella Typhimurium (3/10) and B. pilosicoli (2/10). No herds tested positive for B. hyodysenteriae. Co-infections with L. intrantracellularis, S. Typhimurium and B. pilosicoli were detected in two herds, whereas only one herd was co-infected with both L. intracellularis and S. Typhimurium.

# **Discussion and Conclusion**

Presence of L. intracellularis was demonstrated in all suspected herds. Co-infections were not an unusual event. Further research, including more herds, is warranted to fully understand the role and impact of co-infections on enteric disease.

# PERFORMANCE DATA OF A FARM CLINICALLY INFECTED WITH LAWSONIA INTRACELLULARIS BEFORE AND AFTER INTRODUCTION OF AN INTRAMUSCULAR LAWSONIA VACCINE

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# **Background and Objectives**

Lawsonia intracellularis (LI) is an intracellular predominantly ileal located bacterium causing subclinical performance depression, Porcine Intestinal Adenomatosis (PIA) and Proliferative Haemorrhagic Enteritis (PHE).

# **Material and Methods**

In a closed farm (appr. 300 sows, 1500 nursery pigs, 3500 fatteners) LI related symptoms started 4-5 weeks after beginning of the fattening period (severe bloody diarrhea). Individual tylosin injection was insufficient so feed medication with tiamulin was necessary in nearly all groups. Nevertheless appr. 10 % of pigs developed poorly, 2-3 % very poorly. Diagnostics showed LI seroconversion and high LI loads (PCR >log GE 6/g faeces) in the middle of the fattening period. To control the LI symptoms intramuscular (IM) vaccination with Porcilis® Lawsonia + PCV M Hyo combination (LI vaccine dissolved in PCV M Hyo) was introduced. Performance data from the period without LI vaccination 01.07.-31.12.19 and with LI vaccination 01.04.-30.06.2020 were compared.

# Results

With introduction of the IM LI vaccination the LI morbidity strongly decreased. Only sporadically individual treatment with tylosin, but no more group medication was necessary. Homogeneity was distinctly enhanced with <1% runts. Despite of the present LI load (3.5–5.6 GE LI/g faeces) the clinical situation was clearly improved. Daily weight gain was increased by 21 g. Animal losses decreased by 0.9%. Feed conversion ratio was improved by 0.16. Vet costs for gastrointestinal reasons diminished massively and total treatment days/pig were 91.8 % lowered in LI vaccinated pigs. In total a benefit of 3.96–4.17 €/pig could be calculated with vaccinating the pigs with Porcilis® Lawsonia (vaccination costs need to be considered separately).

# Discussion and Conclusion

In this farm Lawsonia morbidity and animal losses with haemorrhagic diarrhoea were reduced in IM LI vaccinated pigs. Weight gain and feed conversion ratio were improved. Antibiotic treatments for gastrointestinal reason were negligible with LI vaccination. Economically IM LI vaccinated pigs generated lower production costs.

# EFFECT OF A PROBIOTIC ON CLOSTRIDIUM DIFFICILE

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# **Background and Objectives**

Clostridium difficile may be responsible for neonatal diarrhea in piglets and non-antibiotic tools for its control need to be found. The objective of this study was to assess the effect of Bacillus sp. PB6 against Clostridium difficile (CD) in vitro using zones of inhibition in a well diffusion assay (WDA) and areas of clearing in a streak assay and in vivo in a Syrian hamster model of antibiotic-induced CD associated diarrhea (CDAD).

## **Material and Methods**

For the in vitro studies, a feed additive Bacillus sp. PB6 (CLOSTAT<sup>®</sup>, Kemin) was used, two stock cultures of CD were used, using Blood Agar (BA) and Reinforced Clostridial Medium (RCM). For the in vivo study 42 male syrian hamsters, a Culti-loop, toxigenic C. difficile (type strain) (Oxoid), vancomycin (positive control) and Bacillus sp. PB6 (Kemin) were used. For the WDA, zones of inhibition were measured and for the streak assay, presence of clear zones surrounding the junctions of the streak lines was assessed. CDAD was induced using CD and clindamycin. Body weight loss, diarrhea and mortality were assessed.

# Results

On the WDA, average zones of inhibition of 16 and 20 mm for each of the Clostridium difficile isolates and clear zones of inhibition could be observed in between the streak lines. In the in vivo study, diarrhea and mortality was lower in the groups treated with PB6 and positive control (4/ 6 hamsters survived vs 0 on the challenged non treated)) and no significant difference was observed in body weight between between these two groups at the end of the study (P>0,05 Tukey-Kramer, multiple comparison).

# **Discussion and Conclusion**

This data shows that Bacillus sp. PB6 has activity against Clostridium difficile both in vitro and in vivo (in a hamster model) and that this probiotic may aid in controlling neonatal diarrhea caused by CD.
## DISTRIBUTION STUDY OF BRACHYSPIRA HYODYSENTERIAE MEDICATION IN LIQUID FEEDING SYSTEMS

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## **Background and Objectives**

Intra Dysovinol® (ID; Intracare) is a solution for use in drinking water for the treatment and metaphylaxis of swine dysentery. An increasing number of pigs receives liquid feed without additional drinking water. Therefore, we investigated the homogeneity following addition of ID to liquid feed of different compositions.

## **Material and Methods**

Tests were performed on two commercial farrow-to-finish farms that used automated mixing systems. Farm A: cube-shaped tanks mixing 940 – 980 kg dry rations with water; Farm B: round tanks mixing 1770 – 1860 kg combinations of dry rations with multiple by-products. Five different recipes for sows, piglets and fattening pigs were evaluated. All had a dry weight of approximately 25%, converting a product dosage of 0.4 ml ID per liter water to the addition of 0.3 ml ID per kg liquid feed. ID was added to the mixing tank, mixed for 2 minutes, and a random sample was taken from the tank. Product content was determined at least 24 hours after preparation and compared to the expected amount.

#### Results

A recovery was obtained ranging from 87% to 107%.

#### **Discussion and Conclusion**

Liquid feed is a complex analytical matrix, so the observed maximum off-target deviation of 13% (87% recovery) falls within the expected variation. In order to collect background samples, ID was added after addition of dry and raw materials to water. The presented results thus reflect a worst-case, since medicinal additions are generally added to the water-only stage to promote dissolution. The addition of ID to different types of liquid feed formulations and volumes resulted in a homogenous distribution of the product after only 2 minutes of mixing, indicating that ID can be accurately dosed in a liquid feed.

# PK/PD AND CLINICAL RELATIONSHIPS OF PHARMASIN 250MG/G PREMIX (TYLOSIN PHOSPHATE) ADMINISTERED TO PIGS FOR THE TREATMENT OF ILEITIS

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## **Background and Objectives**

The pharmacokinetics (PK) of the tylosin (Pharmasin<sup>®</sup> - Huvepharma NV) ileum contents concentration (ICC) was compared, related to intracellular MICs (iMICs) against Lawsonia intracellularis (Li) strains (pharmacodynamics - PD) and its clinical efficacy was evaluated in artificial infection studies.

## Material and Methods

Tylosin ICC and colon contents concentration (CCC) were determined in a pharmacokinetic study. Ten healthy pigs (bodyweight 20-25kg) were medicated with tylosin phosphate (Pharmasin® 250mg/g Premix) at 10mg/kg bw/24h for 5 consecutive days. The iMICs were derived from studies with Li isolates from Brazil (two isolates) and Thailand (three isolates). In a challenge study (Li challenge strain LR/189/5/83) tylosin phosphate was used in healthy weaned pigs (8 animals/group) at 40ppm (commencing 4 days pre-challenge, continuing for 16 days after infection (D-4-D16), when the dose was reduced to 20ppm, D17-D28) and at 100ppm (commencing 7 days after challenge, medication 21 days, D7-D28).

#### Results

The tylosin ICC was recorded at  $31.4\mu g/g$  (10mg/kg bw) and is estimated to be around  $16\mu g/g$  ( $15.7 \ \mu g/g$ ) at treatment dose (5mg tylosin/kg bw). CCC of tylosin was  $89.4\mu g/g$ . The tylosin iMIC range for the Thailand Li strains was  $2-16\mu g/ml$  and  $2-8\mu g/ml$  for the Brazil Li strains. The tylosin iMICs were far below the ICC and CCC. In the challenge study none of the pigs on the tylosin prevention and treatment programmes showed any gross or histopathological signs of ileitis infection. In the infected untreated group macroscopic lesions in 5/8 pigs (62.5%) and microscopic lesions in 7/8 pigs (87.5%) were found in the ileum and in 3/8 pigs (37.5%) in the caecum.

# **Discussion and Conclusion**

The use of PK/PD relationships is an effective tool in predicting the efficacy of tylosin phosphate to inhibit the growth of Lawsonia intracellularis and to treat ileitis infections in pigs.

STABLE PERFORMANCE WITH REDUCED ANTIBIOTIC USE IN PIGLETS VACCINATED WITH AN E. COLI F4/F18 VACCINE FOR THE PREVENTION OF F18-ETEC POST-WEANING DIARRHEA

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## **Background and Objectives**

Post-weaning Escherichia coli diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD, caused by enterotoxigenic E. coli (ETEC), typically provokes mild to severe watery diarrhea between 5-10 days after weaning. Recently, an oral live bivalent E. coli F4/F18 vaccine (Coliprotec® F4/F18; Elanco) was approved on the European market, which reduces the impact of PWD provoked by F4-ETEC and F18-ETEC. The objective was to compare technical results and antibiotic use following E. coli F4/F18 vaccination with previous standard therapeutic approach under field conditions.

#### Material and Methods

A 1600-sow farm (weaning at 26 days) with diagnosed problems of PWD due to F18-ETEC was selected. Control piglets received the standard treatment protocol with antimicrobials during the post-weaning phase. Vaccinated piglets were immunized at 21 days with the oral live bivalent E. coli F4/F18 vaccine. At weaning, no standard group medication (ZnO and antibiotics) was applied for prevention of PWD. Several performance parameters were collected: treatment incidence (T1100), mortality and days in nursery. Statistical analysis was performed using JMP 14.0 – comparison of means.

#### Results

Oral E. coli F4/F18 vaccination significantly reduced  $TI_{100}$  (7 ± 2 days to 0 ± 1 days; P<0.05). Mortality rate remained stable (2.05% in Control to 1.96% in Vaccinated group; P<0.05). Days in nursery (40 ± 3 days) remained at the same level compared to pre-vaccination.

## **Discussion and Conclusion**

Live E. coli F4/F18 vaccination against PWD resulted to similar technical performance parameters and mortality, in combination with a significant reduction in medication use. In conclusion, control of PWD through oral vaccination is a successful option in order to prevent piglets from the negative clinical outcomes of F18-ETEC infection during the post-weaning period.

# PK/PD AND CLINICAL RELATIONSHIPS OF TIAMULIN AND LINCOMYCIN ADMINISTERED TO PIGS FOR THE TREATMENT OF ILEITIS

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## **Background and Objectives**

The pharmacokinetics (PK) of tiamulin (Vetmulin<sup>®</sup> - Huvepharma NV) and lincomycin (Lincocin<sup>®</sup>) ileum contents concentration (ICC) was compared, related to intracellular MICs (iMICs) against Lawsonia intracellularis (pharmacodynamics - PD) and their efficacy was evaluated.

## Material and Methods

Tiamulin ICC concentration was estimated based on published tiamulin colon contents concentrations at 110ppm (6.6mg/kg bw) & 220ppm (13.2mg/kg bw) - 5 animals (20kg)/dose. Lincomycin ICC concentration was determined in PK experiments at 110 and 220ppm dose - 9 animals (29kg)/dose. The iMICs derived from studies with Li isolates generated worldwide. Artificial infection studies were carried out with tiamulin at 50ppm (2.5mg/kg bw, day - 2 until day 21 PI) and 150ppm (7.5mg/kg bw, onset 7 days PI for 14 days). Li challenge strain LR 189/5/83 (stomach tube). Necropsy: 3 weeks post challenge. Lincomycin was tested (44ppm & 110ppm, day 0-21) after mucosal homogenate gavage challenge (10<sup>8</sup>-10<sup>9</sup>). Necropsy: 1st day after 21-day treatment period.

## Results

Tiamulin ICC was recorded at 0.82µg/g (110ppm) and at 2.33µg/g (220ppm). Lincomycin ICC concentrations of 10.01µg/g (110ppm) and 25.05µg/g (220ppm) were reported. Similar Tiamulin iMIC ranges against US/Europe (0.125-0.5µg/ml) and Brazil/Asian (0.125-2µg/ml). Li strains were determined. Higher iMICs and broader ranges were found for lincomycin (US/Europe Li strains: 8-128µg/ml vs. Brazil/Asian Li strains 16->128µg/ml). Pigs receiving tiamulin at 50ppm pre-challenge and 150ppm post-challenge remained clinically normal, no diarrhoea and gross/histopathological lesions were identified. Pigs either at lincomycin concentration of 44ppm or 110ppm showed lower incidence of clinical impression and diarrhoea and better performance than the untreated controls.

## **Discussion and Conclusion**

ICC and MIC data help to explain the clinical effectiveness of tiamulin seen against ileitis at treatment dosage (7.5mg/kg bw). A prediction of the therapeutic effect of lincomycin due to ileum concentrations and MIC data cannot be given.

## DIAGNOSTIC PERFORMANCE OF THREE MYCOPLASMA HYOPNEUMONIAE ANTIBODY ELISA KITS

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## **Background and Objectives**

ELISA is a rapid, inexpensive and easily automated diagnostic method for Mycoplasma hyopneumoniae (M. hyo) that provides useful information on the presence of antibodies and seroconversion. Differences in sensitivity and specificity of M. hyo ELISA kits have been reported.

This study compares the performance of three commercial M. hyo antibody ELISAs using serum samples from pigs of known status.

## **Material and Methods**

A total 1,216 serum samples were used to evaluate the sensitivity, specificity and onset of detection of the IDEXX M. hyo Ab Test, another commercial M. hyo antibody indirect ELISA (I-ELISA) and a blocking M. hyo ELISA (B-ELISA) according to the manufacturer's recommendations.

#### Results

Both indirect ELISAs, IDEXX M. hyo Ab Test and I-ELISA, correctly identified negative serum samples (Specificity = 100%). The B-ELISA failed to identify four negative serum samples (Specificity = 99.2%).

In experimentally infected pigs, the IDEXX M. hyo Ab Test detected 70% of positive pigs at 14 days post infection (dpi) and 100% at 23 dpi. However, the I-ELISA was only able to detect 20% and 66.7% of positive animals and the B-ELISA 40% and 66.7% at 14 and 23 dpi, respectively. The better onset of detection showed by the IDEXX M. hyo Ab Test, with a higher percentage of positive pigs detected as early as 14 dpi, support its use for early diagnosis of M. hyo infection and detection of index cases compared to the other ELISA kits evaluated. The sensitivity of the IDEXX M. hyo Ab Test was higher than the other indirect ELISA included in this study,

detecting 58 positive samples more (8.1%).

## **Discussion and Conclusion**

In summary, the IDEXX M. hyo Ab Test showed 100% specificity, better than a blocking M. hyo ELISA, higher sensitivity than another M. hyo indirect ELISA and better onset of detection than the indirect and blocking ELISAs evaluated in this study.

## SKIN LESIONS AND FEVER IN FATTENING PIGS - A CASE REPORT

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## **Background and Objectives**

The affected pigs (100 pigs, 130 kg) in this report were the youngest of four age groups in a fattening unit with prolonged fattening duration. The animals exhibited signs of fever, apathy, anorexia. Six animals showed subcutaneous red-to-purple skin lesions, mainly at the hind limbs, perineal region and the ears. The suspected diagnosis was Porcine circovirus type 2 (PCV2), although animals had been vaccinated against PCV2 as piglets and additionally around 23 weeks of age.

#### Material and Methods

Blood samples were collected from clinically affected pigs. One pig that died peracutely was sent to necropsy. Two oral fluid sets were collected in the affected group; however, complete sets were only obtained from 20 pigs, the remainder did not chew on the rope due to disease-related apathy. Sock swabs were collected from all age groups.

#### Results

Blood samples were negative for PCV2 and Porcine Reproductive and Respiratory Syndrome (PRRS). ELISA for Erysipelothrix rhushiopathiae was negative. Oral fluids were negative for PRRS and Influenza. Swine fever and Aujeszky disease were ruled out via negative organ samples. However, cultures from oral fluid material or organ samples were positive for Salmonella Typhimurium, and blood samples were ELISA positive for Salmonella. Necropsy results showed high amounts of Pasteurella multocida in the lung, and detected Salmonella in the intestine. Sock swabs were positive for Salmonella in three of four age groups.

## **Discussion and Conclusion**

The importance of determining the correct diagnosis is critical to ensure appropriate treatment and problem solution: The affected pigs suffered from an acute Salmonella infection, not an infection due to PCV2. The pigs were treated with antibiotics, veterinarian and farmer worked intensively on Salmonella prevention: The production flow was changed to all-in all-out production with thorough cleaning and disinfection, rodent control was intensified, feed was acidified, the farm was environmentally sealed.

## OCCURRENCE OF E. COLI, CL. PERFRINGENS AND ROTA-VIRUS IN NEONATAL PIGS IN DENMARK

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### **Background and Objectives**

E. coli, Cl. perfringens and Rota-virus are considered common infectious agents in neonatal diarrhea in piglets, despite very frequent vaccination of sows against E. coli and Cl. perfringens in Denmark. This investigation was set up to assess the prevalence of these agents and relate them and other risk factors to the extent of neonatal diarrhea.

## **Material and Methods**

In total 60 herds were included in the investigation and visited once. A questionnaire was completed providing information of the frequency of pens and pigs with diarrhea, antibiotic consumption, piglet mortality, age at initial diarrhea, floor type and possible batch farrowing.

One pooled fecal sample per herd was collected from pens with diarrhea. The sample was analyzed for E. coli including F4 and F18 adhesins and virulence factors ST1, ST2, LT and VTe. Also Cl. perfringens including types and Rota-virus A by ELISA were tested for.

## Results

The herd prevalence's of hemolytic and pure culture E. coli were 7 % and 15 %, respectively. No adhesion or virulence factors were detected. Cl. perfringens type A and type C were detected in 70 % and 0 % of herds, respectively.

The herd prevalence of Rota-virus was 22 %. The pen and the pig frequencies of diarrhea were 26.6 % and 20.5 % in virus positive herds compared to 16.7 % and 13.2 % in virus negative herds, respectively (p<0.05).

The pen frequency of diarrhea was 14.6 % in herds with batch farrowing compared to 20.8 % in other herds (p=0.08). The mortality in batch farrowed herds was 12.4 % compared to 14.8 % in other herds (p<0.05). Other factors had no effects.

#### **Discussion and Conclusion**

No effect of E. coli or Cl. perfringens type A on frequency of diarrhea were found. The presences of Rota-virus correlated to the presences of diarrhea, while herds with batch farrowing had a lower mortality.

# PORCILIS® ERY+PARVO+LEPTO VACCINE AGAINST CHRONIC LEPTOSPIROSIS IN THE FIELD AS AN ALTERNATIVE TO ANTIBIOTIC USAGE.

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## **Background and Objectives**

Leptospira is one of the most important worldwide agents causing reproductive disorders. The most prevalent Leptospira serovar in pig population is Bratislava, present at herds in a subclinical way, causing mild reproductive disorders like rise in abortion rate or return to estrus. Besides, reduction in antibiotic usage in EU is cooperating to disease maintenance. The study's aim was evaluating performance of multivalent vaccine to control subclinical Bratislava caused leptospirosis, where antibiotics usage was effective to control the disease.

#### Material and Methods

Early abortions (first gestation period) were described in Iberian genetics farm (1,150 sows). Leptospira Bratislava was diagnosed (MAT technique, 60% positive samples with titres ≥1/100, from sows with reproductive disorders). Since then, oxytetracycline (200ppm, water) was administered in the breeding facilities every two weeks for 7 days. Retirement of the treatment derived in gestation failure in the first 5 weeks post-breeding (early abortions). It was then introduced Porcilis® Ery+Parvo+Lepto, 2 doses 4 week separated, and then the antibiotic treatment was ceased. Three periods were determined in this longitudinal study: no antimicrobials (Group:N, 6 months), use of oxytetracycline (Group:M, 26 months) and vaccinated group (Group:V, 8 months). Data were analyzed in monthly basis, by ANOVA (Fertility %), and by non-parametric test KRUSKAL-WALLIS (early abortions/month).

## Results

Statistical differences were found (p<0.001) in favor of M and V groups, regarding both parameters: fertility N=67.33%, M=87.62%, V=90.00%; and number of early abortions/month N=14.5, M=0.9, V=0.4. Numerical differences were found in favor of V group against M.

#### **Discussion and Conclusion**

In this study Porcilis<sup>®</sup> Ery+Parvo+Lepto vaccine appeared to be a useful tool to control reproductive disorders caused by Leptospira serovar Bratislava, and to reduce use of antimicrobials. As a limitation, we have to consider that animals of the different study groups were bred in different periods of time.

## PERFORMANCE OF PORCILIS® ERY+PARVO+LEPTO VACCINE AGAINST CHRONIC LEPTOSPIROSIS IN THE FIELD.

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## **Background and Objectives**

Leptospira is one of the most important worldwide agents causing reproductive disorders. The most prevalent Leptospira serovar in pig population is Bratislava, followed by others like Pomona and Icterohaemorrhagiae. Bratislava is present at herds in subclinical way, causing mild reproductive disorders like rise in abortion rate or return to estrus. Besides, reduction in antibiotic usage in EU is contributing to disease maintenance. The study's aim was to evaluate performance of multivalent vaccine to control subclinical leptospirosis.

## Material and Methods

Study conducted in 2,900 sows farm. In the past, return to estrus rate was too high, especially in gilts. Lesptospira Bratislava was diagnosed by microagglutination test (MAT, showing titres ≥1/100 against Bratislava) in serum samples coming from sows with reproductive disorders. Two doses schedule of the vaccine was tested. Two periods were analyzed: prior vaccination (P), 21weeks before vaccination; and a vaccinated period (V), 21weeks after the onset of immunity. Reproductive parameters were statistical analyzed ANOVA (parity rate (Pr), total born (TB), born alive (BA), still born (SB)), and non-parametric test KRUSKAL-WALLIS (mummified, abortion rate (Ab), return to estrus (R)). All data analyzed as an average of the events occurred in a weekly batching. Besides, data were analyzed independently considering gilts and multiparous sow.

# Results

Gilts: statistical differences were found in favor of V group in the following parameters: Pr: V=90.7% vs. P=83.3% (p<0.001); Ab: V=1.66% vs. P=3.89% (p=0.009). Multiparous: statistical differences were found in favor of V group in the following parameters: Pr: V=90.8% vs. P=87.1% (p=0.002); R: V=3.68% vs. P=6.55% (p=0.003); TB: V=14.94 vs. P=14.23 (p<0.001).

## **Discussion and Conclusion**

This vaccine appeared to be a useful tool to help controlling reproductive disorders caused by Leptospira, even those cases where subclinical infection is subjacent. We have to consider as a limitation of study that animals were not bred in the same conditions.

## PERFORMANCE OF PORCILIS® ERY+PARVO+LEPTO VACCINE AGAINST ACUTE LEPTOSPIROSIS IN THE FIELD.

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## **Background and Objectives**

There are some serovars of Leptospira affecting pig population, ones adapted (Bratislava, Pomona), others accidentally infecting herds (Icterohaemorrhagiae). Accidental serovars are maintained by other species, like Icterohaemorrhagiae, adapted to rodents. Coexistence in pig farms with rodents is an issue, and sanitary measures are needed to fight against it. Some studies have demonstrated presence of reproductive problems in herds related to the Icterohaemorrhagiae serovar. The aim of this study is to analyze the potential role of sow vaccination against Leptospira in the protection against an acute problem of Leptospirosis caused by Icterohaemorrhagiae.

## Material and Methods

Study conducted in farm operating weekly batch system, where reproductive disorders were reported (abortions, return to estrus, still born). Differential diagnose was done excluding PRRSvirus, but Lesptospira Icterohaemorrhagiae was diagnosed by microagglutination test (MAT) in serum samples coming from sows with reproductive disorders (paired samples showing seroconversion from negative to titres ≥1/100). Then, two doses program of the vaccine was instaured. Two periods analyzed: prior vaccination (P), 19weeks before 1stdose; and a vaccinated period (V), 20weeks after onset of immunity. Reproductive parameters were statistical analyzed ANOVA (born alive (BA), still born (SB)), and non-parametric test KRUSKAL-WALLIS (mummified (Mum), abortion rate (Ab), return to estrus (R), return to estrus primiparous sows (Rp)). Data analyzed as average of events occurred in a batch.

# Results

Statistical differences were found in favor of the vaccinated group in the following parameters: Ab: V=1.00% vs. P=4.74% (p=0.011); R: V=7.00% vs. P=14.74% (p=0.001); Rp: V=10.76% vs. P=22.54% (p<0.001); BA: V=12.82 vs. P=11.39 (p<0.001); SB: V=1.21 vs. P=1.86 (p<0.001); Mum.: V=0.071 vs. P=0.155 (p=0.019).

# **Discussion and Conclusion**

Vaccination was a useful tool to control reproductive disorders caused by Leptospira Icterohaemorrhagiae, especially in those cases where acute outbreak is affecting reproductive performance. Nevertheless, we have to consider, as a limitation of the study, that animals were not bred in the same conditions.

## COULD IT BE GLAESSERELLA (HAEMOPHILUS) PARASUIS? FIELD EXPERIENCE OF ITS POTENTIAL ROLE IN PRDC

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## **Background and Objectives**

Glaesserella parasuis is considered as the causative agent of Glasser's disease, which is dominated by systemic clinical signs, polyserositis, and/or sudden death. Recently, anecdotal reports from several French swine practitioners pointed at the implication of G. parasuis in chronic respiratory conditions, mostly affecting weaners. We describe the presence of G. parasuis in a recurrent episode of porcine respiratory disease complex with digestive involvement post-weaning in a 190-sow farrow-to-finish farm in Brittany (France).

## **Material and Methods**

The farmer started noticing signs of coughing in the post-weaning unit in the autumn of 2016. This triggered several visits of the farm's veterinarian who performed broncho-alveolar sampling on piglets and evidenced the presence of both Mycoplasma hyorhinis and swine influenza virus. The respiratory episode lagged at low level over the following year, until it aggravated in June 2017, with clinical signs of both Streptococcus suis infection and colibacillosis. In-feed antibiotic supplementation controlled both meningitis and respiratory signs post-weaning until July 2018, when both conditions increased in severity. Necropsy allowed the identification of G. parasuis (serotype 4). This prompted the interruption of antibiotic in-feed supplementation and the modification of piglets' and gilts' vaccination programmes, to include Suvaxyn® RespiFend MH/HPS.

## Results

These interventions allowed to control both PRDC and systemic diseases post-weaning, and improved wean-to-finish performance: +20 g/d in wean-to-finish average daily weight gain for the 6 batches produced after vaccination changes, as compared to the 6 previous batches. Also, they allowed to strongly reduce the usage of antibiotics and NSAIDs (from  $0.88 \in$  to  $0.02 \notin$ /piglet).

## **Discussion and Conclusion**

It might be judicious to include G. parasuis in the differential diagnosis of PRDC in France, even though it is more generally considered as a 'potential secondary pathogen' of PRDC. It is possible that the role of G. parasuis in PRDC has so far been under-estimated.

# PREVALENCE OF MYCOPLASMA HYOPNEUMONIAE IN THREE-WEEK-OLD SUCKLING PIGLETS AND CORRESPONDING SOWS IN SOUTHERN GERMANY AND AUSTRIA

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## **Background and Objectives**

Mycoplasma hyopneumoniae (M.hyo) is highly prevalent in the domestic pig population and known as the causing agent of the enzootic pneumonia in pigs. One-shot vaccination of suckling piglets against M.hyo at weaning is one common measure to reduce lung lesions and the economic impact due to M.hyo infections. The present study was conducted to evaluate the prevalence of M.hyo in three-week-old piglets and their mothers directly before vaccination.

## Material and Methods

In total 790 three-week-old piglets and 158 sows from 13 German and 3 Austrian farms were enrolled in this study. Selection criteria were EP-scores at slaughter with different degree within a prescreening (approx. 10 weeks before sampling). Blood samples (piglets and sows) and tracheobronchial- (piglets) or laryngeal- (corresponding sows) swabs were collected. Laboratory diagnostics included ELISA (blood samples; IgG- antibodies against M.hyo) and real-time PCR (laryngeal- and tracheobronchial- swabs; M.hyo-DNA).

## Results

In total, 14 farms were positive for antibodies against M.hyo. On animal base seroprevalence was 50% and 42.0% for sows and piglets, respectively. Five farms were positive for M.hyo-DNA by PCR. 3.8% of the laryngeal swabs (sows) and 0.4% of the tracheobronchial swabs (piglets) were M.hyo-DNA positive. Laryngeal-swabs of gilts had a significantly higher M.hyo-DNA detection rate than swabs of multiparous sows (p=0,008). No significant associations were detected between the detectability of M.hyo and specific antibodies of sows or piglets (ELISA and PCR) and the EP-index.

# **Discussion and Conclusion**

The results of the present study revealed that the M.hyo prevalence was low in the examined study population and the infectious status of sows or piglets did not correlate with EP-index at slaughter. However, EP-indices and samples from study-animals were collected independently from each other. EP-like-lesions are not pathognomonic for M.hyo infections and late infections in the fattening can occur. Nevertheless, gilts had significantly increased M.hyo detection rates compared to older sows.

# ANTIMICROBIAL RESISTANCE IN SEROVAR 8 ISOLATES OF ACTINOBACILLUS PLEUROPNEUMONIAE FROM THE UNITED KINGDOM, DENMARK AND NORWAY: GENOTYPIC AND PHENOTYPIC ANALYSES

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## **Background and Objectives**

Pleuropneumonia caused by Actinobacillus pleuropneumoniae (APP) is a disease of great impact on pig health and productivity globally, and a common indication for use of antimicrobial drugs. Practices of treatment and prophylaxis varies between countries, antibiotic treatment has not been shown effective in eliminating the pathogen from all carrier animals. Monitoring antimicrobial resistance (AMR) in pathogenic bacteria is of value to make informed treatment choices and also contributes to risk assessment of antimicrobial use. The objective of this study was to compare prevalence of AMR genes and phenotypic resistance profiles of UK, Danish and Norwegian isolates of APP serovar 8 (APP8).

## **Material and Methods**

In total, 219 clinical APP8 isolates from UK, Denmark and Norway were collected between 1983 and 2019. For each isolate, genomic DNA was extracted, and quality controlled before sequencing and assembly of reads into draft genomes. The isolates were all confirmed as serovar 8 by comparison of genes in the capsular polysaccharide locus to those found in the APP8 reference strain 405. Antimicrobial resistance AMR genes were investigated using the ResFinder database using k-mer alignment and by ABRicate v0.7 on the assembled genes from ariba. Phenotypic AMR profiles of the isolates were determined using appropriate minimum inhibitory concentration (MIC) agar dilution susceptibility tests.

## Results

The sulfonamide resistance gene, sul2, was the most common AMR gene occurring across countries, being present in 6% of the Norwegian and 66% of the UK isolates. Detailed results and MIC analyses will be presented.

## **Discussion and Conclusion**

Differences in prevalence of AMR genes among the three populations of APP8 isolates might be attributed to different treatment practices in the respective countries. The results are valuable in assessment of current AMR status of a common pig pathogen.

# INVESTIGATION ON THE USE OF SINGLE OR POOLED FAECAL SAMPLES AND COMPARISON WITH GROUP SALIVA SAMPLES FOR LAWSONIA INTRACELLULARIS DIAGNOSTIC IN FATTENING PIGS

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## **Background and Objectives**

Lawsonia intracellularis (LI) is very common in pig farms worldwide. Due to the correlation between faecal LI load and pathological findings of proliferative enteropathy<sup>1</sup> the quantification of LI is a valuable tool to estimate the impact of LI as cause of clinical/subclinical problems. Aim of this study was to evaluate whether single faecal samples, pooled faecal samples or group saliva samples can deliver accurate results and should therefore be recommended for routine diagnosis in vet practices.

## **Material and Methods**

In 15 fattener farms (500-11.000 animals, north west Germany) a total of fifteen faecal samples each (five samples from three different pens) were collected from the floor and one pool was prepared out of the five samples from every pen. Additionally, a chewing rope was offered to the pigs from the same pen to collect saliva. Analyses were performed with the quantitative Lawsonia PCR<sup>2</sup> by Nathues and with the BactoReal Lawsonia kit of Ingenetix. Statistics were performed as correlation and inter-rater reliability by Cohen's kappa calculation.

## Results

There was a high correlation detected between the results of both qPCRs ( $R^2$ =0.940) in pooled faecal samples. The results of single and pooled faecal samples (BactoReal) were also in good agreement (Cohen's kappa 0.71). The correlations between PCRs results of saliva compared to faecal samples was lower but still in strong correlation ( $R^2$ =0.751).

## **Discussion and Conclusion**

For practical reasons the use of pooled samples can be recommended. Due to the close contact between faeces and intestinal mucosa a higher validity can be expected by examination of faecal material. LI load of saliva samples should be tested systematically by qPCR in defined animals to get a correlation to the clinical/pathological impact.

<sup>1</sup>Pedersen, K.S. et al. (2012): BMC Veterinary Research, Vol. 8 <sup>2</sup>Nathues, H. et al. (2009): Journal of Applied Microbiology, Vol. 107

# ORGANIC ACIDS AND NATURE IDENTICAL COMPOUNDS AS POTENTIAL NOVEL STRATEGIES TO MANAGE POST-WEANING DIARRHEA

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# **Background and Objectives**

Post-weaning diarrhea, caused by Escherichia coli K88, is one of the long-standing challenges in the pig breeding industry. Because of the concerns about the threat of antibiotic resistance and the environmental risks arising from zinc oxide pollution, conventional treatments need to be replaced. The aim of this study was to evaluate the antimicrobial activity of several antibiotics, organic acids (OA), nature identical compounds (NIC) and essential oils (EO) against E. coli K88 in vitro.

## **Material and Methods**

A field strain of E. coli K88<sup>+</sup>, LT<sup>+</sup>, STa<sup>+</sup>, STb<sup>+</sup>, obtained from a symptomatic piglet, was tested in vitro for its susceptibility to selected antibiotics and a panel of OA, NIC and EO by assessing the minimal inhibitory concentration (MIC) with microdilution method.

## Results

E. coli K88 was resistant to amoxicillin, ampicillin, lincomycin, neomycin, and penicillin G (MIC > 64 mg/L). Although colistin did inhibit E. coli K88 growth at 4 mg/L, the bacterium had to be considered resistant according to EUCAST breakpoints. Sorbic and benzoic acid were the most effective OA, with MIC values registered at 50 mM. No growth inhibition was found for citric, butyric, formic, fumaric, lactic, malic, propionic acids up to 200 mM. Several NIC proved their efficacy against the pathogenic strain, with MIC values being 1.87 mM for carvacrol and 3.75 mM for thymol and eugenol. No MIC was registered for vanillin, alpha-pinene, eucalyptol, limonene, linalool, and menthol up to 7.5 mM. No MIC was registered for ginger and tea tree EO between 5-100 mg/L and 50-1000 mg/L, respectively.

## **Discussion and Conclusion**

The E. coli K88 tested strain was resistant to all the antibiotics tested, but selected OA and NIC were effective in inhibiting its growth. Therefore, their inclusion in animal diets as feed additives could be proposed as an alternative to control post-weaning diarrhea.

**Bacterial diseases** 

PREVALENCE OF VIRULENCE FACTORS OF ESCHERICHIA COLI ISOLATED FROM PIGLETS WITH POST-WEANING DIARRHOEA IN UNITED KINGDOM AND THE REPUBLIC OF IRELAND

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## **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 entero-toxigenic 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 determine the prevalence ETEC subtypes causing PWD in United Kingdom and Republic of Ireland (UK&ROI).

## Material and Methods

Fifty pig herds distributed throughout UK&ROI showing clinical signs of PWD were sampled (Jan 2018 - Dec 2019). Rectal swab samples (n=5) from diarrheic pigs were 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

In total, 48 non-hemolytic and 93 hemolytic E. coli strains were isolated and subsequently tested by PCR. Seventy-six strains (51.0%) had no virulence factors. The prevalence of the different ETEC subtypes within the strains positive for virulence factors was as follows: F18-ETEC (24.8%), F4-ETEC (12.8%), F4-F18-ETEC (2.0%) and F18-STEC (2.0%). On a herd level, the prevalence of the different ETEC subtypes was as follows: F18-ETEC (24.2%), F4-ETEC (21.2%), F4-F18-ETEC (3.0%) and F18-STEC (3.0%). Ten farms (30.3%) had a mixed infection with different pathotypes.

## **Discussion and Conclusion**

This study confirms that fimbriae type FI8 was twice as prevalent as F4 among E. coli isolates from PWD cases in UK&ROI. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of E. coli in PWD outbreaks and initiate appropriate preventive measures such as live oral vaccination.

# PERFORMANCE AND ANTIBIOTIC USE OF PIGLETS VACCINATED WITH AN E. COLI F4/F18 VACCINATION FOR THE PREVENTION OF F18-ETEC POST-WEANING DIARRHEA

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## **Background and Objectives**

Post-weaning Escherichia coli diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD, caused by enterotoxigenic E. coli (ETEC), typically provokes mild to severe watery diarrhea between 5 and 10 days after weaning. Most common adhesins on ETEC from PWD are the fimbriae F4 and F18. Therapy to combat PWD typically consists of antibiotic treatment in combination with high doses of ZnO (3000 ppm). Recently, an oral live bivalent E. coli F4/F18 vaccine (Coliprotec® F4/F18; Elanco) is available on the European market, which reduces the impact of PWD provoked by F4-ETEC and F18-ETEC. The objective was to compare technical results of E. coli F4/F18 vaccination with previous standard therapeutic approach under field conditions.

## Material and Methods

A 1100-sow farm (weaning at 21 days) with diagnosed problems of PWD due to FI8-ETEC was selected. Piglets were vaccinated at 18 days with the oral live bivalent E. coli F4/ F18 vaccine. At weaning, no standard group medication (ZnO and antibiotics) was applied for prevention of PWD. Piglets were fed a commercial dry feed. Several performance parameters were collected: weight at d0-47, ADG, FI, FCR, Those and mortality.

#### Results

Oral E. coli F4/F18 vaccination significantly reduced the mortality rate (3.56% to 1.46%) and TI<sub>100</sub> (10 to 0 days). All other performance parameters (ADG, FI and FCR) were at the same level compared to pre-vaccination.

## **Discussion and Conclusion**

The results show that live E. coli F4/F18 vaccination against PWD has led to similar technical performance parameters, in combination with a significant reduction in the mortality and medication use. In conclusion, control of PWD through vaccination is a good option in order to prevent piglets from the negative clinical outcomes of F18-ETEC infection during the post-weaning period.

# REDUCED ANTIMICROBIAL USE FOLLOWING THE TREATMENT OF CLINICAL SWINE DYSENTERY WITH A NOVEL ZINC CHELATE IN AN ALL-IN/ALL-OUT FATTENING UNIT IN THE NETHERLANDS

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## **Background and Objectives**

Brachyspira hyodysenteriae is the primary cause of swine dysentery and primarily affects pigs during the grow/finishing stage. A novel non-antibiotic zinc chelate has been reported to demonstrate positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and B. hyodysenteriae excretion after a 6-day oral treatment. The objective was to study the antibiotic use between a standard protocol (tiamulin, Denagard™; Elanco) and a zinc chelate (IntraDysovinol® 499 mg/ml; Elanco) on naturally occurring swine dysentery due to B. hyodysenteriae in a fattening unit in the Netherlands.

## **Material and Methods**

An all-in/all-out fattening unit with consistent clinical signs of swine dysentery was included in the study. Two subsequent production periods were compared in antimicrobial use (animal daily doses over a year; ADD<sub>year</sub>) between a standard therapeutic approach using antimicrobial treatment (tiamulin) and an oral zinc chelate. Statistical analysis was performed using JMP 14.0 - ANOVA.

## Results

A significant reduction in the ADD<sub>year</sub> could be observed between the same period in 2019 (August – October, ADD<sub>year</sub> = 5.42) using the zinc chelate therapy as compared to 2018 (August – October, ADD<sub>year</sub> = 14.0) using the antibiotic therapy. Since July 2019, no group treatment with antibiotics (tiamulin) were performed and only occasional individual antibiotic injections were administered.

## **Discussion and Conclusion**

Zinc chelate is a novel non-antibiotic treatment for clinical swine dysentery. Treatment with the zinc chelate resulted in a reduced antimicrobial use due to lack of group treatments and a significant reduction in individual injections needed. Therefore, based on the ADD measurement of the fattening unit affected with clinical swine dysentery, the use of IntraDysovinol<sup>®</sup> could help in a further reduction of antimicrobial use in herds chronically infected with B. hyodysenteriae.

# BRACHYSPIRA HYODYSENTERIAE ERADICATION WITH A NON-ANTIBIOTIC ZN-CHELATE: THE APPROACH OF THE FUTURE?

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## **Background and Objectives**

Swine dysentery (SD) caused by Brachyspira hyodysenteriae (B. hyo) is an intestinal disease with clinical signs typically consisting of muco-haemorrhagic diarrhea and important economic losses due to decreased performance and mortality. Diagnosis is performed using pooled fecal samples (microbial culture, PCR test). The objective was to eradicate B. hyo from a swine herd using a novel Zn-chelate (Intra Dysovinol<sup>®</sup>; Elanco) in combination with a cleaning and disinfection (C&D) protocol, including improved overall management approach.

## Material and Methods

A closed one-site swine herd with 240 sows had been diagnosed positive for B. hyo in December 2018. A first unsuccessful eradication was carried out in Spring 2019 due to Ab-resistance (tiamulin & valnemulin) and inconsistent internal biosecurity measures. Several steps in C&D, rodent and fly control and biosecurity issues were optimized before a new eradication was started. A second eradication was initiated using a novel Zn-chelate (Intra Dysovinol<sup>®</sup>) for oral drinking water application, which has been proven efficacious in eliminating B. hyo from pigs following a 6-day continuous (24/24h) drinking water administration. Sows were treated for 6 days, followed by a wash & move, and treatment was continued until 6 days after the finalization of the entire C&D procedure.

## Results

Overall, 5 sampling points (pre-treatement, d0, d6, d16 and d60) were identified to check the B. hyo infection status of the animals in scope for the Zn-chelate treatment. Following treatment, in combination with C&D and disinfection, all animals tested PCR-negative in the 4 subsequent samplings.

#### **Discussion and Conclusion**

A novel Zn-chelate has been successfully applied for B. hyo eradication in a closed swine herd infected with an Ab- resistant B. hyo strain. This approach might be interesting for future application under specific circumstances of Ab-resistant B. hyo strains and in the light of further measures concerning continued reduction of Ab-use worldwide.

DETECTION OF MYCOPLASMA HYOPNEUMONIAE IN COUGHING PIGS UNDER FIELD CONDITIONS IN DIFFERENT DIAGNOSTIC SAMPLE TYPES

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## **Background and Objectives**

Mycoplasma hyopneumoniae (M. hyo), the primary pathogen of Enzootic Pneumonia, occurs worldwide and causes major economic losses to the pig industry. Piglets become infected with M. hyo during the suckling period and have been shown positive from weaning onwards. Once infected with M. hyo, animals can excrete the pathogen over a long period of time. Recent research has shown that in pigs experimentally infected with M. hyo, the pathogen recovery by different diagnostic techniques is quite variable. Laryngeal swabs could recover M. hyo earlier as compared to nasal swabs or oral fluids (OF). The objective of the current study was to analyse M. hyo detection from field samples (lung tissue, OF or trachea-bronchial swabs (TBS)) collected on farms with clinical respiratory problems in Belgium and the Netherlands by swine practitioners.

## Material and Methods

During 2018, diagnostic samples for detection of M. hyo were collected in clinically coughing pigs of different ages at 286 swine farms by 52 different swine practitioners in Benelux. Depending the field situation, one of the three different sample types, i.e. trachea-bronchial swabs, lung tissue or OF, was collected. Samples were analysed on presence of M. hyo and results reported as positive/negative.

#### Results

Overall, M. hyo detection was at least 3 times higher in TBS samples (38% M. hyo-positive) as compared to OF (12% M. hyo-positive). Lung tissue samples (18% M. hyo-positive) from pathological lesions were 50% more reliable to detect M. hyo as compared to OF.

#### **Discussion and Conclusion**

The current results are in line with an earlier experimental study by Pieters et al. (2017) demonstrating that sensitivity of OF was lower than other deeper respiratory tract sampling techniques. In conclusion, diagnosis of M. hyo-related respiratory problems, TBS is more appropriate to detect the pathogen with a higher certainty as compared to the use of OF.

# INCREASED PERFORMANCE AND REDUCED ANTIMICROBIAL USE FOLLOWING THE TREATMENT OF CLINICAL SWINE DYSENTERY WITH A NOVEL ZINC CHELATE IN AN ALL-IN/ALL-OUT FATTENING UNIT IN THE NETHERLANDS

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## **Background and Objectives**

Brachyspira hyodysenteriae is the primary cause of swine dysentery and primarily affects pigs during the grow/finishing stage. A novel non-antibiotic zinc chelate has been reported to demonstrate positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and B. hyodysenteriae excretion after a 6-day oral treatment. The objective was to compare performance parameters and antimicrobial use between a standard protocol (tiamulin, Denagard™; Elanco) and a zinc chelate (IntraDysovinol® 499 mg/ml; Elanco) on naturally occurring swine dysentery due to B. hyodysenteriae in a fattening unit in the Netherlands.

## Material and Methods

An all-in/all-out fattening unit with consistent clinical signs of swine dysentery was included in the study. Two subsequent batches of pigs were compared in performance parameters (average daily gain, days in fattening, mortality) and antimicrobial use (TI<sub>100</sub>) between a standard therapeutic approach using antimicrobial treatment (tiamulin) and an oral zinc chelate. Statistical analysis was performed using JMP 14.0 - ANOVA.

#### Results

Average daily gain was significantly higher (735 g/d vs. 711 g/d) in the zinc chelate-treated batch as compared to the standard therapeutic approach. Pigs treated with the zinc chelate product had 6 days less in fattening and had a significant lower mortality (3.5% vs. 6.6%). The  $Tl_{100}$  was numerically lower in the zinc chelate-treated pigs with 9.5 days in comparison to 11.2 days in pigs treated following the standard therapeutic approach.

## **Discussion and Conclusion**

Zinc chelate is a novel non-antibiotic treatment for swine dysentery due to B. hyodysenteriae resulted in a higher average daily gain in combination with lower mortality and a shorter number of days in fattening. Moreover, the Thus was numerically reduced, implying a markedly lower use of antimicrobials to keep clinical signs of swine dysentery under control.

# OPTIMIZATION OF ANTIMICROBIAL TREATMENTS USING PHARMACODYNAMIC PARAMETERS FOR SWINE RESPIRATORY PATHOGENS UNDER FIELD CONDITIONS

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## **Background and Objectives**

Antimicrobials (AB) are essential tools to control clinical outbreaks involving swine respiratory pathogens. The selective pressure exerted by these compounds could contribute to the emergence of antimicrobial resistant (AR) bacteria. There are many guidelines about AB but a more practical approach is urgently needed to put these recommendations into practice. The aim of this research work is to describe a method based on pharmacodynamic determination to select the most suitable AB for swine respiratory pathogens under practical conditions.

## Material and Methods

Samples coming from respiratory clinical cases, collected in the main pig producing areas from Spain since 2018, were cultured on suitable medium culture. After 2–3 days of culture, colonies were selected and cultured again for identification and further analysis using MALDIT-TOF. Antimicrobial susceptibility tests for MIC determination were performed for a battery of twelve AB, using the broth microdilution method, according to CLSI guideline M31–A3 with modifications to automate the procedure (Thermofisher scientific proposal). This MIC value was used to select the most suitable antimicrobial taking into account also pharmacokinetic information, clinical breakpoints and recommendations published by the European Union about the different antimicrobial categories.

## Results

The MIC value was determined for 110 Actinobacillus pleuropneumoniae (APP) and 100 Pasteurella multocida strains. Both bacteria were highly susceptible to many families of antimicrobials with the exception of tetracyclines for both pathogens and amoxicillin for APP. This prediction was checked with clinical information from the field after applying the treatments.

## **Discussion and Conclusion**

These results highlight the relevance of determining pharmacodynamic parameters (MIC) to optimize antimicrobial treatments in pig medicine. The generated information can justify an antimicrobial treatment for the present and future clinical cases if this epidemiological information is linked with the sow origin.

# TREATMENT OF END-OF-NURSERY PIGLETS WITH A NOVEL ZINC CHELATE RESULTS IN LONGER TIME-TO-TREATMENT IN THE FATTENING UNIT

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## **Background and Objectives**

Brachyspira hyodysenteriae is the primary cause of swine dysentery and primarily affects pigs during the grow/finishing stage. A novel non-antibiotic zinc chelate has been reported to demonstrate positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and B. hyodysenteriae excretion after a 6-day oral treatment. The objective was to study the time-to-treatment following introduction of 'clean' piglets treated with a zinc chelate (IntraDysovinol® 499 mg/ml; Elanco) in cleaned and disinfected compartments of a fattening unit with a history of swine dysentery in the Netherlands.

### Material and Methods

A fattening unit with history of consistent clinical signs of swine dysentery was included in the study. All piglets originated from a B. hyodysenteriae-infected breeding herd. In order to eliminate swine dysentery from the fattening unit, a treatment strategy combined with the Intra cleaning and disinfection protocol (Intracare BV; Netherlands) was designed. Piglets were treated with the zinc chelate for 6 days before transport to the fattening facilities and were housed freshly cleaned compartment.

#### Results

Until now, 8 weekly batches of piglets have been transported to the fattening unit. Intensive clinical follow-up has revealed no clinical signs of swine dysentery in any of these newly introduced batches. Currently time-to-treatment interval is at least 8 weeks.

## **Discussion and Conclusion**

Zinc chelate treatment of B. hyodysenteriae-infected piglets prior to transfer to the fattening unit resulted in an extended time-to-treatment interval. Zinc chelate is a novel non-antibiotic treatment for swine dysentery due to B. hyodysenteriae that reduced the excretion of B. hyodysenteriae following a 6-day oral treatment. Treatment resulted in a lower bacterial load and an extended time-to-treatment interval, mainly in combination with cleaning and disinfection prior to restocking and increased internal biosecurity measures to omit potential spread of B. hyodysenteriae from the infected to the non-infected compartments.

# SURVEY ON PRESENCE, PREVALENCE, SEROTYPE, CLINICAL EVIDENCE AND AB SENSITIVITY OF STREPTOCOCCUS SUIS IN A LARGE PIG PRODUCTION SYSTEM IN YEARS 2010-2019

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## **Background and Objectives**

Streptococcus suis is one of the most frequently isolated bacteria inducing severe clinical pictures in the post weaning stage. Although present worldwide with over 30 different serotypes, only few are able to induce clinical pictures like meningitis, septicemia and arthritis. In order to understand if and which were the serotypes circulating in the different flows of a large integrated production system a study was carried out during a ten years long period correlating the origin of the isolates, the clinical pictures, bacteriology results, presence of virulence factors and antimicrobial sensitivity.

## Material and Methods

Using a data extraction system the results, related to the isolations of Streptococcus suis carried out by Laboratorio Tre Valli during the period between 2010 and 2019, were analysed. From every submitted sample, where bacterial isolation was possible, the age and type of the pig (Sows-Weaned-Finishing), the organs and/or anatomical district/tissues of isolation, the possible presence of other bacterial and / or viral pathogens were recorded. It was also possible to note the origin (pig-flow) of the pigs to verify if any serotype could prevail within a specific supply chain.

#### Results

Collected data confirm that in most of the cases it is impossible to determine the capsular type with the available tools/techniques. Among those classified the most frequently isolate is type 9 followed by type 2 though with quite some variation among stages. Most of the isolates showed an Arc/mrp/Sly + profile or anyhow confirmed the presence of at least one or more virulence factors.

## **Discussion and Conclusion**

On the contrary it was impossible to establish a one way correlation with a certain serotype along a determinate flow or even among different flows. Monitoring antimicrobial sensitivity during the last 10 years highlighted how betalactamic antibiotics maintained a very high sensitivity in time confirming these molecules as first choice for therapy.

### DISTRIBUTION OF UREAPLASMA DIVERSUM IN GROWING AND FINISHING PIGS FROM DIFFERENT TYPES OF SYSTEMS

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## **Background and Objectives**

Ureaplasma diversum (U. diversum) is a bovine-origin Ureaplasma that has been associated with a wide range of reproductive failures in cattle. The agent has also been detected in pigs from different clinical specimens such as lung swabs, bronchial mucus and tracheobronchiolar lavages in pigs with and without pneumonia. However, the participation of U. diversum within the porcine respiratory disease complex (PRDC) is unknown, thus, the objective of this study was to estimate nasal swabs PCR prevalence of U. diversum in growing and finishing pigs using pooled samples.

## Material and Methods

For U. diversum detection by PCR a cross sectional study was carried out in 29 indoor and outdoor herds, collecting nasal swabs specimens from four 6-8 and four 15-22 weeks old.

#### Results

U. diversum was detected in 48.3% of the analyzed herds without significant difference (P>0.05) between indoor (32.3%) and outdoor systems (7.3%). The estimated prevalence was 12.6% (CI95%; 5.3-25.5) for 6-8 and 14.4% (CI95%; 6.4-28.6) for 15-22 week-old pigs (p>0.05)

## **Discussion and Conclusion**

It was concluded that U. diversum is present in the nasal cavity of pigs of different ages and, that is widely distributed among different types of analyzed herds.

# PREVALENCE OF MYCOPLASMA SUIS IN GESTATING SOWS USING QUANTITATIVE REAL-TIME PCR, AND IMPACT OF THE INFECTION ON CLINICAL, HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

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# **Background and Objectives**

Mycoplasma suis causes infectious anaemia in pigs and can be very significant, in particular for breeding herds around farrowing with the predominance of a chronic form which results in non-specific signs. Objectives of our study were to assess the prevalence of M. suis infection in French herds using qPCR and to evaluate its impact on the performances and on the haematological and biochemical parameters of sows in the farrowing period.

## Material and Methods

The analyses were carried out on 198 sows from ten farms. Sows randomly selected based on parity were sampled in the week before farrowing. Health and production parameters were recorded for eight days following farrowing. M. suis was detected by qPCR, a complete blood cell count and a blood glucose test were performed for each sow.

## Results

M. suis was detected in 53% of sows and no farm was free by qPCR. Sows of gestation rank ≥5 were significantly less infected than pregnant gilts. There was no statistically significant relation between M. suis status before farrowing and sow hyperthermia, total born, stillbirth and piglet diarrhoea during the first week of lactation. A significantly higher rate of stillbirths was observed in M. suis positive gilts. There was a tendency for haematocrit and lymphocytes to be higher among infected animals. In gilts population, only the lymphocyte count was significantly higher among infected animals.

## **Discussion and Conclusion**

This study confirmed the relevance of qPCR diagnosis of M. suis infection. The bacterium was detected in all farms suggesting that M. suis infection is widespread and enzootic in France. Nevertheless, infection seems to have little impact on health status and technical performance, although relation with stillbirth in gilts has to be investigated.

## COMPARISON OF QPCR AND BLOOD SMEAR MICROSCOPY FOR THE DIAGNOSIS OF MYCOPLASMA SUIS ON SOWS

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## **Background and Objectives**

Mycoplasma suis (M. suis) is an uncultivable haemotropic Mycoplasma that targets red blood cells of pigs and is responsible for infectious anaemia of pigs, historically known as porcine eperythrozoonosis. Clinical consequences of M. suis infection can be significant, particularly for the breeding herd in the period around farrowing. The study aimed to determine the clinical relevance of Giemsa-stained blood smear for the diagnosis of M. suis compared with qPCR results.

## Material and Methods

A total of 199 sows from ten farms were individually sampled in the week before farrowing. qPCR and Giemsastained blood smears were performed from EDTA-anticoagulated venous bloods collected from sows submitted to the diagnostic laboratory within 24 hours under positive-cold conditions. Blood smears were considered positive if the presence of M. suis was clearly identified. If M. suis could not be clearly identified among other artefacts, it was considered doubtful and finally, if no M. suis was observed, the blood smear was considered negative.

## Results

Considering qPCR as the reference standard diagnostic tool for the detection of M. suis, we first determined a specificity of 89%, a sensitivity of 47%, a positive predictive value of 78% and a negative predictive value of 66% for the microscopic observation of Giemsa-stained blood smears. Excluding unquantifiable qPCR results, we found no relationship between qPCR loads and blood smear results. At farm level, two farms out of ten were incorrectly classified as negative based on blood smears.

## **Discussion and Conclusion**

This study confirmed the low sensitivity and the poor clinical relevance of blood smear microscopy for the detection of M. suis. Although more costly, qPCR is probably the best diagnostic tool available today for M. suis diagnosis.

# ANTIMICROBIAL RESISTANCE PATTERNS OF PATHOGENIC AND COMMENSAL E. COLI ISOLATED IN PIGLETS WITH NEONATAL DIARRHOEA

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## **Background and Objectives**

Commensal and pathogenic E. coli can acquire antimicrobial resistance to antibiotics used for the treatment of pig diseases and commensal strains may serve as reservoirs of resistance genes for pathogenic ones. The aim of the study is to evaluate and compare the antimicrobial resistance (AMR) of enterotoxigenic E. coli (ETEC) and commensal E. coli isolated in piglets affected by neonatal diarrhoea.

## Material and Methods

In 2017, one-hundred seventy-six E. coli strains were isolated from intestines and faecal swabs of piglets with neonatal diarrhoea belonging to Italian pig holdings in Northern Italy. Among these isolates, 36 were identified as ETEC by multiplex PCR for virulence factors (F4, F5, F6, F18, F41, STa STb, LT and Stx2e), while 140, negative for virulence factors were considered commensals. Pathogenic and commensals E.coli were analysed for their susceptibility to 10 antimicrobials using the disc diffusion method, following the procedures of Clinical and Laboratory Standards Institute (CLSI). All strains were classified as sensitive, resistant or intermediate to the antimicrobials by interpreting the zones of growth inhibition. Intermediate strains were grouped with the resistant ones.

## Results

The highest resistance rates in ETEC were found for tetracyclin (94%), trimethoprim + sulphametoxazole (69%), ampicillin (67%) and enrofloxacin (64%), while in commensal E. coli the highest resistance rates were found for ampicillin (100%), tetracyclin (94%), enrofloxacin (79%), trimethoprim + sulphametoxazole (74%) and cephazolin (73%). Multi-drug resistance was observed in ETEC (92%) and commensal isolates (94%).

## **Discussion and Conclusion**

AMR of pig's ETEC and commensal E.coli strains represents a concern, especially to critically important antimicrobials. In enterobacteria, one of the major mechanism of resistance to fluoroquinolones, betalactams and cephalosporins is mediated by plasmids, which may be transferable to animals' and humans' pathogenic strains. The results obtained represent a concern not only for animal health, compromising the control of colibacillosis at farm level, but also for human health.

# SHIGA TOXIN ESCHERICHIA COLI (STEC) PREVALENCE STUDY ON ONTARIO FARMS (CANADA)

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# **Background and Objectives**

Edema disease (ED) caused by shiga toxin producing E.coli strains (STEC) is an important disease characteristic causing increased mortality and the reduction of important zootechnical parameters. The prevalence of STEC is unknown in Canada. The aim of this presented study was to assess the presence of STEC strains based on the associated virulence genes Stx2 and FI8.

## **Material and Methods**

Farms (n= 21) with history of unclear clinical signs, possibly related to ED, were included and 15 randomly selected piglets per farm were sampled during the nursery period (4-10 weeks of age). Rectal swabs were inoculated on blood agar plates for haemolysin detection and then pooled 3 to 1 in LB for overnight growth followed by DNA extraction and PCR screening. The DNA samples were evaluated for a panel of virulence factors including F4, F18, EAE, LT, STa, STb, Stx1 and Stx2.

## Results

Five of the 21 farms (23,8 %) were positive for Stx2 gene and 2 of them were also positive for the attachment factor F18. Nineteen farms were positive for various combinations of virulence factors characteristic of ETEC strains usually associated with post- weaning diarrhea in piglets. Only 1 farm was completely negative for all virulence factors.

## **Discussion and Conclusion**

The study confirmed 23,8% presence of STEC strains in Ontario swine herds reporting sub-optimal production results and unclear clinical signs at nursery. The negative effect of STEC colonisation was described in a form of sub-clinical infection as well as negative effects of colonisation on performance on positive farms (Kausche et al., 1992; Leveneu et al., 2018). Further investigation and complete assessment of other bacterial and viral pathogens would be needed to determine the disease status of the farms. The percentage of Stx2 positive farms may have been higher had farms presenting only clinical signs of edema disease been targeted.

## SUSCEPTIBILITY OF ESCHERICHIA COLI AND SALMONELLA FIELD ISOLATES TO PAROMOMYCIN

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## **Background and Objectives**

Paromomycin is an aminoglycide registered in both pigs and cattle for the treatment of gastro- enteric pathogens. As part of the prudent use of antibiotics and to monitor evolution of antimicrobial resistance, susceptibility testing is of great importance and performed on E. coli and Salmonella isolates from diagnostic cases by DGZ.

## **Material and Methods**

Antimicrobial resistance was determined by the disk diffusion method on 2045 isolates of E. coli, hemolytic E. coli and Salmonella strains isolated from pigs and cattle clinical samples over the period of 01/2017 till 08/2019. Cultures were inoculated on Muller Hinton agar plates and susceptibility discs for paromomycin (Mast Diagnostics) 1000 µg were used. Plates and the inhibition zone were read after 24 h of incubation at 37°C. To categorize the strains in susceptible (S), intermediate (I) or resistant (R) the following breakpoints are defined S≥14mm –I: between 13 –11 mm and R<11mm.

#### Results

A total of 2045 isolates of which 1654 E coli strains (19.7 % was hemolytic) and a total of 391 Salmonella were tested. In total 79.80% of the Enterobacteriaceae strains were susceptible, 6.26% of the strains were resistant and 13.94% intermediate to paromomycin. If we look to the different species of origin the highest level of resistance was found in cattle stains 9.95% whereas in strains of pig origin only 0.95% of resistance was observed. Especially the E. coli and hemolytic E. coli isolates from cattle gives the highest level of resistance, 11.40%, while in Salmonella only 0.62% of resistance was noticed.

## **Discussion and Conclusion**

In conclusion we can state that, based on these results, a high proportion of Escherichia coli and Salmonella strains in Belgian farms shows susceptibility to paromomycin, but it is important to analyse the data by animal origin and by bacterial species to evaluate better the real development of resistance.

# DETECTION OF STX2E-ENCODING E. COLI IN WEANING PIGLETS: COMPARISON OF METHODS FOR SAMPLING AND SAMPLE PROCESSING

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## **Background and Objectives**

This study aimed to determine the most sensitive diagnostic approach for detection of Stx2e-encoding E. coli in piglets by comparing different types of samples and sample processing.

## Material and Methods

Five farms (A-E) in Germany with a confirmed history of edema disease were included in this pilot study. On each farm, samples were collected from 4 (A) or 5 pens (B-E), respectively. Rectal swabs, single and pooled faeces from pen floor, and a boot sock swab were taken concurrently in each of these pens. In pens on farms A-D oral fluids were collected additionally by wringing (OF-W) and rinsing (OF-R) cotton ropes having been exposed to the piglets. Depending on the sample type, the specimens were submitted to a stx2e-specific PCR directly or underwent bacterial cultivation for E. coli and/or DNA extraction beforehand.

## Results

Four farms (80 %; B-D) and 12 pens (50 %) proved positive for stx2e. Stx2e-encoding E. coli strains were isolated in all these farms. However, none of the tested methods was capable of detecting all stx2e-positive farms or pens. OF-R was most sensitive, facilitating the detection of 82 % of the stx2e-positive pens and all three stx2e-positive farms sampled with ropes. On the other hand, direct PCR examination of rectal swabs and oral fluids failed to detect any stx2e-positive pen or farm. When the results obtained from rectal swabs were ignored due to high effort and animal welfare, the following combined procedure detected 11/12 stx2e-positive pens (92 %) and 4/5 stx2e-positive farms (80 %): testing E. coli isolates from pooled faeces and from boot socks (8 colonies each) as well as DNA extracts from OF-R by stx2e-specific PCR.

# **Discussion and Conclusion**

Our findings provide valuable information to further improve the sampling and laboratory examination scheme for edema disease (E. coli enterotoxemia) and to better target preventive measures against Stx2eencoding E. coli in piglets.

## INVESTIGATION OF PATHOGENS INVOLVED IN NEONATAL DIARRHOEA IN 134 FARMS IN GERMANY IN 2018

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## **Background and Objectives**

Neonatal diarrhoea is one of the most relevant diseases in early piglets live. Depending on the involved pathogens it can lead to high economic losses due to increased mortality, reduced daily weight gain and a high antimicrobial treatment rate. In 2018 veterinarians were asked to send in diarrhoea samples from new borne piglets (max. 4 days old) from farms having persistent issues with neonatal diarrhoea, to be able to detect involved pathogens.

## Material and Methods

On each attending farm at least 3-5 piglets per litter and minimum 3 litters were sampled. Analyses were carried out for Rotavirus type-A and type-C (RVA/RVC), Porcine epidemic diarrhoea Virus (PEDV), Transmissible gastroenteritis virus (TGEV), pathogenic E. coli, Clostridium perfringens types A (CpA) and C (CpC) and Clostridium difficile. Viruses, attachment factors, toxin genes of E. coli and toxin genes of Clostridium were detected by PCR.

#### Results

338 samples out of 134 submissions could be analysed. In two farms PEDV could be detected, none was positive for TGEV. 75% of the submissions were positive for RVA, 27% for RVC. 389 E. coli were isolated, 21% of these were hemolytic . 310 E. coli colonies were genotyped and 76 pathogenic E.coli (25%) could be identified: 27 EPEC, 25 ETEC and 23 NTEC. In 125 (93%) of the submissions Cp was found in at least one sample. 76% of the tested samples (333) were positive for Cp. 254 CpA and 1 CpC were found. 96% of tested CpA were positive for ß2-toxin-gene.

## **Discussion and Conclusion**

C.perfringens type A, E.coli and RVA were the most prevalent pathogens found in samples collected in farms with ND. Often multiple agents were detected per farm. The role of each pathogen on the course of disease interpreted carefully, as the identification of a pathogen can be of great help but does not give the full diagnose.

IDENTIFICATION OF ACTINOBACILLUS PLEUROPNEUMONIAE (APP) SEROTYPES 4, 5 AND 17 IN SWINE PLEUROPNEUMONIA OUTBREAKS IN PORTUGUESE SWINE HERDS.

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## **Background and Objectives**

Pleuropneumonia remains an important challenge to swine production worldwide. Knowing the current country serotype prevalence of APP informs on herd management. The current serotype prevalence of APP in Portugal is unknown. The objective of this study was to identify the clinically relevant serotypes of APP present in Portugal.

#### **Material and Methods**

Fifty-one samples of lung lesions typical of APP infection were collected during clinical outbreaks of swine pleuropneumonia (from 13 farms) between March 2018 and April 2019. Tissue (25 mg) was homogenized using the Fast Prep Homogenizer (MP Biomedical), and DNA isolated using the QIAamp DNA Mini Kit (Qiagen). Serotyping, based on capsular loci, was carried out by multiplex-PCR as described by Bossé et al. (2018).

#### Results

Three serotypes were detected – 4, 5 and 17. In 8 farms only 1 serotype (Serotype 4, 5 or 17) was found, and in 4 farms at least 2 different serotypes (serotype 5 and 17) were present. No APP was detected in one farm. With one exception, samples from the same farm had a common serotype, and correlated with a common origin of replacement gilts. Farms with more than one serotype had received gilts from at least two different origins in the last 2 years.

## **Discussion and Conclusion**

The results show that at least 3 main serotypes with clinical relevance are present in Portuguese swine herds (serotypes 4, 5 and 17), with some herds positive for more than one serotype. Serotypes 4 and 5 are normally considered of moderate and high virulence, respectively. Serotype 17 was discovered recently but has previously been isolated in neighboring Spain. The results indicate that the source of replacement gilts has a key role in the epidemiology of APP.

# DESCRIPTIVE STUDY OF STREPTOCOCCUS SUIS INFECTION IN SPAIN: PREVALENCE AND SEROTYPES INVOLVED IN CLINICAL CASES.

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## **Background and Objectives**

Streptococcus suis (S. suis) causes septic diseases in pigs characterized by septicemia and acute death, meningitis, polyarthritis, polyserositis, and valvular endocarditis. There are 35 different serotypes, and the objective of this study was to clarify which are currently involved in clinical cases observed in Spain in 2019.

## **Material and Methods**

During 2019, a total of 349 piglets from 3 to 8 weeks of age (196 with arthritis and 153 with meningitis clinical signs) were sampled in 24 Spanish farms. According to these clinical signs, samples from cerebrospinal or synovial fluid were taken and cultured. In positive samples to S. suis, PCR was performed in order to evaluate the serotype of each isolate.

#### Results

Streptococcus suis was detected in 151 samples (43.3%), other bacteria species in 54 piglets (15.4%) and 144 piglets were defined as negativ (41.3%).Regarding arthritis issues, S. suis was detected in 66 samples (33.7%), other bacteria in 42 samples (21.4%) and 88 samples resulted negativ (44.9%).In piglets with nervous signs, S. suis was isolated in 85 piglets (55.6%), other bacteria in 12 piglets (7.8%) and 56 samples resulted negativ (36.6%).Serotype 9 was the most prevalent (26.5%), followed by serotype 1–14(19.9%), 2–1/2 (16.6%) and 7 (15.9%), either in synovial or cerebrospinal fluid samples. Other serotypes detected were 3, 4, 5 and 8, as well as 12 isolates were defined as untypeable (7.9%).Regarding the number of serotypes detected in each farm, 1 only serotype was detected in 6 farms, 2 different serotypes were detected in 10 farms, 3 serotypes in 6 farms and 4 serotypes in 2 farms.

#### **Discussion and Conclusion**

The most prevalent S. suis serotypes in Spain isolated from clinical cases were 9, 1–14, 2–1/2 and 7. Moreover, these results showed clinical cases in herds are usually caused by more than 1 serotype, being most commonly the presence of 2 serotypes.

## SURVEY OF MYCOPLASMA HYOPNEUMONIAE CLINICAL SYMPTOMS AND VACCINATION IN SOWS

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## **Background and Objectives**

Mycoplasma hyopneumoniae (Mhp) is the main pathogen of Enzootic Pneumoniae and an economical relevant infection in major pig producing countries. It can infect all age groups but clinical symptoms are mainly seen in growing pigs. From literature, adult animals are rarely clinically affected except for acute outbreaks in naïve farms but detailed description of clinical symptoms is limited. Therefore, Boehringer Ingelheim developed a survey in Pig333 to get data of clinical symptoms and vaccination strategies against Mhp in sows.

#### Material and Methods

In total, 566 responses from all parts of the world with the majority (493) coming from veterinary practitioners/consultants were analysed.

#### Results

The most interesting findings were that 46,3% of respondents have experienced Mhp outbreaks in sow farms. Unexpectedly, the majority of outbreaks (31 to 40%) has been observed in Mhp positive farms with no sow but piglet vaccination.Clinical symptoms were reported to be **cough in suckling piglets** (36,0% of answers), **cough in sows** (27,5%), **fever in sows** (12,5%), **weak-born piglets/ increased pre-weaning mortality** (12,2%) and **abortions** (6,1%).

## **Discussion and Conclusion**

Mhp outbreaks in sow farms are rarely mentioned in textbooks and are not described at all in scientific papers. However, from this survey, Mhp outbreaks in sows are not unusual and occur not only in naïve farms. Mhp vaccination of only piglets may be due to the missing vaccine label claim for sows in some regions and countries (e.g. Europe) where respondents reported also the highest rate of herds that do not vaccinate sows (30% compared to 17.3% in North America). From literature, sow vaccination can reduce Mhp transmission from sows to piglets. In addition, piglets from vaccinated sows showed a reduction in lungs with lesions as well as a reduction in the severity of lesions.

## EXPLORATION OF STEC GENETIC DIVERSITY IN DIFFERENT REGIONS OF FRANCE

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## **Background and Objectives**

Edema disease (ED) induces high economic losses in pig production due to a high mortality rate and generally poor performance of affected pigs. It is caused by the shigatoxin Stx2e, produced by STEC (shigatoxin producing Escherichia coli). Clinically absent for decades on Reunion Island, ED occurred in early 2014 and spread in a majority of the Reunion pig farms (OI39:K82 serogroup involved). Currently, more than 63% of the Reunion farms successfully use a vaccine against the shigatoxin. One of the explanatory hypothesis is that a high virulent STEC clone infected the island. This study was designed to explore the genetic diversity of the local STEC and to compare them with continental French STEC.

## Material and Methods

STEC genetic diversity was established by using Multi-Locus Sequence Typing with Sanger method (MLST) of seven housekeeping genes (adk, fumC, gyrB, icd, mdh, recA and purA). Ten Reunion Island and 22 continental French O139:K82 isolates were selected, all isolated from clinical ED cases. These strains were compared in a phylogenetic tree built from the MLST results.

#### Results

22 out of these 32 STEC strains belonged to the same cluster. The epidemiological links and relationship of the strains gathered in this unique true cluster were not obvious. Indeed, it encompasses six strains from Reunion Island and 16 strains from continental France. The significant genetic diversity of the Reunion Island STEC was thereby demonstrated.

## **Discussion and Conclusion**

This result is contradictory to the hypothesis, that only a single O139:K82 STEC clone is characteristic for the Reunion island. Therefore, a relevant follow-up study would be necessary to review specific conditions that occurred in the Reunion pig farms and assessed potential risk factors associated with clinical outbreaks since 2014.
# CONTROLLING AN OUTBREAK OF MYCOPLASMA HYOPNEUMONIAE (MHP) AND REDUCING VERTICAL SHEDDING IN A NAIVE GILT POPULATION USING AIVLOSIN® 17% (TYLVALOSIN) MEDICATED PREMIX

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# **Background and Objectives**

This case study assesses the use of Aivlosin<sup>®</sup> 17% Medicated Premix to reduce vertical shedding of Mhp. A 2200 sow farrow to wean farm was repopulating when it was discovered the gilts were infected with Mhp from the the multiplier. The growing pigs in this system are raised without the use of antibiotics, thus a gilt treatment plan was implemented to reduce vertical shedding and prevent disease in finishing.

# **Material and Methods**

Five cohorts, one per month, of 30 pregnant gilts were medicated with Aivlosin® 17% at 5mg tylvalosin/kg BW for 7 days pre-farrow and 7 days post-farrow. Tracheobronchial swabs (TBS) were collected pre- and post-treatment for PCR (Animal Health Laboratory, Canada) identification of Mhp. Once per month, 100 TBS and four oral fluids were collected from one wean group on entry into the nursery and tested for Mhp via PCR (lowa State University). Oral fluids for Mhp PCR were collected and cough scores were evaluated at 4, 8, and 12 weeks post-placement into finisher barns for each nursery cohort.

# Results

The percentage of Mhp positive gilts pre and post treatment, respectively, were as follows: Cohort 1: 53% and 22%; Cohort 2: 57% and 15%, Cohort 3: 26% and 1%; Cohort 4: 1% and 0%, Cohort 5: 12% and 0%. The first nursery cohort was 43% Mhp PCR positive on TBS while the four subsequent cohorts (n=5) were negative. Cough score indexes were low (<2.0) at each finishing site and sample point, indicating no clinical respiratory disease. Mhp prevalence on oral fluid PCR on arrival to finishing barns decreased over time.

# **Discussion and Conclusion**

The Aivlosin<sup>®</sup> 17% Medicated Premix Mhp control program was effective in reducing vertical transmission as shown by the reduction of Mhp positive gilts post treatment and through a decline in Mhp prevalence at nursery and finishing sites.

# ASSOCIATION OF SEQUENCE WITH SEROTYPE, VIRULENCE, AND ANTIMICROBIAL RESISTANCE IN ACTINOBACILLUS PLEUROPNEUMONIAE\*

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# **Background and Objectives**

Actinobacillus pleuropneumoniae (APP) is a predominant etiological source for respiratory disease in pigs, responsible for significant economic losses in pig production worldwide. The clinical manifestation can vary between peracute cases of sudden death, severe pleuropneumonia and subclinical infection with reduced performance and marginal symptoms. Strains are highly variable regarding serotype, virulence and antimicrobial resistance, leading to massive problems in diagnostics, therapy and vaccination. Aim of the present, ongoing study is to develop molecular markers to improve diagnostics, therapy and prophylaxis.

# **Material and Methods**

We determined the genomes of 60 APP strains isolated from lungs of pigs from commercial herds in Germany. Genome sequences were determined using next-generation sequencing. The sequences were compared to 100 publically available genomes. We searched for genes and gene-variants involved in virulence, antimicrobial resistance and serotype formation.

# Results

Together, the sequenced strains reflect the existence of an open pan genome in APP. Any existing serotypes could be determined based on the underlying DNA-sequences. The sequenced strains showed a high diversity for antimicrobial resistance, also well associated with responsible genes and sequences. Genome analyses showed a large number of virulence-associated genes and gene variants which will be associated with virulence phenotypes in the ongoing study.

# **Discussion and Conclusion**

Whole genome sequencing can improve serotyping and help to avoid mistyping when compared to traditional methods. The repertoire of antimicrobial resistance- and virulence-associated genes can be applied to discriminate strains of low and high virulence and for a fast and comprehensive resistance testing. Comparative genome analysis of APP provides a deeper insight into the molecular architecture of APP pathogenesis, virulence and resistance which will lead to faster, more comprehensive and more precise APP diagnostics in the future. It will be the basis for next generation prophylactic and therapeutic measures.

\* This study was funded by MSD animal health.

#### TRIGGER FACTORS FOR SUDDEN DEATHS CAUSED BY STREPTOCOCCUS SUIS IN AN EXTENSIVE HUSBANDRY SYSTEM

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# **Background and Objectives**

In endemically infected swine herds, factors triggering transition from colonizing Streptococcus (S.) suis residing mainly in the tonsils to invading bacteria causing severe disease have not been elucidated so far. Stressors as moving, mixing and coinfecting microorgansims are known to predispose for disease in conventional farms. In contrast to that, risk factors for disease development under extensive husbandry conditions are less understood.

# **Material and Methods**

Two three month old fattening pigs, which had been kept under extensive husbandry conditions in a zoo, died suddenly after transportation to another location. Main necropsy findings were septic shock and disseminated intravascular coagulopathy caused by S. suis capsular type 2 (cps 2), which was cultivated from parenchymatous organs. Remaining pigs in the zoo were healthy and were sampled by tonsillar brushing to cultivate S. suis for performance of functional assays. In parallel, the presence of opsonophagocytotic antibodies was checked in blood samples.

#### Results

Three cultivated tonsillar isolates were not related to the invasive cps type 2 strain isolated from the dead pigs. Neither growth behavior, the capability to induce neutrophil extracellular traps, nor survival in blood was indicative of a high virulence of the invasive cps type 2 strain. Reconstituted blood assays using serum samples from pigs of different age groups revealed varying levels of protecting opsonophagocytotic antibodies in individuals against the invasive cps type 2 strain especially in young pigs.

# **Discussion and Conclusion**

These findings highlight the impact of a high bacterial burden in the environment due to low internal biosecurity and inappropriate development of active immunity as predisposing factors for S. suis related disease in an extensive husbandry system. Results of functional assays with the isolated S. suis strains could not explain the disease picture, so that bacterial factors might have been less important for disease pathogenesis than host factors in this herd.

# EFFECT OF BIVALENT VACCINE FOR F4- AND F18-POSITIVE ETEC ON HEALTH, GROWTH AND FECAL MICROBIAL COMPOSITION OF WEANED PIGLETS.

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# **Background and Objectives**

Post-weaning diarrhoea (PWD), mainly caused by enterotoxigenic Escherichia coli (ETEC) carrying the adhesive fimbriae F4 and F18, remains a major cause of economic losses in pig industry. Coliprotec® F4/F18 is a live E. coli oral vaccine for active immunization of pigs against PWD caused by F4- and F18-positive ETEC. This study investigated the effect of this live E. coli vaccine on the health, performance and faecal microbiota profile of piglet around weaning.

#### **Material and Methods**

A total of 290 piglets (from 48 litters) were randomly distributed into 2 equal groups the day (d) before weaning ( $26 \pm 2$  days of age; d0) and inoculated either with Coliprotec® F4/F18 vaccine (VAX) or with water (CO). Pigs were individually weighed on d0 (VAX:  $6.32\pm1.25$ ; CO:  $6.45\pm1.45$ ) and d17 and weighted by group on d72. Mortality and health were recorded. A faecal sample from a subgroup of 30 piglets per group was collected at d0 and d17 for microbial profile of V3-V4 regions of the 16S rRNA on MiSeq Illumina platform.

# Results

Sixteen piglets were excluded because of health impairment, 7 in VAX and 9 in CO group. No differences in the growth were observed between groups until d17. However, the ADWG was higher in VAX than CO during periods from d17 to d72 and from d0 to d72. As expected, faecal microbiota was affected by age of piglets. Moreover, vaccination reduced the microbial diversity (Shannon and Chao indices) and affected the microbial community profile (Bray curtis distance matrix) compared to CO (P<0.05). VAX was discriminated from CO by beneficial bacteria genera including Prevotella, Blautia, Ruminiclostridium, Parabacteroides and Faecalibacterium.

#### **Discussion and Conclusion**

This study shows that administration of Coliprotec® F4F18 is effective in improving piglet's growth in the postweaning phase by modulating the gut microbial composition, favouring gut eubiosis.

# THE IMPORTANCE OF LT TOXIN ANTIBODIES IN THE SUCKLING PIG'S SERUM TO REDUCE ANTIBIOTIC THERAPY OF NEONATAL DIARRHEA – A FIELD STUDY

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# **Background and Objectives**

Diarrhea caused by Escherichia coli is still one of the major animal health issues on the Hungarian swine farms. In many cases farrowing units try to solve the problem with sow vaccination, good farrowing unit management, and finally treating the piglets with antibiotics. The aim of the study was to collect practical data about importance of LT toxoid antibodies presence in the colostrum.

#### **Material and Methods**

We surveyed a Hungarian farm with 1,500 sows. We vaccinated the sows in two phases: in Phase 1 vaccination without LT toxoid (Gletvax, Zoetis), and in Phase 2 with LT toxoid (Porcilis ColiClos, MSD). We collected blood samples from suckling piglets after both vaccination schemes: in 1, 2, 3, 6 weeks of age. We monitored the prevalence of diarrhea, the number of treatments, and piglet mortality in the farrowing unit. The examinations (ELISA: K88ab, K88ac, K99, 987P, LT) were conducted in Boxmeer R&D Service Lab.

# Results

In Phase 2 the prevalence of diarrhea decreased by 83%, and most importantly the same treatments of these cases were more successful. Summarily, the farm could reduce its antibiotics use by around 30% in the farrowing unit.

# **Discussion and Conclusion**

We found that if a farm uses a sow vaccine with LT toxoid, and the piglets receive the proper amount of colostrum, diarrhea is healing more easily, and treatments are more successful, than farms that do not use LT toxoid vaccines. Good vaccination management, and the selection of more effective sow vaccination protocols can help reducing the antibiotic use in the farrowing units in treating scour problems among suckling piglets. But proper colostrum management is still a key factor.

# ASSOCIATION OF SERUM HOMOCYSTEINE CONCENTRATION AND ILEAL MALABSORPTION DUE TO LAWSONIA INTRACELLULARIS-INFECTION IN PIGS

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# **Background and Objectives**

A stepwise decrease in serum homocysteine (HCY) concentrations has been reported from pigs with chronic, acute, subclinical L intracellularis-infection to control pigs. These results indicate that proliferative changes of the small intestine in pigs with L intracellularis-infection cause altered HCY concentrations due to malabsorption of vitamin  $B_9$  and  $B_{12}$ . The study aimed to quantify L intracellularis in faeces and to evaluate serum HCY concentrations as a biomarker for the detection of malabsorption associated with L intracellularis-infection in pigs.

#### Material and Methods

Serum and faecal samples were collected from 1800 pigs, originating from six countries (i.e., Denmark, France, Germany, Spain, The Netherlands, and United Kingdom), ten herds each country, and 30 pigs per herd (nursery, grower, and finisher). For all pigs, serum and faeces were evaluated by using an immunoassay for HCY and qPCR for L. intracellularis, respectively. HCY concentrations were compared between vaccinated (Enterisol) and non-vaccinated herds, and age categories. Correlation analyses were performed between HCY and L. intracellularis levels for non-vaccinated and nursery, grower, and finisher pigs. Receiver-operating characteristic (ROC) curves were used to determine the optimum cut-off for HCY concentrations that distinguishes vaccinated and non-vaccinated pigs, and L. intracellularis-infected and non-infected nursery pigs.

# Results

HCY concentrations differed between vaccinated and non-vaccinated (p<0.0001; cut-off: 13.7 Mol/L), and nursery, grower, and finisher pigs (p<0.0001), whereas non-vaccinated and nursery pigs had higher HCY concentrations than vaccinated and grower, and finisher pigs, respectively (both: p<0.0001). The strongest correlation was observed between HCY and L intracellularis levels in nursery pigs (M=0.35; p<0.0001; cut-off: 19.2 Mol/L).

# **Discussion and Conclusion**

HCY concentrations could be used to question the L. intracellularis vaccination if the median HCY concentration is >13.7 Mmol/L. Due to the positive correlation of serum HCY and faecal L. intracellularis levels in non-vaccinated nursery pigs, HCY could be a helpful biomarker to diagnose L. intracellularis-infection in nursery pigs.

# PROFILING MYCOPLASMA HYOPNEUMONIAE IN FIVE MACEDONIAN COMMERCIAL PIG FARMS USING SEROLOGY AND LUNG LESIONS SCORING

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# **Background and Objectives**

Mycoplasma hyopneumoniae is a causative agent of enzootic pneumonia in pigs and it has primary role in the porcine respiratory diseases complex (PRDC). The objective of the study was to profile M. hyopneumoniae infections in five Macedonian commercial pig farms by serology and by lung lesions assessment at slaughter.

# **Material and Methods**

Five commercial pig farms (A, B, C, D, E) vaccinating against M. hyopneumoniae were included in the study. During a single visit, blood samples were taken from five different pig categories including 6, 10, 14, 18 and 22 weeks of age. Ten animals per age group from each farm were sampled and analyzed for the presence of antibodies using ELISA. In total 250 lungs (50 lungs per farm) were evaluated and scored for lesions typical of M. hyopneumoniae at slaughterhouse.

# Results

All farms were seropositive to M. hyopneumoniae. Statistical differences in the prevalence of positive animals were detected in 10 weeks (range 0 to 80%), 14 weeks (range 20 to 100%) and 22 weeks (30 to 100%) old groups. Serological trend in farm A was similar with farm E, whilst farm B had similar serological response with farm C. Mean lung lesion score (LLS) for all farms was 11.5, while significantly higher LLS was observed in farms D (14.4) and A (13.6) in contrast to farms C (8) and B (9.5).

# **Discussion and Conclusion**

The results indicate that farms A and E revealed best serological response due to vaccination. Lower LLS found in farms B and C is probably associated with additional managerial practices besides vaccination. Combination of proper vaccination protocol and good managerial practices could improve overall profile in Macedonian commercial pig farms regarding M. hyopneumoniae and PRDC in general.

TEMPORAL ANTIMICROBIAL RESISTANCE PATTERNS OF ESCHERICHIA COLI ISOLATES FROM SWINE HUSBANDRIES IN NORTH WESTERN GERMANY

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# **Background and Objectives**

Since 2011, the use of antimicrobial agents in animals in Germany has been steadily declining. Whether this comes along with reduction of resistance in bacteria has not been assigned yet.

# **Material and Methods**

Antimicrobial resistancy patterns of 3,054 Escherichia coli (E. coli) isolates originating from 2,161 pig farms in North Western Germany in the time period 2006 to 2017 were evaluated retrospectively. E. coli strains were obtained from various organs of diseased pigs and the minimum inhibitory concentrations of selected antimicrobial agents were determined. Susceptibility testing and interpretation of data with respect to clinical cut-offs were done in accordance with the Clinical and Laboratory Standards Institute (CLSI). In case of lack of clinical cut-offs for the combination of host and bacterial species and substance, other published cut-offs were used.

#### Results

For most substances, differences in susceptibility patterns exist between the gastrointestinal and genitourinary tract. While lower percentage of isolates from the genito-urinary was resistant to colistin, ampicillin, apramycin, spectinomycin, neomycin and tetracycline, a higher percentage was resistant to enrofloxacin and florfenicol. For tetracycline, neomycin and spectinomycin a multifactorial logistic regression model showed a time-dependent decrease in percentage of resistant isolates. For colistin, the maximal proportion of 16% of resistant isolates was found in 2015. In 2017 this proportion decrease to the level of 2009-2010. Depending on age-group and time period, the frequency of ampicillin-resistant isolates decreased. In summary less than 15% resistant isolates with respect to colistin, cephalosporins, apramycin, enrofloxacin, neomycin, florfenicol and gentamicin were recorded irrespective of the year.

# **Discussion and Conclusion**

For some substances a time-dependent decrease of resistant E. coli isolates was shown. It could be hypothetised that a general decrease in antimicrobial usage in swine has an effect onto bacterial antimicrobial resistance. The dataset might help swine practitioners to make decisions about antibiotic therapy.

# SIMULTANEOUS DETECTION OF MYCOPLASMA HYORHINIS AND HAEMOPHILUS (GLAESSERELLA) PARASUIS IN PIGS WITH POLYSEROSITIS

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# **Background and Objectives**

The clinical signs and lesions described for Glässer's disease such as fibrinous polyserositis are not pathognomonic of Haemophilus (Glaesserella) parasuis infections. An another important causative agent of fibrinous polyserositis in pig is Mycoplasma hyorhinis. Furthermore this pathogen has been found coinfecting pigs with H. parasuis. The objective of this study was to determine the causative role of M. hyorhinis and H. parasuis and the associations among these pathogens in the etiology of polyserositis in pigs

# **Material and Methods**

A total of 36 samples (lung, heart, liver and spleen) form pigs form 11 Polish herds were examined. All of these samples were obtained from pigs with polyserositis. Presence of M. hyorhinis and H. parasuis DNA was determined in each samples using a real-time PCR methods

# Results

M. hyorhinis was detected in 22 (61.1%) samples form 8 (72.7%) herds and H. parasuis in 16 (44,4%) samples from 9 (81.8%) herds, 13 (36,1%) samples were negative for both of these pathogens. Considering only positive samples, 15 (68,2%) were positive for both M. hyorhinis and H. parasuis simultaneously. Among remaining samples, 7 (31,8%) were positive only for M. hyorhinis and in one sample (4,5%) solely H. parasuis was detected.

# **Discussion and Conclusion**

Because of ambiguous results of previous studies the possible importance of the M. hyorhinis as a cofactor of fibrinous polyserositis in pigs seems to be underestimated. Our partial result shows that M. hyorhinis can be important factor of polyserositis in pigs form Polish pig herds. Further investigation is needed to establish the actual role and the epidemiological importance of M. hyorhinis and H. parasuis coinfections in pigs.

# COMPARISON OF TWO VACCINATION PROTOCOLS AGAINST ERYSIPELAS IN A COMMERCIAL SOW FARM

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# **Background and Objectives**

Despite the intensive vaccination programs applied in the field, erysipelas continuous to be a costly global concern for the swine industry and for public health. Currently, post-farrowing vaccination is routinely applied to protect the breeding stock, however there is a need to vaccinate growing pigs and it is not uncommon to detect outbreaks in the field due to the low levels of antibodies that offspring possesses. Further studies investigating other vaccination protocols that may increase protection against erysipelas remain lacking. Therefore, the specific objectives of this study were two-fold: 1) to study the seroconversion in sows and piglets between pre- and post-farrowing protocols overtime; and 2) to describe the time-to-negative status between protocols.

# Material and Methods

A total of 34 sows and 68 piglets (2/sow) from a farrow-to-wean farm were included in the study. Sows and piglets were assigned into 5 groups considering parity, type of erysipelas vaccine (two commercial vaccines), and vaccination protocol (pre-farrowing vs post-farrowing). Blood samples were collected from sows and piglets at days 7, 14, and 21 of the study, and finally, from piglets at 42, 63, and 84 days. All blood samples were tested for Erysipelothrix rhusiopathiae ELISA test and results analysed by a linear mixed model using R software.

# Results

For objective 1, the mean antibody titer was statistically higher in the pre-farrowing protocol for sows and piglets; only in piglets, this difference was significant overtime and specifically from multiparous sows (p=0.0039). For objective 2, time-to-negative status was significant between sows of different protocols (p=0.049). Piglets from the post- protocol became negative on average 4 days earlier than those in the pre-farrowing protocol (p=0.0037).

# **Discussion and Conclusion**

A new pre-farrowing vaccination protocol in sows, provided higher erysipelas's serum antibodies levels during lactation demonstrating that those piglets were better protected for future challenges.

# KLEBSIELLA PNEUMONIAE SUBSPECIES PNEUMONIAE FROM CLINICALLY DISEASED PIGLETS - WHOLE GENOME SEQUENCING

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## **Background and Objectives**

In 2015, the first cases of septicaemia and sudden death in 2-3 week old suckling piglets, due to Klebsiella pneumoniae subspecies pneumonia (KPP) infection, were found in the Netherlands. Similar cases were reported in pigs in the UK and Australia. This project aimed to gain first insight into the epidemiology of KPP from diseased pigs in the Netherlands by whole genome sequencing (WGS).

#### Material and Methods

KPP isolates were cultured from different tissues (like spleen, brain, lung) at Royal GD from diseased piglets submitted for post-mortem examination. Eight isolates (from 6 different farms) were submitted to the Faculty of Veterinary Medicine (Utrecht University) for WGS. After sequence assembly using SPAdes v3.11.1 and alignment using Parsnp v.1.2, the aligned core genome sequences were used for constructing a maximum likelihood tree and STs were assigned.

#### Results

WGS trees suggested 3 separate clones: clone 1 (1 isolate; ST1480), clone 2 (3 isolates; ST25) and clone 3 (4 isolates; ST25). Clone 1 originated from 1 farm, clone 2 originated from 1 farm (isolates collected in April (n=1) and August (n=2) 2015), clone 3 originated from 4 different farms. Clones 2 and 3 were found in the same geographical region whereas clone 1 originated from a different region.

#### **Discussion and Conclusion**

Although only a limited number of KPP isolates were included, this project provides a first insight into the epidemiology of KPP in the Netherlands. Three different clones were found in two distinct geographical regions, and the persistence of one clone was demonstrated over a short time period. For clone 3 a common source outside the farms is suggested. Seven out of eight isolates showed the same ST (ST25) which has previously been demonstrated in clinical cases in pigs in the UK and in Australia.

CURRENT SITUATION OF ENZOOTIC PNEUMONIA AND PORCINE PLEUROPNEUMONIA IN SPAIN ACCORDING TO LUNG LESIONS SCORED AT SLAUGHTERHOUSE USING THE CEVA LUNG PROGRAM METHODOLOGY.

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# **Background and Objectives**

Evaluating Enzootic pneumonia (Ep) and Actinobacillus pleuropneumoniae (Ap) like lung lesions scores at the slaughterhouse is an efficient method of estimating the incidence of Enzootic pneumonia and porcine pleuropneumonia. The aim of this study was to compare the annual incidence of Ep-like lesions and Ap-like lesions at slaughterhouse from January 2016 until October 2019.

# **Material and Methods**

A total of 3,356 batches and 501.342 lungs (550 in 2016; 1,084 in 2017; 1049 in 2018, and 676 until October 2019) from farms located around Spain were evaluated using the Ceva Lung Program (CLP) score methodology, assisting lung scoring for Ep-like lesions using modified Madec grid , and S.P.E.S system to evaluate Ap-like lesions. For each batch the following parameters were calculated:Percent of affected lungs with Ep-like lesionsPercent of affected surface out of all lungs.Percent of affected surface of pneumonic lungs.Percent of scarred lungsPercent of cranial pleurisy Percent of dorsocaudal pleurisyAPPI (Ap pleurisy index) Results were analysed by a non-parametric test Mann-Whitney.

# Results

Each year scored lungs showed statistically less percentage of affected lungs with Ep-like lesions and less percent of affected surface than the previous year (both p<0.001). The 2018 and 2019 the incidence of dorsocaudal pleurisy and the APPI increased significantly compared to 2016 and 2017 (both p<0.001).

#### **Discussion and Conclusion**

Results of percentage of affected lungs with Ep-like lesions and affected surface suggests that every year more effective programs for the control of Mycoplasma hyopneumoniae (Mh) are implemented.Incidence and severity of Ap-like lung lesions are significantly increasing in 2018-2019, as compared to previous years. The reduction of antimicrobial use over the last years is a likely contributing factor. More appropriate prophylactic measures, like vaccination, should be included in future Ap-control programs.

## EVALUATION OF THE PREVALENCE OF ACTINOBACILLUS PLEUROPNEUMONIAE SEROTYPES IN SPAIN DURING 2018-2019

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# **Background and Objectives**

At the present 18 different serotypes of Actinobacillus pleuropneumoniae (Ap) are described depending on the composition of the capsular polysaccharides. The distribution of Ap serotypes is dynamic and may change over time. Updating the most prevalent serotypes in different geographical areas, even within farms, is important in order to understand clinical Ap dynamics and attend optimal replacement stock control measures. The aim of this study was to describe the most prevalent serotypes of Ap in Spain.

# **Material and Methods**

The study was performed from January 2018 until November 2019 in different farms located in Spain. A total of 133 lungs were taken from 82 respiratory clinical outbreaks and Ap-like lesions. Lungs samples were collected and cultured from pigs at growing and fattening period (10 to 22 weeks of age) and when Ap was isolated, serotyping by PCR was performed.

#### Results

A total of 108 out of 125 (85.37%) bacterial isolates were Ap positive. Nine different Ap serotypes were detected. In decreasing order number, Ap positive samples and serotype prevalence were: Serotype 9/11 (33/108, 30.6%), serotype 2 (33/108, 21.3%), serotype 4 (19/108, 17.6%), serotype 13 (11/108, 10.2%), serotype 17 (10/108, 9.3%), serotype 6 (4/108, 3.7%); serotype 12 (4/108, 3.7%), serotype 5 (2/108, 1.9%) and serotype 8 (2/108, 1.8%).

# **Discussion and Conclusion**

Most of the Ap bacterial isolates results were positive, showing Ap-like lung lesions are a very useful tool to make an Ap presumptive diagnosis. Moreover, the most prevalent serotypes were 9/11, 2 and 4, being involved in 70% of Ap studied cases in Spain during 2018-2019. Comparing with other studies, these Ap prevalence results show an evolution of Ap serotypes in Spain over time.

# SEROTYPE DISTRIBUTION OF ACTINOBACILLUS PLEUROPNEUMONIAE FROM DIFFERENT GEOGRAPHICAL AREAS OF SPAIN DURING 2018-2019

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## **Background and Objectives**

Actinobacillus pleuropneumoniae (Ap) is the etiological agent of porcine pleuropneumonia and cause of important economic losses via acute, subacute and chronic manifestations. Nowadays, Ap is classified into 18 different serotypes (1 to 18) based on the composition of the capsular polysaccharide. Serotypes prevalence varies between regions and can change over time. The aim of this study was to know the serotype distribution in Spain during 2018–2019.

#### **Material and Methods**

This trial was conducted in several farms located in different geographical area in Spain between January 2018 and November 2019. A total of 116 Ap isolates were obtained from lung tissues of pigs which had died from acute respiratory disease during growing and finishing period. Serotyping was performed by PCR.The results from this study was categorized into, three geographical areas including the most important pig production regions in Spain: the Northwest (NW), the Northeast (NE) and the South (S).

#### Results

From 108 Ap isolates, 6 were from NW (5.56%), 73 (67.59%) from NE and 29 (26.85%) from S area. A total of 9 different serotypes were isolated in Spain. The number and percentage of each serotype depending on geographical area were: NW: serotype 9/11 (2, 33.3%), serotype 2 (2, 33.3%), serotype 13 (1, 16.7%), serotype 17 (1, 16.7%); NE: serotype 9/11 (31, 42.5%), serotype 2 (12, 16.4%), serotype 13 (10, 13.7%), serotype 4 (8, 10.9%), serotype 17(6, 8.2%), serotype 12 (4, 5.5%), serotype 5 (2, 2.7%); S: serotype 4 (11, 37.9%), serotype 2 (9, 31.0%), serotype 6 (4, 13.8%), serotype 17 (3, 10.3%) and serotype 8 (2, 6.9%).

# **Discussion and Conclusion**

The prevalence of Ap serotypes detected in this study contrasts with that of other European countries. Serotype 9/11 is predominant in the North of Spain while serotype 4 is more prevalent in the South.

FREQUENCY AND SEVERITY OF PLEUROPNEUMONIC LESIONS IN PIGS VACCINATED AGAINST A. PLEUROPNEUMONIAE WITH A COMMERCIAL TOXOID VACCINE, IN COMPARISON WITH NON-VACCINATED PIGS, IN A GREEK SWINE HERD

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## **Background and Objectives**

A. pleuropneumoniae (A.p) is distributed to swine farms worldwide, with great economic impact, both with its acute and chronic form. Vaccination is one of the measures applied to control the disease and its consequences. In this study, frequency and severity of pleuropneumonic lesions were compared between pigs vaccinated with a toxoid vaccine against A.p and non-vaccinated pigs, in a Greek herd.

#### **Material and Methods**

The study took place from May to September 2019 in a Greek farrow-to-finish farm with 700 sows. The only selection criterion for the farm was the high frequency of dorso-caudal pleurisy found in all lung scorings performed from September 2016 to May 2019. In total, 1168 animals participated in the study. Vaccination with Coglapix® was applied in 576 pigs (treatment group-TG) and 596 were left non-vaccinated (control group-CG). Animals were vaccinated on 70<sup>th</sup> and 90<sup>th</sup> day of life. Pleuropneumonic lesions were scored at slaughter by using the Ceva Lung Program®.

# Results

Lung scoring was performed in 140 lungs in TG and 143 in CG, belonging to 9 different batches. Frequency of lungs with pleuropneumonic lesions was 40.5% for CG and 24.3% for TG (P<0.05). The odds of having pleuropneumonic lesions, adjusted for age effect and clustered for batch, was almost 0.5 times lower (P<0.05) for the vaccinated animals compared to controls. Also, the percentage of lungs with severe SPES score, more than 2, was significantly lower (P<0.05) for TG compared to CG, with 2.9% and 14.7% severely affected lungs, respectively.

#### **Discussion and Conclusion**

In the present study, vaccination with Coglapix® significantly reduced the likelihood for pleuropneumonic lesions and the frequency of lungs with severe lesions, showing that vaccination can be beneficial not only for farms with acute Porcine Pleuropneumonia but also for endemic farms with low mortality due to A.p. (data not shown).

# LEPTOSPIROSIS PREVALENCE IN SWINE FARMS WITH FERTILITY PROBLEMS IN NORTHERN IRELAND IN 2018

J. Borobia-Belsue<sup>1</sup> <sup>1</sup>MOSSVET

# **Background and Objectives**

Leptopirosis is presumed to be the most widespread zoonosis worldwide. It is the cause of reproductive loss in swine breeding herds and has been reported in swine from all parts of the world.

There are very few recent studies of the prevalence of Leptospirosis in pigs worldwide. There are no seroprevalence studies in pigs in the U.K. or Ireland.

# Material and Methods

Eighteen pig farms with fertility problems in Northern Ireland were tested for antibodies against different Leptospira serovars in 2018 using the MAT method. The serovars tested were Australis (AUS), Bratislava (BRA), Autumnalis (AUT), Canicola (CAN), Grippotyphosa (GRI), Copenhageni (COP), Icterohaemorrhagiae (ICT), Pomona (POM), Altodouro (ALT), Hardjo (HAR), Saxkoebing (SAX) and, Tarassovi (TAR). Only, sows with fertility problems were tested.

# Results

Fifteen pig farms had samples with titres of ≥1:100 to one or more Leptospira serovars.There were a total of 137 blood samples taken. Forty four (32.1%) were positive to Leptospirosis and 93 samples (67.9%) were negative. The main serovars detected in these sows were BRA (81.8%) followed by AUS (68.2%), ICT (38.6%), COP (34.1%), POM (34.1%), HARD (27.3%), GRI (22.7%).

# **Discussion and Conclusion**

Leptospira is present in many pig units. The finding of this spirochete by MAT could potentially play a key role in the fertility performance in the herd. Other factors could also alter fertility performance in pig units. These may include infections pathogens (e.g. PRRS, SIV, parvovirus, erysipelas, endometritis, pyelonephritis), management practices, seasonal and/or nutritional imbalances.

Serovars from the serogroup Icterohaemorrhagiae were detected at high levels of frequency after the Australis serogroup. The finding of these serovars in pig units indicates a high level of exposure to rodents in the farm.

Serovar HAR infection is maintained by cattle worldwide, and where cattle and pigs come in close contact. The recognized maintenance host for serovar CAN is the dog.

# PREVALENCE OF VIRULENCE GENES FROM ESCHERICHIA COLI ISOLATES IN AUSTRIAN SWINE STOCKS - A RETROSPECTIVE STUDY (2016 - 2018)

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# **Background and Objectives**

Pathogenic Escherichia (E.) coli are amongst the main causative agents of neonatal diarrhea, post-weaning diarrhea and edema disease in piglets. Their pathogenicity and categorization is based on the occurrence of at least one adherence gene and one toxin gene. The present study aimed to investigate the prevalence of these virulence genes (VGs) of field strains isolated from piglets with diarrhea or the suspicion of edema disease.

# **Material and Methods**

Between 2016 and 2018, a total of 1744 E. coli isolates were obtained from pig stocks all over Austria. 694 strains were isolated from piglets with E. coli – associated diseases. These isolates were further investigated for the presence of 22 VGs by simplex- and multiplex-PCRs. The prevalence of isolates carrying at least one essential adherence gene and one toxin gene was assessed. Pearson Chi<sup>2</sup> test and Fisher Exact Test were used to assess potential significant differences between the occurrence of F4 and fimH as well as papC and cnfl.

# Results

In total, 55 isolates from suckling piglets (12%) and 43 isolates from weaned piglets (18%) could be classified as Enterotoxigenic E. coli due to their VG-profile. The combination of F4 and elt was the predominant one. Potential pathogenic VGs were more frequently detected in isolates showing hemolysis. AidA was detected in the majority of Edema-disease-E. coli. The detection rate of F4 was significantly increased in fimH negative isolates. cnfl was explicitly detected in isolates that were also positive for papC.

# **Discussion and Conclusion**

The frequent combination of F4 and elt was also observed in other European countries. Hemolysis still is a good indicator for pathogenicity, although its absence does not indicate the lack of VGs. AidA and cnfl might play a more significant role in the etiology of colibacillosis than expected. Regarding our data, an indirect correlation between the occurrence of fimH and F4 can be assumed.

# LUNG HOMOGENATE OPTIMIZATION FOR SUCCESSFUL MYCOPLASMA HYOPNEUMONIAE EXPOSURE IN GILTS DURING ACCLIMATION

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# **Background and Objectives**

One method for gilt acclimation to Mycoplasma hyopneumoniae entails the use of herd-specific lung homogenate for intentional exposure. However, characterization of lung homogenate has been minimally evaluated, which poses risks for exposure failure. The aim of this study was to evaluate the bacterial load in different anatomical lung sections by means of real-time PCR.

# Material and Methods

Nine lung donor pigs were selected from three different farms (n=3/farm). Donor pigs were selected based on Ct values from deep tracheal catheters (DTC) tested for M. hyopneumoniae by real-time PCR. Pigs were categorized into the following groups: low (Ct <24), medium (Ct 25-30) and high (Ct 31-39). Selected pigs were euthanized, and their lungs collected. Lung lesions were scored and bronchial swabs from each lung lobe obtained. Each lobe was dissected, and tissue was blended at a 70:30 proportion of tissue and Friis medium. Real-time PCR for M. hyopneumoniae detection was performed on bronchial swabs and lung lobe homogenates (5 replicates each).

# Results

Lesions were observed on all lungs. All lobe-specific bronchial swabs and 85% of lung homogenates were positive for M. hyopneumoniae, regardless of lung lesions. All lung lobe homogenates were positive from pigs with low Ct values, in contrast to pigs with medium and high Ct values, where there were both negative and positive lung lobe homogenates. Mean Ct values in lung lobe homogenates were significantly lower in low Ct pigs compared to medium and high Ct pigs, along with less variation of lung homogenate Ct values across all lobes for low Ct pigs.

# **Discussion and Conclusion**

Our results suggest the selection of donor pigs with low Ct values in DTC, as they correlated with greater and consistent bacterial load in the lung homogenate. Our results indicate that all lung lobes could be employed for lung homogenate preparation, regardless of the presence/absence of lesions.

# COMPARATIVE TRIAL OF TWO DIFFERENT VACCINATION PROTOCOLS WITH HYOGEN®, REGARDING RESPIRATORY HEALTH, IN A GREEK SWINE HERD

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# **Background and Objectives**

Mycoplasma hyopneumoniae is a primary pathogen of the porcine respiratory system. Vaccination, in different protocols, is widely used to control the pathogen. Double vaccination is still favoured in some countries, like Greece, compared to single vaccination. The objective of the present study was to compare the frequency and severity of EP-like lesions between a single and a double vaccination scheme with Hyogen®, in a Greek farm.

#### Material and Methods

The trial took place in a Greek farrow-to-finish farm with 1000 sows, from January 2018 to October 2019. During this period, half of the piglets were vaccinated once with Hyogen® at 21 days of life (group A) and half of them were, per farmer's request, vaccinated twice with Hyogen®(1ml/shot) at 7 and 21 days of life (group B). After weaning, animals from both groups were placed in the same pens in five different buildings. The farm's vet scored pigs' lungs for EP-like lesions by using Ceva Lung Program®.

#### Results

Lung scoring was performed in 525 lungs from group A and 546 from group B. The frequency of Ep-like lesions, adjusted for the possible effect of the different slaughter age and buildings, did not differ between the two groups (P>0.05), with 45% and 45.8% affected lungs in groups A and B, respectively. Similarly, there was non-significant difference (P>0.05) in the percentage of the affected parenchyma among all lungs between the two protocols, with 1.7% ( $\pm$ 0.4) and 1.8% ( $\pm$ 0.4) affected parenchyma in groups A and B, respectively.

#### **Discussion and Conclusion**

In this study, the effect of Hyogen® on pigs' lung health did not differ between the one and the two-shots protocol. This result indicates that a double vaccination scheme would not bring significant benefit for the efficacy of Hyogen®, compared to the single vaccination scheme, in the Greek herds.

## AN OUTBREAK OF CLOSTRIDIAL MYOSITIS AND CELLULITIS IN FINISHING PIGS

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## **Background and Objectives**

An acute disease outbreak involving unusual focal skin lesions and deaths in indoor finishing pigs was investigated.

## **Material and Methods**

Clinical and herd details were obtained and postmortem examinations were undertaken by the Animal and Plant Health Agency (APHA) on four dead pigs. Bacteriology, direct fluorescent antibody tests (FAT) and histopathology were performed. Methylene blue-stained blood smears were examined for Bacillus anthracis.

#### Results

The outbreak occurred in finishers on a well-managed indoor pig unit. Eleven of 160 pigs distributed across three straw-bedded pens within one shed were acutely affected over approximately 36 hours and six died. Affected pigs developed well-defined haemorrhagic patches on the skin of the ears, flanks, ventral abdomen or ventral neck, some with associated swelling, and became lethargic. Four dead pigs were submitted in which smears for Bacillus anthracis were negative. Subcutaneous tissues and musculature were emphysematous and oedematous. Some sections of muscle were emphysematous and dry with dark red-black discolouration. Clostridium septicum was detected by FAT in lesioned muscle from the most freshly dead pigs. Histopathology confirmed acute, necrotising myositis and cellulitis consistent with clostridial disease.

# **Discussion and Conclusion**

Clostridial myositis and cellulitis due to Cl. septicum was confirmed which is uncommon in pigs. As some lesions involved the neck region, anthrax was a possible differential, and was ruled out. Factors that may have precipitated this outbreak were investigated and none were identified. The group had not received recent injections, there was no visible associated skin trauma, their diet and water was the same as that provided to unaffected groups, and there was no ruminant contact. The affected group was promptly treated with amoxicillin in the water and paracetamol and no further pigs or batches developed signs suggesting that there was a transient unidentified point source of infection, such as ingested clostridial spores.

## DETECTION OF BRACHSYPIRA HAMPSONII IN A PIG HERD IN ENGLAND

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## **Background and Objectives**

Diarrhoeic faeces from finisher pigs were submitted to the Animal and Plant Health Agency for diagnostic testing.

# **Material and Methods**

Three pooled faeces were submitted alongside clinical and herd details. Culture and 23sRNA PCR for Brachyspira spp., Lawsonia intracellularis PCR and culture for Salmonella were undertaken. Brachyspira isolates were examined by biochemistry, 23sRNA PCR, cpn60UT-sequencing, whole genome sequencing (WGS), and minimum inhibitory concentrations (MICs) for six antibiotics by broth microdilution. A farm visit and epidemiological investigation with sampling was undertaken.

# Results

Affected finisher pigs on an indoor fattening unit had non-haemorrhagic diarrhoea and uneven growth without associated mortality. A strongly haemolytic Brachyspira was isolated from one faeces. Biochemistry matched that published for B. hampsonii. Although 23sRNA PCR identified the isolate as B. hyodysenteriae, WGS and cpn60UT-sequencing confirmed Brachyspira hampsonii. MICs were less than or equal to the lowest dilution tested for all antimicrobials. No pigs were imported or derived from imported pigs and pig buildings were accessible to wild birds. B. hampsonii was not detected in the next batch of pigs on the farm or in bird faeces. L. intracellularis and B. pilosicoli were also identified in the original samples, with Salmonella Bovismorbificans by enrichment culture.

# **Discussion and Conclusion**

This is the first confirmation of B. hampsonii in GB pigs since retrospective identification of a historic (pre-1994) isolate. The isolate had low MICs for each of the antimicrobials tested, being less than or equal to the lowest dilution tested. Its contribution to the clinical disease is uncertain; other enteropathogens were also identified. It was not detected in the subsequent batch of pigs on the farm and there were no obvious risk factors for its introduction apart from wild bird contact. This finding highlights the importance of using culture, PCR, and WGS to detect new and emerging Brachyspira species or atypical B. hyodysenteriae isolates.

# ANTIBODY RESPONSES TO MYCOPLASMA HYOPNEUMONIAE FROM WEANING UP TO MARKETING IN 14 GROUPS OF PIGS IN ONTARIO

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# **Background and Objectives**

One of the most prevalent respiratory diseases affecting swine farm productivity in Canada is enzootic pneumonia, a relatively mild disease in uncomplicated cases caused by Mycoplasma hyopneumoniae. The objective of this study was to investigate antibody responses to M. hyopneumoniae in pigs from weaning to the end of the finisher stage.

# Material and Methods

Fourteen groups of pigs from eight farrowing sources (n=618) were followed from birth to the end of the finisher stage. Blood samples were collected at weaning and at the end of the nursery, grower, and finisher stages and sera were analyzed by ELISA for the presence of M. hyopneumoniae antibodies. A multilevel mixed-effects regression method was used to analyze the data.

#### Results

In 7 groups, at least 25% of pigs were seropositive for M. hyopneumoniae (high seropositivity groups). Across the high seropositivity groups, 56.0% of pigs at weaning, 41.1% at the end of nursery, 40.6% at the end of grower, and 63.4% at the end of finisher were seropositive for M. hyopneumoniae. In the high seropositivity groups, pigs born between October and January were more likely to be seropositive at the end of the grower (p<0.001) and finisher (p=0.001) stages compared to weaning, while pigs born between May and August were more likely to be seropositive at the end of the nursery (p<0.001) and grower (p=0.001) stages compared to weaning.

#### **Discussion and Conclusion**

These findings suggest that antibody responses to M. hyopneumoniae may vary between different stages of production. In addition, the high proportion of seropositive pigs at weaning indicates the presence of maternal antibodies that decline towards the end of nursery. Although this study was unable to differentiate between antibody responses to natural infections and vaccination, it may help to encourage vaccination in post-weaning pigs, a time in which the interaction between maternal antibodies and vaccine antigens is minimized.

PREVALENCE AND BACTERIAL LOADS OF ESCHERICHIA COLI, LAWSONIA INTRACELLULARIS AND BRACHYSPIRA PILOSICOLI IN 24 DANISH HERDS

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# **Background and Objectives**

During the last 10-15 years, most research concerning intestinal infections in Denmark has focused on the nursery period demonstrating a high prevalence of mixed-pathogen infections. This study's objective was to determine the current prevalence and bacterial loads of E. coli (EC) F4+F18, L. intracellularis (LI) and B. pilosicoli (BP) throughout the nursery-fattening period in herds with a history of diarrhea.

# Material and Methods

Appointed by the herds' veterinarians, 24 herds struggling with diarrhea in the nursery and/or fattening period were included. In each herd, one sock sample in each of the age groups 10-25 kg, 25-40 kg and 40-100 kg were collected minimum 3 weeks after last antimicrobial (including ZnO) batch treatment. Samples were analyzed by qPCR at DTU National Veterinary Institute for EC F4+F18, LI and BP.

#### Results

Across age groups, the herd prevalence of EC-F4, EC-F18, LI and BP was 13%, 33%, 96% and 79%, respectively. For LI, the prevalence was highest at 10-25 kg (75%) and decreased by increasing age (71% and 46%). The opposite was the case for BP (46%, 67% and 79%). Of qPCR-positive samples, the median bacterial loads were 5.79 log(10), 5.83 log(10), 5.39 log(10) and 5.16 log(10) for EC-F4, EC-F18, LI and BP, respectively.

## **Discussion and Conclusion**

Due to the relatively widespread usage of ZnO in Denmark in the sampling period (2018), the pigs sampled in the youngest age group were mostly >15 kg, which is probably the reason for the relatively low occurrence of EC. In concurrence with expectations and previous findings, the prevalence of LI was highest during the nursery period and mixed-pathogen infections, primarily with BP, occurred very frequently.

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# EVALUATION OF THE PREVALENCE AND SEVERITY OF ENZOOTIC PNEUMONIA AND PLEUROPNEUMONIA ON CZECH PIG FARMS BASED ON LUNG LESION SCORING IN 2019

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# **Background and Objectives**

Monitoring of respiratory disease by lung scoring is beneficial to assess the farm health status. 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 2019 compared to the previous periods 2018 and 2015-2017

# Material and Methods

The survey was conducted on conventional pig farms excluding those with the M.hyo and A.p. SPF status and those recently repopulated due to pleuropneumonia. A total of 3819 lungs in 34 batches of slaughtered pigs were scored using the CLP method. Bronchopneumonia lesions(BP), cranio-ventral pleurisy(CP) and scarring were recorded and scored. Dorsocaudal pleurisy(DP) suggestive for previous pleuropneumonia was scored to describe A.p-like lesions. Data were compared to the periods of 2018 and 2015-2017.

# Results

The prevalence of 16,67% of BP was found, compared to 33,85% (2018) and 37,7% (2015-2017) previously. The area of affected surface of lung parenchyma in pneumonic lungs reached 5,34% vs 4,19% and 5,4%. Cranio-ventral pleurisy was recorded in 2,64% (vs 6,31% and 12,9% previously) of total number of lungs. As for pleuropneumonia – 25,69% (vs 10% and 11,1% previously) of lungs were affected by A.p-like lesions with the APPI index 0,59. All values are expressed as median.

# **Discussion and Conclusion**

EP-like lesions have a decreasing tendency compared to previous years. That indicates efficient preventive measures were implemented in the Czech farms. Lesions characteristic for A.p infections were more prevalent than previous years. This result was mostly because some farms (which were A.p positive) were repopulated, and therefore they were not scored. Both types of respiratory diseases deserve high attention to be controlled.

# PREVALENCE EVALUATION OF PORCINE ENZOOTIC PNEUMONIA AND PORCINE PLEUROPNEUMONIA IN ARGENTINA USING THE CEVA LUNG PROGRAM

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## **Background and Objectives**

Slaughterhouse lung evaluations have been widely used to contribute as a monitoring tool for important swine pathogens. The aim of this study was to estimate the prevalence of lesions suggestive of Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae infections in the main pig-producing provinces in Argentina.

#### Material and Methods

The study was conducted throughout the years 2018 and 2019, in which 20.767 lungs from 200 commercial pig batches were scored at the slaughterhouse for EP-like lesions and A.p.-like lesions using Ceva Lung Program scoring methodology (CLP). These batches came from the seven main pork-producing provinces in Argentina. Bronchopneumonia lesions, cranio-ventral 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 mean prevalence of bronchopneumonic lesions was 53,7% with the variation between 42%-63,1% in different provinces. The mean affected surface calculated in sick lungs was 8,3% with the range between 5,6%-11%. The percentage of dorso-caudal pleurisy was remarkably lower with the mean of 8,4% and the range 1,7%-14%. The average APPI was 0,23.

# **Discussion and Conclusion**

The results of monitoring performed during 2018 and 2019 showed a high prevalence of EP-like and A.p.-like lesions, as also observed in similar studies in South America and Europe. These assessments may contribute to estimating the prevalence of the agents involved. As huge differences were observed between regions, further studies are needed to find out the key factors that are influencing these differences and the best control measures that can be taken for these respiratory agents. The CLP methodology is a tool that has been widely disseminated globally to evaluate the effectiveness of control measures of these important swine pathogens.

# OCCURRENCE OF HAEMOLYTIC PATHOGENIC E. COLI STRAINS IN YOUNG DANISH PIGS

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## **Background and Objectives**

The use of medicinal zinc is being phase out by June 2022. In Denmark, classical post weaning diarrhea (PWD) has been handled with medicinal zinc, which is why there has been little focus on diagnostics around weaning. The latest Danish mapping of E. coli strains in relation to PWD is 20 years old. To be able to prevent PWD, it is important to know which E. coli strains are most prevalent in young pigs. The objective of this study was to create an overview of haemolytic E. coli strains found in clinical cases of increased mortality and/or diarrhoea from Danish piglets and nursery pigs.

# Material and Methods

In 2019 from SEGES, Laboratory of Pig Diseases, E. coli strains with haemolytic activity were collected from submitted materials of pig carcasses and faecal samples. The age group from where the strains were sampled was from birth until 12 weeks of age. The selected stains were PCR tested for the presence of the most common fimbria and toxins in pigs: F4, F18, LT, STa, STb and Stx2e.

#### Results

A total of 253 E. coli strains with haemolytic activity was collected and 66% of the strains was classified as pathogenic (fimbriae and toxin PCR positive). The most prevalent fimbriae in the suckling period was F4, whereas F4 and F18 was equally prevalent in weaned pigs. The most prevalent virotype in piglets and nursery pigs was F4+STb+LT which accounted for 43% of all the collected pathogenic isolates.

# **Discussion and Conclusion**

The results from this study show that F4+STb+LT is still the most prevent haemolytic E. coli virotype in young Danish pigs which corresponds well to previous European and Danish studies. This study only evaluated haemolytic strains. Other non haemolytic ETEC virotypes, frequently observed in suckling pigs, was not included in this study.

# IMPACT OF ORAL VACCINATION WITH ENTERISOL ILEITIS® ON GROWTH PARAMETERS AND ANTIBIOTIC CONSUMPTION IN ITALIAN FATTENING FARMS

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# **Background and Objectives**

Subclinical ileitis caused by Lawsonia intracellularis is a common grow-finish enteric disease. It results in reduction of feed conversion and weight gain, often without clinical signs. The objective of this study was to assess the impact of vaccination with Enterisol Ileitis® (Boehringer Ingelheim) on production performance and consumption of antibiotics in Italian fattening units.

#### Material and Methods

The study was conducted in six fattening farms (Italian heavy pigs), AI/AO system, with the same source of weaners, feed and health status. The management practices were comparable. A before/after study was performed on pigs produced in 2018 (6 batches) vs 2019 (6 batches). The vaccination has been applied in 2019. Enterisol Ileitis® was administrated orally into the liquid feed using Thiosulfate Blue (Boehringer Ingelheim) as stabilizer, at the beginning of fattening period (one week after arrival). A total of 16.180 vaccinated and 16.153 non-vaccinated pigs were included in the study. The parameters recorded for each farm were: Weight in/out (Kg), Mortality rate (%), Average daily weight gain (ADWG, kg/d), Feed conversion rate (FCR, Kg) and days of antibiotic treatment for gastro-intestinal diseases. Data were analyzed using Minitab 18.

#### Results

Vaccinated animals shown a reduction of 0,09 kg in FCR (3,23 Kg vs 3,32 Kg; p=0,017; Paired T-Test) and a reduction of mortality (2,82% vs 3,93%; p<0,001; Chi-Square Test). Average consumption of antibiotics was significantly lower in vaccinated pigs (4,67 days vs 23,50 days p=0,005; Paired T-Test).

# **Discussion and Conclusion**

In this field experience, has been shown that vaccination has improved performances reducing FCR and mortality. Oral vaccination into liquid feed as preventive alternative may contribute to reduce the use of antibiotics for gastro-intestinal diseases in the Italian heavy pig production and improves economic results.

# COMPARISON OF PATHOLOGICAL AND SEROLOGICAL PARAMETERS OF PIGS IMMUNIZED WITH TWO DIFFERENT MYCOPLASMA HYOPNEUMONIAE COMMERCIAL VACCINE PRODUCTS

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# **Background and Objectives**

This study aimed to assess lesions and antibody dynamics in pigs immunized with two different commercial vaccine products against Mycoplasma hyopneumoniae (Mhyo).

#### Material and Methods

Sixty 2 to 3 week-old piglets Mhyo seronegative were distributed into 4 groups of 15 animals. At 4 weeks of age animals were intramuscularly injected with 1 mL of Vaccine A, 2 mL of Hyogen® or 2 mL of PBS (Non-vaccinated group [NV] and Non-vaccinated-Non-challenged [NV-NC] groups). Four weeks post vaccination (WPV), pigs were endotracheally inoculated with 5 mL of a  $10^7/10^8$  PCR<sub>50</sub>/mL Mhyo isolate (Vaccine A, Hyogen® and NV-C groups) or with PBS (NV-NC) in two consecutive days. Animals were necropsied at 4 weeks post-challenge (WPC). Parameters studied included weekly determination of Mhyo antibodies and macro and microscopic Mhyo-compatible lung lesions.

#### Results

Animals from the NV-NC group remained seronegative through the study. Hyogen® group had significantly higher number of animals seropositive to Mhyo than the rest of the groups at 3 and 4 (challenge day) WPV. From 1 WPC onwards, both vaccinated groups had significantly higher percentage of seropositive pigs than non-vaccinated groups. The NV-NC and Hyogen® groups had a numerically lower percentage of animals (6.7% and 63.6%, respectively) showing macro and microscopic Mhyo-induced lung lesions compared to Vaccine A (100%) and NV (93.30%) groups. The mean macroscopic score was significantly lower for the NV-NC and Hyogen® groups (0.03±0.1 and 2.96±5.16, respectively) when compared to Vaccine A (11.51±7.28) and NV-C (11.38±7.26) groups.

# **Discussion and Conclusion**

Vaccination with Hyogen® produced significantly higher humoral immune response, numerically lower percentage of animals showing macro and microscopic lung lesions and significantly lower macroscopic scores than Vaccine A and NV-C groups.

# PHYTOGENIC FEED ADDITIVES REDUCED MEDICATION REQUIRED FOR TREATMENT OF POST-WEANING DIARRHEA AND IMPROVE FECAL CONSISTENCY

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# **Background and Objectives**

Weaning is a stressful period in the life of pigs with increased susceptibility to environmental and pathogenic challenges. These challenges can cause severe decrease in growth performance and increase in the need for medications, causing overall considerable economic losses. Phytogenic feed additives (PFA) have the potential to influence palatability of feed and support health and well-being of animals. This study was conducted to investigate the potential of PFA in reducing the incidence of post-weaning diarrhea (PWD) in weaned piglets.

#### Material and Methods

A total of 800 piglets from 25 to 66 days of age were distributed to 32 pens (25 piglets each, 16 pens per treatment). The animals were fed a corn, soybean meal, barley and wheat-based diet either unsupplemented (NC) or with a PFA at 1000 mg/kg feed (Fresta® Protect, Delacon Biotechnik GmbH, Austria). Piglets were housed in a commercial pig breeding farm with recurrent Escherichia coli diagnosed PWD outbreaks. Responses were demonstrated on disease, fecal scoring, antibiotic treatments, and mortality.

# Results

Bacteriological tests showed that toxin-producing E. coli were detected in more than 68% of the randomly collected faecal samples submitted to a commercial microbiological laboratory. Cumulative incidence rate of PWD was reduced by 30.5% (P<0.001) and fecal scores were improved (P=0.006) in comparison to piglets fed corresponding diets without phytogenic addition. This is reflected also in antibiotic treatments required, with 45.1% reduction in the PFA group (n=45) compared to the NC (n=82), while no differences were observed for other diseases and mortality.

#### **Discussion and Conclusion**

The results suggest that feeding diets supplemented with this PFA at the recommended dosage could provide significant benefits on controlling PWD outbreaks in piglets.

# EFFICACY OF ORAL ADMINISTRATION OF A PHYTOGENIC FEED ADDITIVE ON POST-WEANING DIARRHEA IN PIGLETS HOUSED UNDER COMMERCIAL CONDITIONS

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# **Background and Objectives**

Post-weaning diarrhea (PWD) is an important enteric disease. It usually occurs shortly after weaning and twenty to fifty per cent of all weaned pigs may be affected. The aim of the study is to evaluate the efficacy of a phytogenic feed additive (PFA) in piglets during a 6-wk period (from d 25 to d 66 of age) housed in a commercial pig breeding farm with a high incidence of Escherichia coli associated post weaning diarrhea (PWD).

# Material and Methods

Post weaning barrows and gilts (Danbreed x Pietrain) were allocated at random to 20 identical pens with five weaners per pen (10 replicate pens per treatment). The 42-d feeding period consisted of a two-phase feeding with diets based on corn, soybean meal, barley and wheat supplemented either with or without a PFA. Body weight, body weight gain, feed intake, and feed conversion ratio were measured per pen per phase as well as health parameters like diseased piglets, antibiotic treatments, mortality and fecal score.

#### Results

The recorded overall body weight gain in the present study was approximately 10% lower than that recorded in pig farms without remarkable occurrence of E. coli associated post-weaning diarrhea outbreaks. The total PWD incidence rate in piglets fed diets without phytogenic addition amounted to 34.0% whereby 82.4% of the diseased piglets were treated with antibiotics as an injectable. Piglets fed diets containing the PFA showed 30% less incidence of PWD, required 29% less antibiotic treatments and mortality rate decreased by 2%. Dietary supplementation with the PFA during the 6-wk post-weaning period numerically improved cumulative body weight gain by 4.2% in comparison to the control group.

# **Discussion and Conclusion**

These findings suggest that the applied PFA could provide benefits on PWD and performance during a 6-wk post weaning period.

# MANAGING ACTINOBACILLUS PLEUROPNEUMONIA OUTBREAKS IN DIFFERENT ENVIRONMENT CONDITIONS AND WITH DIFFERENT PREVENTION STRATEGIES ON A COMMERCIAL HUNGARIAN FARM – A CASE STUDY

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# **Background and Objectives**

Lung lesions and pleuritis caused by Actinobacillus pleuropneumoniae (APP) are associated with economic losses in the swine industry. In the presence of predisposing factors losses are even higher. The objective of this field study was to find the best APP prevention strategy on a commercial farrow-to-finish farm.

# Material and Methods

In the study two finisher subunits (Farm 1-2) of the same farm were involved: Farm1 - two traditional buildings (old technology and ventilation); Farm2 - 6 modern, renovated buildings with 4 types of ventilation system. The farm had a known history of pleuropneumonia and severe APP outbreaks in finishing pigs. Based on the different types of the facilities we tried to figure out an optimized, cost-efficient treatment strategy, improving the ventilation, hygiene, antibiotic use (AU) and vaccination protocols with two different commercial vaccines (Vaccine 1-2), based on laboratory and slaughterhouse examinations and environmental factor audits.

# Results

We had significantly higher health status, average daily gain (ADG) (+40g) and reduced AU in Farm1, but only when the 2 buildings were populated in the same time. When the stocking was shifted in time, mortality increased (+64%) and ADG decreased (-17g). On Farm1 APP vaccination had no added value. According to the slaughterhouse lung scoring system the APP Index (APPI) as the superscript of pleuropneumonia was significantly higher on Farm2 (Farm1: 0.13-0.67; Farm2: 1.84-2.49). On Farm 2 during application of Vaccine1 APPI was reduced (0.84), but the health status did not change. The best results were obtained with the vaccine2 (APPI: 0.17).

# **Discussion and Conclusion**

The strong effect of environmental factors combined with a farm specific prevention strategy can help to improve the farm health status and reduce the economic losses and AU. Understanding the epidemiology and economic impact of a disease is essential to assist producers in justifying implementation of disease control methods.

# IMPORTANCE OF CONTINUOUS APPLICATION OF A NOVEL ZINC CHELATE FOR THE TREATMENT OF CLINICAL SWINE DYSENTERY TO REDUCE PATHOGEN EXCRETION

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# **Background and Objectives**

Brachyspira hyodysenteriae is the primary cause of swine dysentery and primarily affects pigs during the grow/finishing stage. A novel non-antibiotic zinc chelate demonstrated positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and B. hyodysenteriae excretion after a 6-day oral treatment. This study compared pathogen excretion (analysed by PCR) between treatment protocols according to SPC (24/24h, 6-day treatment) and suboptimal (discontinuous) treatment as occasionally performed under field conditions (due to dosing limitations) with a zinc chelate (IntraDysovinol® 499 mg/ml; Elanco) in the treatment of clinical swine dysentery.

# **Material and Methods**

Several fattening units from one veterinary practices were targeted for the field study. Product application was performed according to SPC (continuous; 24/24h; n = 5 farms) or off-label (discontinuous, 12/24h; due to alternative dosing by farmer; n = 11 farms) for a 6-day treatment period. Following the end of treatment, fecal samples were collected to check for presence of B. hyodysenteriae by real-time PCR based on the nox gene. Results were reported as positive or negative.

# Results

In the fatteners treated according to SPC specifications 1 out of 14 fecal samples (7.1%) was positive for B. hyodysenteriae, whereas in the fatteners treated off-label, due to miscommunications with farmers, 13 out of 36 samples (26.1%) were positive for B. hyodysenteriae.

# **Discussion and Conclusion**

Zinc chelate is a novel non-antibiotic treatment for swine dysentery resulting in a decreased excretion of B. hyodysenteriae following treatment according to SPC specifications (continuous; 24/24h; 6-day treatment period). Discontinuous application (off-label and not according to the SPC) does not results in the same reduction of B. hyodysenteriae excretion following a 6-day oral treatment. In conclusion, correct application according to SPC of the novel zinc chelate for treatment of swine dysentery is crucial to obtain clear reduction in B. hyodysenteriae excretion.

## STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS, AN EMERGING PIG PATHOGEN?

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# **Background and Objectives**

In 2019 two sow herds (approximately 650, herd A, and 1600 sows, herd B) in the Netherlands were confronted with sudden onset of high mortality in gestating sows. Clinical signs were anorexia, pyrexia, ataxia, paresis, and mortality. Herd A lost 27 sows (5.4%) and herd B lost 70 sows (4.4%) within five weeks.

#### **Material and Methods**

Sows were loose housed in groups (200-500 sows per group) with electronic feeding stations (EFS).

#### Results

Post mortem examination of two sows from herd A showed meningitis, pulmonary edema, spleen atrophy, spleen torsion and pleuritis. Streptococcus equi subsp. zooepidemicus was cultured from brain, liver, spleen and pleural cavity. Post mortem examination of six sows from herd B revealed conjunctivitis, sinusitis, meningitis, pulmonary edema and sepsis. Streptococcus equi subsp. zooepidemicus was cultured from brain, sinuses, spleen and liver. WGS of the strain from herd B showed resistance genes for tetracycline, erythromycin, aminoglycosides and streptomycin resistance, while MIC showed resistance to macrolides, lincomycin/clindamycin, tetracycline, aminoglycosides and trimethoprim-sulfonamides.

#### **Discussion and Conclusion**

Streptococcus equi subsp. zooepidemicus is an opportunistic pathogen in different species including humans. It has been suggested that the bacterium is a normal inhabitant of tonsils in swine. Infection pathogenesis in both cases is not clear. In herd A, spleen torsion and spleen atrophy indicated the presence of gastric dilation problems. The outbreak stopped after restoring correct acidification of liquid feed fed in EFS. In herd B, no obvious primary cause was found. In both herds, clinically affected sows were successfully treated with antibiotics. An autogenous vaccine against Streptococcus equi subsp. zooepidemicus was applied in herd B. High-mortality outbreaks have been reported in China, USA, New Zealand and Canada. In contrast to these outbreaks, the herd B strain was not STI94. Whole-genome comparison showed 97.2% similarity between Canadian and Dutch strains (ANIscore).

# MODULATION OF MYCOPLASMA HYOPNEUMONIAE INFECTION USING MULTIPLE VACCINATIONS IN GILTS

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# **Background and Objectives**

Mycoplasma hyopneumoniae (Mhp) infections result in significant respiratory challenges in the industry. Vaccination for Mhp is commonly utilized, as reduction in bacterial loads and clinical severity in vaccinated pigs have been shown. The effect of Mhp vaccinination on the infectious pressure and transmission across different populations has been minimally investigated. The aim of this pilot study was to evaluate the effect of multiple vaccinations on Mhp infection and transmission in seeder and naïve gilt populations.

#### Material and Methods

Naïve gilts (n=36) were allocated into 4 experimental groups: 1) NVI (Non-vaccinated, infected); 2) VI (Vaccinated, infected); 3) NVN (Non-vaccinated, naïve); 4) VN (Vaccinated, naïve). At 5, 7, and 9 weeks of age, VI and VN gilts were vaccinated with a commercial bacterin for a total of 3 doses. At 11 weeks of age, VI and NVI gilts were inoculated with Mhp. At 28 days post-infection (dpi), VI and NVI gilts were relocated and housed with either a NVN or VN gilt (1:1 ratio) for 14 days. Blood samples, deep tracheal catheters, bronchial swabs, and lung lesions were collected/evaluated for Mhp infection. Differences in bacterial load and lung lesions were evaluated using a Mann-Whittney t-test. Force of infection (🛛) and incidence rate were estimated.

## Results

At 28 dpi, 100% and 80% of NVI and VI gilts were PCR positive and all vaccinated gilts were seropositive. Lower Mhp bacterial loads were identified in VI versus NVI gilts (p<0.05). Lung lesions were numerically lower in VI versus NVI gilts (p=0.78). No transmission events occurred between VI and VN gilts, compared to other groups (i.e. 1-2 transmission events/group). Numerical differences in 🛛 and incidence rate were observed among groups.

# **Discussion and Conclusion**

Results from this investigation provided insight in the potential impact of multiple vaccination on Mhp infection modulation. Further research encompassing larger populations is necessary to validate findings.

## ENTEROTOXIGENIC ESCHERICHIA COLI ISOLATED FROM OUTBREAKS OF POST WEANING ENTERIC COLIBACILLOSIS

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# **Background and Objectives**

Post weaning diarrhoea (PWD) due to enterotoxigenic Escherichia coli (ETEC) is one of the most important diseases in swine, resulting in significant economic losses. Knowledge about the diagnostic approach and the epidemiology are of fundamental importance to tackle the disease.

# Material and Methods

A total of 62 outbreaks of post weaning enteric colibacillosis have been observed in 2019 in as many Italian pig farms. The diagnosis of enteric colibacillosis has been confirmed by bacteriological examination on samples of luminal content or rectal swabs, by inoculation onto blood agar and Gassner agar. The semi-quantitative evaluation of pathogenic E.coli, grown in culture, was considered as diagnostic criteria. Strains have been genotyped by multiplex PCR for the detection of genes encoding for toxins (STa, STb, LT and Stx2e) and fimbriae (F4, F5, F6, F18, F41).

# Results

All the pathogenic E.coli strains (68) isolated were haemolytics and the prevalence of fimbriae and toxins was: F4 (42.6 %), F18 (57.4 %), STa (95.6%), STb (55.9%), LT (22%) and Stx2e (2.9%). The most common virotype detected was F18 STa STb (32.4%), followed by F4 STa STb (25%). Mixed infections (ETEC F4 and F18) were observed in 9.7% of the cases.

# **Discussion and Conclusion**

A study published in 2016 by Luppi et al. reported that F4 was the fimbrial type most commonly detected in ETEC isolated from cases of PWD in The Netherlands, France, Germany and Italy. Similarly, a higher prevalence of F4 than F18 fimbriae has been reported before in other European countries including Denmark, Slovakia and Czech Republic. The results reported confirm the importance of PWD due to ETEC in Italy and that F18-ETEC is the main fimbrial type, followed by F4-ETEC. The information presented in this study highlighted the variability of ETEC virotypes prevalence in enteric colibacillosis outbreaks over time.

# COMPARATIVE ANALYSIS OF ANTIBODY DEVELOPMENT AND EFFECTIVITY AFTER HYPER-IMMUNIZATION OF SWINE WITH DIFFERENT MYCOPLASMA HYOPNEUMONIAE VACCINES

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# **Background and Objectives**

Enzootic pneumonia caused by Mycoplasma (M.) hyopneumoniae is a respiratory disease causing economic losses worldwide. Available vaccines reduce the clinical impact of the disease but do not prevent the colonization of the lungs of infected pigs. So far it was not possible to verify supposed differences regarding the efficacy of different vaccines due to the impact of environmental confounders. Therefor the aim of this study was to compare the antibody development and effectivity after multifold vaccination of swine with different M. hyopneumoniae vaccines under controlled conditions.

# Material and Methods

Four commercial available vaccines covering M. hyopneumoniae strains J, 11 and P-5722-3 (NL 1042) were chosen for immunization. Eight M.-hyopneumoniae-free pigs, two for each vaccine, were immunized four times. The presence of specific antibodies in serum and BALF was evaluated by western immunoblot. Quantification of specific IgG and IgA was performed by ELISA. Impact of hyper-immune sera and BALF on in vitro growth of M. hyopneumoniae was tested in ML media.

# Results

ELISA S/P ratios in sera ranged from 1.58 to 1.86 depicting clear positive test results for all tested vaccines. Growth of M. hyopneumoniae in ML media was significantly inhibited by the addition of BALF and serum samples. Although this effect was seen in all vaccinated animals there were significant differences between the used vaccines. For one vaccine there was also a significant difference between the two vaccinated pigs.

# **Discussion and Conclusion**

Although all M. hyopneumoniae vaccines led to seroconversion, clear differences in growth retardation by the added antibodies were depicted for the different tested vaccines but also a pig-dependent effect was shown for one vaccine. Therefor it can be concluded that, if seroconversion occurs, with the currently available methods it will not be possible to evaluate the real degree of the protective effect of the vaccination under field conditions.
# EVALUATION OF STOMACH LESIONS IN HUNTED WILD BOAR IN THE NETHERLANDS, FALL 2019

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# **Background and Objectives**

In the Netherlands the prevalence of stomach lesions in sows and finisher pigs at slaughter has been evaluated in different years; no clear change in prevalence of the different types of lesions found in the pars oesphagea (hyperkeratosis, erosions and ulcers) was observed. From a welfare point of view it's questioned to what extent stomach lesions might be encountered in pigs living in their natural habitat. Wild boar are considered as the best model for pigs in their natural habitat. Therefore the aim of this project was to evaluate the prevelance/severity of stomach lesions in wild boar in the Netherlands.

#### **Material and Methods**

100 buckets were distributed among hunters in the provinces Noord-Brabant, Limburg and Gelderland in the Netherlands in September 2019. From each wild boar that was shot, general data (geographical area, age, body condition score, sex) and the stomach (including content) were collected. Buckets with stomachs were submitted to the pathology department of GD. Stomachs were weighed, opened and both stomach contents (type of contents and consistency (firm, pasty, watery)) and lining (pars oesophagaea, 5-point scoring system) were evaluated by one person. Data were analysed descriptively.

#### Results

In totaal 89 stomachs were collected from September-November 2019. 84 stomaches scored '0' (no lesions), 4 scored '1' (slight hyperkeratosis), 1 stomach scored '2' (clear hyperkeratosis; the highest score observed). In 75 stomachs a 'firm' content was found, 12 scored 'pasty', 2 scored 'watery'.

# **Discussion and Conclusion**

In stomachs of wild boar, shot in a nutrient-rich season, hardly any lesions are found. This might be related to feeding behaviour (rooting for several hours) and type of feed (fiber-rich). As it is known that mild changes such as hyperkeratosis may diseappear within 6 weeks, it is advised to repeat the research in a season with a limited feed availability to confirm the low prevalence of stomach lesions.

# LOCAL PAIN DURING PIGLET CASTRATION - INVESTIGATION ON THE DISTRIBUTION OF LOCAL ANESTHETICS FROM DIFFERENT INJECTION SITES

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# **Background and Objectives**

As of 2021, in Germany surgical castration of male piglets aged less than eight days is no longer allowed without anesthesia, i.e. the total elimination of pain during the surgery is required. Local anesthetics are used in different countries for pain relief during castration, but studies show a varying analgesic effect. To eliminate pain, local anesthetics must block sensory innervation of the cremaster muscle, the tunica vaginalis and the scrotum. As part of a study assessing the efficacy of local anesthetics applied before castration, drug distribution from different injection sites was visualized by computed tomography (CT).

#### Material and Methods

Ten piglets aged less than eight days were anesthetized with isoflurane and fixed in dorsal recumbency. A mixture of an iodinated contrast agent and lidocaine 20 mg/ml (diluted 1:8, volume: 0.3 ml/location) was injected in two piglets, each: into the testicles (IT), into the spermatic cord (IF), subcutaneously into the scrotum (SC), and combined IT & SC and IF & SC. CT scans were performed five times every two minutes.

#### Results

CT images demonstrate the dispersal of the diluted contrast medium. Within two minutes after injection into the testicles, it distributed into the spermatic cord. Injected subcutaneously into the scrotum, the contrast medium spread around the testis. After administration into the spermatic cord, the solution's dispersal was limited to the region of the puncture site. Over the course of time no further spread of the contrast medium was observed.

# **Discussion and Conclusion**

Combined administration into the testis and subcutaneously into the scrotum resulted in a widespread distribution of the diluted contrast medium beneath the scrotal skin, in the testis and in the spermatic cord. Despite the fast distribution into the tissues within two minutes, the onset of anesthesia depends on the local anesthetic. Whether pain elimination can be reached must be proven in further investigations.

APPLICATION OF HEMICELL HT<sup>™</sup> – A I<sup>™</sup> – RESTORES POST-WEANED PIGLET PERFORMANCE IN THE PRESENCE OF CHALLENGING PROTEIN SOURCES

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# **Background and Objectives**

Ø-Mannans are strongly anti-nutritive polysaccharide fibres found in most vegetable feed ingredients. Estimated content of soluble Ø-mannans in common nursery diets is only 0.15-0.35%, and in vitro studies have demonstrated that as little as 0.05% soluble Ø-mannan in feed can elicit a strong innate immune response. This innate response is referred to as a feed induced immune response (FIIR). Hemicell<sup>™</sup> HT (Elanco) is a Ø-mannanase enzyme for animal feeds breaking down Ø-mannans, thereby preventing economic losses from this wasteful immune response to Ø-mannans.

#### Material and Methods

A seven weeks feeding trial was conducted with 896 pigs in two rotations of 448 piglets in 32 pens of 14 pigs. Standard three-phase control diets were compared to similar iso-nutritive diets with 300 g/tonne of Hemicell<sup>™</sup> except for following changes: Phase-1 (weeks 1-2): 1.14% potato protein concentrate and 1.00% Forcital (extruded soya product), was replaced with soybean meal. Phase-2 (weeks 3-4): 0.46% potato protein concentrate and 0.68% Forcital was replaced with soybean meal. Phase-3 (weeks 5-7): Hemicell<sup>™</sup> was formulated to replace 63 kcal/kg NE. Standard production and health data were collected. Data were analysed using JMP 14.0 statistical program – one-tail t-test.

#### Results

In phase-1, pigs on Control feed gained 8 g/day more than on Hemicell<sup>™</sup> (P<0.05) and both feed intake and FCR were similar (P>0.05). No significant (P>0.05) performance differences were observed in phase-2, phase-3 or overall. Mortality was numerically (-0.90 %) lower on Hemicell<sup>™</sup>. This correlated with a significant reduction in antimicrobial use (P<0.01).

# **Discussion and Conclusion**

Degradation №-mannans in nursery diets by Hemicell<sup>™</sup> made it possible to reduce feed cost by replacing part of the expensive proteins and reducing dietary energy by 63 kcal/kg NE in phase-3 without reducing nursery pig performance or health.

BEHAVIOURAL RESPONSES OF 26-DAY-OLD PIGLETS TO VACCINATION: COMPARISON OF IM AND ID INJECTION ROUTES

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# **Background and Objectives**

Animal welfare issues are seen as fundamental for the general public. Accordingly, attention to reducing pain in routine procedures in farms has grown in the recent years. The aim of this study was to **evaluate piglet behavioral response during vaccine administration, by comparing intramuscular injection with needle (IM) and intradermal injection without needle (ID).** 

#### **Material and Methods**

In a commercial farm, 490 three weeks old piglets were divided into two comparable groups. At 26 days, one group received intramuscular vaccination (IM group, n=247), the other received intradermal vaccination (ID group, n=243). At the time of injection, pain expression was described for each individual piglet (vocalization, struggling, stiffening, defecation) and recorded. Two subgroups of 100 piglets were randomly formed in each group. These subgroups were filmed during vaccination to record pain related facial grimaces, according to Viscardi 2017. For each pig a score of pain expression and pain facial grimaces was calculated. These scores were compared between IM and ID groups.

#### Results

Pain expressions average scores are 5.6 and 1.5 respectively for IM and ID groups (p<0.0001). **The proportion of pigs expressing signs of moderate to severe pain are 23.1 and 2.9% respectively** (p<0.0001). Average scores for fascial grimaces of pain are respectively 1.3 and 0.45 for the IM and ID subgroups (p=0.023). **The proportion of pigs expressing grimaces of moderate to severe pain are respectively 25 and 2.3%** (p=0.0059).

# **Discussion and Conclusion**

Injection by IM route appears more painful than ID route with both methods of measurement. Scoring pain expressions and pain facial grimaces gives very similar overall results. However, on an individual scale, they are complementary as a shouting and struggling pig may not make a grimace, and vice versa. These two methods of pain evaluation appeared to be quite easy to implement in field conditions.

# ASSESSMENT OF RUBBER RINGS ON PIG TAIL IN CASE OF TAIL BITING OUTBREAK

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# **Background and Objectives**

Tail biting is an important animal health and welfare issue. Numerous risk factors have been identified, and their control is the main method of prevention. However, in case of tail biting outbreak, a rubber ring is sometimes put on the injured tail (i) to stop bleeding and thus reduce cannibalism and (ii) to prevent ascending infections. There is currently no scientific data that confirm the interest of this method. Recently, a survey documented by 74 swine French veterinarians demonstrated that 49% of them have ever used it. A trial in commercial pig farms was then carried out to assess the advantage of this intervention on pig health and welfare.

# **Material and Methods**

Five farms exhibiting tail biting outbreak were then selected by veterinarians in charge of their health monitoring. In each farm, four pairs of bitten pigs were formed, each comprising a control and a treated animal (i.e. with a rubber ring at the limit between the healthy and the injured parts of the tail). Pigs were matched according to lesion stage, room and pen. Behavioural and clinical observations were carried out during four weeks, respectively at d0, d1, d7 and d28, to assess pain and to compare healing process between groups.

#### Results

We observed that even if the rubber ring caused acute pain, chronic pain signs were less clear one week later. Fromdl rubber ring-treated piglets exhibited less blood at the hound suggesting that the biting process had been stopped. At d28 lesion scores were lower for treated piglets.

# **Discussion and Conclusion**

The rubber ring seems to reduce tail biting and to favour wound healing. Further investigations including more animals are necessary to confirm the benefit of this method on pig health and welfare.

# SUBSTITUTION OF EXPENSIVE PROTEIN SOURCES BY SOYBEAN MEAL SUPPLEMENTED WITH A M-MANNANASE ENZYME RESULTS IN IMPROVED GENERAL CLINICAL HEALTH SCORE DURING THE POST-WEANING PERIOD

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# **Background and Objectives**

Ø-Mannans are strongly anti-nutritive polysaccharide fibres found in most vegetable feed ingredients. Estimated content of soluble Ø-mannans in common nursery diets is only 0.15-0.35%, and in vitro studies have demonstrated that as little as 0.05% soluble Ø-mannan in feed can elicit a strong innate immune response. This innate response is referred to as a feed induced immune response (FIIR), which suppresses growth to protect the liver and reserve energy/nutrients for high priority immune functions. Hemicell<sup>™</sup> HT (Elanco) is a Ø-mannanase enzyme for animal feeds breaking down Ø-mannans, thereby preventing economic losses from this wasteful immune response to Ø-mannans.

# Material and Methods

A seven-week feeding trial was conducted with 320 pigs in two rotations of 160 piglets in 20 pens of 8 pigs. Standard two-phase control diets were compared to similar iso-nutritive diets with 300 g/tonne of Hemicell<sup>™</sup> except for following changes: Phase-1 (weeks 1-3): 0.15% potato protein concentrate and 2.00% Danex (extruded soybean meal (SBM)), was replaced with SBM 48%, Phase-2 (weeks 4-7): Hemicell<sup>™</sup> was formulated to replace 63 kcal/kg NE. Standard production and health data were collected. Data were analysed using JMP 14.0 statistical program.

#### Results

Piglets on Hemicell<sup>™</sup> diet performed equal to Control diet (P > 0.05). No antimicrobials were used in neither of the treatment groups. General clinical condition was scored significantly (P<0.05) better in the Hemicell<sup>™</sup> as compared to the Control group. Fecal clinical scores did not significantly (P>0.05) differ among treatment groups.

# **Discussion and Conclusion**

In conclusion, the use of an exogenous heat-tolerant ⊠-mannanase allowed reduced levels of expensive protein sources to be used in Phase-1 and a 63 kcal/kg NE reduction in Phase-2 without adverse effects on intestinal health or overall piglet performance. General clinical scores were significantly (P<0.05) better in the Hemicell<sup>™</sup> group.

# EFFECTS OF FEEDING HIGH AND LOW PROTEIN DIETS TO EARLY WEANED PIGLETS ON OXIDATIVE STRESS AND GUT HEALTH PARAMETERS EVALUATED IN PLASMA, LIVER AND ILEAL TISSUES

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# **Background and Objectives**

A study was conducted to evaluate the effects of two dietary protein level on oxidative stress and gut health parameters evaluated in plasma, liver and Peyer's patch in weaned piglets.

# **Material and Methods**

A total of 88 male pigs weaned at 21 days old with initial BW of 5.4 ± 0.44 kg were allocated to 10 pens of 8 animals per pen in a 2 week trial. There were two treatments that consisted of 1) low protein diet (LP; 18.3% CP) and 2) high protein diet (HP; 24.7% CP), but both including the same level of lysine (15 g/kg of feed). Faecal scores were assessed daily by pen. At days 0, 7 and 14 of the study individual piglet weights and plasma samples were collected to analyse concentrations of plasma urea nitrogen (PUN), cortisol, vitamins A and E, antioxidant enzymes (SOD, CAT and GPx) and oxidative stress index. At the end of the study, liver and Peyer's patch tissue were collected to analyse antioxidant enzymes and genes linked to oxidative stress and gut health, respectively. Differences between treatments were statistically analysed by one-way ANOVA using Graphpad Prism software.

# Results

There was no difference in body weight between treatments. There was a tendency (P=0.07) for higher faecal score in HP piglets compared to LP piglets. HP piglets had higher PUN (P<0.001), and a downregulation of genes related to gut integrity (TFF2, OCLN, SI) reflecting higher gut permeability compared to LP piglets and on SLC15A1, a peptide transporter gene. In addition, HP piglets had higher GPX enzymatic activity in plasma and liver, and also an upregulation of SCARA3, GSTI, GPX3 and SOD3 genes related to oxidative stress.

# **Discussion and Conclusion**

In conclusion, feeding high protein diets to 21 day-old weaned pigs increases gut permeability and oxidative status at the gut level.

# WELFARE EFFECT OF INTRADERMAL NEEDLE-FREE VACCINATION ON PIGLETS

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# **Background and Objectives**

Nowadays, society demands high welfare standards for farm animals. Vaccination is crucial for a better health status on farms, but it can represent a stressful event for animals. Different vaccination approaches could lead to improved animal welfare. This study analysed whether the Hipradermic<sup>®</sup> intradermal needle-free device (ID) improves welfare compared to intramuscular injection with a needle (IM) in piglets.

# Material and Methods

Seventy-two PRRS-naïve healthy piglets, 4-weeks-old, were randomly allocated to three groups: ID group (n=24, UNISTRAIN® PRRS 0.2 ml/dose ID), IM group (n=24, UNISTRAIN® PRRS 2 ml/dose IM) and group C (n=24, no vaccination). An aversion test (time taken to cross 4 metre raceway) and vocalisation were evaluated at different times using video and audio recordings, and cortisol analyses were performed on oral fluid. Statistical analysis was carried out using SAS (p-value<0.05).

#### Results

At the time of the injection, the ID group had a lower incidence of piglets vocalising than IM (52% ID vs 88% IM) and the number of high pitch vocalisations (> 1000 Hz) was also lower in ID than IM piglets (0.35 and 1.24 vocalisations/animal, respectively). At 10 minutes post-vaccination, the group IM took longer to cross the raceway compared to the ID and group C. No significant difference was observed for salivary cortisol.

# **Discussion and Conclusion**

The increased times taken to cross the raceway and higher vocalisation (presence and power) shows that IM injection was more aversive for piglets than being injected ID. These behavioural consequences had a low physiological impact if the results of salivary cortisol are considered, the latter also being found in previous studies on commercial farms. It can be concluded that Hipradermic<sup>®</sup> reduce behavioural indicators of pain compared to IM vaccination with a needle, and represents an alternative for improvement of animal welfare on swine farms.

IRON DEFICIENCY ANEMIA IN PIGLETS: PROBLEM SOLVED OR HIDDEN? ITALIAN SURVEY ON THE HEMOGLOBIN LEVEL OF PIGS AT WEANING AND COMPARISON OF AN INJECTABLE COMBINATION OF GLEPTOFERRON + TOLTRAZURIL VS. INJECTABLE DEXTRAN IRON PREPARATIONS IN PREVENTING AN

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# **Background and Objectives**

Iron Deficiency Anemia (IDA) is a pathological condition of great importance for pig production since it causes reduced growth performance and impairment of swine immune system. Prevention of this condition by Iron (Fe) administration is essential. Objective of this study is to determine the IDA's incidence in Italian farms and to evaluate the efficacy of an injectable product based on Gleptoferron+Toltrazuril in comparison with different preparation based on Iron dextran.

# Material and Methods

381 piglets (23 days of age) coming from 10 Italian farms were included in the survey. On 3rd day of life, 150 animals had been treated with Forceris® (Ceva Sante Animale) (200 mg of Fe as Gleptoferron), whereas 230 piglets received a preparation based on 200 mg of Fe dextran (products B, C, D, E). Hemoglobin (Hb) levels were measured on farms using the Hemocue® Photometer and piglets were classified as follow: 1) Healthy (Hb > 11 g/dl), 2) Sub-anemics (Hb between 9 and 11 g/dl) and, 3) Anemics (Hb < 9 g/dl). Average Hb values with corresponding 95%-CI were calculated for the whole population and compared between Iron preparations and farms.

# Results

The overall average Hb value was 10.67 [10.52; 10.81] g/dl, with 13% of anemic piglets and 45% of sub-anemic ones. Piglets treated with Forceris® had an average Hb value of 11.3 [11.1; 11.5] g/dl, statistically higher than those treated with Iron dextran-based products (10.3 [10.1; 10.4] g/dl) (p>0,05). Percentage of anemic piglets was 1% and 20% for animals treated with Gleptoferron+Toltrazuril and Fe dextran products, respectively.

# **Discussion and Conclusion**

IDA is a problem still present in the Italian pig farms and the preventive use of Forceris®, the first associated product based on Gleptoferron+Toltrazuril, showed a higher efficacy than competitors products based on Fe dextran.

TIME ASSESSMENT OF THE ADMINISTRATION OF TWO ALTERNATIVES TO PREVENT ANAEMIA AND COCCIDIOSIS IN PIGLETS

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# **Background and Objectives**

Porcine coccidiosis is caused by Cystoisospora suis, one of the most common enteric pathogens in suckling piglets. Piglets are born with very low iron storage, therefore to avoid anaemia exogenous iron supplementation is required. To optimize labour and improve piglet welfare it is important to reduce the number of times piglets are handled. The aim of this study was to assess time to apply two different options to prevent anaemia and coccidiosis.

#### Material and Methods

The study was carried out in a total of 1325 piglets. Depending on treatment to prevent anaemia and coccidiosis, piglets at 24 h of age were divided into two groups. Group 1: Piglets treated with an injectable toltrazuril-gleptoferron (Forceris™)Group 2: Piglets treated with an oral toltrazuril and an injectable gleptoferron. The time required for the treatment with these products and for the full piglet management at this age were recorded. Results were analyzed by a non-parametric test Mann-Whitney.

#### Results

A total of 18 litters were treated with injectable toltrazuril-gleptoferron (group 1) and time per piglet was 4,71±1.72 sec (p= 0.0274) vs 6.48±2.61 sec (15 litters). Moreover, when time full piglet managing was recorded time per piglet was better in group 1 (25 litters) by 22.63±3.33 sec (p=0.0003) vs 26.99±3.87 sec (19 litters).

#### **Discussion and Conclusion**

The results between groups showed that treatment with a combo injectable gleptoferron+toltrazuril (Forceris<sup>™</sup>) required significantly lower time than treatment with an oral toltrazuril and an injectable gleptoferron. Therefore a combo product to prevent anaemia and coccidiosis in piglets is an optimal tool to save labour and labour costs and improve animal welfare.

# INFLUENCE OF BACKFAT AND PARITY ON L-CARNITINE, CHOLINE CHLORIDE AND SORBITOL SUPPLEMENTATION 4-5 DAYS PRE-FARROWING

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# **Background and Objectives**

L-carnitine and choline chloride are essential for fat metabolism and transport of fatty acids into the mitochondria. Piglet survival depends partially on parity and backfat status of the sow. This case describes the intervention with a supplementary feed, Carnitol-L®, in a Danish commercial herd with high prolificacy and backfat measurement prior to farrowing.

#### **Material and Methods**

A commercial SPF herd of 1100 sows (Danbred LY) with approximately 50 farrowings per week and elevated number of stillborn piglets was selected. 267 farrowings (control) from January 1<sup>st</sup> to February 9<sup>th</sup> were compared to 211 farrowings (Carnitol-L®) February10<sup>th</sup> to March 10<sup>th</sup>. At entry into the farrowing unit, sows were supplemented with 40 ml Carnitol-L® per sow per day until farrowing (4-5 days). Daily dose per sow corresponded to 1200 mg L-carnitine, 1500 mg choline chloride and 11,4 g sorbitol. Backfat (mm) was measured at entry in the Carnitol-L®-group. Efficacy data from last quarter prior to the trial showed 18,9 live born – and 2,3 stillborn piglets / litter. Student's t-test with unequal variances were calculated for live- and stillborn piglets.

#### Results

# After supplementation:

32 sows, backfat group 9–12 mm, average parity 2,4, average 18,4 liveborn / litter, 1,78 stillborn / litter. 91 sows, backfat group 13–16 mm, average parity 3,4, average 19,2 liveborn / litter, 1,84 stillborn / litter. 48 sows, backfat group 17–20 mm, average parity 3,2, average 19,2 liveborn / litter, 1,73 stillborn / litter. 40 sows, backfat group >21 mm, average parity 4,2, average 19,2 liveborn / litter, 2,20 stillborn / litter.

Stillborn/litter was 2,24 in the control group versus 1,87 in the Carnitol-L® group (p=0,07). 1,71 in the Carnitol-L® group (p=0,03).

#### **Discussion and Conclusion**

The number of stillborn piglets per litter in this herd was lowered by 0,37 (P=0,03 1-tailed; P=0,07 2-tailed). Fat sows and high parity sows had numerically more stillborn piglets.

# INFLUENCE OF A BACILLUS-BASED PROBIOTIC ON VIABILITY AND PERFORMANCE OF SUCKLING PIGLETS WHEN FED TO HYPERPROLIFIC SOWS

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# **Background and Objectives**

This study aimed to investigate the effect of a bacillus-based probiotic on zootechnical and health parameters of hyperprolific sows and their piglets when added to the sow diet.

# Material and Methods

116 sows and their piglets (Danbred x Pietrain (BHZP77)) were divided into two treatment groups (n=58). Both groups received a diet either with or without probiotic supplementation (BioPlus® YC, 1.3x10<sup>9</sup> CFU/kg) from d7 before until d26 after farrowing. Diets were based on wheat, barley and soybean meal (13.2 MJ ME/kg, 16.5% CP, 1.05% Lys). All litters were balanced to a size of 13 piglets by cross-fostering within the first 24h after farrowing. Following parameters were observed: body weight of sows and piglets at trial start and end, piglet body weight gains, piglet mortality rates, sow fecal consistency (1=normal, 1.5=hard, 2=very hard, obstipation). Statistical analyses were performed by Oneway-ANOVA and Person correlation analysis using SPSS-software.

# Results

A higher number of weaned piglets (11.7 vs. 12.1; P<0.01) as well as higher litter weaning weight (84 vs. 91 kg; P<0.01) and higher individual piglet body weight at weaning (7.2 vs 7.5 kg; P<0.01) were observed. Piglets from probiotic-fed sows showed higher body weight gain (228 vs. 236 g per d; P<0.05) and lower mortality rates (10.1 vs. 6.8%; P<0.001). Reduced need of antibiotic treatments in piglets were observed in probiotic group. No differences in sow body weight losses during trial period and in sow fecal scores were observed. However, fecal scores correlated with different parameter such as sows body weight at farrowing, number of stillborn piglets, and piglet birth weight and were numerically lower in probiotic-fed sows.

# **Discussion and Conclusion**

The supplementation of the bacillus-based probiotic to diets of hyperprolific sows improved performance and health status of sows and their piglets. Fecal scores of sows correlated with different parameters which indicates a smoother farrowing process.

# TAIL BITING REDUCTION IN NURSERY PIGLETS: ROLE OF MATERNAL NUTRITION

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# **Background and Objectives**

In order to improve animal welfare, tail restriction of pigs has been banned from authorized routine procedure in Europe. However, despite enrichment of the living area, outbreaks of tail biting may happen, altering pig and farmer welfare, and responsible for economic losses. Swine Inflammatory and Necrotic Syndrome (SINS) has been recently described, based on visible skin lesions on ears, feet and tail of newborn piglets, and likely associated to sow's pro-inflammatory status. Association between presence of SINS and tail biting behavior remained to be described. A trial was conducted to measure the consequence of gestating and lactating sow feed composition on tail biting of nursery piglets raised without caudal restriction.

# Material and Methods

In order to create divergent pro-inflammatory status, sows were fed diets either enriched in net energy with vegetable oil (PIS+), or reduced in net energy and including live yeast (PIS-). At weaning, 256 piglets were allocated to 32 pens based on their maternal origin. Along the trial, piglets were weighed individually, and feed intake was determined per pen. Tail, ears and general health status were recorded daily.

# Results

The risk of tail injury was multiplied by 1.6 (P<0.10) comparing PIS+ vs. PIS-. Tail damage was observed at least once on 18.7% of PIS- vs. 27.3% PIS+ piglets (P<0.10). Tail docking interventions needed to be performed on 5.4% PIS+ piglets only. In addition, higher number of PIS+ piglets were excluded from the trial because of severe tail or ear injury (10% vs. 2% P-Chi<sup>2</sup><0.05). Piglets from PIS- had a higher growth (P<0.05) and a higher feed intake (P<0.05) than piglets from PIS+.

# **Discussion and Conclusion**

In conclusion, impact of maternal nutrition on the risk of tail biting should be considered, and it underlines the importance of accurate management from the early age to increase pig welfare.

## EFFECTS OF DIFFERENT SOURCES OF SOY PROTEIN CONCENTRATE ON PERFORMANCE RESULTS IN WEANED PIGLETS

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# **Background and Objectives**

The remaining antinutritional factors of soybean meal (SBM) such as oligosaccharides may limit its usage in practical weaning piglet diets. The processing of Soy protein concentrate (SPC) leading to a significant reduction in the content of trypsin-inhibitors and oligosaccharides. Aim of this study was to investigate different commercially available sources of SPC's and their effect on the performance of piglets as compared to control piglets fed with conventional SBM.

# Material and Methods

384 piglets [topig x pietrain] (6.30 ± 1.11 kg body weight) were randomly allocated to 4 dietary treatments, with 8 replicates each. The control diet consisted of corn, barley, wheat, and soybean meal (TI). In the experimental groups, soybean meal was replaced at rates of 5 % against one of three different commercially available soy protein concentrates (T2, T3 and T4). The total trial duration of 42 days was divided in a pre-starter period (0 – 14 d), a starter period (15 – 29 d) and a grower period (30 – 41 d). Statistical analysis was done by SPSS, GLM procedure (Tukey's test, p<0.05).

# Results

The piglets which received one of the SPC diets (T2-4) showed a slightly improved final body weight as well as increased feed intake in comparison to the diet based on SBM (TI). FCR ranged between 1.36 and 1.39. Numerically the average highest body weight at the end of the trial was observed in T4 (T4: 22.0 kg; TI: 20.96 kg) as well as the highest average daily feed intake (T4: 122.48 g; TI: 119.36 g).

# **Discussion and Conclusion**

Based on performance results, piglets showed tendency of improved performance in treatments with 5 % SPC, however, the differences were statistically non-significant (p<0.05). The exact magnitude of this performance change, however, depends on the SPC source.

# THE CAPABILITY OF BIMULAC® WEANER TO IMPROVE FECAL CONSISTENCY AND REDUCE THE APPLICATION OF ZINC-OXIDE IN WEANED PIGLETS

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# **Background and Objectives**

There is a growing pressure to wean piglets without the use of antibiotics or pharmacological levels of zinc oxide. The aim of our study was to investigate effects of a complex synbiotic-based product on fecal consistency and growth performance in weaned piglets by avoiding prophylactic use of antibiotics and zinc oxide.

# Material and Methods

702 weaned piglets (DanBred x PIC 408) were involved in the study. Average age was 27 days, average weaning weight 5.58 kg. Piglets were housed and fed under commercial standards in Germany.The control group got a combination of zinc oxide and antibiotic (2g / 10kg body weight) against recurring diarrhea, from day one to five after weaning. **Bimulac® Weaner** (5kg / ton) was administered in the test group, day one to 15 after weaning.Average daily weight gain (DWG) and feed take (FI) was documented and feed conversion (FCR) was calculated (phase 1: day 1-19; phase 2: day 19-35). Mortality was documented on a daily base.Feces were scored six times (day 1-15) using the following scoring system: 1 = severe diarrhea; 2 = moderate diarrhea; 3 = pasty feces; 4 = ideal feces.All data were subjected to statistical analysis using one-way ANOVA (SPSS Vers. 24).

#### Results

No statistically significant differences in performance and mortality were observed between the treatments. No additional diarrhea treatment was needed in both groups. The fecal consistency of the trial group was more closely corresponding to the ideal consistency compared to the control group. Moderate diarrhea (Score 2) was observed more frequently in the control group (9 times vs. 3 times).

#### **Discussion and Conclusion**

**Bimulac® Weaner** prevents diarrhea after weaning and reduces zinc oxide and antibiotic treatment. Costs per piglet are slightly higher but longterm benefits on gut function, immune system, life performance are expected and investigated in further studies.

# CONTACTLESS VIDEO-BASED RESPIRATION RATE MONITORING OF THE PIG IN RESTING STATUS

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# **Background and Objectives**

Respiration rate (RR) is a vital physiological parameter that is related to a pig's state of health, stress and wellbeing. Real-time and continuously monitoring of RR can help timely detect respiratory diseases and heat stress in pigs. However, the methods commonly used to measure RR requires human interaction, and they normally are time-consuming and labour-intensive. Thus, automatically monitoring of RR using contactless video combined with computer vision approaches would be beneficial to both stockmen and animals, as it not only frees up human labour but also is non-invasive to pigs.

# Material and Methods

Video data were collected from a Large White × York pig which was placed in a PigTurnTM experimental pen under ambient light. A Sony HandyCam HDR-SR5 camcorder was positioned on a Manfrotto Autopole at a height of 2.5m and 2.0m from the centre of the pen. 5 periods of the video with a mean value of 149.8s and standard deviation (SD) of 35.29s were chosen from the video data. To extract RR from the videos, a bandpass filter was first used to remove noise. Then short-time Fourier transform (STFT) with sliding windows were adopted to accurately identify the RR.

# Results

The gold standard (GS) RR measurements for the chosen videos had a mean value of 27.70 beats per minute (bpm) and SD of 1.72 bpm. Compared to GS, the estimated RR was promising to keep Mean Absolute Error (MAE) and Root Mean Square Error (RMSE) under 3bpm.

# **Discussion and Conclusion**

If the developed method works well, it will be promising to consider implementing video-based contactless physiological monitoring to further applications, e.g., illness diagnose and stress detection.

# SHORT- AND MEDIUM-CHAIN FATTY ACID SUPPLEMENTATION IMPROVES SOW AND PIGLET PERFORMANCE DURING LACTATION PHASE.

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# **Background and Objectives**

Organic acids are known to enhance performance, intestinal morphology and microbiota in pig production. Newborn piglets are exposed to a wide variety of microorganisms, mainly transferred from the sow and farrowing environment. We hypothesized that supplementing the sow pre- and post-farrowing with a blend of short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) until weaning can modulate the early microbial colonization of newborn piglets, thus improving the intestinal health and preventing intestinal disturbances during pre- and post-weaning phases.

# Material and Methods

A total of 72 (Landrace x Large White) sows with an average parity of 3.3 were fed two different diets: control diet (CON; 36 sows); and CON + 0.3% of a mixture of SCFA and MCFA (SCFA-MCFA; 36 sows) from 3 days prefarrowing until weaning (21d). Sow and piglet performance were recorded individually. Fecal samples were taken before transferring sow to farrowing crates, at 7d post-farrow, and at weaning.

# Results

At weaning, no significant differences between treatments were observed on sow BW or ADFI. However, sows fed SCFA-MCFA diet showed lower backfat loss than sows fed CON diet (-2.94 vs -3.75 mm; P=0.037). While, piglets from SCFA-MCFA sows had higher individual BW (6.01 kg vs 5.61 kg; P=0.013) and lower CV (15.8% vs 18.7%; P=0.022) than piglets from CON sows.

Regarding microbial counts, the supplementation of SCFA-MCFA resulted in an increase of fecal lactic acid bacteria in sows at weaning and in piglets at 7d post-farrowing and weaning. Moreover, at 2ld, piglets from sows fed SCFA-MCFA had lower S. suis counts than those fed control diet. No differences were observed for E.coli and C. perfringens.

# **Discussion and Conclusion**

The dietary sow supplementation with a blend of short-chain fatty acids and medium-chain fatty acids during peripartum and lactation periods enhanced intestinal microbial colonization and performance in suckling piglets.

# CONTROLLING PRE-WEANING MORTALITY CAUSED BY E. COLI WITH PROBIOTICS

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# **Background and Objectives**

Pre-weaning mortality is seen on all farms and affects the profitability of the sow unit. It's caused by many factors such as poor viability in piglets, crushing, starvation or disease. This study looked at the effect of feeding probiotics on pre-weaning mortality and the requirement for antibiotic treatments in sucking piglets affected by E. coli.

#### **Material and Methods**

The study was conducted at a Danish breeding farm found positive for E. coli (F4) and lasted for 32 weeks of which the first 19 weeks were the control period (involving 9502 sucking piglets) and the last 13 weeks (involving 6792 sucking piglets) were the trial period. Milk replacer was fed ad libitum to all piglets immediately after farrowing and until weaning at day 33 in both periods. In the trial period a probiotic blend consisting of B. subtilis (DSM 25841), B. amyloliquefaciens (DSM 25840) and LACTIFERM® (Enterococcus faecium (NCIMB 11181)) (2.67x10<sup>7</sup> CFU/g milk powder) was added to the milk replacer. Performance in the control period was compared to performance in the trial period. Data was analyzed using GLM (SAS Institute) with a model using probiotic as fixed effect (yes/no).

# Results

Pre-weaning mortality overall (n/week) was significantly reduced (P=0.01) and pre-weaning mortality caused by E. coli (n/week) was also significantly reduced (P<0.0001) in the trial period compared to the control period. In the trial period antibiotic treatments per week for E. coli infection was significantly reduced (P=0.005). Furthermore, the weaning weight of the piglets was significantly higher when the probiotic blend was fed (P=0.003).

# **Discussion and Conclusion**

The reduced mortality, both overall and mortality caused by E. coli, together with a lower number of antibiotic treatments indicate the health and viability of the sucking piglets was improved when the probiotic blend of B. subtilis, B. amyloliquefaciens and LACTIFERM® was fed to sucking piglets.

# EFFECTS OF HERBAL DERIVED VITAMIN D ORALLY ADMINISTERED IN PIGLETS

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# **Background and Objectives**

Vitamin D has multiple biological effects. It is not only critical for the homeostasis of calcium, phosphorus and magnesium but also affects skeletal structure, immune response and performance. Active form of vitamin D is the hydroxylated 1,25 Dihydroxycholecalciferol (1,25D<sub>3</sub>). Solbone®, a water-soluble extract from the plant Solanum glaucophyllum or waxy-leafed-nightshade, naturally contains 1,25D<sub>3</sub>-glycosides. Aim of the study was to compare a single dose of plant derived 1,25D<sub>3</sub>-glycoside with chemically synthesised free 1,25D<sub>3</sub> to evaluate serum kinetics.

# Material and Methods

Twenty-one piglets were randomly allocated to Solbone<sup>®</sup> groups (20 and 60mg/kg BW; SOL20 and SOL60) or control group (20mg synthetic 1,25D<sub>3</sub>/kg BW; CON). For repeated blood sampling, central venous catheters were surgically inserted. Blood samples, before and 8 times after receiving the test substance orally, were analysed for calcium, phosphorus and 1,25D<sub>3</sub>.

#### Results

In all groups serum concentrations of 1,25D<sub>3</sub> increased clearly above preprandial values, showing bioavailability of both sources. Concentration-time-curves revealed significantly higher 1,25D<sub>3</sub> values in SOL60 than in CON group. Moreover, kinetics showed differences: CON group peak concentrations were reached 2h after application followed by a steep decline directly afterwards and reaching pre-supplementation values after 12 to 24h. Especially in SOL60 group serum 1,25D<sub>3</sub> concentrations increased significantly after 8h and stayed above initial values for up to 48h. Serum concentrations of total calcium and phosphorus were not affected by both substances.

# **Discussion and Conclusion**

The results demonstrate that Solbone<sup>®</sup> effectively increases serum concentrations of the metabolically active form of vitamin D without impacting concentrations of phosphorus and calcium. It also showed that in piglets the ingestion of the glycosidic form results in a delayed and prolonged increase of serum 1,25D<sub>3</sub> compared to free 1,25D<sub>3</sub>. As 1,25D<sub>3</sub> is not only related to calcium but phosphorus metabolism, Solanum glaucophyllum could be a promising tool helping pigs cope with low phosphorus diets.

# GLUCONIC ACID IMPROVES PERFORMANCE OF NEWLY WEANED PIGLETS ASSOCIATED WITH ALTERATIONS IN GUT MICROBIOME AND FERMENTATION

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# **Background and Objectives**

Gluconic acid was shown to be poorly absorbed in the gut but readily fermented to butyrate which may improve gut function, which has particular relevance for the weaned piglet.

# Material and Methods

144 weaning piglets were fed 3 treatments (8 pens with 6 piglets/treatment): T1, control diet; T2, gluconic acid at 0.9%; and T3, gluconic acid at 1.8%, for 42 days. After 21 days, one piglet from each pen was sampled for blood hematology and biochemistry, and gut digesta characteristics and metagenomics.

#### Results

Feeding gluconic acid increased performance in the immediate postweaning period, particularly feed intake (P<0.05). Overall, feed intake increased (P<0.05), which resulted in numerically higher final body weight. Piglets from T3 had fewer total white blood cells (P=0.060), caused by particularly lower numbers of lymphocytes than those from T1 (P<0.05). Compared to T1; T2 and T3 had lower plasma urea (3.8, 2.6 and 2.6 mmol/L, respectively, P<0.05). Feeding gluconic acid provoked substantial changes in the relative abundance of lactic-acid-producing and acid-utilizing bacteria. The abundance of Lactobacillus amylovorus and Veillonella AY445131.1524 in the distal small intestine were increased and decreased, respectively (P<0.05), whereas in mid-colon, a reduction in Prevotella 9 HQ716341.1469 (5.9 vs. 10.0% for T3 and T1, P<0.05), and an increase in Faecalibacterium prausnitzii and L amylovorus (7.2 vs. 2.8% for T3 and T1, P<0.05) and 5.6 vs. 1.1% for T3 and T1, P<0.05) were found. Finally, butyrate producers Megasphaera elsdenii and Mitsuokella multacida were dose-dependently elevated, in particular M. elsdenii (1.7, 1.6 and 3.7% for T1, T2 and T3, respectively). Consequently, in caecum and mid-colon, increased relative molar percentage of butyrate were found, e.g. 10.0, 12.9 et 14.7% in caecum for T1, T2 and T3, respectively, P<0.05).

# **Discussion and Conclusion**

Feeding gluconic acid reduces the postweaning growth-check in piglets mediated by altered fermentation and buytrate production.

# TREATING DRINKING WATER WITH SODIUM HYPOCHLORITE: EFFECTS ON BACTERIA AND ENDOTOXIN CONCENTRATIONS

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#### **Background and Objectives**

Low drinking water quality can affect the health and performance in pig production. Disinfection of provided water may enhance the microbial quality and may also affect concentrations of endotoxins. In this study, bacteria and endotoxin concentrations were examined in sodium hypochlorite treated and untreated water on a pig rearing farm.

# **Material and Methods**

Water samples were taken from incoming water and from sections with treated and untreated water at the beginning, from nipples and at the end of pipes. The rearing farm was visited 14 times to measure the concentrations of the total bacteria count, Pseudomonas spp. and endotoxins at all sampling points. Additionally, the occurrence of coliform bacteria was examined. Data analysis was performed with SAS (SAS Institute Inc., Cary, USA), using a mixed model analysis.

#### Results

The results showed significant reductions of total bacteria and Pseudomonas spp. in treated water at the beginning of pipes and at nipple drinkers (P-values < 0.0001). Differences between bacteria concentrations at the end of pipes showed no clear trend and were mainly not significant. In contrast, endotoxin concentrations were approximately equal at the beginning of pipes and at nipple drinkers and showed different trends at the end of pipes. The occurrence of coliform bacteria was significantly reduced in treated water (Fisher's exact test, p = 0.0001).

#### **Discussion and Conclusion**

The application of sodium hypochlorite can significantly reduce bacteria including potential pathogens and biofilm forming bacteria in drinking water pipes of pig barns. Disinfection of the dead-end sections failed and these parts must be regarded as potential contamination sources. Endotoxin concentrations remained rather unaffected. However, concerning their adverse effects on pig health, the uptake of endotoxins via drinking water should receive attention.

# BEHAVIOURAL EVALUATION AND STRESS INDICATORS AFTER INTRADERMAL OR INTRAMUSCULAR VACCINATION IN THREE WEEKS OLD PIGLETS.

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# **Background and Objectives**

The aim of this study is to evaluate piglets' behavioral and physiological responses during administration of vaccines comparing intramuscular injection with a needle (IM) and intradermal injection without needle (ID).

#### **Material and Methods**

3-4 weeks old piglets have been vaccinated simultaneously against PCV2 and Mycoplasma hyopneumoniae either using needle syringes (IM, n=53) or a needle free device (ID, n=53), according to manufacturers' indications. Piglets were video and audio recorded at the time of vaccination to evaluate reactions (attempts to flight) and vocalizations (number and intensity), while analysis of cortisol were performed on blood samples taken 30min and 2 hours after vaccination from 18 piglets per treatment.

#### Results

No flight reactions have been shown by 90% of the ID piglets at the time of vaccination while only 55% of the IM piglets did not react (P < 0.001). Moreover 18% of the IM pigs continued to fidget after vaccination, while the ID ones did not show this behavior (P < 0.01). Following ID administration, 55% of piglets did not vocalize and only the 13% of them emitted repeated screams, compared to the 25% and 50% of the IM group respectively (P < 0.001). Analyzing in detail the vocalizations it has also emerged a longer duration (IM = 588 ms vs ID = 352 ms, P = 0.010) and a higher maximum peak (IM = 99.8 dB vs ID = 90.7 dB, P < 0.001) of IM Group's vocalizations compared to the ID one. No differences were shown in cortisol concentration at 30 minutes, 2-hours data is pending.

# **Discussion and Conclusion**

This study showed a statistically significant reduction of stress responses. This suggests that the intradermal route of vaccination decreases negative behavioral indicators of animal welfare during and immediately after the administration of the vaccine.

# SHORT CHAIN FATTY ACIDS ADMINISTRATION VIA A WATER ACIDIFIER IMPROVED GROWTH PERFORMANCE AND MICROBIAL BALANCE IN NEWLY WEANED PIGLETS.

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# **Background and Objectives**

During the first days post-weaning changes in environment and diet cause a reduced feed intake, increased diarrhea incidence, gastrointestinal (GI) microbiota dysbiosis and higher susceptibility to infections, all of which can negatively affect survival and long-term growth performance of piglets. Due to the low feed intake, drinking water acidification can be used as an effective and efficient delivery medium for short chain fatty acids (SCFA) helping the piglets through the weaning process. The objective of this study was to investigate the effect of a water acidifier containing free and buffered SCFA on growth performance and GI microbiota of piglets during first 42 days post- weaning.

# Material and Methods

In total 192, 28 day old, newly weaned piglets were divided into groups of eight animals per pen. Each pen received either clean drinking water (control), or drinking water with 2.0L/1000L water acidifier (SCFA-WA) (Selko B.V., the Netherlands), with 12 replicate pens per treatment. Body weight and feed intake were measured per pen on days 0, 21, and 42. Feces were collected from three piglets per pen on days 14 and 42 and analyzed for microbiota composition using 16S rRNA gene sequencing.

#### Results

SCFA-WA improved feed efficiency (P<0.05), whereas no significant differences were observed in body weight, weight gain, feed intake, and diarrhea between treatments. Compared to control group, the SCFA-WA treatment was associated with higher microbial diversity (number of observed genera), lower Simpson index, and significantly higher relative abundance (RA) of Clostridium\_Sensu\_strico\_1 and lower RA of Streptococcus on day 42 (P<0.05). In addition, higher fecal RA of beneficial, SCFA producing genus Butyricicoccus was associated with improved feed efficiency on days 14 and 42.

# **Discussion and Conclusion**

This study confirms that SCFA-WA drinking water acidification post weaning has a beneficial effect on piglet growth efficiency and gut microbiota balance.

SULFUR AMINO ACIDS REQUIREMENTS INCREASED DURING A LIPOPOLYSACCHARIDE INFLAMMATORY CHALLENGE IN PIGLETS

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# **Background and Objectives**

Methionine is an essential amino acid for livestock. It plays roles in protein synthesis, antioxidant defense and immune functions. This study aimed to determine whether increased consumption of total sulfur amino acids (TSAA) by both sows (late gestation and lactation) and weaned piglets could improve their resistance to inflammatory stress induced by lipopolysaccharide (LPS).

### Material and Methods

Thirty primiparous sows were fed three treatments from gestation d85 to postnatal d21: a control diet (CON) adequate in TSAA, CON + 25% TSAA supplemented via DL-Methionine or OH-Methionine. Piglets were weaned at 21 d old and were allocated to the experimental treatments from 21 to 63 d old. Post-weaning diets were either adequate in TSAA (CON) or above (+25% DL-Met and +25% OH-Met). After 2 weeks (35 d old), 20 male piglets from each treatment were divided in two groups and were injected with saline or LPS solution (100  $\mu$ g/kg BW E. coli 0111: B4, Sigma).

# Results

The LPS challenge altered significantly ( $P \le 0.05$ ) piglets' growth performance from 35 to 63 d old. The plasma levels of aspartate aminotransferase, total bilirubin, IL- $\mathbb{N}$ , IL-6, TNF- $\mathbb{N}$  and malondialdehyde increased in the LPS group at 4 h and 12 h post-challenge, as response to the inflammation. Plasma albumin (4 h), total protein (12 h), total antioxidant capacity (12 and 24 h) and glutathione peroxidase (GPx, 24 h) decreased with the inflammation. The increase of TSAA, particularly as OH-Met, reduced the pro-inflammatory cytokines (IL- $\mathbb{N}$  IL-6 and TNF- $\mathbb{N}$ ). The GPx remained the same between the LPS and saline treated piglets with the increase of TSAA. Consequently, the growth performance of piglets fed high levels of TSAA was partially restored.

#### **Discussion and Conclusion**

Overall, the increase of TSAA consumption, by both sows and piglets improved the biomarkers of the inflammation and thereby increased piglet's growth performance.

# THE PREVALENCE OF GASTRIC LESIONS IN SOWS.

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# **Background and Objectives**

In pigs, gastric lesions are observed in pars oesophagea covered with stratified squamous epithelium which does not produce mucus and has no capability of buffering the aggressive influence of low pH stomach content. Etiology of the disease is multifactorial and pathological changes evolve from hyperkeratosis, through erosion to ulceration. Such lesions may lead to increased mortality rate. They also affect animal welfare. The objective of this study was to evaluate the prevalence of gastric lesions in slaughtered sows.

# Material and Methods

The prevalence of gastric lesions in 95 culled sows (due to lameness, age or reproductive failure) from 5 large farms located in northern Poland (1600-4000 sows; managed with accordance with Danish production standards) was investigated during abattoir survey. Genetic line (DanBred), health status (PRRSV-negative), feeding (pelleted feed) and housing system were the same in every location. In order to perform the morphological scoring, a four-grade system was adopted. Grade 0 - no change, grade 1 - hyperkeratosis, grade 2 - erosion, grade 3 - ulcer. Stomachs were examined within up to 20 minutes post-slaughter, after their emptying and gentle rinsing with running water.

#### Results

Pathological lesions, ranged from hyperkeratosis to ulceration, were found in 70.5% of the sows. From a total of 95 stomachs examined, 49 (51,6%) had gastric ulcers. In the rest, hyperkeratosis and erosions were observed in 6 (6,3%) and 12 (12,6%), respectively.

# **Discussion and Conclusion**

The prevalence of gastric lesions observed in the present study was similar to the reported previously: 60– 98% (Christensen et al., Dansk Veterinaertidsskrift Nr. 9824; Carstensen et al., Proc. 19<sup>th</sup> IPVS). It indicates that the efforts to understand the pathogenesis of gastric ulceration in sows, and to prevent it, are largely inefficient. Therefore, and considering growing importance of animal welfare, studies to identify risk factors of gastric ulceration in sows should be intensified.

# APPLICATION OF RNA SEQUENCING TECHNOLOGY TO STUDY THE INFLUENCE OF PHYTOGENIC FEED ADDITIVES IN PIGLETS

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# **Background and Objectives**

Phytogenic feed additives (PFAs) describe plant-derived compounds affecting beneficially livestock production through manifold biologically properties. However, underlying PFA's mechanisms are yet to be unraveled in order to deliver animal industry efficacious and reliable products to support animal health. Recent advances in molecular biology confer us the ability to monitor all gene expression simultaneously by high throughput sequencing of mRNA transcripts (RNA-Seq). Although RNA-Seq is becoming the predominant transcriptomics tool, its use in animal science is still limited. Here we present the application of RNA-Seq in both intestinal and liver tissues from a swine experiment (oxidative stress model).

# Material and Methods

Thirty-two piglets were allocated for 28 days to four experimental diets (2x2 factorial design), a standard diet (CTR), the same diet supplemented with PFA (PFA), or the same two diets with inclusion of oxidized oil (OO and PFA\_OO). RNA-Seq was done on samples from the ileum (CTR and PFA; 5 pigs per diet) and from the liver (all 4 diets; 6 pigs per diet). An average of 50 million of reads per sample (2\*75 bp) were generated and processed in our Snakemake-embedded bioinformatics pipeline (STAR, HTSeq-counts and DESeq2). Over representation analysis was used to identify the enriched biological pathways.

#### Results

RNA-Seq analysis showed that PFA supplementation downregulated the expression of several genes related to inflammation, and matrix degradation in the ileum of pigs. As expected, the oxidized oil challenge strongly induced the expression of hundreds of genes in the liver of pigs, and PFA was partly able to neutralize the effects, especially on the genes associated with fatty acid Ø-oxidation, peroxisome and lipid metabolic process. Validation of the data with qPCR confirmed the results of RNA-Seq.

# **Discussion and Conclusion**

The results obtained showed that RNA-Seq is a useful approach to elucidate plant-derived feed additives' molecular mode of action, and therefore guide the effective use of such compounds.

# USE OF ACETOMINOPHEN IN THE FARROWING PERIOD IN SOWS: EFFECT ON HEALTH AND CONDITION OF THE SOW AND ON MORTALITY, WEIGHT AND WEIGHT GAIN OF THE PIGLETS

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# **Background and Objectives**

Pain and fever in the periparturient period can lead to prolonged farrowing and can slow down the recovery of the sow, which will have effect on vitality and survival of the piglets. This study investigated the use of orally administered Acetominophen in sows in the periparturient period.

#### **Material and Methods**

Fourty-four sows were stratified based on parity and then randomly allocated to either the Control group (n=21) or the Acetominophen (Pracetam<sup>®</sup>) group (n=23). Acetominophen was administered from 3 days before to 2 days after farrowing. IgG concentration was measured in 6 litters per treatment using the Immunocrit method.

#### Results

Mortality did not differ in piglets during the first week, nor during total lactation (P>0.10). No difference was found in weight or weight gain of the piglets during lactation. Coefficient of variation of piglet weight was smaller in the Acetominophen group at day 7, day 14 and at weaning, but not at birth. So, variation within litters was smaller in Acetominophen treated sows. No difference in mean IgG concentration was found between treatments, but coefficient of variation was too high (>40) in 50% of the C-litters and not in Acetominophen treated sows lost less backfat than Control sows. No effect was found on temperature in sows, but fever was rare in both groups.

#### **Discussion and Conclusion**

Acetominophen results in less variation of body weight of piglets during lactation, seems to have an effect on distribution of IgG within litters and has a positive effect on back fat loss. Effects of Acetominophen might be even more pronounced in farms with high mortality (this farm 8%) or with a high incidence of agalactia, fever after farrowing or piglet diarrhoea, which was not the case in this farm. Acetominophen is a promising product for increasing welfare in lactating sows and optimising production in the farrowing stable.

# INCREASED SURVIVAL CHANCES OF LOW BIRTH WEIGHT PIGLETS AFTER APPLICATION OF PIGLET PROTECTOR®

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# **Background and Objectives**

Weak and underweight piglets need intensive support, to reduce post weaning mortality. The susceptibility to diseases in low birth weight piglets is high and concepts like **Piglet Protector®** are developed to support them.

# **Material and Methods**

The trial was conducted in the Philippines on a commercial sow farm. 58 piglets from 15 sows were included. Piglets of each litter were divided into trial and control group and only piglets with a low birth weight between 0.7 kg and 1.0 kg were observed. Sows from second and third parity were selected and the lactation period lasted over 29 days in average. **Piglet Protector®** was given to the trial piglets within six to 12 hours after birth, a second dose was given after another 12 hours. Piglets with diarrhea were treated with antibiotics in both groups. Measured parameters were birth and weaning weight, mortality and necessary number of antibiotic treatments. All data were subjected to statistical analysis using one-way ANOVA or T-test (SPSS Vers. 24).

#### Results

The trial group started with a lower average birth weight (0.826g vs. 0.899g; p=0.000) and finished with a higher average weaning weight (7.88kg v. 7.15kg; p=0.000), which is ensured by better average daily weight gain (0.241g vs. 0.214g; p=0.000). The mortality of the trial piglets was much lower (12.9% vs. 33.33%) and also the application of antibiotic treatments (6.5% vs. 14.8%), because of diarrhea incidence, was strongly decreased.

#### **Discussion and Conclusion**

The supporting ingredients in **Piglet Protector®** like probiotics and colostrum fosters the intestine, which results in less antibiotic treatments. Additional energy, vitamins and trace minerals, strengthen the animal and enhance the chances for sufficient intake of colostrum and milk from the sow. A healthy piglet shows better performance like daily weight gain and reduced mortality. **Piglet Protector®** promotes low birth weight piglets in critical phases

# ZINC SUPPLEMENTATION WITH HIGHLY BIOAVAILABLE SOURCES IN PHYTASE-FREE DIETS CAN IMPROVE FEED INTAKE AND OXIDATIVE STATUS OF WEANED PIGLETS

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# **Background and Objectives**

After weaning, piglets need several days to recover from stress and reach to the normal feed intake levels. Therefore, more bioavailable feed components should be fed during this period, e.g. trace elements. Zinc sulfate (ZnSO<sub>4</sub>) is considered more bioavailable than oxides (ZnO), because of its solubility in water. However, some recent studies have shown that a potentiated ZnO (HiZox®, Animine, France) has higher bioavailability than ZnSO<sub>4</sub>. The aim of this study (Project E!11780) was to evaluate the effect of ZnSO<sub>4</sub> and HiZox® at 60, 120, 180 mg/kg on performance and antioxidant status in phytase-free diets.

# **Material and Methods**

At 28 days of age, 384 piglets were weaned and allotted to 48 pens (8 piglets/pen). Performance was measured on day 7, 14 and 35. At the end of the trial, four piglets per treatment were slaughtered for evaluation of oxidative status. Zn source, dose and its interaction were included as fixed effects in the statistical analysis.

# Results

After 7d, a source effect was observed for ADFI (HiZox® = 150, ZnSO4 = 125g/d; P<0.05) and a tendency for ADG and BW. The differences on ADFI were also observed at the end of the trial (497 and 464g/d; P<0.05), although no differences were observed on final BW (mean 19.4 kg). Hepatic Zn was higher in the HiZox group both at the lowest (19.8%) and at the highest (22.9%) Zn doses, while no differences were found at 120 mg/kg (Pinteraction<0.05). Glutathione peroxidase (GSH-Px) activity in the liver linearly increased (P<0.05) by HiZox® supplementation (from 4 to 6U/g) while decreased with ZnSO4 (from 6 to 3U/g) but no differences on hepatic malondialdehyde concentration were observed.

# **Discussion and Conclusion**

The Zn supplementation through highly bioavailable sources increases feed intake immediately after weaning and improves antioxidant activity of GSH-Px enzyme.

# ASSESSMENT OF SOW MORTALITY IN A LARGE PRODUCTION SYSTEM.

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# **Background and Objectives**

Sow mortality has a great economic impact on production. It is also an important animal welfare parameter. Despite profound interest elucidation of the causes of sows mortality is still challenging and seems to be multifactorial. The greatest limitation of examining dead sows is that sow necropsies are difficult to perform on a daily basis. Only a few studies involved large-production systems in a long-term perspective. The objective of this study was to re-evaluate sow mortality to identify the most problematic periods during production cycle.

# **Material and Methods**

The analysis was conducted between 2013 and 2019 in 5 large Polish farms (1600-8000 sows). Sow mortality was recorded and analysed in relation to the stage of reproduction cycle (W – deaths percentage distributed by weeks since served) of dead animals were collected.

#### Results

In total 13634 death cases were analysed. Obtained data indicate following pattern of mortality: W1 – 0.5%, W2 – 1.2%, W3 – 1.8%, W4 – 2.3%, W5 – 3.1%, W6 – 2.3%, W7 – 2.4%, W8 – 2.5%, W9 – 2.5%, W10 – 2.3%, W11 – 2.4%, W12 – 2.2%, W13 – 2.3%, W14 – 2.3%, W15 – 3.1%, W16 – 4.9%, W17 – 16.4%, W18 – 12.7%, W19 – 7.0%, W20 – 7.3%, W21 – 7.2%,  $\geq$ W22 – 11.3%.

#### **Discussion and Conclusion**

In this database approximately 40% of deaths took place during lactation. These proportions clearly identify the most risky stage in the whole production cycle. The fact that 1 out of 8 of dead females were not inseminated successfully after weaning is also indicative and might be associated with underestimated infections related to farrowing or multisystemic problems connected with poor body condition. Going forward plans for reduction of female death loss, particularly in post farrowing period, need to be implemented.

# THE USE OF CRUDE FIBRE (TRITICALE-WPS) FOR CONTROL OF DAILY FEED INTAKE OF FOUR DIFFERENT TYPES OF FATTENING PIGS ACCORDING TO THEIR BODY COMPOSITIONS

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# **Background and Objectives**

Present feeding concepts pay insufficient attention to the various needs of different types of fattening pigs. The animals differ in their performance potential and thus in their nutrient requirements. Pigs with below or above average feed intake capacity are not optimally supplied in terms of resource efficiency. A high feed intake capacity can result in excessive fatness and a high degree of nitrogen excretion. Diets rich in crude fibre should prevent this excess feed intake and cause better nitrogen fixation in the animals' colon. Therefore, the hypothesis was to control in a first step the feed intake of group housed fattening pigs by the use of Triticale–whole–plant silage selectively.

# Material and Methods

The study was performed in a conventional large group-housing barn (n=660 pigs) with sorting gates and automatic body weight recording. The animals were grouped (Ø BW 50kg) according to the body weight and by ultrasound examination of the back. Based on the weighing data all animals were subdivided into "light" and "heavy". In addition, all pigs were categorized as "fat" and "lean" based on ultrasound data. The feed was offered to the four feeding groups ("LF","LL","HF","HL") ad libitum, the diets were designed on an equal amount of energy and protein, just the amount of crude fibre differs significantly (2,5-10% in 88%DM). The feed intake was calculated on a body weight of 100kg

#### Results

The average daily feed intake decreased in all four groups with an increased use of triticale WPS (⊠ HF -0,48kg, HL -0,36kg, LF -0,18kg, LL -0,22kg).

# **Discussion and Conclusion**

It can be said that the use of whole plant silages with a high crude fibre content increases the feeling of satiety and thus has an influence on feed intake. Therefore it shout be possible to feed fattening pigs more individually and according to their nutrient requirements.

# EFFECTS OF ALTERNATIVE HOUSING SYSTEMS ON THE WELFARE AND HEALTH OF WEANER AND FATTENING PIGS

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# **Background and Objectives**

In this study effects of early socialization of piglets in farrowing units and effects of mixing pigs at different ages on their welfare and health were examined.

# Material and Methods

Piglets were raised either in single-litter-systems with conventional farrowing crates (FC) or free-movementpens (FMP) or a multi-litter system with group-housing (GH). At weaning piglets were distributed to different systems: a standard system with mixing and regrouping at weaning and in the fattening unit (control group, CG), a system where the weaners were left in their farrowing system (weaning-in-farrowing unit, WiFU) followed by mixing and regrouping only in the fattening unit, and a wean-to-finish system (WetoFi, mixing and regrouping only at weaning). Eight consecutive batches were performed, where piglets were tail-docked or undocked batchwise. Scorings for skin lesions, tail lesions, claw health, lameness, diarrhea and coughing bouts were performed regularly and cortisol levels were determined.

# Results

Shortly after weaning GH-piglets had less skin lesions than the FC- or FMP-piglets. Leaving the piglets in their farrowing crate (WiFU) was the least stressful kind of weaning. During rearing, skin lesions decreased in the CG and the WetoFi-group in contrast to the WiFU-group. In the WetoFi-group significantly more claw lesions developed after weaning than in the other groups. Claw lesions increased in the WiFU-group after regrouping for the fattening period.Tail lesions increased in all weaning-systems, especially in undocked pigs. A lower incidence was observed in undocked WiFU-pigs during rearing and in undocked WetoFi-pigs compared to the conventional system.

# **Discussion and Conclusion**

Early socialization (GH) had a positive effect on skin lesions after weaning. Development of claw lesions shortly after weaning depended on the occurrence of agonistic behavior after regrouping and on floor conditions. Keeping pigs in stable groups (WiFU, WetoFi) for a longer period, reduced tail lesions in undocked pigs, but could not prevent them completely.

# EARLY DETECTION OF ANESTRUS OF SOWS BY FEEDING INTAKE PATTERN DURING LACTATION

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# **Background and Objectives**

New technologies and innovative farm's equipment allow massive data collection that properly used can help to optimize production. In particular, electronic sow feeding is helping to understand better sows' needs and performance. This study aimed to predict the sows that are going to show an anestrus (estrus absence within 10 days after weaning) based on lactation feed intake pattern.

# Material and Methods

The study was carried out in a commercial farm in Segovia (Spain). A total of 181 sows were included in the trial. Data collection of feed intake during lactation period were performed using the farm equipment Gestal Solo (Jyga Technologies, SaintLambert-de-Lauzon, QC, Canada). Reproductive data were collected from PigCHAMP software. Sows were categorized in two groups based on estrus presence (ESTRUS): sows which showed estrus (n=162) and sows which did not (ANESTRUS) (n=18). Statistical differences were sought in the average daily feed intake and considering two periods: the whole lactation period and divided by weeks. Normality and homocedasticity of variables were proved and the Kruskal-Wallis test or the ANOVA test was applied to find the differences between feeding curves.

# Results

Feed intake during lactation period was significantly different between the ANESTRUS and ESTRUS groups. The lactation period was analyzed by weeks to be able to early detect a possible anestrus. A statistical trend (p=0.06) was detected in the second week, when ESTRUS sows ate 0.41kg more than ANESTRUS sows (7.08kg vs 6.67kg, respectively). These differences increased in the latter part of lactation. In the third week estrus sows ate 1.07kg more than anestrus sows (8.21kg vs 7.14kg, respectively) whilst in the fourth estrus sows ate 0.85kg more than anestrus sows (7.63kg vs 6.78kg, respectively).

# **Discussion and Conclusion**

In conclusion, it has been proved that it is possible to detect the anestrus from second week of lactation knowing the feed intake pattern of sows.

# FEED INTAKE OF SOWS THE LAST WEEK OF GESTATION AFFECTS THEIR PERFORMANCE DURING LACTATION

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# **Background and Objectives**

Electronic sow feeders allow providing more controlled daily feed administration individually assigned according to the feeding behavior of each sow. In addition, the feed can be administered in higher number of times, thus mitigating the feeling of hunger of sows and obtaining a more distributed consumption throughout the day. The aim of this study was to prove if an increase in feed intake at the end of gestation, improves the body weight (BW) of piglets at birth and at weaning.

# **Material and Methods**

A total of 69 sows were distributed in two groups according to their voluntary average daily feed intake (ADFI) from day 107 of gestation to farrowing day: high feed intake group (HFI; n=35 sows; ADFI=3.66kg/d) and low feed intake group (LFI; n=34; ADFI=2.11kg/d). Feed intake data from day 107 of gestation to weaning were collected from the electronic feeder equipment Gestal Solo (Jyga Technologies, SaintLambert-de-Lauzon, QC, Canada). The Kruskal-Wallis and Mann-Whitney U tests were applied to find the differences between groups.

#### Results

The amount of feed offered to sows the last week of gestation did not affect the BW of piglets at birth (1.48kg; p>0.05). However, the HFI sows tended to have higher ADFI than LFI ones in the lactation peak (from day 18 to 22 of lactation) (8.80kg/day vs 8.42kg/day, respectively; p=0.10). This difference probably supposed a greater mammary gland development and, consequently, was translated in higher BW of piglets at weaning, where the piglets from HIF sows were heavier than those from LFI sows (9.21kg vs 8.46kg; p<0.01).

# **Discussion and Conclusion**

In conclusion, although offering higher amount of feed to the sows during the last week of gestation did not affect BW of piglets at birth, it implied an increase in their ADFI in the lactation peak that translated into heavier piglets at weaning.

# QUANTIFICATION OF THE DIFFERENCES OF FEED INTAKE BETWEEN MULTIPAROUS AND PRIMIPAROUS SOWS DURING LACTATION PERIOD

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# **Background and Objectives**

The use of sow electronic feeders in swine production helps to better understanding of the needs of sows and, in consequence, improves their performance. It is already described that multiparous sows have a higher feed intake than primiparous sows during lactation period. However, to the best of our knowledge, these differences are not quantified.

# Material and Methods

Therefore, the aim of this study was to quantify the differences of feeding intake between primiparous and multiparous sows during lactation period. The study was conducted in a commercial farm in Segovia (Spain). A total of 214 sows were included in the trial (the average of length lactation period was 29.1 days). Data feed intake during lactation were collected from Gestal Solo (Jyga Technologies, SaintLambert-de-Lauzon, QC, Canada) farm equipment, an electronic feeder which gives the opportunity to the sow of self-fed wet-dry consumption. Reproductive data were obtained from PigCHAMP software, and the total of sows were categorized in two groups: primiparous (n= 70) and multiparous (n= 144). Regarding statistical analysis, firstly, the normality and homocedasticity of the feeding intake curves between groups were tested. Then, the Kruskal-Wallis test was used to detect statistical differences.

#### Results

Feed intake curves of multiparous and primiparous sows were different (p< 0.05) during the whole lactation period. Average daily feed intake of primiparous sows was  $5.78 \pm 0.67$  kg (3.41kg, 6.20kg, 6.96kg, 6.33kg averages per week), while that of multiparous sows was  $6.98 \pm 0.84$  kg (4.18kg, 7.44kg, 8.63kg, 7.68kg averages per week).

## **Discussion and Conclusion**

In conclusion, the average daily feed intake of multiparous sows was 22.5% higher than of primiparous sows during the lactation period. This study helps us to better understanding of voluntary feed intake of sows. Electronic feeders allow a more precise adjust of curves of electronic feeders and, in consequence, sows waste less amount of feed, specially primiparous ones.

# EFFECTS OF LIDOCAINE-HYDROCHLORIDE AND PROCAINE-HYDROCHLORIDE COMBINED WITH TWO INJECTION TECHNIQUES ON PAIN RESPONSES INDUCED BY PIGLET CASTRATION

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# **Background and Objectives**

From January 1, 2019, all piglets in Denmark must be treated with local anesthesia prior to castration due to an initiative by the Danish pig industry. The adopted procedure for local anesthesia is a 3-step injection technique with procaine-hydrochloride. However, a definition of the most suitable methods is not well-documented. This study aimed at investigating combinations of injection techniques and local anesthetic agents that provide the most efficient pain relief during castration.

#### **Material and Methods**

The study was carried out in a conventional Danish pig production herd with 770 sows. 200 male piglets were evenly and randomly assigned to 1 of 5 groups at 3-7 days of age and injected: without local anesthesia (CON), with a 3-step procaine-hydrochloride injection technique (PRO3), a 2-step procaine-hydrochloride injection technique (LID3), a 2-step lidocaine-hydrochloride injection technique (LID3), a 2-step lidocaine-hydrochloride injection technique (LID3), a 2-step lidocaine-hydrochloride injection technique (LID2). PRO2 and LID2 piglets were injected with 0,3 ml in each testicle, PRO3 and LID3 piglets were injected with 0,5 ml in each testicle, and CON piglets were only manipulated (shamhandled). Surgical castration was performed, respectively 5, 3 and 3 minutes after treatment with procaine-hydrochloride, lidocaine-hydrochloride or sham-handled. During the local anesthesia injection and castration, the equivalent continuous sound level ( $L_{eq}$ ) and the maximum peak ( $L_{max}$ ) were measured with a sound level meter and the movement intensity and time consumption were scored.

# Results

The local anesthesia reduced the sound and movement intensity during castration compared to controls (p<0,001). There was no difference during injection or castration between the groups PRO2, PRO3, LID2 or LID3. Due to the time consumption, the 2-step method was faster to perform than the 3-step method.

#### **Discussion and Conclusion**

Based on this study, the use of local anesthetic significantly reduces pain during castration whereas product and method were insignificant.
## PRELIMINARY EVALUATIONS OF CLAW LESIONS IN ITALIAN SOWS REARED ON DIFFERENT TYPES OF FLOOR

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## **Background and Objectives**

Claw lesions in sows are both a welfare and an economic issue with a multifactorial aetiology linked to nutritional, genetic, structural and management factors. Aim of this study was to evaluate claw lesions in Italian culled sows which were housed on different types of floor.

#### Material and Methods

Overall, 368 claws of 23 culled sows, housed in two Italian farms (A= concrete slatted floor; B=concrete continuous floor) were evaluated at slaughter for eight types of lesions. Namely, cracked wall (vertical and horizontal), heel-sole crack, white line damage, heel overgrowth and erosion, overgrowth (toe and dew claw), coronary band damage, sole ulcer. Differences between farms were assessed using a Fischer's exact test.

#### Results

Overall prevalence of lesions at the level of claws was 54.2% in farm A and 43.8% in farm B. Significant differences between farms were found for overgrowth (4.6% in farm A versus 0.0% in farm B, P=0.010) and coronary band damage (7.5% in farm A versus 0.0% in farm B, P<0.001). Prevalence of heel overgrowth and erosion was significantly higher (P=0.037) in farm B (25.0%) if compared to farm A (15.8%).

#### **Discussion and Conclusion**

Even though these results should be considered preliminary, significant differences between different floors were found for some specific claw lesions. Nonetheless, no difference was observed regarding the overall prevalence of all lesions. Overgrowth and coronary band damage were more frequent in farms with slatted floor, suggesting a potential link respectively with scraping quality of the concrete and openings in the floor. Furthermore, heel overgrowth and erosion were more frequent in the farm with continuous floor, suggesting that the scraping quality of the concrete floor may be inadequate. Nevertheless, these results should be interpreted with caution considering the limited sample. Further studies are required to investigate the influence of floor types on sows' claws in Italian farms.

# GENOMIC ANALYSIS FOR THE IDENTIFICATION OF GEN LOCI ASSOCIATED WITH SWINE INFLAMMATION AND NECROSIS SYNDROME (SINS)\*

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# **Background and Objectives**

The Swine Inflammation and Necrosis Syndrome was described in 2016 as a multifactorial complex, responsible for lesions of tail, ears, teats, coronary bands, claws, soles and heels in pigs. Preliminary results indicate a genetic influence on the degree of symptoms. The aim of the present study is to identify gene loci associated with SINS.

# Material and Methods

Degrees of inflammation and necrosis of tails, ears, teats, coronary bands, claws, soles and heels were characterised in 395 suckling piglets and 372 weaners. All piglets were offspring of 27 Dansk Yorkshire x Dansk Landrace crossbred sows and eight Pietrain boars. DNA was isolated from the tips of the docked tails. Genotypes were analysed with the Illumina BeadChip Porcine60SNP. Genome wide association studies were done with the R-pakage (rr-BLUP).

## Results

A total of 23 significant associations between lesion scores and genotypes were identified. The score for teatlesions of suckling piglets was significantly associated with nine markers on the chromosomes (SSC) 1, 6, 14 and X. Two markers on SSC 9 and X were associated with face lesions in weaners. Lesion scores of the suckling piglets' coronary bands correlated significantly with three markers on SSC X. The ear-lesion scores of suckling piglets were associated with two marker on SSC 2 and two markers on SSC 15 correlated with tail base lesions. Heel lesion scores of weaners were linked to a marker on SSC 7. Tail base lesions in weaners were associated with a marker on SSC 16.

# **Discussion and Conclusion**

Swine inflammation and necrosis Syndrome is a multifactorial complex, influenced by a polygenetic background. The Results of the present study show that genetic selection is an important opportunity for controlling SINS.

\*This study is funded by Akademie für Tiergesundheit, Bonn, Landesamt für Natur, Umwelt und Verbraucherschutz, Düsseldorf and Tönnies Forschung, Rheda-Wiedenbrück

# BOAR GENETICS AFFECTS SUSCEPTIBILITY TO SWINE INFLAMMATION AND NECROSIS SYNDROME (SINS)\*

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# **Background and Objectives**

SINS has been comprehensively characterized in recent years (1, 2). Symptoms occur on the tail, ears, teats and in the area of the coronary bands and claws. Although modified by environmental influences, they can already be observed in newborn piglets directly under birth (2). An overloading of the intestinal and liver metabolism, which is accompanied by considerable shifts in the metabolome and transcriptome, form the etiological basis. Measures that relieve both systems can considerably improve the symptoms and significantly increase the pigs' welfare (2). However, practical conditions point towards significant effects of boars' genetics. Herd observations suggest that Pietrain boar offspring are more sensitive to SINS than Duroc boar offspring. There also appears to be significant boar effects within Pietrain.The goal of the present study was to characterize these effects and to point out the metabolic pathways involved.

# **Material and Methods**

A set of Duroc and Pietrain boars was mated to a Yorkshire x Landrace sow herd. One semen portion each of one boar supposed to be more sensitive and one more resistant were mixed into one insemination portion. So piglets of both boar breeds were born in the same litter and were genetically assigned to their father only after completion of the clinical, SINS-specific examinations.

#### Results

The offspring of Duroc boars showed hardly any signs of SINS, while those of Pietrain boars were severely affected. Additionally, there were significant differences in offspring sensitivity between the Pietrain boars. Genome-wide Association Studies revealed first insights into possible genetic mechanisms.

# **Discussion and Conclusion**

The results open the way to the selection against SINS as an essential contribution to the improvement of animal welfare in pigs.

\*funded by: Akademie für Tiergesundheit, Bonn; LANUV, Düsseldorf; Tönnies Forschung, Rheda.
1: Reiner and Lechner, 2019, CAB Reviews 14, No. 040.
2: Reiner et al. 2019, Animal 13, 9, 2007-2017.

# EVALUATION OF ANAESTHETIC EFFICIENCY AND POST-OPERATIVE PAIN-MANAGEMENT DURING PIGLET CASTRATION UNDER ORGANIC FIELD CONDITIONS\*

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# **Background and Objectives**

The EU animal welfare act prohibits castration of male piglets without anaesthesia. Three alternatives are available: boar fattening, immunocastration and the castration with anaesthesia. Two methods are approved for the castration of suckling piglets under anaesthesia: inhalation anaesthesia with isoflurane and injection anaesthesia with ketamine and azaperone. In any case, controlling of post-operative pain by NSAID, e.g. meloxicam is indicated. The aim of the present study was to evaluate the efficiency and post-operative pain-management of both anaesthetic methods under organic field conditions.

# Material and Methods

On each of seven organic farms in Germany, 12 litters with at least six male piglets were included. Two-thirds were castrated with inhalation anaesthesia and one-third with injection anaesthesia. All piglets received meloxicam, half of both anaesthesia groups were additionally administered metamizol. In order to assess the pain during castration, the operation was recorded by camera. The postoperative pain behaviour of the piglets was filmed for 72 hours and subsequently evaluated with an ethogram. After 72 hours the castration wounds were scored.

#### Results

Our previous results demonstrate that the efficiency of anaesthesia by injection is much more variable than anaesthesia by inhalation. Piglets show more defensive movements at a higher intensity, more vocalisation and a higher decrease in body temperature. Both methods are equivalent in terms of postoperative haemorrhages and wound healing. The additional administration of metamizol had no significant effect on the clinical parameters. Both methods are massively affected by handling and farm management.

# **Discussion and Conclusion**

If the methods are well trained on farms, both can guarantee perioperative pain relief and good anaesthesia efficiency. However, postoperative pain and a prolonged recovery phase during injection anaesthesia, both remain. Therefore, both methods cannot be regarded as a last resort to castration of male suckling piglets.

\*This study was funded by the Bundesamt für Landwirtschaft und Ernährung, Bonn, Germany

# VALIDATION OF BIOFILM MEASUREMENT INDICATORS IN WATER LINES IN PIG FARMS AND COMPARISON OF DIFFERENT PIPE CLEANING PROTOCOLS

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# **Background and Objectives**

Pipe cleaning protocol is essential to limit biofilm development. This study aims to validate biofilm measurement indicators in water pipes of pig farms and to compare different cleaning protocols.

# **Material and Methods**

Three biofilm cleaning protocols were tested in 30 post-weaning rooms in 30 farms: FLUSHPIPE® (Successive of water and air under pressure), HYDROCARE® (silver chelate stabilized hydrogen peroxide) and succession of base and acid were tested each of the ten rooms. Four indicators were compared, before and after cleaning protocols at the end of pipes: bacteriological analysis of the water with colony count at 22°C (G22) and 37°C (G37), visual level of cleanliness of internal surface of pipes by endoscopy (END), measurement of ATPmetry on internal surface of pipes (ATPsurf) and color of water (COUL). G22 and G37 were compared at beginning and end of pipes.

# Results

Before cleaning, G22 and G37 showed a significant difference between beginning and end of the pipe in postweaning, indicating an increasing level of biofilm along pipes.All indicators showed also a significant difference before and after cleaning pipes. ATPsurf, END and COUL allowed an immediate result to objectify the presence of a biofilm which is not the case for G22 and G37.There is no statistical difference between the success rate of different protocols. But the results showed a trend to a better success of the base-acid protocol.

# **Discussion and Conclusion**

This study has shown the interest of several indicators to raise awareness of presence of biofilm in water line and usefulness of a cleaning protocol.

# IMPACT OF FUSARIUM MYCOTOXINS ON PIG PERFORMANCE AND THE EFFICACY OF A NOVEL ZEARALENONE-DEGRADING ENZYME IN VIVO

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# **Background and Objectives**

The mycotoxins deoxynivalenol (DON) and zearalenone (ZEN) are secondary metabolites of Fusarium fungi. In pigs, DON mainly influences feed intake and daily weight gain (DWG), while ZEN affects the reproductive tract. This study aimed to investigate the correlation between different concentrations of mycotoxins in feed and their effects on the reproductive tract and growth performance in pigs. Further, it aimed to prove the ability of a novel enzyme by BIOMIN to reduce the estrogenic effects of ZEN.

# **Material and Methods**

60 gilts were divided into six groups with different concentrations of mycotoxins in their diet; one control group, two groups with 0.9 and 3.0 ppm DON, three groups with 0.5 ppm ZEN, 1.5 ppm ZEN and 1.5 ppm ZEN plus the novel enzyme (ZENzyme<sup>®</sup>). Piglets were clinically examined daily and body weights were recorded weekly. Vulva sizes (VS) were measured twice a week. After 4 weeks, piglets were euthanized and dissected to determine uterus weight (UW).

# Results

The DWG did not differ significantly, however diarrhea was more frequently observed in the DON groups compared to the other groups. VS and UW were increased (P≤0.05) in the 1.5 ppm ZEN group compared to all other groups. VS and UW in the enzyme group was reduced to the size of the 0.5 ppm ZEN group indicating a reduction of toxicity of approximately 1 ppm. Both groups (0.5 ppm and enzyme) had increased (P≤0.05) VS compared to the control group, while no differences were discernable regarding UW.

# **Discussion and Conclusion**

This study showed the efficacy of the enzyme for decontamination of ZEN and the correlation between the severity of the effect of ZEN on the size and weight of the female reproductive organs and the concentration of ZEN in feed. Unexpectedly, there were no differences in DWG while there were indications for affected gut health in the DON-exposed groups.

# SEVERE VULVA BITING IN GESTATING SOWS; A CASE REPORT

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# **Background and Objectives**

Vulva biting is a welfare impeding behaviour especially seen in group housed sows fed with electronic sow feeders (ESF's). At a 1200 head SPF sow farm in the Netherlands, where 800 sows were housed in one dynamic group with 14 ESF's, periods with severe vulva biting were observed. This study aims to describe the incidence of vulva biting and associations with parity and production stage as well as the diagnostic process to detect potential causes and improvements in the farm.

# Material and Methods

Continuous video capture of the area near the entrance of 5 feeding stations was analysed in order to determine when, where and which sow(s) performed vulva-biting and what the possible causes are. Periods in which less than four animals were waiting to enter the ESF were excluded from the analysis. For a two-day period, every hour during seven minutes, vulva biting, and related behaviours and clinical signs, were quantified. Next risk factors were investigated systematically.

#### Results

Damaged vulvas were observed in 69% of the gestating sows, but not in gestating gilts. Biting was performed in the area near the entrance and in the feeding station. Vulva biting was only seen just after a new feeding day was started, particularly in the first nine hours. The analysis showed that overcrowding, residual feed (due to no water supply and or feed disposal rate ) in the feeding station and malfunction of the entrance gate were evident risk factors which contributed to vulva biting. The suspicion was raised that biting was performed by the older and thus dominant sows. We did not observe gilts vulva biting. Finally, diet contained too low dietary fibre.

# **Discussion and Conclusion**

Video analyses showed pivotal to diagnose the problem and define interventions, such as removal of old sows, adequate repair of ESF, adequate fibre intake and prevention of overcrowding.

# DENTAL HEALTH - LESIONS INDUCED BY TEETH GRINDING IN SUCKLING PIGLETS

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# **Background and Objectives**

Piglets are born with eight sharp needle teeth that can cause facial lesions on littermates and teat lesions on the sow, especially in large litters or in case of hypogalactia. Teeth grinding of the incisors (Id3) and canines (Cd) is frequently practiced to reduce these lesions. As the teeth are different in length and the dental adamantine is only 1.3 mm thick, grinding with the roller head is nearly inevitably resulting in opening the pulp cavity of the longer tooth. In a previous field study more than 95% of the pigs showed at least one opened tooth after grinding (Hessling-Zeinen 2014). The aim of this study was to evaluate the short- and long-term effects of grinding on tooth lesions.

# **Material and Methods**

The study comprised approximately 700 teeth of suckling up to slaughter pigs. First, the teeth have undergone a morphological examination. Thereafter, they were preserved in formalin and processed by decalcification, embedding in paraffin and staining with haematoxylin and eosin for histopathological examination.

#### Results

Most of the teeth with opened pulp cavities showed pathological findings like hyperaemia, purulent pulpitis with or without intralesional bacteria, pulp necrosis, fractures, irritation of dentin formations and foreign material like plant fibres in the pulp cavity.

# **Discussion and Conclusion**

Opened pulp cavities are frequent findings in pigs getting grinded their teeth just after farrowing. Open pulp cavities are associated with various pathological findings clearly indicating that grinding with the commonly used roller head frequently induces long-term damage. To avoid tooth damage by grinding the procedure routinely performed in all piglets as well as the used grinding head need a critical re-evaluation. Instead of the roller grinding head, a so-called teacup grinding head (Ellert 2017) for the grinding of single tooth is recommended.

# POSITIVE EFFECT OF CALSEAGROW®, A COMBINATION OF ALGA AND PLANT EXTRACT, ON THE MICROBIOTA OF POST-WEANING PIGLETS

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# **Background and Objectives**

Microbiota plays an essential role for animal well-being, nutrition and performance. Antimicrobials decrease the incidence of digestive disorders during the piglet post-weaning period. However, antibiotic resistance is a major issue. CalSEAGrow® is a natural combination between an alga and a standardized lemon extract rich in pectic oligosaccharides and flavonoids. In this study, its effect on post-weaning piglets microbiota has been investigated as an alternative to antimicrobials.

#### **Material and Methods**

Two groups of 20 post-weaning piglets, from the same batch in a French farm, were studied : TI – Control (Basal Diet), T2 – Basal Diet + tested product (1.1kg/ton of feed). The supplementation was given to the piglets from the 21st until 37th days of age. A feces anal sample was taken from each piglet and 16S rDNA Microbiota diversity has been determined.

#### Results

The supplemented group showed a significant decrease (p.value <0.05) of the reads corresponding to the genera Selenomonas, Dialister and Helicobacter by factors of 17, 33 and 3 respectively. Moreover, a decrease in Campylobacter genus 16S rDNA sequences (-22%, p.value = 0.24) was observed, especially for Campylobacter lanienae species (-44%, p.value = 0.13).

#### **Discussion and Conclusion**

This study showed a positive impact of supplementation on post-weaning piglets microbiota by exposing a significant decrease in Selemonas, Dialister and Helicobacter and a reduction in Campylobacter lanienae, that can cause such a broad spectrum of gastrointestinal diseases without clinical signs.

These results suggest that supplementation may improve the gut health status of post-weaning piglets and counteract some of the negative effects that occur when piglets are more challenged.

# AUTOMATED LESION SCORING IN SLAUGHTERED PIGS USING CONVOLUTIONAL NEURAL NETWORKS

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# **Background and Objectives**

The abattoir is recognized for its role in herd health monitoring, therefore many systems have been developed to score diseases, particularly respiratory conditions, in slaughtered pigs. Unfortunately, the collection of such data is expensive and time-consuming, making a systematic recording difficult. Conversely, automated systems, using algorithms such as "convolutional neural networks" (CNNs), would enable the recording of large amounts of data inexpensively. We aimed to develop a CNN capable of automatically scoring pleurisy in slaughtered pigs.

#### **Material and Methods**

A total of 5902 half-carcasses were inspected, photographed and pleurisy scored by veterinarians, using a simplified version of the "pleurisy evaluation on the parietal pleura" (PEPP) method. Thereafter, all pictures were "segmented", labeling 7 features including the cranial and caudal chest wall areas and lesions. Segmented pictures were used to train a CNN, the performance of which was evaluated by an independent test set of 200 images, measuring sensitivity, specificity, and accuracy rates.

#### Results

The average accuracy of the CNN was 85.5%, recognizing healthy and severe cases particularly well (accuracy rate = 96% and 92% respectively). Accuracy rates for less severely affected half-carcasses were slightly lower, ranging from 70% to 84%. The sensitivity rate for the recognition of diseased half-carcasses, regardless of severity, was 92% whereas the specificity rate was 96%.

# **Discussion and Conclusion**

Overall, the trained CNN was able to distinguish between healthy and diseased half-carcasses, performing particularly well for the recognition of healthy and severe cases. More mild cases showed slightly lower accuracy rates, likely due to the presence of small lesions mostly located in the cranial part of the chest wall. In conclusion, we found that automation technology such as CNNs can be used to score lesions in slaughtered pigs, potentially making it possible to gather massive quantities of data, useful for monitoring herd health.

# HEMOGLOBIN CONCENTRATIONS IN NURSERY, GROWING AND FINISHING GILTS INTENDED AS REPLACEMENTS IN BREEDING HERDS

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# **Background and Objectives**

The association between anemia in replacement gilts before selection and their subsequent first litter performance has not been evaluated. The objectives of this study were to establish parameters of hemoglobin (Hb) levels in nursery, grower and finishing stages, and to examine the effects of Hb status upon gilt reproductive performance.

# Material and Methods

Gilts (n=600) were identified at 4 weeks of age, ear tagged, and blood samples collected from ear veins for analysis with a HemoCue 200+ instrument. Hemoglobin concentrations were then determined for pigs at 9, 15, and 28 weeks of age. Replacement gilts were selected by standard farm protocol, and not on hematological status. Farm records were used to analyze gilt reproductive success. Farm staff determined stillbirths visually. For Hb concentrations, the ANOVA included age and selection with means compared with Tukey's test. Pearson correlation coefficients assessed the relationship among Hb concentrations and litter characteristics.

#### Results

Only 317 animals were selected as replacement gilts. At all days, Hb concentrations were similar between selected and non-selected gilts. The Hb status increased (P<0.05) from early nursery to late finishing stage. For selected gilts, litter characteristics included 12.8+0.16 liveborn, 0.74+0.16 stillborn, 0.29+0.04 mummies per litter. Hb concentrations at 28 weeks of age were correlated (coefficient 0.2332, P<0.0003) to the number of stillborn pigs. Number of stillborns was correlated (coefficient 0.312, P<0.0001) to total born.

#### **Discussion and Conclusion**

The results indicate that as gilt Hb concentrations increase, the number of stillbirths in the first litter increases accordingly. This result was not expected as previous studies reported the opposite relationship. The increase in stillborns may be related to the total litter size. Further investigation is needed to elucidate the relationship between Hb and stillborn pigs. Finally, the Hb concentrations collected at each life stage can serve as reference values.

## OCCURENCE OF CYSTOISOSPORA SUIS ON PIG FARMS IN BELGIUM AND THE NETHERLANDS

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# **Background and Objectives**

Despite high efficacy of toltrazuril against Cystoisospora suis the parasite still seems to be prevalent on farms in Europe.

## Material and Methods

We investigated 9 Dutch farms and 11 Belgian farms with an average of 982 sows/farm (min 230, max 4000) with (n=11) or without (n=9) oral based toltrazuril application regime to determine the influence of treatment on the prevalence of parasite excretion and diarrhoea. Ten litters per farm were sampled twice (12±2 and 16±3 days of life) and faeces were examined for the presence of oocysts by autofluorescence (semiquantitative evaluation) and faecal scores 1-4 with 3 and 4 considered as semi-liquid or liquid diarrhoea. 110 Belgian and 70 Dutch litters were included.

# Results

On farm level 12 farms were positive for C. suis (5 without and 7 with toltrazuril treatment), 15 (5 with and 10 without toltrazuril treatment) had diarrhoea. The mean toltrazuil treatment age was 4 days (standard deviation: 0.94). Only 2 farms treated at the 3<sup>rd</sup> day of life or earlier. These farms were negative for C. suis and had only few litters with diarrhoea. Overall, 18.3% of the litters showed diarrhoea at least once, and 38.9% of them excreted C. suis oocysts. One third of the litters (n=60) was untreated, of these 16.7% were diarrhoeic and 51.7% shed oocysts, while 19.2% of the treated litters (n=120) had diarrhoea and 32.5% showed excretion of oocysts. Medium to high oocyst shedding was detected in 56.8% of the samples from untreated and 40.0% of the samples from treated litters.

#### **Discussion and Conclusion**

Although toltrazuril application seems to reduce the prevalence of C. suis somewhat, treatment on these farms could not effectively interrupt the life cycle of the parasite or reduce diarrhoea. Early treatment seems recommendable for effective control of C. suis in suckling piglets.

## VITAMIN D STATUS IN DANISH ORGANIC OUTDOOR SOWS

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# **Background and Objectives**

Vitamin D is essential for a well-functioning body. It affects bone formation, the immune system, reproduction and several other body functions. There are no reference values for serum vitamin  $D_3$  levels in pigs and the knowledge on serum levels under natural conditions is limited. The aim of this study was to determine the serum vitamin  $D_3$  levels (measured as 25-hydroxyvitamin  $D_3$  (25(OH) $D_3$ )) in sows housed under outdoor conditions.

# **Material and Methods**

In August 2020 a study was conducted in Danish organic outdoor herds to establish the mean serum vitamin  $D_3$  levels for outdoor sows at the approximate peak levels. Blood samples from 97 sows from 5 different herds were obtained between the 11<sup>th</sup> and 28<sup>th</sup> of August. All sows were sampled within 3 days after weaning. Data on parity, body condition score 1-3 and meteorological data of sunlight hours in from June-August 2020 was obtained. Blood samples were analyzed twice by liquid chromatography coupled with tandem mass spectrometry.

# Results

A linear mixed model did not show any significant effect of farm, parity, body condition score, or hours of sunlight on serum levels of 25(OH)D<sub>3</sub>. Values of 25(OH)D<sub>3</sub> was normally distributed with levels ranging from [32.29; 133.79] mean level was 67.02 ng/ml (SD±16.53).

# **Discussion and Conclusion**

Since the serum levels found was obtained under natural conditions vitamin  $D_3$  production was regulated by the UVB light from the sun, the levels found can be assumed to be non-toxic. The results from this study could possibly help to define the reference intervals for optimal vitamin  $D_3$  levels in sows.

# PLASMA DISPOSITION KINETICS AND DISTRIBUTION OF TOLTRAZURIL AND ITS MAIN METABOLITE IN INTESTINAL TISSUES AND CONTENTS OF PIGLETS AFTER ORAL AND INTRAMUSCULAR ADMINISTRATIONS

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# **Background and Objectives**

Porcine coccidiosis caused by Cystoisospora suis is a major cause of diarrhea and poor growth in piglets worldwide. The only effective chemotherapeutic drug available for the control is oral toltrazuril. Recently, the first toltrazuril-iron based combination for injection has been developed for the concomitant prevention of coccidiosis and IDA in piglets (Forceris®, Ceva, France). This study aimed to evaluate, the disposition kinetics of toltrazuril and its main metabolite in the plasma and target tissues for Cystoisospora suis infection after oral (Baycox®) or intramuscular (Forceris®) application of toltrazuril in piglets.

# Material and Methods

56 piglets from 4 litters were included and randomly allocated to two treatment groups. Piglets in Group A were treated by injection on the second day of life (24h+). Piglets in Group B were treated with intramuscular iron dextran on the second day of life (24h+) and oral toltrazuril on the third day of life (48h+). Samples were collected at 1, 5, 13 and 24 days post-treatment. Concentrations of toltrazuril and its active metabolite (toltrazuril sulfone) were determined by HPLC analysis.

# Results

On overall, intramuscular (IM) application of toltrazuril resulted in significantly higher and more sustained concentrations in blood plasma, intestinal tissue (ileum and jejunum) and intestinal content. In comparison after oral dosing, higher tissue concentrations were observed only immediately after dosing (day 1). Remarkably, toltrazuril and toltrazuril sulfone accumulated more in proximal intestinal segment (jejunum), independently of the administration route. Strong correlations (R<sup>2</sup>>0.9) were observed between plasma and tissue concentrations.

# **Discussion and Conclusion**

Significant drug concentrations at the site of action where C. suis, an intracellular parasite, reproduces and multiplies, are important for its desired therapeutic effect. Higher and more sustained concentrations were observed following IM application, which may be responsible for its higher anticoccidial activity due to the intensity and duration of its pharmacological effect.

# COMPARISON OF INJECTABLE TOLTRAZURIL-GLEPTOFERRON AND ORAL TOLTRAZURIL+ IRON DEXTRAN FOR THE PREVENTION OF ANAEMIA UNDER THE FIELD CONDITIONS

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# **Background and Objectives**

Iron Deficiency Anemia (IDA) causes poor immune response and growth in suckling piglets. Recently, a single dose combination product (Forceris<sup>®</sup>) has been developed for the concomitant control of piglet cystoisosporosis and IDA. The aim of the present study was to confirm the efficacy of injectable toltrazuril-gleptoferron under field conditions in Chile.

# Material and Methods

148 piglets were randomly allocated to 2 treatment groups: Piglets in injectable toltrazuril- gleptoferron group (n=74) received a fixed IM dose of 45 mg toltrazuril + 200 mg iron- gleptoferron per piglet on the 3<sup>rd</sup> study day (SD). Piglets in the Iron Dextran+Toltrazuril group (n=74) received 200 mg of parenteral iron dextran on the 3<sup>rd</sup> SD + 20 mg toltrazuril/kg body weight per os on the 4<sup>th</sup> SD. Body weight and haemoglobin were determined on 3<sup>rd</sup> and 20<sup>th</sup> SD. Total Iron content (TOT-Fe), Iron Repletion Efficiency (IRE) were calculated according to Patterson et al, 2008. The % of anaemic piglets was calculated by considering Hb levels < 90 g/l as anemic,  $\ge$  90 g/l and < 110 g/l as suboptimal and  $\ge$  110 g/l as optimal. Groups were compared by using unpaired t-test for normally distributed data or Kruskal-Wallis test. (GraphPad Prism 8.4.2.).

# Results

BWs were comparable at the beginning of the study 3<sup>rd</sup> SD (P= 0.1291). The body weight gains and final weaning weight were significantly higher in injectable toltrazuril- gleptoferron group (6.70 kg vs. 6.15 kg) (P= 0.0075). The % anaemic piglets (17% vs. 74%, P <0.0001), the TOT-Fe and IRE were also significantly different at the end of the study.

# **Discussion and Conclusion**

Forceris<sup>®</sup> was safe to use and a single application resulted in better growth and higher iron replenishment efficiency than the standard iron dextran + oral toltrazuril.

## PRODUCTIVE IMPACT OF SOW VACCINATION DURING LACTATION ON SOW AND OFFSPRING PERFORMANCE

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# **Background and Objectives**

Post-farrowing vaccination in sows is routinely applied to protect the breeding stock against Parvovirus sp. and erysipelas. The impact that this strategy may have on sow and piglet's production performance parameters when applied during their lactation period have been poorly studied. For that, the objectives of this study were to evaluate the potential impact that vaccination during the lactation period may have in sows and their offspring's performance.

#### **Material and Methods**

A total of 61 sows and 654 piglets were included in the study. Sows and piglets were aleatory assigned into 2 vaccination protocol groups; 1) Vac: Sows vaccinated at day 10 (d10) post-farrowing with a bivalent Parvovirus sp. (PPV) and erysipelas (ERY) commercial vaccine. 2) No Vac: Sows were inoculated at d10 with 2 ml of a physiological saline solution.Temperature (T<sup>a</sup>, °C) and daily feed intake (DFI, Kg/day) were recorded daily in sows, weight was registered in piglets at birth (Bdw, Kg), day 10<sup>th</sup> (Bd10w, Kg), and weaning (Bdwe, Kg). Average daily weight gain was calculated at d10 of lactation or vaccination day (ADG-B:10, kg/d), and then at weaning (ADG-10:W, kg/d).All results were analysed using R software.

#### Results

Results from the model showed that the average weight gain difference in the piglets **control group was 8 grs higher** than in the treatment group (p-value=0.0411). However, no differences in temperatures and intake percentages after vaccination were found between such groups. Interactions between these covariates and the group factor were also not statistically significant.

#### **Discussion and Conclusion**

Results from this study demonstrated that vaccination protocols post-farrowing had an impact in average daily gain of piglets. Under the conditions of this study, variables such us sow feed intake and temperature in sows were not significant. Therefore, more studies are needed to understand if more variables can be affected.

# SPECIFICITY AND SENSITIVITY OF PRRS XR ANTIBODY ELISA (BIOCHEK) AND COMPARISON WITH OTHER COMMERCIAL KITS

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# **Background and Objectives**

Since no clinical signs are typical for PRRS, antibody, RNA and antigen testing are necessary for diagnose and follow up of the formulated interventions. Antibody tests can be used for PRRS diagnosis, and to assess exposure status of pigs. Testing cross sectional and longitudinal samples allows to estimate the time when infection occurs in farrow to finish farms. There is a number of ELISA kits available in Europe that differ in sensitivity and specificity. The aim of the study was to compare sensitivity and specificity of a new kit recently introduced on the market, PRRS XR antibody ELISA (BioChek, the Netherlands), with 7 commercial ELISA's.

# **Material and Methods**

For comparing 8 ELISA's, a samples set from 5 PRRSV positive pig farms (3 stable and 2 unstable) and 5 PRRSV negative sow farms were used. Regarding the specificity the amount of false positives was determined in sow farms. The sensitivity was measured based on detection of seroconversion in different age groups in relation to virus detection.

#### Results

Regarding the specificity the BioChek ELISA scored 99.3%. Two other ELISA showed a poor specificity (<96%). The BioChek PRRS ELISA early detected active antibody immune responses with a comparable sensitivity (up to 100%) to most other ELISA's The sensitivity of most ELISA's is high but there are differences in response time. MDA's were less and shorter detected by the BioChek ELISA on PRRS stable farms without herd infection in the nursery.

# **Discussion and Conclusion**

The new BioChek PRRS ELISA has a good specificity and sensitivity which makes it suitable for diagnose and monitor PRRS on both SPF and conventional farms. The apparently lower sensitivity towards MDA's in the stable farms is difficult to explain and warrants further studies. However, as the ELISA measured PRRSV antibodies have no or limited correlation with protection, the practical significance of this phenomenon is limited.

# ATYPICAL ULCERATIVE DERMATITIS IN A SPANISH FARM: A CLINICAL CASE

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# **Background and Objectives**

Porcine Ulcerative Dermatitis Syndrome (PUDS) is a sporadic and chronic disease affecting only to single animals and it has not been associated to evident negative effects in their health or productivity. These could be the causes why there is very little literature or information about this disease. This abstract shows the clinical, laboratory and pathological findings in an atypical case of ulcerative dermatitis occurred during Autumn 2019 at a commercial farm in South-East Spain.

# **Material and Methods**

In a 1,550 sows commercial farm, one sow appeared with skin lesions in the mammary region and flanks, and 10 more showed similar lesions but only in vulvar area appeared simultaneously. They didn't show any other clinical symptoms. A biopsy from the flank skin was obtained for histopathological diagnosis, and blood samples for haematology and biochemistry analysis were obtained. Ten normal sows were sampled as negative control. Immunohistochemistry to detect apoptosis markers was developed.

#### Results

The lesions resolved into 1.5 months, but not immediately to the treatment. The histopathology showed areas of necrosis of the epidermis with ulcerated zones and under them abundant mixed inflammatory infiltrate. Adjacent to the ulcerated zones there were hyperplasia of the epithelium with hyperkeratosis. Under the areas of hyperplasia of the epithelium the inflammatory infiltrate, principally lymphocytes, was concentrated in the dermal-epidermal junction. The haematology and biochemistry showed differences for Basophil count, AST and albumin, all decreased in dermatitis group.

# **Discussion and Conclusion**

This abstract report a rare disease that never has been never described previously in vulvar area. Based on the clinic and the lack of response to treatment this could be a case of PUDS. The lack of increase of CRP contributed to exclude an acute inflammation and an infectious origin. However, the pathological findings pointed out other possibilities such as interface dermatitis.

# TEMPERATURE CIRCADIAN CYCLE IN SOWS IN POSTWEANING DURING SUMMER IN SOUTH-EAST SPAIN

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## **Background and Objectives**

There is a lack of information regarding temperature circadian cycle in pigs. The ability to thermoregulate depends sometimes on the environmental factors such as temperature and relative humidity. And it is known that environmental temperature can influence the appearance of oestrus, resulting in anoestrus during summer in warm countries. This work has as objective to describe some parameters related to temperature circadian cycle in sows afterweaning, comparing summer with all the other seasons.

# Material and Methods

The skin temperature was assessed in 65 sows, measured by iButton dataloggers (Maxim Integrated Products, USA) fixed in the cervical region. The devices registered 2,049 temperature values over one week after-weaning. The parameters calculated were stability (IS), fragmentation (IV), amplitude (RA), daytime and night-time values (M8 and L8), their timing (TM8 and TL8), and mean temperature (Tm) over the whole period. For analysis data were grouped in summer (August; n:21) and rest of the year (n:44).

#### Results

There were differences for IS comparing rest of the year with summer (0.38±0.03 vs. 0.74±0.04, p<0.001), IV (0.08±0.01 vs. 0.04±0.01, p<0.01), RA (0.01±0.00 vs. 0.02±0.00, p<0.001), M8 (37.9±0.2 vs. 39.3±0.1°C, p<0.001), L8 (37,5±0.2 vs. 38.1±0.1°C, p<0.05), TM8 (17:47±00:48 vs. 18:04±00:21 h, p=0.82) TL8 (05:00±00:52 vs. 08:13±00:09 h, p<0.01) and Tm (37.7±0.2 vs. 38.7±0.1°C, p<0.001).

# **Discussion and Conclusion**

There are differences during the summer period, including an increase of body temperature of 1°C. The increased IS and IV means that the body temperature during summer is more related to environmental temperature. The sows delayed their temperature rhythm more than 3 hours the cycle during summer, related, probably with the sleep accommodation.

# SPECIFIC REAL-TIME PCR ASSAY FOR DETECTION OF CYSTOISOSPORA SUIS IN PIGLET FECAL SAMPLES

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# **Background and Objectives**

Cystoisospora suis is an important coccidial protozoan parasite causing severe diarrhea in piglets. Detection and enumeration of the oocystes by autofluorescent microscopy and flotation techniques are widespread standard methods to identify the agent (oocysts) in the faeces. Furthermore, PCR assays have also been developed for sequence-based identification. Here we describe a new real-time PCR assay for rapid detection and quantification of C. suis.

# Material and Methods

Faecal samples of diarrheic piglets from experimental as well as field circumstances were collected. DNA was extracted using QIAGEN Power Fecal DNA Kit. Cytochrome c coding gene and ribosomal ITS region of C. suis genome were amplified and sequenced. Based on sequence data 4-4 primer combinations were designed and tested. Thermal profile of the assay was optimized by gradient real-time PCR. Similarly, parameters of mechanical disruption and enzymatic lysis were also optimized. To validate the assay, autofluorescent and flotation based detection of oocystes was carried out from the same faecal samples.DNA extraction was optimized for silica spin filter tubes as well as 96-well plate design. The ITS-specific real-time PCR was developed and optimized for different thermocyclers.

#### Results

A ITS-specific primer-probe combination was found to be the most sensitive assay. Parallel sample analyses resulted in a good reproductivity of the assay; average Ct difference among parallels is only 0.7 without contradiction in positivity. Results of autofluorescent detection have a good correlation with Ct values.

# **Discussion and Conclusion**

The developed qPCR assay provides a specific and sensitive tool for diagnosing C. suis infection. Larger scale evaluation of the assay on field samples is necessary to evaluate its usefulness in high throughput sample processing.

# TWO UNUSUAL CLINICAL CASES OF BALANTIDIUM COLI IN SWINE FARMS IN ISRAEL

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# **Background and Objectives**

We describe two unusual outbreaks of Balantidium coli in several swine farms, causing chronic enteric symptoms, growth delay, and mortality increase, in the same growing phase.

# Material and Methods

In 4 close-cycle farms, 4,050 sows in total (200 to 2,000), an estimated 13,700 to 18,300 growers (4th to 6th months age) were affected. 28 pooled fecal samples from diarrheic pigs from all farms were collected; submitted to bacteriological examination and smear technique for parasites at low x100 or high dry x400 magnifications. Feed samples, supplied from two feedmills, were examined for levels of mycotoxins potentially inducing enteric clinical signs and grinding size of grains (excluding soy).

#### Results

Samples resulted negative to pathogenic bacteria (E.coli; Salmonella spp) routinely investigated in these cases. Lawsonia intracellularis, Brachispira hyodisenteriae or pilosicoli, Porcine Epidemic Diarrhea Coronavirus, were never evidenced in Israel. All the four farms resulted positive to Balantidium coli, at its trophozoite mobile phase, in both outbreaks. 23 out of 28 samples resulted positive: 66,7% in the first event; 100% in the second. The concentration of grain-origin mycotoxins (Aflatoxin, Vomitotoxin-Deoxynivalenol (DON), T-2 toxin) resulted well below toxicity levels. Municipality water supply excludes contaminations. Grinding resulted too coarse in both feeds (feed A: >50% grains ranged 840⊠ to 2380⊠; feed B: >31% ranged 1000⊠ to >1400⊠).

# **Discussion and Conclusion**

Balantidium coli is commonly found in the large intestine of pigs without particular clinical relevance; it feeds mainly on starch. Starch should not be found in abundance in the large intestine, as almost all of its digestion (up to 98%) occurs along the small intestine at a suggested grain size of 640-650<sup>II</sup> in growers. Starch may escape digestion in coarsely grinded grains, reaching the large intestine undigested, inducing massive B.coli multiplication. Feed grinding resulted too coarse in both feeds. Therapy consisted of Oxytetracycline at 1000ppm in feed/7 days; feeds were changed.

## RARE EARTH ELEMENTS AS GROWTH PROMOTERS IN PIG FEEDING

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# **Background and Objectives**

Purpose of this study was to measure the efficacy of lanthanide citrate, a rare earth element (REE) compound, for its growth promoting effects in weaned piglets.

# Material and Methods

1) In an experimental study with 192 piglets (12 replicates, 8 piglets/replicate, 8 kg start bw), a control and a treatment group with 250 mg Lanthanum-Cerium-citrate/kg feed were fed for 42 days. Bw and feed intake were measured weekly, the feed conversion ratio (FCR) was calculated.

2) In a field study with Lanthanum-Cerium-citrate, fattening pigs were fed from 39 kg bw till slaughter at 105 kg. FCR was calculated, carcass and meat quality parameters were analysed.

#### Results

1) The body weight of the treatment group (20.1 kg) was 6.9% increased compared wih control (18.8kg). FCR improved significantly by 15.3% from 2.29 kg feed/kg bwg (control) to 1.92 in the treatment group.

2) FCR improved significantly by 3.8% for animals (male,female) from 2.93+0.06 kg feed/kg bwg (treatment group) to 2.82+0.07 in the control, and by 5.7% calculated only for male. At slaughter, lean meat content of the carcasses in the treatment group was significantly 3.6% higher than in control. Furthermore, the classification after E.U.R.O.P. system resulted in a 23% higher rating in "E" (best class, lean meat >55%) for lanthanide-citrate as compared to control.

# **Discussion and Conclusion**

REE have been known for a long time in China as growth promoters in pigs. In 2019, the EFSA concluded that lanthanide citrate, given to piglets up to 120 days of age, is efficient and does not present a safety concern for consumer or environment. Since October 2020, Lanthanum-Cerium-citrate. (Lancer®) is authorised as a zootechnical additive in pig nutrition. REE are supposed to excert an influence on gut microbial population. Especially, fecal Lactobacillus counts are reported to increase under the influence of REE in pig diet.

# THE EFFECT OF RESPIRATORY DISEASE ON ANTIMICROBIAL USE IN IRISH PIG PRODUCTION

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#### **Background and Objectives**

Respiratory disease has been suggested as a major reason for antimicrobial use (AMU) in pigs although supporting quantitative scientific evidence is scarce. This study aimed to quantify the effect of respiratory disease on AMU in Irish pig farms.

#### **Material and Methods**

Fifty-four farrow-to-finish herds were enrolled in the study. AMU data comprised medicated feed and prescription records for 2016 and is expressed as mg/PCU (ESVAC). Data on pluck (heart, lungs, liver) lesions and blood samples (32finishers/farm) were collected at slaughter. An average of 162 (range 55-308) plucks/herd were assessed for pleurisy, pneumonia, lung abscesses, pericarditis, and milk spots. Serological testing for swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus, Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae (APP) was performed using IDEXX ELISA kits. Vaccination data was obtained via telephone contact. The effect of respiratory disease on AMU was modelled using multivariable linear regression. A forward regression approach was used with a 0.10 cut-off for inclusion of predictors in the model. Predictors are presented as coefficient±SE.

#### Results

The respiratory disease model explained 31% of the variability in AMU(mg/PCU). AMU increased with APP prevalence ( $1.7\pm0.49$ mg/PCU, P=0.001); with abscess prevalence ( $31.1\pm9.08$ mg/PCU, P=0.001), in farms vaccinating sows for SIV ( $87.4\pm28.94$ mg/PCU, P=0.004) compared to non-vaccinating farms, and tended to decrease with the prevalence of pneumonic lesions(- $2.1\pm1.11$ mg/PCU, P=0.07).

## **Discussion and Conclusion**

Respiratory disease was confirmed as a major risk factor for high AMU in Irish pig farms. Vaccination for SIV, lung abscesses at slaughter, and exposure to APP were the main indicators of AMU. SIV vaccine is typically used on Irish farms with more severe flu problems. APP prevalence is related to higher exposure, once APP vaccination is not routinely practiced on Irish farms. The link between pneumonia and AMU requires further research.

## LAMENESS IN GROWING PIGS IN FLANDERS, BELGIUM

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# **Background and Objectives**

Lameness in growing pigs is often reported and include infectious, traumatic or metabolic causes. In Flanders, Belgium, locomotion disorders are also often encountered, however thorough causal examination is limited. Therefore, Veepeiler Varken started a project to gain insights about lameness in growing pigs in Flanders.

# **Material and Methods**

In seven farms in which lameness in growing pigs was a consistent finding, a maximum of 5 lame, nontreated pigs were euthanized. Most of the joints were macroscopically inspected. Swabs of both tarsi and carpi were collected for cultivation and PCR. Right and left humerus and femur condyls were histologically examined. Control animals, non-lame non-treated animals from other farms were included.

#### Results

In total, 25 lame and 15 non-lame animals were examined. Gross lesions were observed in 40 joints (31%) of lame animals only. Cultivation was positive in 7 of 42 swabs. A total of 138 PCRs from lame animals were performed, of which 29% detected genetic material of swine pathogens. Haemophilus parasuis and vtaA10 were rarely found (13.0% and 4.3%, respectively). Mycoplasma hyosynoviae was found in half of the swabs examined (52.2%). Mycoplasma hyorhinis was detected in 17.4% of the swabs. No bacterial growth and/or genetic material was found in control animals, except for one (Mycoplasma hyosynoviae).Histological abnormalities were found in 11 of the 25 lame pigs examined, and at each farm there was at least 1 animal with bone defects. Further, histological examination revealed 17 and 6 affected epiphyseal plates and growth plates, respectively. In control animals, 60 histological examinations were performed. In 6 and 3 control animals affected epiphyseal plates and growth plates, respectively, were found.

# **Discussion and Conclusion**

We have demonstrated a high number of Mycoplasma hyosynoviae in joints of growing pigs in Flanders, as well as histological changes in growth plates. Obviously, M. hyosynoviae provokes lameness in growing pigs.

Miscellaneous

# EFFECT OF DIFFERENT METHODS OF ADMINISTRATION OF TOLTRAZURIL-IRON BASED COMBINATION FOR INJECTION ON THE PK OF TOLTRAZURIL AND THE PREVENTION OF IRON DEFICIENCY ANAEMIA (IDA)

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# **Background and Objectives**

The needle free injection systems were developed for mass administration of iron preparations in piglets. Forceris® is the first combination product registered within EU for the concomitant prevention of IDA and prevention of clinical signs of coccidiosis (diarrhoea). The aim our study was to investigate and compare the pharmacokinetics of toltrazuril and hematinic activities following intramuscular application by injection with needle and Needle free device (MS Pulse) of Forceris.

# Material and Methods

10 randomly selected piglets per group were treated with injectable toltrazuril+ gleptoferron on the 2<sup>nd</sup> DOL (group A- needle free, group B- injection by needle), were blood sampled for assay of Toltrazuril and Toltrazuril sulfone (HPLC) on 5 time points (1, 5, 13, 19, 24 days post treatment). A maximum 4 piglets per one litter were selected. Safety and hemoglobin (Hb) levels of piglets were evaluated at birth and at weaning by using HemoCue (39 piglets A, 40 piglets B).

# Results

The two application methods yielded similar plasma profiles for toltrazuril and its main metabolite toltrazuril sulfone. Both route of administration resulted in similar time -concentrations profiles, AUC and Cmax. The mean haemoglobin levels at weaning were not significantly different with 109.6 g/L in group A and 111.1 g/L in group B. Only one (1) anaemic (Hb < 90 g/L) piglet was observed at weaning (24 DOL) in both groups. No local/general safety issue was recorded.

# **Discussion and Conclusion**

This exploratory study provides the first positive data about injectable toltrazuril+ gleptoferron needle-less injections on piglets and its effect on toltrazuril PK and haematinic effect.

# PHARMACOKINETIC STUDY OF PARACETAMOL FOLLOWING ORAL APPLICATION IN SOWS

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# **Background and Objectives**

Thanks to its favourable safety profile, Paracetamol is frequently used in swine medicine together with other NSAID's for treatment of fever and pain during flu syndromes or acute respiratory disorders, as well as for the control of postpartum disorders (MMA). The aim of this study was to determine the plasma and colostrum/milk kinetics of paracetamol following application of 30 mg/kg/day divided in two administrations (morning, evening) for 3 consecutive days in sows, starting at farrowing.

# Material and Methods

Paracetamol (Pracetam 40%, Ceva) was administered per-os to 4 different parity sows according to the following protocol: syringe application of the dose at the base of the tongue. Animals received the treatment as follows:  $D_{0_b}$ ,  $D_{0_b}$ +12hrs,  $D_{0_b}$ +24hrs,  $D_{0_b}$ +36hrs,  $D_{0_b}$ +48hrs,  $D_{0_b}$ +60hrs and  $D_{0_b}$ +72hrs 30 minutes after their food ration dispensed. Two milk samples were collected per day for 3 days. 12-hours time-interval was retained between samplings and UPLC-MS-MS method was used for the assays. To compare the concentrations of parent drug (4-acetanominophen) in milk and plasma, plasma concentrations were obtained from the publication in minipigs. PK curve in milk was extrapolated from plasma concentration using a plasma/milk ratio of 0.94 from human data.

# Results

Overall (n=24) mean concentration (SD) of acetaminophen was 6.89 (3.13)  $\mu$ g/mL, with relatively high variability (2.3 - 14.30). Means obtained for individual sows were as follows: 4.05 (1.46), 8.40 (3.81), 5.70 (1.23), 9.40 (2.30)  $\mu$ g/mL.

# **Discussion and Conclusion**

Based on PK profiles simulated from plasma and sparse data obtained from sows, ratio close to 1 was obtained and transfer of the active into milk/colostrum of sows confirmed. Future study exploring potential effect of transmammary delivery of paracetamol to piglets needs to be performed.

## AHI PIG HEALTHCHECK PROGRAME

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# **Background and Objectives**

Animal Health Ireland (AHI) is a public private partnership between private sector organisations and businesses in the agri-food sector and the Department of Agriculture, Food and the Marine (DAFM) in Ireland. The Pig HealthCheck is an AHI-led programme co-funded by pig producers and DAFM, with the aim of improving the profitability and sustainability of the Irish pig industry through improved animal health. The programme has started in 2019 and it is focused in providing a holistic picture of animal health and welfare status at farm and national level, through the use of bench-marking tools to allow farmers to monitor their status for a range of measures and to compare this with the national profile.

# **Material and Methods**

The programme is based on 5 areas of activity: the delivery of assessments of 1) herd biosecurity and 2) risk factors for tail biting; the 3) capture, analysis and reporting of abattoir data from ante and post mortem meat inspection; and the integration of 4) health data with antimicrobial usage (AMU) data and of 5) AMU/health data with the National Salmonella Control Programme data.

# Results

As of 31st October 2020, 185 and 126 units (49.6% and 33.8% of the commercial pig units in Ireland) have been reviewed in terms of their biosecurity and risk factors for tail biting respectively. The median score for biosecurity was 70% (77% for external biosecurity and 61% for internal biosecurity). For tail biting, the lack of provision of proper enrichment materials was the main risk factor identified.

# **Discussion and Conclusion**

These results suggest that Irish pig farmers put more emphasis on protecting themselves against external hazards (such as African Swine Fever) than avoiding the spread of endemic diseases within their farm and that there is scope to implement measures to assist farmers to progress to rearing pigs with intact tails.

# CONGENITAL ORAL SQUAMOUS CELL CARCINOMA IN A SUCKLING PIGLET

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## **Background and Objectives**

Cancer is rarely detected in pigs, likely due to their short lifespan. Lymphosarcoma, nephroblastoma, melanoma and hepatic tumors are considered to be the most common porcine neoplasms, while other tumors are sporadically observed in this animal species. The present report aims to describe the main pathological features of a case of oral cancer recently observed in a suckling piglet.

# Material and Methods

The event occurred in a small farrow-to-finish pig herd located in central Italy, which experienced a severe episode of colibacillosis in suckling piglets, with a relevant increase of mortality. Several piglets were autopsied for diagnostic purposes. One of them showed necrotic and ulcerated lesions affecting the oral mucosa on the inner surface of the lower lip, close to the incisors. Tissue samples were collected and routinely processed for histopathological investigation.

#### Results

The microscopic examination of oral lesions demonstrated the presence of a neoplastic proliferation, consisting of large, polygonal epithelial cells, provided with a single nucleus with a prominent nucleolus and abundant cytoplasm. Neoplastic cells were arranged as cords and islands, leaking from the epithelium and infiltrating the underlying lamina propria. Keratinization of individual epithelial cells and horn pearls were detected throughout the tumor. A very high number of mitoses were observed. In addition, desmosomal junctions were clearly seen between adjacent neoplastic cells. On the basis of the above findings, taking into account that tumors are defined as congenital when detected in fetuses and newborns until 2 months of age, the final diagnosis of congenital and multiple oral squamous cell carcinoma (SCC) was made.

#### **Discussion and Conclusion**

To the best of our knowledge, only two cases of oral SCC have been described thus far in pigs, both affecting aged pot-bellied pigs. Therefore, the present report describes an exceptionally rare disease condition, also representing the first case of a congenital oral SCC ever described.

# BACILLUS-BASED PROBIOTICS CAN DECREASE VARIATION AMONG WEANED PIGLETS

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# **Background and Objectives**

Every producer experiences variation in the weaning weight of piglets. Therefore, sorting the piglets for weight is often done which requires extra work, especially for the smallest pigs that might require special diets and handling. If the variation continues throughout the nursery phase additional work and sorting of pigs is required when the pigs enter the grower phase. Therefore, if the weight variation can be decreased the workload in handling the flow of pigs through the production system can be eased.

The aim of this study was to investigate whether a dual-strain probiotic product could have an effect on reducing weight variation among piglets in the nursery phase.

# **Material and Methods**

The study was carried out at a European research facility where 384 weaned piglets were divided evenly into two treatment groups (control (TI) and probiotic group (T2)). The trial lasted from weaning until 42 days postweaning (BW 8 – 26 kg).

Two probiotic strains, B. subtilis (DSM 25841) and B. amyloliquefaciens (DSM 25840) were included only in the diet for T2 for all 42 days at an inclusion rate of 1.1E+09 CFU per kg feed.

The weight variation among the piglets in both treatment groups were monitored three times during the trial period: at weaning, 14 days after weaning and at the study end 42 days after weaning.

#### Results

The weight variation amongst the piglets in TI and T2 did not differ significantly at the start of the trial or after 14 days on trial. However, at day 42, T2 had a statistically significant lower weight variation among the piglets compared to TI.

# **Discussion and Conclusion**

This study shows that this dual strain probiotic combination can reduce weight variation significantly in postweaned piglets when fed throughout the nursery phase, resulting in a more homogenous group entering the grower phase.

# CONGENITAL MALFORMATION OF THE FRONT CLAWS OF A PIG

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# **Background and Objectives**

During a sow farm visit, a lame piglet was noticed with a malformation of both claws of both front legs. Other pigs in the litter and in the compartment did not show abnormalities. The pig was transported to the Farm Animal Health clinic to exactly diagnose which abnormalities were present.

# Material and Methods

After arrival, the pig was examined clinically and X-ray examination was performed. After four days, the pig was euthanized and necropsy was performed.

# Results

Clinically, breathing frequency, and heartrate were slightly elevated during the 4 days, whereas body temperature was only elevated on day 1. Because of the fever on D1 Meloxicam was administered on D1 and D3.

All four hoofs in the front legs were absent and the dew claws were absent or distorted. The legs were, however, not painful.

X-ray examination revealed that left front leg missed phalanges 2 and 3 of digit 3 and 5 and phalanges 1, 2, and 3 of digit 4.

The right front leg missed phalanx 3 of digit 3 and phalanges 3 and 4 of digit 4.

In addition, the middle phalanx of the fifth toe had signs of osteomyelitis.

At necropsy, in addition to the missing bones in the left front leg an abscess was observed, originating from a pododermatitis as well as signs of an osteomyelitis.

# Discussion and Conclusion

This case study describes a malformation of the claws of both front legs combined with missing phalanges on both front legs. The malformation is probably due to a defect of the dermal ossification of the phalanges. As far as the authors know, this anomaly has been described for foals, but not for pigs.

#### ADENOCARCINOMA IN A SLAUGHTERED PIG

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## **Background and Objectives**

Naturally occurring neoplasia in pigs is rare due to the short life span. Pig tumors do not frequently cause clinical signs and are typically found as incidental lesions in diagnostic specimens or carcass at slaughter. Lymphosarcoma including leukemia and lymphoma is most common tumor in pig, followed by nephroblastoma, melanoma. hemangioma, rhabdomyosarcoma. Adenocarcinoma in pig has rarely been reported. We detected a adenocarcinoma in a slaughtered pig, and describe gross and histopathological lesions.

# Material and Methods

Many nodules were incidentally found in a slaughtered pig in December, 2018 Korea. The pig was female, 6 month old. Diaphragm and abdominal muscles with nodules were submitted for diagnosis. Histopathological examination, bacterial culture, and polymerase chain reaction (PCR) for Mycobacterium tuberculosis complex were performed.

#### Results

Grossly, many round red or gray nodules, 0.5 - 1.0 cm in diameter were observed in diaphragm and abdominal muscles in a slaughtered pig. Some parts of the nodules was caseous. Histopathologically, the nodules were consisted of numerous neoplastic tubular or papillary glands. The lumen of the glands was empty. The glands were consisted of one to five layer columnar cells. The nuclei of it were round or spindle. Mitotic figures were frequently observed. Necrosis and calcification were found in the center of the nodules. Tumor emboli were found in lymphatics. The glands had invasiveness and were surrounded by connective tissues or eosinophils.No pathogenic bacteria was isolated from diaphragm and abdominal muscles. And PCR for Mycobacterium tuberculosis complex was negative.

#### **Discussion and Conclusion**

This report might be valuable because adenocarcinoma is very rare in pig. The adenocarcinoma in this study should be differentiated from tuberculosis and other tumors. Based on histopathological and bacterial tests, this case was confirmed as adenocarcinoma in diaphragm and abdominal muscles.

# EFFECT OF DUST REDUCTION IN ANTIBIOTIC POWDERS (MEDICATED PREMIXES)

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# **Background and Objectives**

Antibiotic powders release dust each time they are handled (opening the container, dosing, weighing, mixing). This dust always contains a certain amount of active ingredient. Farmers and treated animals can absorb this dust via the respiratory tract. Depending on the active ingredient, also penetration of the skin is possible. Environment and equipment may be contaminated. To what extent this diffusion of dust contributes to the emergence of antibiotic resistance is largely unknown. Certain a small amount of active substance gets into the environment each time the powder is used. Therefore contact with skin and mucous membranes should avoided and wearing protective equipment is recommended. But users often neglect. From use in technical installations (e.g. feed mill) it is known that the more the drug raises dust, the higher the risk of an active ingredient carryover. We investigated the dust emission of important medicated premixes on the Swiss market. On the example of chlortetracycline we also tried to detect how much of antibiotic this dust contains.

# **Material and Methods**

Dust emission by medicated premixes containing dust binding substances (Premixes A) was compared with emission by medicated premixes without such substances (Premixes B) using standard Heubach method. The active ingredients, concentration and carrier material were similar in Premixes A and B. For chlortetracycline analysis, a routine HPLC content analysis method was modified for trace detection.

#### Results

Premixes A raised at minimum 3 times lesser dust than Premixes B. The amount of chlortetracycline in the dust corresponds to the dosage of the drug.

# **Discussion and Conclusion**

The use of dust binding substances in the production of antibiotic powders or medicated premixes reduces effectively dust emission. The fraction of antibiotic in the dust may be similar to the concentration in the drug. Prudent use is also to select powders that do raise lower dust.

# CYSTOISOPORA SUIS PREVALENCE IN FRANCE - AN UPDATE FROM A FIELD SURVEY

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# **Background and Objectives**

Porcine neonatal coccidiosis caused by Cystoisospora suis (C. suis) is one of major causes of diarrhea in piglets worldwide and cause substantial economic losses in the pig breeding industry. To update the overview prevalence in France, a field study was conducted in ten farms, accompanied by a questionnaire assessing farm management and performances.

# **Material and Methods**

Ten farms (76-600 sows), which didn't apply toltrazuril treatment on piglets, were included and pooled samples of one hundred litters were examined twice: in the second and third week of life (n=199 samples) by flotation and MacMaster count: detection limit: 500 OpG (oocysts per gram of faeces). The faecal score of litter was graduated each day from 0 to 2 (0 was no signs of diarrhea in the crate, 1 was one piglet with clinical signs and 2 was more than one piglet showing diarrhea).

#### Results

Overall, 70% of the farms and 35% of the litters were positive at least once. 15% of the samples were positive at first sampling (2<sup>nd</sup> week of age) and 27% were positive (3<sup>rd</sup> week of age). All farms reported history of diarrhea in gilt's litters and 80 % in case of sow's litters. Twenty-six percent (11/42) of positive samples were diarrheic (faecal score 2).

# **Discussion and Conclusion**

C. suis is highly prevalent in French farms not using toltrazuril (64% positive farms). Within farm prevalence was high on average half of litters were positive. A crucial factor to diagnose C. suis is repeated sampling and not to focus only on diarrheic litters at the time of sampling.

# ESTABLISHMENT OF A SKILLS LAB FOR LARGE ANIMAL MEDICINE

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# **Background and Objectives**

The veterinary curriculum in Switzerland is based on lectures, seminars, further theoretical sessions and practical exercises involving patients in the clinics. At the end of their studies, graduates should be proficient in practical skills such as performing specialized examinations (e.g. rectal exams in cows, obstetrics), simple surgical procedures like castrations, injections or adequate sampling techniques. Unfortunately, the ratio of students to number of patients, as well as animal welfare considerations only allow for few opportunities per student to practice these skills. Therefore, the Vetsuisse Faculty in Bern established a skills lab for large animal medicine in 2018 for hands-on training in different practical clinical abilities.

# Material and Methods

Ten different training systems were developed, where students can perform practical exercises on simulators, models and carcasses of pigs and cattle. The systems include a dystocia simulator, two gynecological models and an artificial insemination simulator for cattle, a collection of gynecological and obstetrics tools, two injection models for pigs, carcasses for zootechnical measures such as castration, teeth shortening of piglets and ear tagging and tattooing of pig ears, a hygiene sluice model and a model for the correct aspiration of one or several drugs into a syringe. The acceptance and advancement in skills of students were evaluated through a survey among students and teaching staff.

#### Results

The students spent three to five hours to complete all ten training systems. Acceptance was high, as most students estimated the systems to be relevant to their later work in large animal medicine. Students advanced their practical skills by practicing individually on all training systems and discussing key points in groups.

# **Discussion and Conclusion**

A skills lab significantly contributes to the improvement of practical skills in the veterinary curriculum and is well accepted by students. However, this initiative is regarded as an addition to exercise on animals and not as a complete replacement.

# EFFECTS OF GARLIC (ALLIUM SATIVUM L) IN POSTWEANING PIGS - A PLACEBO CONTROLLED STUDY

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# **Background and Objectives**

Postweaning diarrhoea (PWD) due to Escherichia coli is an economically important disease in pig production. The preventive oral administration of medicinal plants like Allium sativum L. (AS, garlic), showing antimicrobial, anti-inflammatory, immunomodulatory and antidiarrheal properties, might be an option to improve health and performance in postweaning (p.w.) pigs. In this placebo-controlled study the effects of oral supplementation of AS on performance, health and faecal parameters were investigated in p.w. pigs.

# Material and Methods

Piglets (n=600, all half-siblings) of a Swiss farm were within each litter randomly assigned to the treatment groups and observed from birth until three weeks p.w.. For the first two weeks p.w., the piglets received either 0.3 g dried AS-powder/kg body weight/day, 6 mg colistin-sulphate/kg body weight /day or a placebo as top dressing divided to two daily administrations.

For three weeks p.w., body, weight, daily weight gain, body condition and a clinical score were measured individually and faecal dry matter and coliform bacteria were measured weekly on pen level. Data were analysed using generalized mixed effect models in R.

#### Results

Body weight and daily weight gain of the AS group were significantly higher compared with placebo in the third week p.w. No differences in body condition and faecal dry matter were observed as well as no significant difference was found for the faecal coliform bacteria count when AS was compared to placebo. The clinical score of AS-treated animals was significantly better compared with the colistin group. About 33 per cent of the piglets of the AS and the placebo group had to be treated with antibiotics due to the occurrence of severe PWD.

# **Discussion and Conclusion**

In conclusion supplementation of dried garlic powder shows a high potential as a feed additive to improve performance and overall clinical health of postweaning pigs but does not save them from severe PWD.

## ENDOPARASITES IN SWINE IN DIFFERENT AGE GROUPS AT FARM BREEDING IN SERBIA

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## **Background and Objectives**

Parasitioses present a permanent health and economic problem in farm breeding swine in Serbia.

#### **Material and Methods**

During 2018 we examined 200 fecal samples from ten farms. Samples were examined using standard coprological methods.

## Results

In different age groups endoparasites are of varying importance. Suckling piglets are predominantly infected with Balantidium coli (95-100%), Cryptosporidium spp. (17-32%), Eimeria perminuta (27-31%), E.debliecki (3-24%), E. polita (4-9%) and Isospora suis (3-13%). Weaners and fatteners are infected with Eimeria perminuta (27-31%), E.debliecki (3-24%), E. polita (4-9%) Balantidium coli (90-95%), Ascaris suum (9-16%), Oesophagostomum dentatum (2-8%), Hyostrongylus rubidum (3-6%), Strongyloideus ransomi (1-17%) and Trichuris suis (1-7%). Sows and boars are predominantly infected with Balantidium coli (95-99%), Eimeria perminuta (17-21%), E.debliecki (12-23%), E. polita (14-19%), Cryptosporidum parvum (3-12%), Ascaris suum (2-16%), Oesophagostomum dentatum (2-16%), Hyostrongylus rubidum (2-15%), Strongyloideus ransomi (1-3%), Trichuris suis (2-13%) and Metastrongylus pudendotectus (1%).

# **Discussion and Conclusion**

Damage induced by helminths is mainly due to reduced performance, in the case of ascarosis to reduced carcass value. Therefore planned antiparasitic measures should be taken at all stages of pig keeping. Hygienic measures can reduce infection pressure; however parasite elimination is difficult to obtain under conventional management conditions. In order to solve this problem, preventive coprological examinations should be introduced, which should cover all animals on the farm and are performed at least twice a year in all age and production categories. Newly procured breeding animals during quarantine must be controlled twice parasitologically. In addition, it is necessary to carry out all the prescribed zoo-hygiene measures on farms that, with adequate medicament prevention, largely solve this problem.
## A SURVEY ON EXTERNAL BIOSECURITY IN COMMERCIAL PIG FARMS IN SERBIA

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## **Background and Objectives**

The epidemiological situation in Europe regarding the spreading of african swine fever (ASF), fomented the Veterinary Directorate of Serbia to order in 2017 implementation of a set of control and preventive measures throughout country and at state borders. In the last two years an intense work in order to increase awareness and disseminate informations to all subjects involved in pig production was intensively undertaken. Unfortualtely, ASF was officially recognized in Serbia in 2019 and biosecurity has become the most relevant issue for the sustainable pig production. The aim of the paper was to investigate the external biosecurity measures applied in commercial pig holdings in the Vojvodina region.

#### **Material and Methods**

In order to evaluate external biosecurity measures on commercial pig farms, a biosecurity questionnaire was applied. Epizootiologists from the veterinary institute participated in the data collection on existing external biosecurity measures on pig farms.

## Results

According to the results of the biosecurity questionnaire, it can be concluded that significant risks regarding external biosecurity are related to different transport vehicles that enter the farm perimeter and lack of adequate sanitary facilities for employees and visitors. However, farm workers in contact with backyards pose a foremost biosecurity risk for disease introduction.

### **Discussion and Conclusion**

According to the level of biosecurity, five different pig production types can be distinguished: commercial farm, family farm types A and B, backyards and free-range. The backyards are common practice in villages and city outskirts and account for most of the pig production in Serbia (82.7%). In conclusion, we need to have in mind that maintaining biosecurity is not of importance only in case of epidemic outbreak, but should be routinely applied measures to prevent the swine diseases introduction.

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## CHANGES IN HEMATOLOGICAL PROFILES OF PIGS ON DIFFERENT FARMS REFLECT THEIR HEALTH STATUS

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## **Background and Objectives**

Hematological testing is still rarely performed in the routine evaluation of pig health status. This study aimed to evaluate the influence of the health status of pigs on different farms on the hematological profile.

### **Material and Methods**

The samples were collected from three large farms (600 – 1900 breeding sows), and three small farms with less than 100 breeding sows in Slovenia. From each category (5, 7, 9–10, 11, 12–13 week-old, fatteners, sows), individual blood samples and a group sample of oral fluid (OF) and feces were collected. The PCR and RT-PCR were used for detection of PCV2 and PRRSV respectively, whereas qRT-PCR was used for HEV. Three hundred seventy blood samples were obtained for determining complete blood count and white cell differential count using an automated laser-based hematology analyzer ADVIA 120. Statistical analysis of hematological data was performed using one-way and two-way analysis of variance (ANOVA), the Tukey's honestly significant difference (HSD) test and the Bartlett's test.

### Results

PRRSV was detected on farm 5 (OF), PCV2 on farm 2 and 6 (OF and feces) and on farm 4 (OF). HEV was detected on farm 6 (OF and feces). PRRSV significantly lowered lymphocyte (%), MCV and HCT values, and increased platelet count and basophil (%, x10<sup>9</sup>/L), neutrophil (%, x10<sup>9</sup>/L) and 'large unstained cell' (%, x10<sup>9</sup>/L) values. PCV2 significantly lowered lymphocyte (%) and increased neutrophil (%, x10<sup>9</sup>/L), eosinophil (%, x10<sup>9</sup>/L) and basophil (%, x10<sup>9</sup>/L) values. HEV significantly lowered MCV and lymphocyte (%) values and increased RBC and hemoglobin concentration, and basophil (%, x10<sup>9</sup>/L) and neutrophil (%) values. Increased neutrophil (%) values, as well as decreased lymphocyte (%) values have been detected in animals with PRRS, PCV2, and HEV.

## **Discussion and Conclusion**

Hematological profiles of pigs differ significantly among farms. Changes of hematological parameters accurately reflect the health status of pigs on different farms.

## MICROGRANULATED PREMIXES ENSURE SUPERIOR CLINICAL EFFICACY

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## **Background and Objectives**

The formulation of a medicated premix determines at large extent the clinical outcome of an antimicrobial treatment. The clinical efficacy of 2 premixes, both containing the same active compound at the same concentration, was evaluated.

# Material and Methods

Pigs (n=32) free from Actinobacillus pleuropneumoniae were divided in 4 groups and intranasally challenged with Actinobacillus pleuropneumoniae serovar 7 (2 x 10<sup>8</sup> CFU, tiamulin MIC 16  $\mu$ g/ml). Two groups were infected and treated with 2 different premix formulations, both containing 100 g tiamulin hydrogen fumarate/ kg: a powder based formulation and Vetmulin<sup>®</sup> microgranulated premix (Huvepharma<sup>®</sup>). The unique microgranulation technology of Vetmulin<sup>®</sup> premix ensures superior homogeneity of the active ingredient in the medicated feed and logically, results in correct dosing of all treated pigs. The pigs in both groups were treated at 10 mg tiamulin hydrogen fumarate/ kg bodyweight/ day for 15 consecutive days, starting 18 hours after the challenge. An uninfected and infected control group without treatment were also included. The clinical efficacy was evaluated daily by 3 parameters: a respiratory score, a decrease of locomotory activity score (0 = no signs observed  $\rightarrow$  4 = severe symptoms) and the number of days with fever ('39.8°C). The evaluation was based on the average results over the 15 days treatment period.

# Results

When comparing the Vetmulin<sup>®</sup> group with the powder formulation group, a significant reduction (p<0.05) of 83% of the respiratory score (0.10 and 0.57 respectively) and 93% of the decrease of locomotory activity (0.03 and 0.43 respectively) was observed. Furthermore, the number of days with fever was 0.6 days less (5.80 and 6.40 days respectively).

## **Discussion and Conclusion**

The formulation of medicated premixes determines the clinical outcome under field conditions. The superior homogeneity of Vetmulin<sup>®</sup> microgranulated premix in medicated feed results in optimal efficacy.

## PIGFEN® ORAL SUSPENSION ENSURES OPTIMAL EFFICACY WHEN USING DOSING PUMPS

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## **Background and Objectives**

From nature, benzimidazoles are poorly water soluble. To enable drinking water application in a convenient and effective way, Huvepharma® developed a unique nanosuspension based on a particle size reduction of fenbendazole crystals. Pigfen® 200 mg fenbendazole/ ml Oral Suspension was administered via a dosing pump at different flow rates and durations to investigate the concentrations of fenbendazole in the final drinking water at the nipples. Simultaneously, the potential for blocking dosing pumps, water supply lines and nipples was evaluated.

## Material and Methods

Pigfen<sup>®</sup> was administered via a stock container and a dosing pump at 2.5 mg fenbendazole/ kg bodyweight/ day for 2 days. Two administration protocols were tested without a pre-dilution step or additional stirring. In a first test ( $\Pi$ ) the product was administered for 24h at a setting of 5% to evaluate the longest possible treatment period. In a second test (T2), a 3h administration at 1% setting was installed to investigate the highest concentration. The fenbendazole concentrations in the final drinking water were determined by High Performance Liquid Chromatography immediately after the preparation of the stock suspension (0h) and at the end of the administration period (at 24h for  $\Pi$  and 3h for T2). These data were compared with the targeted fenbendazole concentrations of 25 and 125 µg/ ml for  $\Pi$  and T2 respectively.

## Results

In T1 the determined fenbendazole concentration was 25.2 and 25.1  $\mu$ g/ ml at 0 and 24h respectively. Regarding T2, a concentration of 118.1 and 121.1  $\mu$ g/ ml at 0 and 3h respectively was noted. No plugging of the water delivery system was encountered at all.

## **Discussion and Conclusion**

Pigfen® Oral Suspension used in dosing pumps ensures correct dosing and subsequent optimal efficacy as targeted concentrations in the final drinking water are achieved, even after 24h. The product offers maximal ease of use, even at high concentrations.

## STABILITY OF PIGFEN® ORAL SUSPENSION IN THE DRINKING WATER

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## **Background and Objectives**

The efficacy of a veterinary product for use in the drinking water largely depends on the solubility and stability. Pigfen® 200 mg/ ml fenbendazole Oral Suspension (Huvepharma®) is developed by a unique nanosuspension technology and ensures optimal solubility and ease of use in the field. In this study, the stability was investigated in 2 different qualities of drinking water and for 2 different kinds of materials used in the water delivery systems.

## **Material and Methods**

Pigfen® was dissolved in soft water/ low pH (19.9 mg Calcium carbonate/ I, pH 5.5) and hard water/ high pH (227.6 mg Calcium carbonate/ I, pH 8.5) without a pre-dilution step or additional stirring. For both water qualities, the medicated drinking water was stored in plastic and metal buckets at 25°C, not protected from direct sunlight. Samples of these 4 conditions were taken at 0h (= just after the preparation) and 24h later. The content of fenbendazole was investigated by a validated High Performance Liquid Chromatography test and a recovery of 95–105 % versus initial value was considered to indicate excellent stability. In the meantime, the appearance of the solution was also checked.

## Results

Compared to the original content in the medicated drinking water, the recovery of fenbendazole in soft water/ low pH at 24h after the start of the administration was 99.0 and 99.5 % for plastic and metal buckets respectively. Similarly, the recovery of fenbendazole in hard water/ high pH was 102.1 and 99.0 % for the plastic and metal buckets respectively. The appearance of the solution remained constant throughout the 24h study period without visual sedimentation.

## **Discussion and Conclusion**

Pigfen® Oral Suspension demonstrates an excellent stability in the drinking water for 24h after the preparation. This stability is independent on the quality of the water and the storage conditions.

## CYSTIC HYGROMA IN PIETRAIN PIGS - A RARE GENETIC DISEASE?

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## **Background and Objectives**

Cystic hygroma is a malformation of the lymphatic and vascular system and it is recognized as benign congenital tumour that affects humans and animals in the perinatal period. This congenital disorder is rarely described in animals and until today, cystic hygroma in pigs has not been described in the literature.

## **Material and Methods**

In a purebred Pietrain litter with twelve live-born piglets, cystic hygroma on the limb was noticed in two male pigs within the first week of their life. To rule out the common differential diagnoses, e.g. abscess or haematoma, further investigations were conducted. Furthermore, all littermates and the parents were sampled for molecular genetic analysis. Subsequently SNP array genotyping and whole-genome sequencing was carried out.

#### Results

During a clinical examination, a painless and soft mass, which was compressible, was detected on the limbs of both animals. The ultra-sonographic examination revealed a fluid-filled and cavernous subcutaneous structure. In addition, a puncture of the cyst was conducted, revealing a serosanguinous fluid with negative bacteriological culture. In both animals, a necropsy was performed showing that the animals had fluid-filled cysts. Based on the results of the clinical examination and the further investigations cystic hygroma was diagnosed. Genetic analysis revealed nine genome regions, which occur homozygous in both cases in accordance with a Mendelian recessive inheritance although a dominant acting de novo germline variant could also explain the cases. Genome sequencing revealed a short list of putative candidate variants causing this disease.

#### **Discussion and Conclusion**

Systematic surveillance is needed to identify genetic defects as early as possible and to avoid the occurrence of further losses in the pig population. Until today, no single causative genetic variant could be revealed. Further genotyping, ideally after adding further affected piglets, is needed to finally confirm a genetic aetiology.

# EVALUATION OF DIFFERENT TEACHING INTERVENTIONS TO MOTIVATE AGRICULTURAL STUDENTS IN THE LECTURE OF ANIMAL HEALTH

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## **Background and Objectives**

Student motivation, both intrinsic and extrinsic motivation, is an important factor for successful learning. Therefore, teachers should try to engage both motivation patterns during their lectures; because motivated students work more actively during this time and thus tend to learn more efficiently. Hence, the aim of this study was to describe and evaluate specific teaching interventions (identifying interesting topics for classes, pig herd examination, group work during classes, excursion to the federal food safety and veterinary office) used in teaching animal health to students of agronomy.

#### Material and Methods

For evaluating the motivation of students, the MUSIC® Model from Jones was used, which measures five primary components of motivation; namely empowerment, usefulness, success, interest, and caring. By means of the MUSIC® inventory and open questions at the end of the semester, students were asked about their perception of different interventions during the course. All variables of the MUSIC® Model were rated on a 6-point Likert type scale.

#### Results

In total 36 students out of 48 students opened the link to the questionnaire and 27 completed the whole survey. The caring component of the lecturer was evaluated at highest (5.7), followed by success (5.1), interest (4.8), usefulness (4.6) and empowerment (4.5) of the topics in this class.

## **Discussion and Conclusion**

Each of the implemented interventions increased the motivation of the students in the class. As a result of the open questions, usefulness and interest seem to be the main driver for motivation among students in this study. Therefore, interventions, which activate these two components such as field trips with exercises and group discussions, should be regularly used in teaching at the university to improve the motivation of students.

## LIVER TORSION AS A COMMON CAUSE OF SPONTANEOUS SOW DEATH IN FARROWING UNITS

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## **Background and Objectives**

The highest prevalence of spontaneous sow deaths is in the farrowing unit, but knowledge about the cause of death is limited. Thus, in order to prevent these deaths, we need to know more about the possible cause of death and management-related risk factors.

## Material and Methods

We included 10 sow herds, agreeing to deliver the next six spontaneously dead sows from their farrowing units to the laboratory. Macroscopic and histologic examinations were performed on a total of 53 sows. Apart from delivering the sows, the farmers provided anamnestic information related to the dead sows and information in relation to other sows dying in the farrowing unit during a six months period.

#### Results

Twenty-five % of deaths occurred on day 0 - 5 after farrowing, but we also found a high prevalence (21%) day 21-30. Forty-two % of the necropsied sows succumbed to a torsion of the outer left liver lobe, 17% died due to complications related to farrowing (primarily decomposing fetuses in the uterus) and 17% showed signs of infections (infections in lungs, bones, liver, bladder and udder). Torsion of the left liver lobe were seen throughout the farrowing period, but the majority of cases seemed to be around 3 weeks postpartum. The histologic examination indicated that the torsion of the liver lobe happened up to 24 hours prior to death. In some but not all liver torsion cases, the farmers had noticed a lack of appetite the day before death.

### **Discussion and Conclusion**

The high prevalence of liver lobe torsion as cause of spontaneous death in sows is surprising, as previous Danish and international studies have shown a prevalence of just 5-12%. The study, however, did not indicate any management related risk factors associated with these mortalities. From literature and abattoir observations it seems likely that an anatomical predisposition is the major risk factor for liver torsion.

# FIELD EVALUATION OF HAEMOGLOBIN (HB) LEVEL AND INFLUENCE OF APPLICATION METHOD ON HB STATUS IN PIGLETS AT WEANING ON DUTCH FARMS

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## **Background and Objectives**

Iron deficiency anaemia (IDA) is an important health problem in piglets, it is controlled by routine application of iron. The aim of our study was to evaluate the haemoglobin (Hb) of piglets at weaning in farms in the Netherlands and to assess influence of route of application and type of iron.

## Material and Methods

Twenty-one randomly selected farms using different forms of iron (dextran, gleptoferron) and route of administration (injection-504 piglets x needleless-126 piglets) were included. Within each farm, ten randomly selected litters from different parity sows have been assessed (30 piglets/farm, 630 piglets in total). One small, medium and large piglet per litter were sampled, Hb levels were measured (HemoCue<sup>®</sup>). Piglets were classified as follows: Hb levels < 90 g/l-anemic, Hb levels  $\geq$  90 g/l and < 110 g/l-suboptimal and Hb levels  $\geq$  110 g/l-optimal.

## Results

The type of iron treatment has effect on the percentage of anaemic piglets (6,5% for gleptoferron vs 12,4% for dextran). Application method (IM injection x needleless) has low effect on % of anaemic piglets (9,9% vs 8,7%), the main difference was found in % of optimally supplied piglets (Hb  $\ge$  110 g/l) (53,4% vs 38,1%) as well for median Hb values (111 vs 105 g/l). Slight numerical difference in effect of the type of product administered by needleless application was observed with more optimal piglets treated by gleptoferron (38,5% vs 36,7%).

## **Discussion and Conclusion**

There was visible evidence of influence of different route of administration of iron supply efficiency. The percentage of non-anaemic piglets (Hb >9 g/dL) was variable from one farm to another depending on product used and this categorisation seems to be sensitive criterion beside Hb levels. Gleptoferron based products administered by intramuscular injection tend to have better hematinic efficiency in the field.

# DETECTION AND CHARACTERIZATION OF MICROBIAL FLORA AND HOST METABOLITES IN SYNOVIAL FLUIDS OF PIGS WITH CLINICAL SIGNS OF LAMENESS

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## **Background and Objectives**

The existence of microbiota in joints of humans suffering arthritis has been confirmed, suggesting a potential correlation with degree of disease. To date, the potential role of inherent microbial flora in pig joints has not been evaluated in understanding lameness development. Thus, this study sought to characterize the synovial microbiota and host metabolic changes in joints of pigs with clinical signs of lameness.

## **Material and Methods**

Five wean-to-finish farms reporting recent history of lameness were enrolled in the study. Three pigs from each farm were selected based on lameness scores: one healthy (score 0) and two lame pigs (score  $\ge$ 3). Synovial fluid (SF) samples were obtained from eight joints per pig (n=128). Due to low-biomass composition, only 21 SF samples passed the quality control to perform the 16S rRNA sequencing to characterize the microbiota in pig joints. However, the SF from all joints were analyzed for determining host metabolite composition using liquid chromatography-mass spectrometry (LC-MS).

### Results

The dominant phyla were Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria. Abundance of erysipelas and genus from the family of Pasteurellaceae were observed in 57% and 43% of the analyzed SF samples, respectively. These taxa could potentially include bacteria associated with lameness such as Haemophilus parasuis and Actinobacillus suis. Metabolomics analysis revealed that within each farm, there was a significant difference in amino acid composition profiles in SF between the healthy and lame pigs. The altered amino acid biomarkers identified in each farm were found to be associated with the Krebs cycle as well as amino acid metabolism pathways, indicating increased protein catabolism in the affected joints.

## **Discussion and Conclusion**

Overall, this study confirms existence of bacterial nucleic acids in SF samples from both healthy and lame pigs. The potential association between joint microbiota and lameness remains uncertain. Besides, lameness may result on metabolomic changes in SF from pigs of the same farm.

# RAISED WITHOUT ANTIBIOTICS (RWA) PROGRAM - EUROPEAN LEADER'S PERSPECTIVE.

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## **Background and Objectives**

For many years, the antibiotic use in feed and water have been a tool in pig farming worldwide. Nowadays, the improvement of animal welfare, awareness of antibiotic resistance, availability of advanced diagnostic and prophylaxis methods led the best producers to the concept of RWA pigs. RWA production might be associated with increased risk for exposure to numerous pathogens. The data about the impact of shifting standard production to RWA are scarce. The aim of this paper is to present basic of the procedure, its success rate and typical health problems.

## Material and Methods

As a first step, Goodvalley farms minimised pathogens exposure by improving management strategies and reducing pigs density. Parallel with these changes there were taken attempts to maximise immunity by use of autogenous vaccines for sows. To prove traceability, all animals excluded from the program receive additional ear-tag in the opposite ear. RWA runs in 2 production lines (total 8200 sows).

### Results

Typical success rate in piglets is 75%. In weaner and finisher phase it is 85 and 93%, respectively. The main reason for suckling piglets, weaners and finishers to lose RWA status is neonatal diarrhoea, diarrhoea/respiratory signs and lameness, respectively. RWA practices did not impact animal health significantly. However, after implementing RWA some pathogens are increasingly gaining in importance. The main problems are associated with Trueperella pyogenes and Mycoplasma suis subclinical infections, which may negatively impact carcass quality by abscesses and jaundice in up to 1% of pigs slaughtered. Strategies for eradication of these pathogens are not available. Recommendations included cessation of teeth clipping and surgical castration, needles management, prevention of cannibalism by using enriched environment and better climate control.

## **Discussion and Conclusion**

Successful transitioning from conventional to RWA production is possible. The success rate analysed since over 4 consecutive years has growing tendency. Nevertheless, keeping standards is still challenging.

## ENZYMATIC DEGRADATION OF ZEARALENONE IN THE GASTROINTESTINAL TRACT OF PIGS

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## **Background and Objectives**

Zearalenone (ZEN), one of the major mycotoxins frequently occurring in feedstuffs, can negatively affect animal performance already at moderate concentrations. Efficient biological detoxification of this estrogenic Fusarium toxin is a key step to prevent clinical and subclinical effects. Zearalenone hydrolase ZenA (ZENzyme®), a newly developed enzyme, is capable of biotransforming and therefore detoxifying ZEN in the gastrointestinal system of animals.

## **Material and Methods**

Weaning piglets were divided into three experimental groups (12 piglets; 6 male, 6 female per group) and kept in floor pens for 42 days. The animals received grower diet over the whole trial period. One group received an untreated, uncontaminated diet (control group "CG"); the second group was fed a diet containing 200 µg/kg ZEN (toxin group "TG"); the third group received a diet containing 200 µg/kg ZEN and 10 U ZenA/kg feed (treatment group "TRG"). Performance parameters (body weight, feed intake, FCR, body weight gain) were recorded regularly. ZEN and its metabolites hydrolyzed zearalenone (HZEN), decarboxylated HZEN (DHZEN) and alpha-zearalenol ( $\boxtimes$ -ZEL) were analyzed in feces on days 21 and 42.

## Results

ZenA significantly reduced the levels of ZEN and a-ZEL in feces, both after 21 days (ZEN: TG: 347.6 ng/g, TRG: 127.2 ng/g, P=0.025; &-ZEL: TG: 134.5 ng/g, TRG: 56.9 ng/g, P=0.025) and 42 days (ZEN: TG: 354.6 ng/g, TRG: 200.7 ng/g, P=0.025; &-ZEL: TG: 208.5 ng/g, TRG: 114.1 ng/g, P=0.025). At the same time, fecal HZEN levels were significantly elevated on day 21 and day 42. No significant differences were observed among the three groups with regard to performance (final body weight: CG: 34.85 kg, TG: 34.95 kg, TRG: 35.60 kg; P=0.670).

## **Discussion and Conclusion**

Results of this study revealed that ZenA effectively biotransformed zearalenone to the non-toxic metabolite hydrolyzed zearalenone, reducing the overall risk for intoxicated animals.

## COMPARISON OF DIFFERENT SWINE IGG MEASURING TECHNIQUES

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## **Background and Objectives**

Evaluation of colostrum management is possible by measuring maternal derived antibodies (MDA) in new born piglets. This study compared different techniques on the same set of serum samples for measuring MDA.

## Material and Methods

Serum samples obtained from newborn piglets (n= 36, 6 litters with 6 piglets, age 24h) submitted for routine analysis of MDA, were combined with 4 negative control sera, collected from still born piglets. Serum samples were all analyzed with several available techniques: RID (triple J Farms), Gel Electrophoresis (GE), Zinc turbidity (ZT), Elisa (El. Bethyl swine IgG), immmunocrit by two labs (ICa and ICb) and for BRIX% using a simple refractometer (RF, Sper scientific).

### Results

All four negative control samples appeared negative in the RID assay (not any precipitation visible). The range of IgG concentration in the 36 serum samples was normally distributed and had a mean of 28.2 mg/ml (min 9.46; max 39.3). RID and GE were high correlated (R<sup>2</sup> =96.4%). When RID is considered as the gold standard, the other methods R<sup>2</sup> (in order of correlation): ICa 94.0%; RF 86.8%; ICb 81,1%; ZT 80.0%; El 57.0%.

### **Discussion and Conclusion**

ZT was relative inaccurate in the low range of IgG concentrations (< 15 mg/ml). Elisa has considerable higher variation and contained outliers exceeding the IgG concentrations that were determined by the other techniques. RID and Gel electrophoresis are well correlated to each other and either one can be used for validation purposes. The immunocrit assay, however, is relatively cheap, can be performed under field conditions and has accurate results but needs to be validated by each laboratory since differences in centrifugation forces can exist, resulting in less preciptation and accuracy. In conditions of low available (technical) resources, the use of a refractometer (BRIX%) is a simple but quite accurate way of measuring MDA in newborn piglets

# PREVALENCE OF DILATED CARDIOMYOPATHY IN PIGLETS DIAGNOSED WITH PFTS.

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# **Background and Objectives**

Periweaning Failure to Thrive Syndrome (PFTS) has been reported in nursery piglets since 2011.

In our practice in Portugal we find it since 2012 with an increase in prevalence in the last 2 years.

Besides the typical symptoms of PFTS, we found consistent heart lesions compatible with dilated cardiomyopathy in piglets from several farms. This heart condition can cause some of the symptoms of PFTS, so we suspect that this might be a primary cause for the depressed behavior, anorexia, general weakness and weight loss.

The objective of this study is to determine the prevalence of heart lesions (dilated cardiomyopathy) in piglets with PFTS symptoms.

# Material and Methods

2 farms with PFTS diagnosed in nursery piglets (piglets sent to CRESA in Barcelona, confirmed PFTS) were selected for this study.

Records of the necropsies of dead and euthanized PFTS-like piglets from Jan-Nov 2019 were analyzed. Nursery monthly mortality rates ranged from 3,6% to 12,1%.

Piglets and organs were sent to diagnostic laboratory.

## Results

Lesions found at necropsy:

None of the organs affected (typical PFTS);

Poliserositis (pericarditis, pleurisy, peritonitis, all with abundant fibrin); Arthritis; Pneumonia; Enteritis; Heart lesions (bilateral dilatation of the 4 chambers);

% of each type of lesion:

None of the organs affected (typical PFTS) and Cardiac dilatation: 64%

Poliserositis and Arthritis: 23%

Enteritis: 9%

Pneumonia: 4%

Affected hearts were sent to histopathology and virology studies, but were negative to myocarditis and no viral material found.

# **Discussion and Conclusion**

In these 2 farms most of the PFTS-like piglets necropsied had bi-lateral heart dilatation.

In the same piglets there were no more lesions in other organs.

Piglets with other lesions than PFTS had normal hearts.

We suspect that this dilated cardiomyopathy can be a cause for the PFTS-like symptoms in these cases. Prevalenece increased from 2018 onwards.

Further investigation is needed to find the causal agent for the cardiomyopathy.

# TRANSFER OF CELL MEDIATED IMMUNITY AGAINST MYCOPLASMA HYOPNEUMONIAE TO TWO-DAY-OLD PIGLETS UPON VACCINATION OF SOWS AGAINST MYCOPLASMA HYOPNEUMONIAE

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# **Background and Objectives**

Maternal immunity protects piglets against diseases in the first weeks of life. Vaccination against M. hyopneumoniae induces a shift in the different T-cell subpopulations in blood and stimulates the cells' ability to produce cytokines. Transfer of maternal antibodies and lymphocytes to the offspring has been shown. However, transfer of vaccine-induced M. hyopneumoniae T-cells is less clear.

## Material and Methods

This study investigated TNF-🛛 and IFN-🖾 production by CD8<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>CD4<sup>+</sup> and CD8<sup>-</sup>CD4<sup>-</sup> T-cells in blood of two-day-old piglets (n=23) from M. hyopneumoniae vaccinated sows in an endemically infected farm and in blood of two-day old piglets (n=24) from non-vaccinated sows on a M. hyopneumoniae negative farm. Peripheral blood mononuclear cells were isolated, in vitro stimulated with M. hyopneumoniae J strain bacterin, a 5-step 5-colour flow cytometry staining protocol was performed and data were acquired with a flow cytometer.

## Results

In sows vaccination induced M. hyopneumoniae specific antibodies, which were transferred to the piglets. On the M. hyopneumoniae negative farm antibody concentrations were very low. A decrease in CD8<sup>+</sup> T-cells and increase in CD8<sup>+</sup>CD4<sup>+</sup> T-cells was observed after vaccination. Piglets from vaccinated sows had more CD8<sup>+</sup>CD4<sup>+</sup> T-cells, which correlate with a memory function, in their blood (0.9±0.7%), than piglets from the M. hyopneumoniae negative farm (0.07±0.05%). Furthermore, piglets from vaccinated sows had more TNF-<code>X<sup>+</sup>CD8<sup>+</sup></sup> and TNF-<code>X<sup>+</sup>CD8<sup>-</sup>CD4<sup>-</sup></sup> T-cells, more IFN-<code>X</code> producing T-cells and more TNF-<code>X<sup>+</sup>IFN-<code>X<sup>+</sup>CD8<sup>+</sup></sup> and TNF-<code>X<sup>+</sup>IFN-X<sup>+</sup>CD8<sup>+</sup></sup> and TNF-<code>X<sup>+</sup>IFN-X<sup>+</sup>CD8<sup>+</sup></sup> and TNF-X<sup>+</sup>IFN-X<sup>+</sup>CD8<sup>+</sup></sub> and TNF-X<sup>+</sup>IFN-X<sup>+</sup>CD8<sup>+</sup> and TNF-X<sup>+</sup>CD8<sup>+</sup> and TNF</code></code></code></code></code></code>

## **Discussion and Conclusion**

Mycoplasma hyopneumoniae vaccination in sows induced M. hyopneumoniae specific cytokine producing T-cell subsets which were transferred to and found in the blood of their offspring.

# SAFETY AND SEROCONVERSION OF NEW INTRAMUSCULAR VACCINE AGAINST LAWSONIA INTRACELLULARIS (LI) UNDER FIELD CONDITIONS

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## **Background and Objectives**

Li. is a worldwide pathogen affecting swine population. Study aim: evaluate safety characteristics and seroconversion provided by a new intramuscular vaccine available in EU market.

### Material and Methods

The study was carried out in 2 commercial farms producing 10 weeks of age piglets, and with the same vaccination protocol. Piglets between 3-4 weeks of age were selected preweaning (Farm1: 30; Farm2: 60), all individually identified and their rectal temperatures recorded (°C) just prior to vaccination. Different treatment categories were designed to have a control group (C: not vaccinated), a group vaccinated with Porcilis® Lawsonia (MSD A.H.) alone (PL), and combination of Porcilis® Lawsonia reconstituted in Porcilis® PCVMhyo (MSD A.H.) (PLP). Rectal temperatures were recorded individually at 6 and 24h postvaccination to assess safety of vaccination. Besides, piglets were blood sampled at vaccination and 4 weeks postvaccination (Farm 1), and at vaccination and 3 and 6 weeks postvaccination (Farm 2). The diagnostic kit SVANOVIR® L intracellularis/lleitis-Ab was used to analyze all serum samples under the same conditions. Two-way mixed ANOVA (95% CI) were done to analyze data for both farms.

### Results

Average rectal temperature: at vaccination moment no statistical differences were found in Farm 1 between groups, but +0.6°C in Farm 2 appeared in control group. Statistical differences appeared at 6 hours postvaccination in all vaccinated groups (+0.8°C Farm 1; +0.6°C Farm 2). These statistical differences disappear 24 hours postvaccination.

Seroconversion: a statistically significant seroconversion was shown in both vaccinated groups compared against control (Farm 1: PL 82% seroconversion vs. C 8%; Farm 2: PL 78%, PLP 68%, vs. C 0%). No statistical differences between vaccinated groups were found in Farm2.

## **Discussion and Conclusion**

In the conditions of this study, this new vaccine has proved safe and showed seroconversion against a control group.

# ONE DOSE OF A NEW VACCINE INCLUDING TWO GENOTYPES OF PORCINE CIRCOVIRUS (PCV2A AND PCV2B) AND MYCOPLASMA HYOPNEUMONIAE CONFERRED A DURATION OF IMMUNITY OF AT LEAST 23 WEEKS

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# **Background and Objectives**

Porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhyo) are major swine pathogens for which vaccination is the main control strategy. The continuous genetic evolution of PCV2 suggest the need to update vaccines for broader coverage. The aim of this research was to study the duration of immunity (DOI) of a novel vaccine combining PCV2a/PCV2b genotypes and Mhyo.

# Material and Methods

Three separate studies, each testing the DOI of one vaccine fraction were performed. In each study, threeweek-old piglets naïve to PCV2 or Mhyo were vaccinated and challenged 23-weeks later with either PCV2a, PCV2b or Mhyo. Pigs were euthanized 3-4-weeks post-challenge (PC). In PCV2 studies, blood and fecal swabs were collected prior to challenge, twice a week PC and prior to necropsy, when a selection of lymphoid tissues was collected. In Mhyo study, serum, body weights and oral fluids were collected prior to challenge and necropsy, when lung lesions were assessed, and lung tissues collected.

# Results

Vaccinated pigs showed significantly lower PCV2a ( $P \le 0.0011$ ) and PCV2b ( $P \le 0.0029$ ) viremia (qPCR) from day 168 until day 175 or until euthanasia, respectively. Fecal shedding (qPCR) was significantly lower for PCV2a-challenged ( $P \le 0.002$ ) from day 171 until day 178 and for PCV2b-challenged ( $P \le 0.0001$ ) from day 172 until euthanasia. Differences between percentage of positive pigs to PCV2a immunohistochemistry, histiocytic replacement or lymphoid depletion were not significant (P > 0.05) compared to controls. However, significant differences were noted between PCV2b-challenged and control groups for immunohistochemistry (P = 0.0098) and histiocytic replacement (P = 0.0213), not for lymphoid depletion (P > 0.05). Vaccination reduced significantly the mean percentage of Mhyo-like lung lesions (P = 0.0196) compared to controls. Percentages of lung tissues positive for Mhyo via immunohistochemistry were 49.3% and 67.1% for vaccinated and control groups, respectively.

# **Discussion and Conclusion**

One dose of the PCV2a/PCV2b/Mhyo vaccine conferred 23-weeks of DOI for all three fractions.

# CLINICAL EFFICACY OF A NEW INTRADERMAL VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE AND PCV2 IN THE CONTROL OF M.HYO-LIKE LUNG LESIONS AND PCV2 VIRAEMIA UNDER FIELD CONDITIONS

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## **Background and Objectives**

MHYOSPHERE® PCV ID (Vaccine A) is a new intradermal vaccine against M.hyo and PCV2, all in one. The aim of this study was to assess its efficacy in the reduction of M.hyo-like lung lesions and PCV2 viraemia under field conditions.

## **Material and Methods**

Seven commercial farms with M.hyo and/or PCV2 circulation were included in a multicentre, randomized, negative-controlled and blinded field trial. In total, 2,507 healthy 21 days-old piglets were distributed between two groups. One group (n = 1,253) was vaccinated with Vaccine A, whilst the other group (n = 1,254) received a placebo. A single dose of 0.2 ml was administered intradermally to both groups using a needle-free device (Hipradermic<sup>®</sup>) and they were then monitored up to slaughter. The primary efficacy variables were M.hyo-like lung lesions at slaughter, evaluated in all the animals, and PCV2 viraemia, evaluated by qPCR (log10 PCV2 genomic copies/ml of serum) in 30 animals/group/farm periodically sampled during the study.

## Results

With regard to the M.hyo-like lung lesions at slaughter, the mean percentage of affected lung surface was significantly lower in the Vaccine A group than in the Placebo group (10.94 % vs 13.52 %, respectively; p < 0.0001), corresponding to a 19.1 % reduction. In addition, the incidence of pigs with at least one M.hyo-like lung lesion was also significantly lower in the Vaccine A group (p < 0.0045). With regard to the PCV2 viraemia, the mean PCV2 viral load (in terms of Mean Area Under the Curve from weaning to slaughter) was significantly lower in the Vaccine A group.

## **Discussion and Conclusion**

The novel intradermal vaccine MHYOSPHERE® PCV ID is efficacious in reducing the incidence and severity of M.hyo lesions and control of PCV2 viraemia, eliciting long-lasting protection against M.hyo and PCV2-related diseases.

# ASSESSMENT OF THE EFFICACY OF A PRRSV-1 SUBTYPE 1-BASED MODIFIED LIVE VIRUS ATTENUATED VACCINE IN FRONT OF THE CHALLENGE WITH SUBTYPE 2 AND SUBTYPE 3 PRRSV-1 STRAINS

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## **Background and Objectives**

The ability of Porcine Reproductive and Respiratory Syndrome (PRRS) modified live virus (MLV) attenuated vaccines to induce cross-protection is considered to be limited. Two studies were designed to test the efficacy of a PRRSV-1 subtype 1-based MLV to protect pigs in front of PRRSV-1 subtype 2 and subtype 3.

## Material and Methods

In both studies, I day-old piglets were divided into two groups (22 to 29 pigs each): one group vaccinated with Suvaxyn PRRS MLV, and one control group. Four weeks later, pigs from one study were challenged with the subtype 2 strain BOR-57 and pigs from the second study were challenged with the subtype 3 strain Lena. Clinical signs were recorded during 10 days; body weight was taken before challenge and before necropsy. PRRSV viremia and shedding (nasal and oral) were measured by RT-qPCR. Lung macroscopic lesions were evaluated at necropsy.

## Results

Subtype 2 study: non-vaccinated piglets showed a higher incidence of demeanor and abnormal respiration after challenge. Body weight and ADWG were significantly higher in vaccinated pigs. Viremia and oral shedding were significantly reduced in vaccinated pigs between days 2-8 and at 6 and 10 days after challenge respectively. The percentage of lung lesions in vaccinated pigs (3%) was significantly lower than in control pigs (15%).

Subtype 3 study: non-vaccinated piglets showed a higher incidence of depression, bad general condition and conjunctivitis after challenge. ADWG was significantly higher in vaccinated pigs. Viremia was significantly reduced in vaccinated pigs after challenge. The percentage of lung lesions in vaccinated pigs (2.8%) was significantly lower than in control pigs (6.2%).

## **Discussion and Conclusion**

Vaccination of 1 day-old pigs with Suvaxyn PRRS MLV was able to reduce the severity of the disease and the infection after challenge with PRRSV-1 subtype 2 and subtype 3 strains.

# EFFECT OF THE VACCINATION AGAINST SHIGATOXIN 2E IN A PIGLET PRODUCING FARM WITH HISTORY OF EDEMA DISEASE: CASE STUDY

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# **Background and Objectives**

Edema disease (ED) is one of the major diseases in pigs during nursery caused by Stx2e+/F18 Escherichia coli . The goal of this study was to test the efficacy of a vaccination on a farm with a history of ED caused by atypical STEC-2e strain.

# Material and Methods

The study was carried out on a farm with 1100 sows and known history of ED. The status of STEC-2e occurrence was confirmed before start of the trial, as well by serum neutralization test (SNT). 113 piglets from 13 randomly selected litters were included in the study. The litters were equalized (max. 12–13 piglets per litter). In the group A, piglets (51) were vaccinated at 4 DOA (days of age) using (Ecoporc Shiga®, Ceva), 62 piglets (group B) were non-vaccinated. The parameters: mortality, weight gain (nursery 21 – 69 DOA), antibiotic treatment (ATB) were recorded. Detection and characterization of STEC was performed.

# Results

Groups didn't differ in weight at inclusion and at weaning (p= 0.6980). Mortality was better in group A (1/51, 2.0 %) vs. group B (4/62, 6.5%) in nursery. Weight gain was statistically better in group A (p= 0.03438) with difference between the means in kg (B-A) ± SEM = 1.614 ±0.7526 kg. Injection treatment required 1 piglet, group A (2%) vs. 6 (9.7%) group B. E.coli strains carrying stx2e genes from group B were detected. Selected strain was characterized by sequencing as O100:H30, stx<sub>2e</sub>, iha. The ability to cause cytotoxic effect on Vero cell was confirmed in vitro (IC<sub>50</sub> 8000).

# **Discussion and Conclusion**

Numerical benefit of vaccination in reduction of mortality and ATB treatment was confirmed on the farm, where atypical STEC strains were repeatedly isolated. STEC strain belonged to serotype O100:H30, negative for typical adhesins, positive for iha, adherence factor till now reported only in pathogenic E. coli O157:H7.

# EFFICACY OF A NEW VACCINE TO PROTECT PIGLETS AGAINST A CLOSTRIDIOIDES DIFFICILE AND CLOSTRIDIUM PERFRINGENS TYPE A CHALLENGE

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## **Background and Objectives**

The aim was to evaluate the efficacy of a basic vaccination and revaccination scheme of a new vaccine (Diff/A) administered intramuscularly (IM) in pregnant sows to protect their progeny against a Clostridioides difficile (Cdiff) and Clostridium perfringens type A (CpA) challenge.

# Material and Methods

Ten pregnant sows, seronegative against both pathogens, were used for the basic vaccination study. Group A (n=5) was vaccinated IM with two doses of Diff/A (6 and 3 weeks prior to farrowing). Group B (n=5) was injected PBS IM with the same schedule. Twenty-five 24h-colostrum-fed piglets were challenge with each pathogen (15 vaccinated / 10 control). For the revaccination study, six pregnant sows that received the basic vaccination scheme in the previous gestation were vaccinated with one dose of Diff/A (3 weeks prior to farrowing; group C). Four non-vaccinated pregnant sows were included as control group (D). Twenty-four 24h-colostrum-fed piglets were challenged with Cdiff (12 vaccinated / 12 control) and twenty-three with CpA (12 vaccinated / 11 control). Piglets were monitored for 5 days after challenge. Piglet mortality was analysed using  $\mathbb{Z}^2$  test and clinical signs using t-test (p<0.05).

## Results

After Cdiff challenge, vaccinated groups demonstrated a prevention of mortality compared with controls (A and C: 0% vs B: 100% and D: 92%). After CpA challenge, vaccinated groups had a significantly reduced mortality when compared with control groups (A: 13% and C: 8% vs B: 100% and D: 82%). Clinical signs were significantly reduced in vaccinated groups after challenges (Cdiff A: 0.8 and C: 1.3 vs B: 3.2 and D: 2.5; CpA A: 1.7 and C: 2.1 vs B: 3.5 and D: 3.9).

# Discussion and Conclusion

Diff/A vaccination prevents mortality and significantly reduces clinical signs after Cdiff challenge and reduces clinical signs and mortality after CpA challenge. This vaccine is a useful tool to control these important neonatal diarrhea agents in piglets.

## THE IMPACT OF DIFFERENT COMBINATIONS OF SOW AND PIGLET VACCINATION AGAINST PCV2

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# **Background and Objectives**

Porcine circovirus remains one of major infection agents impacting pig performance in industrial farms. Vaccination has proven to reduce direct and indirect losses due to PCVD. The interference between high levels of maternal antibodies (MDA) and piglet vaccination was described previously. The aim of this study was to assess the impact of different vaccination protocols in sows and piglets on PCV2 circulation in pigs and their performance

## Material and Methods

In total 36 gilts were split into three groups and vaccinated with Circovac® before first farrowing or at first weaning or not vaccinated (Groups A, B and C). Their offspring were vaccinated at 3(R1), or 4(R2) or 6(R3) weeks of age (WOA) or not vaccinated(R4) with the same vaccine. Antibody profiles in sows, PCV2 circulation patterns and performance of those pigs were measured in three consecutive reproduction cycles.

## Results

The mean titers of PCV2 IgG in sows of group A, B and C at 107 days of gestation were  $1,73^{\alpha}$ ;  $1,17^{b}$  and  $1,15^{b}$  respectively(p<0,001) and at weaning  $1,42^{\alpha}$ ;  $1,22^{b}$  and  $1,14^{b}$  respectively(p<0,05). Numerically, piglets R3 showed higher proportion of pigs PCR-negative at the end of the study (85,4%), than R1 pigs (64,1%). No statistical difference was observed in the growth performance among groups R1-R4. Piglets of 2nd and 3rd parities born to vaccinated sows A, B had lower early mortality (7,35%<sup>b</sup> and 8,02%<sup>b</sup>) than those born to non-vaccinated sows C (15,72%<sup>a</sup>).

# **Discussion and Conclusion**

Sow vaccination at weaning avoided high peaks of antibodies during lactation. If sows are vaccinated prefarrow, it is advisable to vaccinate their piglets not earlier than at 4WOA, better at 6 WOA, which is coherent with previously published reccomendations. Sow vaccination against PCV2 can reduce early piglet mortality

## CLINICAL SAFETY OF A NEW VACCINE AGAINST C. DIFFICILE AND C. PERFRINGENS TYPE A

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# **Background and Objectives**

Piglets' passive immunization is a key point to control enteric pathogens. In order to achieve it, vaccination of sows during gestation is highly recommended. During this period, it is very important to use vaccines that cause little increase in temperature so that the sow's feed consumption or reproductive disorders are not affected and consequently, neither are the piglets' development. The objective of this study was to assess the safety of a new vaccine against C. difficile and C. perfringens Type A (Diff/A) under field conditions.

# Material and Methods

A multicentre, randomized, blinded and controlled field trial was performed in Hungary. A total of 311 pregnant sows were randomly distributed into two treatment groups. One group was vaccinated with Diff/A (n=155), whilst the other group received a placebo (n=156). Both groups were injected twice intramuscularly 6 weeks prior to farrowing (VI) and 21 days after the first dose (V2). Adverse reactions and reproductive parameters were recorded for all the included sows. Rectal temperatures were recorded individually in a subgroup of 58 sows starting the day before vaccination and up to 2d post-vaccination (pv).

## Results

No significant differences in adverse reactions nor abortions related to the administration Diff/A compared to the placebo were observed during the study (p > 0.05). A slight transient rise in temperature, clinically irrelevant, was observed at 4h pv with Diff/A vaccine (0.22°C at VI and 0.17°C at V2), returning to normal values after 24h. The maximum individual temperature increase was at 4h after VI (0.95°C). No significant differences between groups were observed in the mean number of piglets born, liveborn, stillborn and mummified piglets (p > 0.05).

## **Discussion and Conclusion**

The new vaccine Diff/A can be safely used in pregnant sows in terms of adverse reactions, reproductive disorders and rectal temperature increase.

EFFECTIVENESS OF TWO INTRAMUSCULAR COMBINED VACCINES FOR THE CONTROL OF MYCOPLASMA HYOPNEUMONIAE AND PORCINE CIRCOVIRUS TYPE 2 IN GROWING PIGS: A RANDOMISED NON-INFERIORITY FIELD TRIAL

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# **Background and Objectives**

Vaccination is commonly used to control Mycoplasma hyopneumoniae (Mhyo) and Porcine circovirus type 2 (PCV2) around weaning and considered as an efficient tool to control these diseases. The aim of this trial was to compare the efficacy of two vaccines against M.hyo and PCV2 with a ready-to-use (RTU) PCV2+M.hyo vaccine.

# **Material and Methods**

A commercial farm with the history of enzootic pneumonia(EP) and PCV2-associated disease(PCV2AD) in France was selected for this trial. A total of 822 pigs were included: G1 piglets were vaccinated at 21 days of age simultaneously with Circovac® and Hyogen®, G2 with Vaccine A (PCV2+M.hyo RTU) and 20 piglets (G3) were not vaccinated. Clinical signs, mortality, weight gain, EP-like lung lesions using Ceva Lung Program method and both pathogens infection dynamics using qPCR were examined and compared. Statistical analysis were carried out using R Studio version 4.0.2.

# Results

Clinical signs, mortality (4.2% vs 5.1%, p=0.31) and growth performances (ADG inclusion-slaughter 723.3g vs 727.9g, p=0.36) didn't differ statistically between G1 and G2. The average lung lesions score (3.2 vs 4.2, p=0.04) and the percentage of pigs with extensive EP-compatible lesions (lung score<sup>2</sup>6) (35.4% vs 45.9% respectively, p=0.0007) were significantly lower in G1 than G2. M.hyo loads in lower respiratory tract increased with the age and the percentage of pigs coughing. M. hyo loads in G1 were lower than in G2 and both were lower than in G3 but these differences were not significative (p=0.82). Both vaccines protected by reducing PCV2 viremia (PCV2 load was quantifiable only in G3).

## **Discussion and Conclusion**

In this study, both vaccination strategies provided efficient protection against infection with the two pathogens. Circovac® and Hyogen® vaccination resulted in stronger reduction of EP-like lung lesions than combined vaccine.

CONTROLLING PORCINE CIRCOVIRUS TYPE 2 (PCV2) EARLY INFECTIONS IN PIGLETS USING MASS VACCINATION OF SOWS.

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# **Background and Objectives**

Piglet vaccination is considered the most efficient tool to control PCV2 infections. Sow vaccination is also frequently applied before farrowing to prevent vertical transmission. Nevertheless, information is limited regarding the effect of mass sow vaccination for controlling early infections. Therefore, the objective of this study was monitoring the effect of mass sow vaccination against PCV2 in terms of piglet viremia during nursery.

# Material and Methods

This field trial was performed in a farrow-to-wean herd in which PCV2 early infections were detected at nursery and sow vaccination was not used. To assess PCV2 early infections, blood samples were taken from 30 animals at 3 weeks of age (woa) and 10 animals at 6 and 9 woa. Moreover, three piglets at 4 woa with growth retardation were sacrificed for PCV2 diagnosis. Upon farmer's request, all sows received one-dose commercial PCV2 vaccine (2ml; Circovac®, Ceva) independently of their reproductive status and piglet vaccination was maintained at 3 woa. To monitor the new scenario, the same sampling was repeated after six months of mass sow vaccination.

## Results

Piglets coming from non-vaccinated sows were PCV2 negative by qPCR at 3 and 9 woa, whereas 1 out 2 (50%) pools resulted positive at 6 woa with viral loads of 10<sup>4</sup>. Sacrificed piglets were positive to PCV2 viral loads of 10<sup>10</sup>, but no clinical signs were observed after sow vaccination. All piglets coming from vaccinated sows were PCV2 negative at all sampled ages after mass sow vaccination.

## **Discussion and Conclusion**

Mass sow vaccination against PCV2 at weaning reduced the number of PCV2 infected piglets. This strategy, together piglet vaccination, could represent an effective approach for decreasing PCV2 infectious pressure in farms. Additionally, it is important to note other factors (e.g. Seasonality) might influence the results. Notwithstanding this aspect, these results could be a useful starting point for further field studies.

# FIELD EVALUATION OF SAFETY AND EFFICACY OF NEW VACCINE AGAINST NEONATAL DIARRHEA CAUSED BY ESCHERICHIA COLI AND CLOSTRIDIUM PERFRINGENS A, C IN GILTS

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## **Background and Objectives**

Neonatal diarrhea causes major losses in pig herds worldwide. Enteroporc COLI AC (Ceva) is the new, registered vaccine containing inactivated fimbrial adhesins of Escherichia coli (F4ab, F4ac, F5, F6) and toxoids of Clostridium perfringens type A (alpha toxin, beta2 toxin) and C (beta1 toxin). The objective of study was to investigate the safety and efficacy of the vaccine in gilts under field conditions.

### Material and Methods

Seronegative or low positive gilts for the vaccine antigens were divided into a vaccinated (n=30) and a control group (saline, n=30). Gilts were treated at 5 and 2 weeks prior to farrowing. Efficacy was determined as % of gilts with antibody titer ≥ the protective titer in colostrum. The protective titer was calculated on the basis of experimental challenge studies and represents the colostral antibody titer above which the majority of piglets were protected and the majority of piglets below the cut-off level was not protected. Seroconversion in blood was determined at 14 days after second vaccination (dpv2). Safety was determined on the basis of rectal temperature, local and systemic reactions and reproductive performance.

#### Results

At 14 dpv2, a significant higher percentage of vaccinated gilts had colostral antibodies above the protective titer than control gilts. Also antibody titers in blood were significantly increased for all 7 antigens. No systemic reactions and differences in reproductive performance were observed. Transient local reactions were recorded within 7 days after each vaccination. The rectal body temperature in vaccinated gilts reached the peak with a mean increase from the baseline of 0.20 °C at 6h after first and 0.25 °C at 6h after second vaccination.

## **Discussion and Conclusion**

The efficacy of the vaccine was demonstrated based on a significant higher percentage of gilts with protective titers in colostrum for all tested antigens. No relevant differences in safety between the vaccinated and control group were recorded.

# EFFECTS OF A NEW VACCINE AGAINST CLOSTRIDIOIDES DIFFICILE AND CLOSTRIDIUM PERFRINGENS TYPE A ON THE INCIDENCE OF DIARRHOEA AND ANTIBIOTIC TREATMENTS UNDER FIELD CONDITIONS

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## **Background and Objectives**

Enteric pathogens such as Clostridioides difficile and Clostridium perfringens Type A are responsible for significant production and economic losses in piglets during the first week of life. Due to the restriction of antimicrobials in animal production systems, the control of enteric, pathogen-induced diarrhoea in piglets should be based on passive immunization. The objective of the study was to evaluate the passive protection of neonatal piglets by means of sows' vaccination with a new vaccine (Diff/A), assessing the incidence of diarrhoea and antibiotic treatments needed under field conditions.

## Material and Methods

Two farms suffering from neonatal diarrhoea from Hungary were included in a randomized, negative controlled and blinded field trial. C. difficile, C. perfringens Type A and Rotavirus were detected from faeces of sick animals. In total, 210 sows were distributed into two groups. One group (n = 109) was vaccinated with Diff/A, whilst the other group (n = 101) received a placebo. Products were intramuscularly administered at 6 and 3 weeks prior to farrowing. Mean incidence of neonatal diarrhoea (expressed as the percentage of piglets affected during the first 7 days of age) was compared between groups. The percentage of animals treated with antibiotics against diarrhoea up to 28 days old was recorded in one farm.

## Results

The incidence of neonatal diarrhoea was significantly lower in the vaccinated group than in the placebo (15.2% vs. 23.0%; p<0.05) corresponding to 34% decrease. Moreover, a significant reduction of 23% in the percentage of animals treated with antibiotics was detected (12.7% vs. 16.4% in vaccinated and placebo groups; p<0.05).

## Discussion and Conclusion

These findings indicate that immunization of sows with the vaccine Diff/A minimizes the productive losses caused by C. difficile and C. perfringens Type A reducing the diarrhoea incidence (Risk Ratio=0.66) and the percentage of animals treated with antibiotics (RR=0.77) despite Rotavirus circulation.

# FIELD EVALUATION OF SAFETY AND EFFICACY OF NEW VACCINE AGAINST NEONATAL DIARRHEA CAUSED BY ESCHERICHIA COLI AND CLOSTRIDIUM PERFRINGENS A, C ON SOWS

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## **Background and Objectives**

Neonatal piglet diarrhea has a worldwide and high prevalence. One of the most frequently associated pathogens are Escherichia coli and Clostridium perfringens type A and C. Enteroporc COLI AC (Ceva) is the new, registered vaccine containing 7 antigens: fimbrial adhesins of Escherichia coli (F4ab, F4ac, F5, F6) and toxoids of Clostridium perfringens type A (alpha toxin, beta2 toxin) and C (beta1 toxin). The objective of the presented study was to investigate the safety and efficacy on sows under field conditions following booster vaccination after initial (twofold) vaccination.

#### **Material and Methods**

38 second parity sows were allocated into 2 groups: (A= vaccinated, 20) and (B= saline, 18) and treated 2 weeks before expected farrowing. Before all sows from group A were initially vaccinated according to SPC (5 and 2 weeks before expected farrowing) whereas Group B was previously treated by saline solution. Safety was determined on the basis of local and systemic reactions, rectal temperature and reproductive performance. Blood samples were taken at 14 days post vaccination and colostrum on the day of farrowing for the assessment of the antibody titers.

#### Results

At the start of the study, sows of group A had significantly higher median antibody titers against the respective antigens (F4ab, F4ac, F5, F6, beta 1 toxin) than group B. At 14 days post vaccination a further significant increase of antibodies was observed in sows of group A in blood and colostrum which were equal or higher than after basic vaccination. No systemic reactions or differences in reproductive performance were recorded. Local reactions were observed in 70% of the sows with complete resolution by day 7. The maximum mean increase in rectal temperature was 0.24 °C.

## **Discussion and Conclusion**

Third administration of Enteroporc COLI AC was efficacious in sows and had an excellent safety profile.

## OUTBREAK OF PORCINE PARVO VIRUS NEW CLUSTER IN THE NETHERLANDS

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## **Background and Objectives**

Porcine Parvo Virus (PPV) infections are controlled by vaccination. Within PPV there is evolution and in Europe PPV strains are shifting from cluster A to cluster D. Recently evidence for the presence of a cluster D strain in the Netherlands was found in a PPV outbreak. This is a summary of the case and consequent findings.

## Material and Methods

A herd of 325 sows in the Netherlands showed clinical signs of still-born, mummification, embryonal death and infertility (SMEDI). Over a 9 weeks period the average number of live born piglets per week was 81 compared to 208 in the 9 weeks before the onset of the problems. Additionally the piglets in the week groups that showed the SMEDI-problems suffered higher pre-weaning mortality. Pooled samples of fetuses and mummies were tested by PCR for PCV2, PRRSV and PPV. PPV positive samples were tested by nanopore sequencing. The vaccine used in this farm was a registered PPV-Erysipelothrix rhusiopathiae vaccine administered according to the label.

#### Results

PPV was shown in the fetuses and mummies by PCR and by nanopore sequencing. The PPV VP2 region sequence was analyzed and the result showed clustering with recent European strains of cluster D.

# **Discussion and Conclusion**

Often used PPV-vaccines are based upon cluster A antigen that do not fully protect against the PPV-27a (cluster D) strain. The presence of PPV cluster D in the Netherlands aligns with previous studies that show an increase of PPV cluster D in Europe. We conclude that the presence of a PPV cluster D strain in the Netherlands is relevant and may lead to a different choice for PPV vaccine.

EFFICACY OF A NEW VACCINE INCLUDING TWO GENOTYPES OF PORCINE CIRCOVIRUS (PCV-2A AND PCV-2B) AND MYCOPLASMA HYOPNEUMONIAE WHEN ADMINISTERED TO PIGLETS AT 3 DAYS AND 3 WEEKS LATER UNDER FIELD CONDITIONS

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# **Background and Objectives**

This study aimed to evaluate the efficacy of a new trivalent vaccine containing inactivated Porcine Circovirus (PCV) Type 1-Type 2a chimera (cPCV-2a), PCV-1/2b chimera (cPCV-2b) and Mycoplasma hyopneumoniae (Mhyo) administered in a split dose regime in a commercial farm.

# Material and Methods

A total of 2.038 pigs (1.017 vaccinated and 1.021 non-vaccinated) were enrolled. Vaccine and placebo were administered at 3 days-of-age (DOA) and 3 weeks later. PCV2 antibodies and viraemia were assessed before first vaccination by ELISA and qPCR, respectively. Same parameters, including PCV2 qPCR faecal excretion, were evaluated monthly since vaccination until slaughter. Body weights (BW) were recorded before vaccination, at 16 weeks of age (WOA) and before slaughter. Lymphoid tissues were collected from euthanized and from spontaneously dead pigs. Histopathology and immunohistochemistry were performed to detect PCV2. PCV2 genotypes circulating in the farm were determined by sequencing.

## Results

Vaccinated group showed statistically lower PCV2 load in sera ( $p\le0.0446$ ) and faeces ( $p\le0.0039$ ) from 11 until 25 WOA and at 20 and 25 WOA, respectively. Proportion of pigs viraemic at least once (p=0.0343) and proportion of viraemic pigs ( $p\le0.0003$ ) were statistically lower in vaccinated groups compared to controls. Significant reduction in PCV-2 presence in lymphoid tissues (p=0.0176) was also observed in vaccinated pigs. PCV2b and PCV2d genotypes were isolated from study pigs. PCV2 antibody levels from vaccinated pigs were significantly higher ( $p\le0.0392$ ) from 7 to 20 WOA compared to controls. Vaccinated pigs had significantly higher BW ( $p\le0.0444$ ) at 16 WOA and at time of market, as well as average daily weight gain (ADWG) (p=0.0195) over the entire study.

# Discussion and Conclusion

Double immunization with the cPCV-2a/cPCV-2b/Mhyo vaccine reduced histopathological lymphoid lesions and PCV2 detection, as well as reduced losses in production variables (BW and ADWG), in pigs from a commercial farm experiencing a mixed PCV2 genotype infection.

## IMPACT OF PCV2 VACCINATION ON GILTS SEROCONVERSION

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## **Background and Objectives**

Porcine circovirus 2 (PCV2) has been shown to be involved in reproductive disorders in sows. This study sought to evaluate the effect of gilts whole virus vaccination against Porcine Circovirus 2 (PCV2) on seroconversion and to compare two boosting protocols on maternal derived antibodies (MDA) on their piglets at first farrowing.

## **Material and Methods**

The trial was performed in a PCV2 subclinical infected breeding herd in Brittany, in France. Twenty-six gilts were primo-immunized in quarantine with a commercial PCV2 vaccine, Circovac®. Because of field conditions, no control gilt remained un-vaccinated in this commercial farm. Then, the same animals received a booster either 5 weeks before farrowing (G1) or 3 weeks after (G2). Blood samples were taken throughout the study to assess PCV2 antibodies (semi-quantitative Elisa adapted from Synbiotics) on vaccinated animals and on 2 piglets of their litters at weaning age (3woa) (PCV2 Ingezim IgG).

### Results

At arrival in farm, gilts' PCV2 profile was heterogeneous and low (61% of titers <5000 and coefficient of variation (CV) of 80%). Four weeks after primo-vaccination, the titers were higher and homogeneous (81% of titers > 10000 and CV of 26.5%). At weaning, on piglets born from G1 animals, 45% showed Ingezim S/P > 1.2 whereas on piglets born from G2, only 4% showed Ingezim S/P > 1.2 (threshold for potential interference established in Martelli and al., 2016).

## **Discussion and Conclusion**

In this study, primo-vaccination of gilts with Circovac® enabled to reach high and homogeneous PCV2 antibodies titers lasting until first weaning. When a booster is applied at weaning on sows, less MDA are found on piglets at weaning; That could indicate a probability to have less interference with an early PCV2 piglets' vaccination. Specific studies would be necessary to confirm this hypothesis.

# EFFICACY OF DIFFERENT COMBINED OR SIMULTANEOUSLY ADMINISTERED VACCINES AGAINST MYCOPLASMA HYOPNEUMONIAE INFECTION

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## **Background and Objectives**

Enzootic pneumonia and PCVD remain a severe health problem in pig farms. Vaccination against Mhyo and PCV2 helps to reduce corresponding losses. Several commercial mono- or bi-valent vaccines are available. The aim of this study was to evaluate the efficacy of different PCV2 and M.hyo vaccines in the experimental M.hyo challenge models.

## **Material and Methods**

In two different experiments three-week old piglets (20per group) were vaccinated either with Circovac® plus Hyogen®(CH) - both Ceva simultaneously or various PCV2+M.hyo RTU or RTM vaccines. In the trial 1)vaccines RTUA and RTUB were used and pigs were challenged at 7 weeks of age(WOA). In the trial2)vaccines RTUA and RTMC were used and the challenge was performed at 12WOA. Serum samples were collected prior to vaccination, challenge, and slaughter, and measured by BioChek and IDVet Mhyo ELISAs. Pigs were always euthanized 4weeks later and lung lesions scored according to the European Pharmacopoeia 9.0.

## Results

In both trials CH induced always higher M.hyo seroconversion prior to challenge than any other vaccines. Group mean lung lesion scores (LLS) in groups CH, RTU A, RTU B and positive control in trial 1)were as follows: 0.16; 0.38; 0.23; and 0.68. Only CH and RTUB differed significantly from the control. In the trial 2) the results for CH, RTUA and RTM and positive control were as follows 0.72; 0.98; 1,2; and 1.22, respectively. Histopathology confirmed the macroscopic scores.

## **Discussion and Conclusion**

This study demonstrated that vaccines CH administered simultaneously outperformed the already combined PCV2+M.hyo RTU and also the RTM vaccines concerning the protection against the development of lung lesions due to M.hyo. Some of the combined PCV2 and M.hyo vaccine may provide sub-optimal protection against M.hyo infection and thus the convenience of such use doesn't correspond with the expected efficacy

## CEVA LUNG PROGRAM IN EVALUATING THE LUNG HEALTH AFTER TWO DIFFERENT MHYO VACCINES WERE USED

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# **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is part of the Porcine Respiratory Disease Complex at finisher farms. Prophylactic solutions exist such as Mhyo vaccines. The objective was to compare the lung health after two different vaccination strategies against Mhyo were used in a commercial pig farm.

# Material and Methods

Lung lesions were assessed in pigs from a Danish 600 sow to 30 kg and a finisher farm, positive for Mhyo, Actinobacillus pleuropneumoniae 6 and 12. Batch 1 of piglets was vaccinated on day 7 of age with a 2 mL Mhyo vaccine registered for day 3, and batch 2 was vaccinated on day 7 after weaning with Mhyo vaccine Hyogen, Ceva. Both batches were also vaccinated with a pcv2 vaccine on day 7 after weaning. The scores were evaluated using the Ceva Lung Program (CLP) method. 100 set of lungs of batch 1 and 122 set of lungs of batch 2 were randomly chosen and were evaluated by the independent vets at the Laboratory of swine in Kjellerup, Denmark.

## Results

The prevalence of broncho pneumonic lungs in batch 1 and 2 was 30% vs. 4% and the affected surface of the broncho pneumonic lungs was 7% vs. 2% resulting in an Enzootic Pneumonia like lesions (EP) index of 1.27 vs. 0.1 with a statistically significant difference (p=0.0000). Scarring was 21.0% vs. 3.28% with a statistically significant difference (p=0.000). Cranial pleurisy was 8.0% vs. 12.3% with no significant difference (p=0.378). Dorsocaudal pleurisy in Slaughter Pleurisy Evaluation System (SPES) in batch 1 and 2 was in a score 0=85%, 2=8%, 3=6%, 4=1% vs. 0=80%, 2=9%, 3=6%, 4=5% with no statistically significant difference (p=0.439).

## **Discussion and Conclusion**

Batch 2 had a statistically significant lower EP index and statistically significant fewer lungs containing Scarring. No statistically significant difference was found between the 2 batches regarding cranial pleurisy and dorsocaudal pleurisy.

# EFFECT OF A NEW INTRADERMAL VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE AND PCV2 ON GROWTH PERFORMANCE UNDER FIELD CONDITIONS

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## **Background and Objectives**

Mycoplasma hyopneumoniae (M.hyo) and Porcine circovirus type 2 (PCV2) are responsible for significant production and economic losses worldwide. The objective of the study was to evaluate the effect on growth performance of the new intradermal vaccine MHYOSPHERE® PCV ID (Vaccine A) under field conditions.

## **Material and Methods**

Seven farms with M.hyo and/or PCV2 circulation in previous batches were included in a multicentre, randomized, negative-controlled and blinded field trial. In total, 2,507 healthy 21 day-old piglets were distributed between two groups balanced by weight before vaccination. One group (n = 1,253) was vaccinated with Vaccine A, whilst piglets from the other group (n = 1,254) received a placebo. A single dose of 0.2 ml was administered to both groups intradermally using Hipradermic<sup>®</sup>. 100 animals/group/farm were weighed (21 days old, 9 weeks old, before slaughter). Growth performance was evaluated by analysing: average daily weight gain (ADWG), body weight and culling rate (<75 kg).

## Results

M.hyo and/or PCV2 circulation was confirmed on all farms during the study. ADWG was higher in the Vaccine A group from weaning to the end of fattening, with an improvement of 18 g/day (p = 0.0004). Consequently, at the end of the fattening period the mean body weight in the Vaccine A group was higher, 2.61 kg heavier than the controls (p = 0.0005). Finally, statistically significant differences were detected in the culling rate just before slaughter with 5.2 % fewer underweight pigs in the Vaccine A group (p = 0.0003).

## **Discussion and Conclusion**

The new intradermal vaccine MHYOSPHERE<sup>®</sup> PCV ID is a useful tool to minimize productive losses due to M.hyo and/or PCV2-related diseases because it has a beneficial effect on growth performance, by reducing the loss of daily weight gain and culling rate due to M.hyo and/or PCV2 on commercial pig farms.

## COMPARISON OF THE EFFICACY OF TWO DIFFERENT PCV2 VACCINES UNDER DUTCH FIELD CONDITIONS

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# **Background and Objectives**

The aim was to evaluate the efficacy of inactivated whole virus PCV2 vaccine against clinical problems related to PCV2 compared to an intradermal (I.D.) PCV2 vaccine in the nursery and finisher period under Dutch field conditions.

## Material and Methods

At two conventional herds with 200 and 185 sows, piglets were vaccinated at 3 WOA, either with Circovac®(VaccineC,0.5mL;I.M.) or I.D.PCV2 vaccine(Vaccine ID,2mL;I.D.). Both farms were vaccinating against M. hyopneumoniae with Hyogen® at the same age. Piglets were randomized per pen and housed in the same environmental conditions. At the age of 12 weeks piglets were moved to a 2000 finisher farm. The growth performance (ADWG), feed conversion (FCR) and mortality were measured of 4 batches of piglets and 4 other batches of finishers. Per treatment group, the weighted average ADWG, FCR and mortality was calculated.

## Results

In total 989 nursery piglets were included in the study. The Vaccine C vaccinated piglets showed an ADWG of 479 g/d (n=443) and the Vaccine ID group showed 481 g/d (n=546). The FCR was 1,93 and 1,92 respectively and the mortality was 3,16% and 2,2% respectively. No significant differences were found in the nursery. In total 838 finishers were included in the study. The Vaccine C vaccinated pigs showed a FCR of 2,33 (n=410) compared to the Vaccine ID group which showed a FCR of 2,41 (n=428). No differences were found for the ADWG which was 858 g/d for both groups and for the mortality which was 0,73% and 0,93% for Vaccine C and Vaccine ID group respectively. Only a significant difference was found in the FCR (p<0,05).

## **Discussion and Conclusion**

In conclusion, the 2 piglet vaccines demonstrated very similar efficacy in terms of ADWG and mortality. The FCR was significantly better for the Vaccine C group in the finishing period.

# VALIDATION OF THE TECHNICAL CHOICE OF VETERINARIANS FOR THE INTRADERMAL ROUTE WITH IDAL IN MYCOPLASMA VACCINATION VIA ANALYSIS OF 5 YEARS LUNG SCORING

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## **Background and Objectives**

Many vaccines against Mycoplasma hyopneumoniae, a major concern in respiratory pathology, are available. Good adequacy of vaccine schemes, biosecurity, or absence of iatrogenic risks due to needles are selection criteria for swine practitioners to maximize the benefits of vaccination. In this context some veterinarians decided to propose a vaccine by intradermal route with the IDAL<sup>®</sup> injector : Porcilis<sup>®</sup> M Hyo ID Once, and to make a follow up of lung lesions.

## Material and Methods

Lung scorings based on Madec system reduced to 6 lung lobes (score from zero to 24) were performed at slaughter between 2014 to 2019 on a total of 22907 lungs, while a switch from different intramuscular (IM) mycoplasma vaccines, to Porcilis<sup>®</sup> M Hyo ID Once, intradermal (ID), gradually occurred in 30 farms. It led to a comparison of an IM group of 10309 scorings, to an ID one of 12598 scorings performed after ID vaccination.

## Results

Mean pneumonia score calculated in the ID group were significantly better than in the IM group: 1,66 versus 1,98 with p value of 2,2.10<sup>-16</sup>. Repartition of individual pneumonia scores into 3 levels of severity show for the ID group, compared to the IM group, a significantly higher percentage of lungs without lesion (score of zero): 61,32% versus 53,61% (p=2,2.10<sup>-16</sup>), a significantly lower percentage of lungs with moderate lesions (score from 1 to 7): 31,55% versus 38,07% (p=2,2.10<sup>-16</sup>), and a significantly lower percentage of lungs with high lesions (score over 7): 7,13% versus 8,31% (p=0,00079).

## **Discussion and Conclusion**

This follow up of 5 years on 30 farms highlights a lower pneumonia score and higher proportion of lungs without lesion when the intradermal route is used in mycoplasma vaccination, compared to the intramuscular. It reinforced the veterinarians in their choice, initially made to limit infection risks due to needles.
# DEVELOPMENT OF A REAL-TIME RT- PCR ASSAY ABLE TO DIFFERENTIATE BETWEEN PRRS VACCINE STRAIN AND FIELD VIRUS STRAINS

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#### **Background and Objectives**

The aim of this study was to develop a new real-time PCR assay able to differentiate between vaccine and field virus, to facilitate monitoring of PRRSv.

## **Material and Methods**

The vaccine used was Porcilis® PRRS (MSD Animal Health). The PCR (PCR-DV) amplifies exclusively a 221 bp fragment from the ORF5 region of the vaccine virus. Based on the primers previously described by Harder and Huebert (2004), a probe (Werfer) was designed for use in real-time RT-PCR. Initial tests with the vaccine virus were performed and the detection limit was 10 PFU.

A total of 96 serum samples, previously tested and positive for PRRS (VetMAX PRRSV EU&NA 2.0 kit, Life Technologies), coming from 37 farms with known PRRS situations (field virus recirculation or animals just vaccinated), were analyzed to determine the sensitivity (Se) and specificity (Sp) of the PCR-DV, following the recommendations of the NF U47-600. Sequencing of ORF5 was performed in 19 cases to confirm the results.

#### Results

Of the 96 samples tested, one showed false positive and two false negative to the PCR-DV.

The calculated value of the estimated sensitivity was 94,3% (79,5%–99,0%, with a 95% C.I).

Estimated specificity was 98,4% (90,0%–99,9%, with a 95% C.I.).

In the two false negative cases, Ct values in the commercial PCR were 32,9 and 34,0, indicating some lack of sensitivity when PRRSV RNA quantity is low.

# **Discussion and Conclusion**

The new PCR-DV has shown to be a valid tool to differentiate between vaccinated and infected animals in the field. Nevertheless, further validation of the assay is needed to improve its sensitivity and specificity

# COMPARISON OF THE EFFICACY OF PCV2 WHOLE VIRUS INACTIVATED VACCINE WITH ANOTHER COMMERCIAL VACCINE UNDER FIELD CONDITIONS

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# **Background and Objectives**

Vaccination against PCV2 is standard practice in pig production and a number of different vaccines are available. The aim of the study was to evaluate the efficacy of a whole virus PCV2 vaccine against the effects of a PCV2 infection compared to a subunite PCV2 vaccine, in terms of growth performance and mortality under Dutch field conditions

## **Material and Methods**

At a conventional health 500 sow herd, with a known PCV2 infection, piglets were vaccinated at 3 weeks of age either with Circovac® (Vaccine C,0.5 mL, I.M.) or Vaccine A (1mL, I.M.). Two batches of piglets were included in the study. Piglets were randomized per pen and housed in the same environmental conditions. The 9-10 weeks old piglets were moved to a 4000 finisher farm. Growth performance (ADWG) and mortality were measured

## Results

In total 572 pigs were included in the study. In the first and second batch the Vaccine C vaccinated pigs showed an ADWG of 871 (n=102) and 841 g/d (n=143) and a mortality of 1.90% and 3.90% respectively. The Vaccine A group showed an ADWG of 857 (n=151) and 843 g/d (n=158) and a mortality of 2.58% and 1.27% respectively. There was no statistical differences for the mortality ( $c^2$  test, p>0,05) between the groups.

## **Discussion and Conclusion**

In conclusion, the 2 piglet vaccines demonstrated the same efficacy in terms of ADWG and mortality in the finishing herd. This study performed under Dutch conditions showed that Circovac® piglet vaccination leads to a solid protection against PCVD and its impact on performance.

# DURATION OF IMMUNITY OF A NOVEL INTRADERMAL VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE AND PCV2 INFECTION

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## **Background and Objectives**

Mycoplasma hyopneumoniae (M.hyo) and PCV2 are two of the most significant infectious agents causing economic losses in the weaning to slaughter period. The objective of this study was to establish the duration of immunity (DOI) of MHYOSPHERE<sup>®</sup> PCV ID (Vaccine A) against M.hyo and PCV2.

# Material and Methods

The DOI was evaluated in independent experimental M.hyo and PCV2 challenge studies. Within each experimental challenge, 3-week-old piglets were randomly divided into two groups (vaccinated and control). A single dose of 0.2 ml was administered intradermally to the vaccinated and control (PBS) pigs using Hipradermic<sup>®</sup>. M.hyo challenge was performed intranasally on three consecutive days at 23 weeks postvaccination. Three weeks after M.hyo challenge, the pigs were necropsied to evaluate lung lesions. PCV2b challenge was done by the intranasal route 22 weeks postvaccination. Four weeks after challenge, all the pigs were necropsied and the mesenteric and inguinal lymph nodes, tonsils and lungs were collected for PCV2 quantification by qPCR.

#### Results

With regard to the M.hyo lung lesions, the mean percentage of affected lung surface was significantly lower in the Vaccine A group than in the control group (1.96% vs 4.52%, respectively; p<0.05). In the PCV2 experiment, the mean AUC viral load in serum was significantly lower in the Vaccine A group (0.0 Log<sub>10</sub> genomic copies/mL vs 40.8 Log<sub>10</sub> genomic copies/mL; p<0.05). Vaccination also resulted in a significantly lower duration of viraemia in the Vaccine A group (0.0 days vs 17.1 days p<0.05). PCV2 tissue load was significantly lower (p<0.05) in the Vaccine A group than in the control group for lymphoid tissues, tonsil and lung tissues.

## **Discussion and Conclusion**

These results show that a single dose of MHYOSPHERE® PCV ID provides protection until at least 23 weeks (M.hyo) and 22 weeks (PCV2) postvaccination, which are typical slaughter ages.

# EVALUATION OF CROSS-NEUTRALIZATION BY ERYSENG® PARVO AGAINST NEW HETEROLOGOUS PORCINE PARVOVIRUS GENOTYPES

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## **Background and Objectives**

The Porcine Parvovirus (PPV) genome is a single-stranded DNA molecule of about 5000 bases. Sequence analysis of recent isolates suggests active evolution of PPV. Thus, phylogenetic studies of the capsid protein gene (VPI) have revealed a new cluster of PPV viruses characterized by specific nucleotide and amino acid changes. In this study, the virus neutralizing (VN) activity with post-vaccination sera was investigated.

## **Material and Methods**

Post-vaccination sera from 6 gilts vaccinated twice, 3 weeks apart, with Eryseng® Parvo (vaccine containing inactivated PPV strain NADL-2 and inactivated Erysipelothrix rhusiopathiae strain R32EII, adjuvanted with an aqueous adjuvant based on ginsenosides), and PPV-negative serum from a PPV-naïve gilt were serially diluted and used in a standard VN assay. Test antigens consisted of homologous (NADL-2) and heterologous (27a, 143a and Kresse) PPV reference strains. The geometric mean neutralization titres (GMT) and confidence interval (CI) at 95 % in post-vaccination sera were calculated for each antigen.

#### Results

All post-vaccination sera showed neutralizing capacity against both homologous and heterologous antigens, although with a trend towards being higher against homologous antigens with some within-group variation. The GMTs and CIs were (806; 150-1462), (570; 290-851), (403; 233-573) and (63; 26-101) for NADL-2, 27a, 143a and Kresse antigens, respectively. Meanwhile, the control serum did not show any type of neutralizing activity against the antigens tested.

## **Discussion and Conclusion**

Previous challenge experiments with PPV in pregnant sows have shown that low VN antibody titres on the day of infection were enough to confer clinical protection to the foetuses, avoiding death and mummification. Therefore, this in-vitro study suggests cross-protection of the post-vaccination sera of vaccinated animals with homologous and heterologous strains.

# INTERFERENCE OF MATERNALLY-DERIVED ANTIBODIES WITH SUVAXYN PRRS MLV VACCINATION IS DEPENDENT OF THE ANTIBODY LEVEL

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## **Background and Objectives**

We previously showed that maternally-derived neutralizing antibodies (MDNA) could interfere with the Porcilis PRRS vaccine by decreasing the post-vaccination (PV) immune response and the vaccine efficacy. Suvaxyn PRRS MLV is a new vaccine which is intended to be used in piglets from the first day of life onward, in presence of maternal antibodies. The objective of the study was to assess the sensitivity of Suvaxyn to MDNA interference in piglets with moderate (A+<sup>mod</sup>; mean NA titer=3.5 log2) or high antibody level (A+<sup>high</sup>; mean NA titer>5 log2).

#### **Material and Methods**

Specific Pathogen Free (SPF) piglets derived from Suvaxyn vaccinated (A+) or unvaccinated (A-) SPF sows, were vaccinated or not at 2 weeks of age. The PV response was followed (vaccine viremia, PRRS ELISA, virus neutralization test and ELISPOT IFNg) until PRRSV challenge at day 45 PV. The vaccine efficacy was then assessed at the virological level (challenge strain specific RT-qPCR).

#### Results

In A+<sup>mod</sup> piglets, the vaccine viremia was 2 week-delayed and 1000-fold-reduced, the seroconversion was postponed by 1 week but the cell-mediated immunity (CMI) induction was preserved compared with A-vaccinated piglets (270 and 253 IFNg secreting cells/million PBMC at D42 PV, respectively). After challenge, the vaccine efficacy was decreased with a PRRSV viral load 10 to 100-fold higher compared to A-vaccinated piglets. In A+<sup>high</sup> piglets, the Suvaxyn viremia and the antibody response were abolished and only a very low CMI (60 IFNg-secreting cells/million PBMC at D42 PV) was detected leading to a very limited vaccine efficacy (0.5log10 eqTCID50/ml decrease of the PRRSV viral load compared to unvaccinated pigs).

#### **Discussion and Conclusion**

This study demonstrates that Suvaxyn, as Porcilis, is susceptible to interference for high MDNA levels. In case of moderate MDNA levels, partial interference is evidenced for Suvaxyn with incomplete vaccine efficacy.

# COMPARISON OF FOUR VACCINE COMBINATIONS ON GROWTH PERFORMANCE AND ACUTE PHASE PROTEINS AT WEANING

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# **Background and Objectives**

PCV2, Mhyo and Lawsonia intracellularis are pathogens that play a primary role in the porcine respiratory or enteric complexes in pigs. Several vaccination strategies are available in the market to protect piglets that may differ in their adverse responses. This study compared the impact of 4 different vaccination combinations on Average Daily Gain (ADG) and acute phase proteins (APP) levels.

## **Material and Methods**

A total of 64 piglets were randomly selected and assigned to treatment groups: Group A was vaccinated with FLEXCOMBO®, Group B with FLEXCOMBO® + Enterisol Ileitis®, Group C with Porcilis PCV MHyo® and Group D with Porcilis PCV MHyo® + Porcilis Lawsonia®. All groups were vaccinated two days before weaning. Individual blood samples and weight were taken at time 0h, 24h, and 48h after vaccination. Serum Haptoglobin (HP) and C-Reactive Protein (CRP) were determined using an automatic biochemical analyzer (Olympus 2700, Germany). Data were analyzed using R 3.6.2. software. Linear mixed models with both fixed and random effects (intercept) were used to study the evolution of ADG and HP levels from vaccination to weaning depending on the treatment group.

#### Results

No difference in the ADG over time was observed between the Group A and B (p=0.1702). However, the ADG decreased over time in C and D groups by 2,5 and 2,1 gr/dia compared to animals from group A (p=0.0137 and p=0.0264). Additionally, Hp levels were also significantly higher in C and D groups compared to A group (p=0.0020 and p=0.0001, respectively).

## **Discussion and Conclusion**

Results from this study suggested that different types of vaccine combinations applied to control PCV2, Mhyo and Lawsonia intracellularis differ in the evolution of inflammatory markers and key economic production parameters of piglets. Further studies are needed to compare the long-term impact of these vaccine combinations in the growing-finishing phase of piglets.

## EFFICACY OF A NEW INTRADERMAL VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE AND PCV2 INFECTION

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# **Background and Objectives**

MHYOSPHERE<sup>®</sup> PCV ID (Vaccine A) is a novel ready-to-use vaccine against Mycoplasma hyopneumoniae (M.hyo) and PCV2 consisting of a new biological entity as the active substance. The aim of this study was to determine the efficacy (onset of immunity (OOI)) against M.hyo and PCV2 infections under experimental challenge studies.

## **Material and Methods**

The OOI for each pathogen was evaluated in independent experiments. In each experiment, 3-week-old piglets were randomly divided into two groups (vaccinated and control) at the time of vaccination. A single dose of 0.2 ml using Hipradermic<sup>®</sup> was administered intradermally to the vaccinated pigs and the control pigs (PBS). M.hyo challenge was performed intranasally on three consecutive days at 3 weeks postvaccination. Three weeks after M.hyo challenge, the pigs were necropsied to evaluate lung lesions. PCV2b challenge was done by the intranasal route 2 weeks postvaccination. Four weeks after PCV2b challenge, all the pigs were necropsied and the mesenteric lymph nodes, tonsils and lungs were collected for PCV2 quantification by qPCR.

## Results

With regard to the M.hyo lung lesions, the mean percentage of affected lung surface was significantly lower in the Vaccine A group than in the control group (8.26% vs 12.89% p<0.05). In the PCV2 experiment, the mean AUC viral load in serum was significantly lower in the Vaccine A group (10.4 Log<sub>10</sub> genomic copies/mL vs 24.2 Log<sub>10</sub> genomic copies/mL p<0.05). Vaccination also resulted in a significantly lower duration of viraemia in the Vaccine A group (6.0 days vs 13.0 days p<0.05). PCV2 tissue load was significantly lower (p<0.05) in the Vaccine A group than in the control group for mesenteric lymph node, tonsil and lung tissues.

## **Discussion and Conclusion**

The experimental challenge studies indicate that the efficacy (OOI) of MHYOSPHERE® PCV ID occurs as early as 3 weeks postvaccination for M.hyo and 2 weeks postvaccination for PCV2.

# CASE OF REPRODUCTIVE FAILURE IN GILTS CHARACTERIZED BY DELAYED DELIVERY AND MUMMIFICATION OF FOETUSES

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## **Background and Objectives**

The porcine parvovirus (PPV), more correctly named Ungulate protoparvovirus I species, is a single-stranded DNA virus classically leading to stillbirths, mummification, embryonic death and infertility (SMEDI). PPV viruses are prone to constant mutations and are classified into several strains of variable pathogenicity, some of them with very high virulence. Here we report a recent clinical case of SMEDI in vaccinated gilts.

## **Material and Methods**

In May 2020, an Austrian PRRS-free farm counting around 200 sows (farrow-to-finish) with fair reproductive performances reported multiple reproductive disorder. For repro-prophylaxis, the gilts were vaccinated twice pre-mating and boosted every six months by a commercial PPV-NADL2 + Erysipelas rhusiopathiae (Ery) combo-vaccine. After being one day late, farrowing was induced in one gilt which then delivered 5 mummies. Mummies and serum from selected gilts/ sows, as well as boar semen, were submitted for laboratory investigations. Two other farms receiving gilts from last facility experienced similar reproductive issues but were not available to investigation.

#### Results

Pooled organ samples from the mummified foetuses (10, 12, 12, 12, and 30 cm) contained high PPV viral load by real-time PCR (Cq values of 9.5) while resulted negative to Chlamydia, Leptospira, PCV2 and PRRSV. Furthermore, no antibodies against PPRSV were detected in serum. Vaccine management and application was controlled and found to be up to good standards (vaccinated by veterinarian). Application of a PPV-K22 + Ery combo-vaccine reversed the reproductive disorders.

### **Discussion and Conclusion**

Vaccination against PPV and Ery is one of the most basic protocols for breeding stock worldwide. As mentioned previously, PPV strains can have different levels of pathogenicity, genetic clusters, and variable antigenicity. When vaccinating against PPV there are indications that the PPV-Kresse-like strain K22 as an antigen confers a wider and more efficient clinical PPV protection than the NADL-2 strain, particularly against virulent strains.

SEROCONVERSION PROFILE OF WEANED PIGLETS AFTER VACCINATION WITH 3 DIFFERENT COMMERCIAL MYCOPLASMA HYOPNEUMONIAE VACCINES

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# **Background and Objectives**

Mycoplasma hyopneumoniae (M.hyopneumoniae) is a cell wall-free bacterium and one of the primary agents causing Porcine Respiratory Disease Complex (PRDC) [1]. Circulating antibodies (IgG) against M.hyopneumoniae can be detected after maternal absorption, infection or vaccination [2]. Interpretation of the latter could be a useful tool in M.hyopneumoniae-unsuspicious herds, after the implementation of vaccination protocols.

## **Material and Methods**

Present trial was conducted in a M.hyopneumoniae-unsuspicious piglet producing farm counting 540 sows. At weaning, piglets were separated into four different groups with 20 piglets each. Group A (GA) was treated with a M.hyopneumoniae + PCV2 vaccine (2ml), group B (GB) with a one-shot M.hyopneumoniae formulation (1 ml), group C (GC) with another one-shot vaccine (2ml) and group D (GD) was left unvaccinated. Blood samples were taken at weaning and repeated every two weeks. A commercial competition ELISA was used for seroconversion evaluation and values expressed in serum/negative control in %.

## Results

Surprisingly, seroconversion was measured in all four groups. GA and GD had on average 10% of positive animals throughout the trial. GB had 40% on all three sampling occasions. Highest seroconversion was observed in GC, with 60%, 75% and 65% of positive animals per sampling. Inversely correlated values were lowest in GC (42.0), followed by GB (53.1), GA (82.1) and GD (82.7) respectively.

# **Discussion and Conclusion**

Even though, the detection of antibodies by ELISA is not an evidence of protection. In this present study, it has been shown that in terms of seroconversion induction, there are major differences between tested commercial products. Whereas GA did not show differences to unvaccinated animals, GB presented a seroconversion in 40% of all animals. Animals treated with vaccine from GC (Hyogen, Ceva Santé Animale) had the highest probability of seroconversion as well as the highest titres, indicating that serology might be used as a tool to check performed vaccination and its potency.

# DECAY OF MATERNALLY DERIVED ANTIBODIES AGAINST PORCINE CIRCOVIRUS 2 (PCV-2) IN PIGLETS FROM EUROPEAN FARMS USING DIFFERENT VACCINATION PROTOCOLS IN SOWS

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# **Background and Objectives**

The objective of the study was to evaluate the decay of Porcine Circovirus 2 (PCV-2) maternally derived antibody (MDA) titers in piglets from 109 European farms using different PCV-2 sow vaccination strategies.

# **Material and Methods**

Farms from different countries (France, n=30; Germany, n =27; Italy, n=22; and Spain, n=30) were selected based on the following PCV-2 vaccination strategies in sows: during sow pregnancy (n=29), during gilt development (n=33), as piglet only (n=36) and none (n=11). All licensed PCV-2 vaccines in Europe were represented in the study. Within each herd and in a single visit, thirty 3-day-old and thirty 21-day-old piglets were sampled from sows of different parity number. Sera were tested using SERELISA® PCV2 Ab Mono Blocking kit. The MDA decay was calculated as the reduction (in percentage) of MDA titers from 3 to 21 days of age.

## Results

In all countries and for all vaccination regimes, the highest PCV-2 MDA titers were detected in 3-day-old piglets and the lowest in 21-day-old ones (MDA decay). The highest titers were detected in piglets coming from sows vaccinated during pregnancy in all countries. In France, Germany and Spain the lowest antibody titers belonged to piglets from sows vaccinated as piglets; in Italy in those coming from vaccinated gilts. In France, Germany and Spain, the lowest reduction of MDA levels was observed in those farms vaccinating sows during pregnancy (21, 33 and 46%, respectively). In Italy, the lowest reduction occurred in farms vaccinating during gilt development (32%).

## **Discussion and Conclusion**

This study illustrates the overall decay of MDA levels in piglets from farms using different PCV2 sow vaccination strategies in 4 European countries. PCV2 MDA levels and decay dynamics vary depending on the vaccination strategy used.

# EFFECT OF ALGAE DERIVED BETA-(1,3)-GLUCAN ON HUMORAL RESPONSE TO PRRSV VACCINATION IN WEANED PIGS

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# **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) is affecting pigs worldwide and has a huge economic impact. Vaccination is used to control this disease. Therefore, any tool supporting vaccination is welcome. Previous studies have shown that algae derived beta-(1,3)-glucan is increasing humoral response to several vaccines. The aim of this trial was to test if the administration of beta-(1,3)-glucans in feed to weaned piglets results in a higher humoral response after PRRSv vaccination.

## Material and Methods

A trial was performed at the farm of the university of Barcelona (Spain) and consisted of 3 groups of 17 piglets each, aged 35 days at start. Animals were fed diets containing 0, 100, and 200 g/t beta-(1.3)-glucan (Aleta<sup>™</sup>, Kemin) for 14 days before vaccination: group 0, 1 and 2 respectively. At day 14 of the trial, all pigs received a dose of an attenuated live vaccine (Porcilis PRRS, MSD animal health). Antibody titers were measured at 14 and 21 days after vaccination, expressed as sample to positive (s/p) ratio.

## Results

Piglets of group 1 and 2 showed significant higher levels of specific PRRSv antibodies (s/p ratio was 0.91 and 0.90 respectively) and increased proportion of seroconverting animals (75% and 70.6% respectively) at 14 days after vaccination, compared to the non-supplemented animals (47.1% of animals seroconverting with an average s/p ratio of 0.52). At 21 days after vaccination, both group 1 and 2 showed numerically higher levels of specific antibodies (s/p ratios were 1.170 and 1.011 respectively) compared to group 0 (s/p ratio=0.991). More animals seroconverted in both group 1 (93%) and 2 (94%), compared to group 0 (76%).

## **Discussion and Conclusion**

Findings from this trial show that beta-(1.3)-glucan supplementation can be used to improve and accelerate humoral response to PRRSv vaccination in weaned pigs.

# ASSESSMENT OF PORCINE LUNG LESIONS AT SLAUGHTER FROM BATCHES IMMUNIZED WITH DIFFERENT CONVENTIONAL MYCOPLASMA HYOPNEUMONIAE VACCINES AND UNVACCINATED ANIMALS

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## **Background and Objectives**

Mycoplasma hyopneumoniae (MH) is the primary agent of enzootic pneumonia (EP), which in case of viral co-infections can lead to the Porcine Respiratory Disease Complex. Vaccination against MH is applied worldwide to control possible infections. The goal of this study was to compare lung lesions at slaughter from fattening pigs vaccinated with six different conventional MH vaccines or unvaccinated.

## Material and Methods

Slaughter lung checks were performed in Germany and Austria from 2017 until 2019, for 19238 lungs from 174 batches. Lungs were assigned to each one of the following groups according to the MH vaccine and protocol used: unvaccinated piglets (0), Hyogen® (1), single-shot (SS) 2ml vaccine (2), SS 2ml vaccine (3), combined MH + PCV2 (COM) 2ml vaccine (4), COM 2ml vaccine (5) and SS 1ml vaccine (6). The prevalence of bronchopneumonia (BP) and % of affected lung surface (LS) was calculated and compared among groups.

# Results

BP% for each of the seven groups was, 0: 42.8%, 1: 31.6%, 2: 41.3%, 3 :66.1%, 4 :44.2%, 5 :57.9%, 6 :56.8%. BP% was negatively associated only with group 1 compared to unvaccinated animals, although the association was non-significant (p>0.05). Regarding comparison among vaccine groups, BP% was positively associated (p<0.05) with each of the other MH vaccines compared to group 1. In terms of LS, group 1 had numerically lower values than all other groups and significantly lower (p<0.05) compared to 0 (4.3% vs. 6.8%) and 3 (4.3% vs. 8.7%).

# **Discussion and Conclusion**

In this study, lungs from pigs vaccinated with Hyogen® showed superior lung health in terms of EP lesions compared to unvaccinated animals and the rest of MH vaccines. However, unvaccinated animals did not perform worse than most MH vaccine groups, probably due to the high respiratory health status of those herds.

# COMPARISON OF INTRADERMAL AND INTRAMUSCULAR INJECTION TO CONTROL RESPIRATORY PROBLEMS CAUSED BY MYCOPLASMA AND CIRCOVIRUS

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## **Background and Objectives**

Respiratory pathology is still a major issue in pig farms, especially in high pig density area, such as Brittany, and the biosecurity measures recommended with the vaccination program by the veterinarian, are decisive for the success of the control

# Material and Methods

Five hundred piglets, random allocated in 2 groups at weaning, 2 times were followed from weaning to slaughterhouse to evaluate the benefit of Porcilis M Hyo ID ONCE and Porcilis PCV ID vaccines, injected by the intradermal way with IDAL injector for ID group (ID) and Ingelvac Mycoflex and Circoflex mixed vaccines injected intramuscularly for IM group (IM).

## Results

The differences obtained between ID group and IM group are presented below. The percentage of losses over the period was 13% for ID group compared to 20% for IM group (p=0.0074, chi<sup>2</sup> test), at slaughter the pigs of ID group was 116,4 kg at 118,9 days and 114,1 kg for IM group at 118,2 days (p=0.0018, Kruskal Wallis test). The economical added-value (lean and homogeneity) was  $0,148 \in$  / kg for ID group and  $0,132 \in$  / kg for IM group (p=0.01, Kruskal Wallis test). A lung lesion score was performed on 86% of the slaughtered pigs and revealed a very significant difference between the two groups. 50% of the lungs from the IM group show large lesions (note > 7 in Madec scale) while for the ID group it was 20 % (p= 3.475<sup>-15</sup>, pearson's chi<sup>2</sup> test)

## **Discussion and Conclusion**

In a severe respiratory context, the Porcilis M Hyo ID ONCE and Poriclis PCV ID injected with IDAL have demonstrated significantly higher efficacy compared to the Ingelvac Mycoflex-Circoflex IM vaccination.

# RU COMPARATIVE STUDY TO EVALUATE THE EFFICACY OF DIFFERENT VACCINATION STRATEGIES AGAINST PCV2 AND ENZOOTIC PNEUMONIA

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#### **Background and Objectives**

A controlled trial was performed on a conventional farrow-to-finish farm in Russia. The goal was to compare the efficacy of different vaccines against PCV2 and enzootic pneumonia (EP further) under field conditions.

### **Material and Methods**

The study was performed on a farm with 5000 sows. The farm was PRRS, EP and Actinobacillus pleuropneumoniae positive. 2 months prior the moment of slaughter pooled serums were investigated for the presence of PRRS and PCV2 antigens and all of them were negative at middle and the end of nursery and finishers (cross sectional sampling) and there was no clinical observation of PRRS and PCV2 during the trial. The same should be mentioned about Actinobacillus pleuropneumoniae, groups were not affected by that pathogen during the trial. Group 1 was vaccinated with commercial vaccine Porcilis PCV M.Hyo and group 2 was vaccinated with freshly mixed PCV and M.hyo vaccines, at 3 weeks of age. Comparison of production parameters was done out of 1530 finisher pigs from group 1 and 1324 finisher pigs from group 2, also 250 lungs were checked from group 1 and 250 lungs checked from group 2 with Porcus Lab Application®.

#### Results

Total results of evaluating lungs and production data per 500 pigs vaccinated with 2 different strategies.

Healthy lungs: Group 1: 65.6% (164) Group 2: 26.8% (67)

Mild damage: Group 1: 33.6% (84) Group 2: 56.8% (142)

Serious damage: 0.8% (2) 16.4 (41)

## **Discussion and Conclusion**

Objective of the study was to compare different vaccines and Mycoplasma-associated pneumonia at slaughter using Porcus Lab Application®. Severity of EP can be reduced by usage of different vaccines, since Porcilis PCV M.hyo group showed better results in comparison with group vaccinated with freshly mixed vaccines.

# EVALUATION OF LUNG HEALTH IN PIGS VACCINATED AGAINST MYCOPLASMA HYOPNEUMONIAE BY TWO DIFFERENT ROUTES

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## **Background and Objectives**

Mycoplasma hyopneumoniae (Mhyo) is the etiological agent of Enzootic pneumonia, a pig disease worldwide distributed. This respiratory disease leads to important economic losses. Vaccination has been shown to reduce the severity and the impact of the disease. The aim of this study was to evaluate the lung health in pigs vaccinated by two different routes.

## **Material and Methods**

In total 200 pigs from a commercial herd in UK were included in the study and allocated in two groups: group ID and group IM. All the pigs were vaccinated against Mhyo and PCV2 at 21 days of age: ID (Porcilis PCV ID & Porcilis M Hyo ID Once); IM (Porcilis PCV M Hyo). A control non-vaccinated group was not included due to ethical considerations. Lung tissue was collected at slaughter and lung health evaluated by the following parameters: presence of Mhyo (PCR; n=10), microscopic Mhyo-like lesions (semiquantitative score 0-3 according to Redondo; n=10) and macroscopic Mhyo-lung lesions (Godwin score; n=200).

## Results

There was no DNA of Mhyo present on the lung tissue. Bronchoalveolar cell infiltration was absent in both groups, whereas BALT hyperplasia was mild in ID group (1/5) and moderate in IM group (4/5). A low prevalence and severity of macroscopic Mhyo-like lesions was recorded at slaughter. The number of animals affected with mild pneumonia lesions was very limited in both groups (ID: 6%; IM: 11%). Goodwin score confirmed this low severity (ID: 0.15; IM: 0.28).

# **Discussion and Conclusion**

In conclusion, these results together suggest that both intradermal and intramuscular vaccination against Mhyo were equally efficacious in controlling Mhyo infection as shown by the absence of bacterial colonization in lung tissue, the low severity of microscopic lung lesions and the low prevalence and severity of macroscopic lung lesions.

# EFFICACY OF A NEW VACCINE AGAINST ERYSIPELAS, PARVOVIRUS AND LEPTOSPIRA TO CONTROL ENDEMIC LEPTOSPIROSIS

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#### **Background and Objectives**

The aim of this trial was to evaluate the efficacy of a new Leptospira vaccine to control an endemic infection of leptospirosis in a commercial sow's farm

#### Material and Methods

The trial was conducted in a 1200 sow's farm, previously diagnosed as positive of Leptospira interrogans, serovar Bratislava. The farm had high percentage of returns in estrus, low farrowing rate, and high percentage of vulvar discharges. To control Leptospira impact, antibiotic treatment was used in a routine basis (oxytetracycline via feed, for 15 days, every 2-3 months), despite reproductive performance was still below objectives. In September 2018, all sows were vaccinated and revaccinated against Leptospiras, followed by a revaccination scheme every 6 months. The vaccine used was a trivalent vaccine against Erysipela, Parvovirus and Leptospira. Gilts were also vaccinated and revaccinated prior to its introduction in the sow's farm. Antibiotic treatment was stopped 2 weeks after sow's revaccination. To evaluate the efficacy of vaccination, reproductive data were analyzed, comparing data from 6 months prior to vaccination against 5 months after onset of immunity of vaccination (sept and oct 2019 were excluded of the study). Reproductive data were statistically analyzed (one-way ANOVA).

#### Results

Farrowing rate (FR) was significantly higher in the vaccinated batches (V) than in the not vaccinated ones (NV) (FR: V 90,13% vs 81,96%; p=0,025). No statistical differences were found in fertility until 40 days of gestation, nor in prolificity data (total born, born alive, still births). It was observed a clear reduction in vulvar discharges, that almost disappeared after vaccination. Additionally, none antibiotic treatment was needed after vaccination implementation.

#### **Discussion and Conclusion**

In this study, vaccination against Leptospira with a trivalent vaccine showed to improve the reproductive impact of leptospirosis in an endemic infected farm, as well as to reduce the use of antibiotics destined to its control.

## EFFICACY OF VACCINATION AS A TOOL TO CONTROL AN ACUTE LEPTOSPIROSIS INFECTION

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## **Background and Objectives**

Leptospirosis can have a big impact on reproductive parameters. Its control in Europe has classically been faced via antibiotic treatments. Recently a new vaccine against Leptospira was registered in Europe. The aim of this trial was to evaluate the efficacy of vaccination as a tool to control an acute infection of leptospirosis in a sow's farm.

## **Material and Methods**

The study took place in a 600 sow farm. Between April and June 2018, a reduced fertility rate was detected, reaching values of 78%. Diagnostics proved the presence of an infection with Leptospira interrogans, serovar Bratislava. Antibiotic treatment was initiated with Oxytetracycline, and, despite some improvement, fertility was still low. A sow vaccination with a trivalent Erysipela, Parvovirus and Leptospira vaccine was implemented. All sows were vaccinated in February 2019 and revaccinated 4 weeks later. Re-vaccinations were done per group 10 days post-farrowing. Efficacy of vaccination was evaluated comparing fertility rates from outbreak to vaccination implementation (April 18 to Jan 19) versus the one obtained in the following months after onset of immunity (April to September 2019). The months when basic vaccination was stablished were excluded from the analysis (February and March 2019). Reproductive data were statistically analyzed (one-way ANOVA and Mann Whitney test).

## Results

Fertility was significantly higher in the vaccinated batches (V) than in the not vaccinated ones (NV) (V 91,67% vs NV 83,5%; p=0,007). Variability between batches was also reduced (standard deviation V 1,96 vs NV 6,13). Also, a clear reduction of antibiotic use was detected after vaccination. No statistical differences were observed in other parameters such as total born, born alive or still births.

## **Discussion and Conclusion**

In this study, vaccination against Leptospira showed to be an effective strategy to improve fertility and reduce its variability after an acute infection.

#### INFLUENCE OF DIFFERENT VACCINES ON THE BEHAVIOR OF THREE WEEK OLD PIGLETS UNDER FIELD CONDITIONS

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## **Background and Objectives**

Adjuvants have an important function driving the type of immune responses, and also an impact in the safety profile of each vaccine. The impact of a new PCV2-M.hyo combination vaccine on piglet activity and milk uptake, in comparison to other vaccines was assessed in this study.

## Material and Methods

120 suckling piglets from ten litters were assigned randomly to four groups within each litter at three weeks of age, and treated as follows: A) M. hyo vaccine only (2 ml), B) M. hyo vaccine (2 ml) and NaCl (2 ml), C) M. hyo vaccine (2 ml) and PCV2 vaccine (2 ml), D) Suvaxyn Circo+MH RTU vaccine (Zoetis, 2 ml). Individual piglets' behavior was recorded hourly during 43 hours after vaccination as a binomial parameter as either active or non-active. Piglets were weighed before, 20 and 43 hours after vaccination.

## Results

Active behavior recorded in the 43 hours after vaccination differed among the four treatment groups (GLMM, vaccine treatment  $\chi^2$ =17.733, df=3, p=0.0004), was significantly different in piglets from group C. Piglets in Group C were more often recorded to be less active. Furthermore, they spent more time in the heated creep area (Fisher's Exact Test, p=0.03). Piglets of group C showed the slightest increase in weight until 43 hours after vaccination, suggesting lower milk uptake compared with the other groups.

## **Discussion and Conclusion**

The behaviour of piglets vaccinated at weaning can be impacted by the type of PCV2 vaccine, vaccine combination and adjuvant. In this study, a vaccine containing a mineral oil-based adjuvant (group C) administered simultaneously with an M. hyo vaccine led to a reduction in piglet activity and milk uptake post-vaccination. This may have a negative impact in performance and well-being of vaccinated piglets at the critical time of weaning.

## APPLICATION OF PCV2 VACCINES IN A COMMERCIAL SWINE FARM: A COMPARATIVE FIELD TRIAL USING CIRCOVAC®.

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# Background and Objectives

Vaccinating against Porcine Circovirus type 2 (PCV2) helps to reduce the clinical manifestations of this infection and the losses associated with the subclinical infection. Several commercial vaccines are available: from whole virus vaccines to sub-unit and recombinant vaccines. The purpose of this study was to evaluate the efficacy of Circovac® (whole virus inactivated vaccine) in a commercial farm in comparison with a competitor PCV recombinant vaccine (Vaccine A).

# Material and Methods

In a commercial farrow-to-finish farm, 3 consecutive weaning batches were vaccinated with vaccine A and the following 3 batches were vaccinated with Circovac® (approximately 660 piglets per group, all vaccinated at 3 weeks of age). In each group, 60 random piglets were ear tagged and weighted at 54 and 165 days of age. Blood samples were collected at 4, 6, 8, 12, 16, 20 and 24 weeks of age and submitted to qRT-PCR for PCV2 (LSI VetMAX<sup>TM</sup> PCV2 – Quantification kit). Mortality was recorded and the animals were regularly checked for PCVD compatible clinical signs.

## Results

A relevant viral load of PCV2 was observed between the 12 and 24 weeks of age (maximum viral load: 10<sup>7</sup> copies/ml in the Circovac® group versus 10<sup>8</sup> copies/ml in Vaccine A). No clinical signs or differences in the mortality rate were observed (1,48% in Circovac® group versus 1,61% in Vaccine A group) and the average daily weight gain was similar (0,710g in Circovac® group versus 0,711g in Vaccine A, p>0,05).

## **Discussion and Conclusion**

These results showed a similar protection against PCV2 provided by both vaccines: despite de viral load found, no signs of disease or losses related to subclinical infection were detected. The vaccination with Circovac® granted a protection at least as good as the competitor vaccine, with no differences in mortality, clinical signs or ADWG.

# EFFICACY OF CIRCOVAC® AND HYOGEN® VACCINES ON THE CONTROL OF PORCINE CIRCOVIRUS AND MYCOPLASMA HYOPNEUMONIAE INFECTION UNDER FIELD CONDITIONS.

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#### **Background and Objectives**

The objective of this study was to compare the efficacy of two commercial vaccines, administered simultaneously, on the control of Mycoplasma and Porcine Circovirus infection under field conditions.

## Material and Methods

A total of 1,295 weaned pigs (at 4 weeks of age) were divided into two groups. Group A (n=700) was vaccinated with a commercial porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo) combination vaccine at 4 weeks of age. Group B (n=595) was vaccinated with Circovac® and Hyogen® (0.5 mL and 2.0 mL), at 4 weeks of age. Lung lesions scores were measured for enzootic pneumonia-like lesion (EP-like) using B.E.Straw's method at slaughter. Pig performance parameters including mortality rate, average daily gain (ADG), weight at slaughter were recorded and calculated.

#### Results

Result showed that prevalence of EP-like lesion in group B was 72.2%, compared with 79.3% in group A. Average EP-like lesion affected lung surface was 9.3% and 11.7% in B and A groups, respectively. In the fattening period, pig mortality rate was significantly reduced in group B (2.6% VS. 7.0%, p=0.002). Average daily gain (ADG) in group B was significantly higher compared with that of group A (755 g/d VS. 695 g/d, p<0.001). Furthermore, pigs in group B had significantly shorter average days to slaughter compared with group A pigs (117.2 days VS. 121.9 days, p<0.001). However, there were no differences in pig performance during nursery period.

## **Discussion and Conclusion**

In conclusion, the use of Circovac® and Hyogen® vaccines showed better efficiency in growth performance under field conditions. The use of this combined vaccine can reduce mortality rate and EP-like lesion, and improves growth performance during the fattening phase comparing with conventional PCV2/M. hyo combied vaccine.

# EFFECT OF SOW PORCINE CIRCOVIRUS 2 (PCV2) VACCINATION WITH CIRCOVAC® ON PIGLET ANTIBODY TITRE AND VIRAEMIA AT WEANING.

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## **Background and Objectives**

Commercial vaccines against Porcine circovirus 2 (PCV2) are an efficient strategy to control PCV2 infections. Concretely, sow vaccination increases PCV2 antibody titres, reduces viral shedding and improves production parameters. Therefore, the objective of the present study was to assess the effect of sow vaccination against PCV2 with Circovac® on maternally-derived antibodies (MDA) and viraemia in piglets.

# **Material and Methods**

This trial was performed in five farrow-to-finish herds, where PCV2 sow vaccination was not used. A sow batch per farm was selected and blood samples of ten piglets from different multiparous non-vaccinated sows (NV) were collected at weaning. Sows were vaccinated based on farmer's demand (V) with 2 mL of Circovac® at weaning (21d post-farrowing). For monitoring this new scenario, blood samples from ten piglets from different multiparous V sows were collected at weaning. Blood samples were tested by ELISA and qPCR (5 samples/pool). MDA titre of piglets at weaning from NV and V sows were compared using Kruskall-Wallis test. Chi-square test was used to compare number of piglets with PCV2 viral loads between V and NV groups.

## Results

Piglets from V sows showed lower MDA, but more homogeneous levels in farm A(0.35±0.2), B(0.35±0.2), C(0.41±0.23) and D(0.62±0.31) compared to the NV sows in each farm (A=0.60±0.30; B=0.71±0.33; C=0.53±0.34; D=0.96±0.51). Piglets from farm E showed lower MDA levels in NV (0.90±0.35) than those coming from V sows (1.00±0.39). All piglets from V sows were PCV2 negative by PCR, whereas 5/10 pools from those coming from NV sows resulted positive (p<0.05), with viral loads from  $10^3$ - $10^4$ 

## **Discussion and Conclusion**

Sow vaccination against PCV2 significantly reduced the number of PCV2 infected piglets, representing an effective tool for decreasing the PCV2 infectious pressure in farms.

# IMPROVEMENT OF NURSERY PRODUCTIVE PERFORMANCE AFTER PRRS AND PCV-2 WHOLE-HERD PREVENTION APPROACH IN SPAIN

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# **Background and Objectives**

This is a summary of a field trial designed to evaluate the impact of the whole-herd-approach concept (defined as sows and piglets disease prevention strategy) and the 5 step process on controlling PRRS and PCV-2 related diseases, assessed by nursery improvement performance.

## Material and Methods

The study was conducted in a 1000 sows farrow to wean production system. Before the trial sows were vaccinated with another type-1 modified live PRRSv vaccine strain every 4 months. Whole-herd approach prevention strategy was implemented by mass vaccination of sows with 2 ml of Reprocyc PRRS EU and 1 ml of Ingelvac CircoFLEX®. Piglets were vaccinated at weaning with the FLEXcombo® protocol (Ingelvac Circoflex®Iml+Ingelvac Mycoflex®Iml) from week 3 (2018) and from week 11 with 1ml IM of Ingelvac PRRSFLEX EU at 17 days of age on regular basis. A before and after treatment approach, including 3 (31343 pigs) and 4 (22398 pigs) batches respectively, was adopted. Final nursery weight, medication cost per pig, mortality rate, economical feed conversion ratio (eFCR), and average daily weight gain (ADWG) were compared.For statistical analysis, Kruskal-Wallis test was performed and BECAL calculator for economics calculation.

## Results

All the analyzed data numerically improved after de implementation of the whole-herd approach prevention program. The magnitude of the reduction was 52% for mortality (p=0.03) and 41 % for medication costs (p=0.15). The improvement of ADWG was 29% (p=0.03) and 10% for final weight (p=0.07). Final weight, and the improvement in FCR showed a statistical trend (p=0.07).Calculated return on investment (ROI) was **3.19:1**.

## **Discussion and Conclusion**

Disease prevention applied both in sows and piglets is a valuable approach for controlling PRRS and PCV-2 impact in swine herds consistently. The whole-herd vaccination program implemented in this system, had a significant positive impact on nurseries performances and economic data.

# LEPTOSPIROSIS CONTROL: A FIELD CASE REPORT OF COMPARISON OF THE RESULTS OBTAINED WITH ANTIBIOTIC TREATMENTS VERSUS VACCINATION OF SOWS.

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#### **Background and Objectives**

Leptospirosis is described in pigs worldwide. It most often causes reproductive disorders, such as infertility, abortions, and weakness in newborn piglets. Leptospirosis control has long relied on antibiotic treatments and rodent control. A vaccine has been authorized in the European Union since the end of 2018. This case report describes the implementation and results of antibiotic treatments initially, in 2017 and 2018, followed by the new vaccine alone in early 2019, on a farm with leptospirosis from 2016.

#### **Material and Methods**

Reproductive disorders appeared in a farrow-to-finish pig farm in the fall of 2015 and lasted until the diagnosis of leptospirosis was made in 2016. Between the beginning of 2017 and the end of 2018, each batch of sows and gilts was systematically treated at each cycle with oxytetracycline. From 19/12/2018, antibiotic treatments were stopped. Each sow batch received a vaccination for leptospirosis with Porcilis Ery+Parvo+Lepto. Fertility rate, farrowing rate, and prolificity are presented and compared over 4 successive periods: before leptospirosis contamination (base period), during clinical leptospirosis outbreak ("leptospirosis"), during antibiotic treatments ("ATB"), and during vaccination ("PorcilisEPL").

#### Results

The base period lasted from weeks 30-2013 to 28-2015. "Leptospirosis" period runs from 31-2015 to 01-2017. "ATB" period runs from 04-2017 to 08-2019 and "PorcilisEPL" period runs from 11-2019 to 38-2019.For these 4 successive periods, fertility was respectively 88.8; 80.9; 88.7 and 92%.For the "ATB" and "PorcilisEPL" periods, farrowing rate was 73.4 and 84% respectively. The prolificity was respectively 14.9 and 16.1 total born piglets per farrowing, and 14.1 and 14.6 live born piglets per farrowing.

#### **Discussion and Conclusion**

These initial results must be confirmed over time and in other farms. However, it appears that for all parameters measured, vaccination with Porcilis Ery+Parvo+Lepto is superior to antibiotic treatments and is as such a clear alternative to these treatments.

# LOCAL AND SYSTEMIC CYTOKINE GENE EXPRESSION MODULATED ON NURSERY PIGS FED FUNGI FERMENTED FEED ADDITIVE

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## **Background and Objectives**

Edible mushrooms and derivate products are promising solutions with prophylactic and therapeutic properties such as antimicrobial and immunomodulatory. Bioactive components include 1,3-1,6- $\boxtimes$ -glucans, glycoproteins, vitamins, prebiotic oligosaccharides, phenolic compounds, triterpenoids. This study determines intestinal tissue and peripheral blood mononuclear cells (PBMCs) cytokine gene expression in nursery pigs supplemented with fungal fermented feed additive (FFA).

#### **Material and Methods**

Post-weaning, 70 nursery pigs were randomly assigned to control (n = 40) or 2 kg/mt FFA (n = 30; Trouw Nutrition, the Netherlands) provided ad libitum for 45 days. Blood and tissue samples were collected from 10 control pigs after weaning (basal group), and 10 pigs per treatment at days 15, 30 and 45 post-weaning. Samples from jejunum, ileum and colon and PBMCs were analysed for gene expression of cytokines IL-10, IL-12p35, IL-12p40, TNF-Ø, IFN-Ø and TGF-Ø and housekeeping genes. For days 15, 30 and 45, gene expression was analysed per tissue including control and FFA treatment by means of Kruskal-Wallis test.

#### Results

Gene expression for cytokines was downregulated (P<0.05) in FFA compared to control for different tissues and time points; IFN- $\boxtimes$  in jejunum and ileum d15 and colon d42, IL- $1\boxtimes$  in ileum d15 and colon d42, TNF- $\boxtimes$  in jejunum d15, and IL-10 in jejunum d15 and colon d42. IFN- $\boxtimes$  IL- $1\boxtimes$  IL-6, IL-10, IL-12, and TGF- $\boxtimes$  showed upregulated (P<0.05) gene expression compared to control at d15 on PBMCs. Several differences indicated that postweaning d15 and d30 had downregulated cytokines compared to basal (d0). An interaction showed IL- $1\boxtimes$ downregulated at d30 for control compared to basal, while FFA was upregulated.

#### **Discussion and Conclusion**

The use of fungal fermented feed additive resulted in an immunomodulatory effect with downregulation of cytokine gene expression in the intestine and upregulation systemically as PBMCs, suggesting local antiinflammatory response.

# SALMONELLA TYPHIMURIUM-BASED INACTIVATED VACCINE CONTAINING WIDE SPECTRUM OF BACTERIAL ANTIGENS WHICH MIMICS PROTEIN EXPRESSION CHANGES DURING DIFFERENT STAGES OF AN INFECTION PROCESS

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## **Background and Objectives**

Salmonella infections are still considered as a persistent problem in veterinary medicine. Vaccination is one of the tools for decreasing the burden of animals by many pathogens. However, the efficiency of available commercial or experimental vaccines against non-typhoid Salmonella strains is not yet sufficient. We followed the path of inactivated vaccine that is safe and well-accepted but the antigen offer is limited. We improved this by diverse cultivation conditions mimicking bacterial protein expression during the natural infection process.

## **Material and Methods**

Salmonella Typhimurium cultivation process was set up to simulate the host environment to enhance the expression of SPI-1 (Salmonella pathogenicity island) proteins, SPI-2 proteins, siderophores and flagellar proteins. Three different cultivation media were used and subsequent cultures were mixed together, inactivated and used for immunization of post-weaned piglets. A mixture of recombinant Salmonella proteins was also used as a recombinant vaccine for comparison. Clinical symptoms during the subsequent experimental infection, antibody response and organ bacterial loads were examined.

## Results

One day after the infection, we observed an increased rectal temperature in the group of unvaccinated animals and animals vaccinated with the recombinant vaccine. The increase of temperature of pigs vaccinated with inactivated Salmonella mixture was statistically lower. In the same group, we also found lower bacterial counts in the ileum content and the colon wall. IgG response to several Salmonella antigens was enhanced in this group but did not reach the titers of the group vaccinated with the recombinant vaccine.

## Discussion and Conclusion

Pigs vaccinated with an inactivated mixture of Salmonella cultures mimicking protein expression changes during the natural infection, exhibited less serious clinical symptoms and lower bacterial load in the body after the experimental infection compared to unvaccinated pigs and pigs vaccinated with a mixture of recombinant Salmonella proteins.

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## SURVEY OF LUNG LESIONS OF PIGS AT SLAUGHTER WITH THE CEVA LUNG PROGRAM IN GERMANY AND AUSTRIA

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# **Background and Objectives**

Lung scoring in the abattoir is a useful method to gain information about respiratory health in swine herd. Lung lesions caused by various pathogens are not pathognomonic, however results can clearly indicate if lungs are more affected by bronchopneumonia, pleurisy or both. The aim of the study was to show the distribution of lung lesions in various parts of Germany and Austria over a period of 2 years.

## **Material and Methods**

The Ceva Lung Program is a scoring method used to assess lung lesions at slaughterhouse (Cvjetkovic et al., 2018). 36.080 lungs belonging to batches of ≥60 lungs from 4 geographical regions were assessed and the mean values compared: North Western Germany (NWG), n=21.422; Eastern Germany (EG), n=4.123; Southern Germany (SG), n=6.574; Austria (A), n = 3.961. In total 272 lung scores were included.

## Results

In terms of bronchopneumonia, the mean EP-Index was highest in batches from A (2,28<sup>b</sup>), followed by SG (2,01<sup>b</sup>), NWG (1,32<sup>a</sup>) and EG (0,90<sup>a</sup>) (a:b, p<0,01). Mean prevalence of cranial pleurisy was highest in A (26,05%<sup>b.d.f</sup>), followed by SG (17,96%<sup>b.e.</sup>), EG (12,33%<sup>c</sup>) and NWG (8,9%<sup>a</sup>) (a:b; c:d; e:f; p<0,01). Scars were most prevalent in A (14,61%<sup>b</sup>), followed by SG (13,46%<sup>b</sup>), EG (7,00%<sup>a</sup>) and NWG (6,81%<sup>a</sup>) (a:b, p<0,01). For pleurisy, the APP-Index was highest in batches from NWG (0.70<sup>a</sup>), followed by EG (0.58), A (0.54)and SG (0.44<sup>b</sup>) (a:b, p<0,05).

# **Discussion and Conclusion**

Although very high vaccination rates against Mycoplasma hyppneumoniae lungs still show associated lesions with highest mean in A and SG. Lungs from EG scored the lowest in this category, reflecting the high health status of large and isolated herds. The App-index was found highest in NWG which can be explained with the high pig density and high animal traffic in the area, including the importation of pigs from abroad.

## AUTOGENOUS SOW VACCINE : COMPARISON OF TWO FORMULATIONS

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# **Background and Objectives**

The aim of this study is to compare two formulations of autogenous sow vaccines including Streptococcus suis serotype 4 (Ss4) and Actinobacillus pleuropneumoniae biovar 1 serovar 2 (App2) which differs by the type of adjuvants and the volume of injection (2 and 5ml). The 2ml is more adapted to the usual volumes in sow vaccines and to animal welfare.

## **Material and Methods**

The study took place in a two site farm. Site n°1 is a 240 sow farrowing unit seronegative for App2 and with Ss4 clinical cases on piglets. Site N°2 is a post-weaning-growing unit with clinical symptoms of App2. Thereby, an autogenous sow vaccination with Ss4 and App2 was implemented. Fifty-six sows were divided in three groups: one vaccinated with the 2ml formulation (2ml), one with the 5ml (5ml) and one non vaccinated (NV). Sows were vaccinated 7 and 4 weeks before farrowing. App2 seroconversion was used as vaccine marker and was evaluated at three time points: before primary vaccination (TP1); before booster vaccination (TP2); 10 days before farrowing (TP3). Clinical symptoms compatible with Ss4 infection were recorded.

## Results

No seroconversion was observed in NV. After the first injection more animals from 2ml shown a seroconversion compared to 5ml with higher antibodies titers. After the booster 2ml titers were still higher than 5ml but the ratios of seropositive animals were not significantly different between the two groups. Only 2,7% of all piglets were treated for Ss4 (6 animals in 2ml, 2 in 5ml, 13 in NV).

## **Discussion and Conclusion**

With both formulations App2 seroconversion was observed and their use resulted in a decrease of Ss4 symptoms. Antimicrobials were stopped. The higher antibodies levels in 2ml doesn't mean that this formulation is more efficient: to our knowledge no relationship between Elisa App antibody levels and clinical protection has been demonstrated.

## HUMORAL RESPONSE IN PIGLETS UPON DIFFERENT VACCINATION STRATEGIES WITH HYOGEN® - A PILOT STUDY

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# **Background and Objectives**

In this pilot study we investigated in one farm the serological profiles against M. hyopneumoniae of 60 piglets from different sows after different vaccination regimes.

# Material and Methods

The piglets were divided into three groups: a non-vaccinated group (NV), a group vaccinated 1 day before weaning (V1) and a group vaccinated 4 weeks after weaning (V2). Piglets were weaned at 20 days of age. The sows had not been vaccinated against M. hyopneumoniae during this study. Blood samples were taken from piglets and analyzed serologically for M. hyopneumoniae (IDEXX Herdcheck). Those results were compared to serological results of piglets (n=20) from the same farm three months before, when sows were vaccinated against M. hyopneumoniae 3 weeks before farrowing and piglets at weaning. All vaccinations have been performed with Hyogen®.

## Results

All piglets from non-vaccinated sows showed significantly lower S/P ratios (average  $\pm$  SD) at birth (1.29 $\pm$ 0.30), at weaning (0.86 $\pm$ 0.40) and 4 weeks after weaning (0.38 $\pm$ 0.27) compared to piglets from the previous study of vaccinated sows (2.33 $\pm$ 0.18, 1.71 $\pm$ 0.22 and 1.22 $\pm$ 0.31, respectively). There were significant differences in S/P ratios between VI (0.54 $\pm$ 0.27) and NV (0.30 $\pm$ 0.24) and V2 (0.30 $\pm$ 0.22) four weeks after weaning. Eight weeks after weaning, S/P ratios of VI (0.64 $\pm$ 0.38) and V2 (0.82 $\pm$ 042) were significantly higher than those of NV (0.12 $\pm$ 0.10).

## **Discussion and Conclusion**

In conclusion, piglets of sows vaccinated at the end of gestation had significantly higher antibody levels until at least 6 weeks of age compared to piglets from non-vaccinated sows. The antibody levels in 10 weeks old piglets either vaccinated either at weaning or at 4 weeks after weaning were not statistically different. More studies with a larger number of animals are needed to confirm these results.

# EFFICACY OF DIFFERENT MYCOPLASMA HYOPNEUMONIAE BACTERINS AGAINST EXPERIMENTAL MYCOPLASMA HYOPNEUMONIAE INFECTION

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## **Background and Objectives**

Vaccination against Mycoplasma hyopneumoniae is used worldwide to control enzootic pneumonia. However, protection is incomplete and varies between pigs and herds. We assessed the efficacy of two bacterins using an experimental infection model.

## **Material and Methods**

In total, 53 4-week old M. hyopneumoniae-free piglets were divided into 5 groups: V1 Hyogen® (n=12), V2 another commercial bacterin (n=12), V3 same as V1 but without antigen (n=12), V4 non-vaccinated challenged group (PC) (n=12), V5 non-vaccinated non-challenged group (NC) (n=5). After 9 days of acclimation, pigs in V1, V2 and V3 were vaccinated intramuscularly. PC and NC received intramuscularly a physiological saline solution. At D21, all animals (except NC) were endotracheally inoculated with two different M. hyopneumoniae strains (7 ml 10<sup>7</sup> CCU/ml per strain per pig). Clinical signs were assessed daily using a respiratory disease score (RDS) and blood samples were taken to determine serum antibodies (IDEIA<sup>™</sup> M. hyopneumoniae, Oxoid). Animals were euthanized four weeks after challenge and macroscopic lung lesions were scored (score 0 to 35).

#### Results

The mean RDS values were: VI 0.24, V2 0.60, V3 0.46, PC 0.60 (p>0.05). The macroscopic lungs lesions (mean ± SD) were: VI  $0.05^{a} \pm 0.11$ , V2  $3.52^{b} \pm 4.10$ , V3  $4.52^{b} \pm 5.24$ , PC  $7.60^{b} \pm 5.15$  (p<0.05). Serum OD-values at challenge were: VI  $0.60^{a} \pm 0.20$ , V2  $0.73^{a} \pm 0.18$ , V3  $1.69^{b} \pm 0.22$ , PC  $1.97^{b} \pm 0.59$  (p<0.05).

## **Discussion and Conclusion**

Only numeric differences were found for RDS whereas the lung lesions were significantly lower in V1 than in the other groups. Both V1 and V2 induced a significant serological response three weeks after vaccination. These preliminary data suggest differences in the protective efficacy of both bacterins. Numeric improvements in RDS and lung lesions were seen in V3 compared to the PC, pointing to a possible role of the adjuvant.

COMPARISON BETWEEN DIFFERENT COMMERCIAL MYCOPLASMA HYOPNEUMONIAE VACCINES UNDER FIELD CONDITIONS.

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## **Background and Objectives**

Vaccination against Mycoplasma hyopneumoniae (Mh) is a common tool used for prevention and control of Enzootic pneumonia (Ep). Evaluation of lungs in slaughterhouse and reduction of affected lungs with Eplike lesions it is a common method to assess efficacy of vaccination. The aim of this study was to investigate the prevalence and extension of lungs with Ep-like lesions observed at slaughter in pigs vaccinated with either Hyogen® (vaccine H), other 9 commercial Mh vaccines or unvaccinated pigs.

## Material and Methods

From January 2016 until October 2019 a total of 1.701 batches within 256.344 lungs from different farms from Spain were scored at the slaughterhouse using the Ceva Lung Program (CLP) score methodology, which assists lung scoring for (EP)-like lesions using modified Madec grid. For each batch, the following parameters were calculated: Percent of affected lungs with Ep-like lesions Percent of affected surface out of all lungs and percent of affected surface of pneumonic lungs. The statistical analysis was performed using the Mann-Whitney test.

## Results

Pigs vaccinated with Vaccine H showed statistically (p<0,001) less percentage of affected lungs with Ep-like lesions and less percent of affected surface than lungs from unvaccinated pigs or vaccinated with other one-dose vaccines, combo vaccines or bi-dose vaccines. Batches vaccinated with Vaccine H showed statistically lower percentage of affected lungs with Ep-like lesions than vaccines 2,3,4,6,7,8 (p<0.01) and vaccine 9 (p<0.05) and statistically lower percentage of affected surface than vaccines 5 and 9 (p<0.05).

## **Discussion and Conclusion**

Vaccination of piglets against Mh reduced the severity of Ep-like lesions. 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. In this study, the vaccine H showed its superiority in the reduction of lung lesions over the rest of the vaccines included in.

# EFFECT OF DIFFERENT SOW VACCINATION PROTOCOLS AGAINST PRRSV ON MATERNALLY-DERIVED HUMORAL IMMUNE RESPONSE IN PIGLETS AT WEANING

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## **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSv) is one of the most economically significant pathogen for the swine industry worldwide. Vaccination protocols against PRRSv based on the combination of modified live (MLV) and killed (KV) vaccines in sows are frequently used to maintain the PRRS immunity and stability within herds. However, information on the effect of those combined protocols on piglet's immunity is limited. Therefore, the objective of this study was to assess the effect of different sow vaccination schedules against PRRSv on maternally-derived humoral immune response in piglets at weaning.

## Material and Methods

A total of 120 sows distributed into three consecutive weekly batches (40 sows/batch) of a PRRSv positive farm were selected. Sows were vaccinated with a MLV vaccine against PRRS according to the farm's protocol. Four weeks later, sows were divided into 4 groups (A, B, C and D). Group A was vaccinated with a commercial KV vaccine (Progressis®) at 6 and 3 weeks pre-farrowing (wpf). Group B and C received Progressis® at 3 or 6 wpf, respectively. Group D was not vaccinated. At weaning, blood samples of four piglets coming from five randomly selected sows per group and batch were taken (n=240). These were tested by RT-PCR and ELISA. Proportions of seropositive piglets by group were compared using Fisher's exact test.

## Results

Proportion of seropositive piglets coming from vaccinated sows from group A (49/60-82%), B (49/60-82%) and C (54/60-90%) were significantly higher (p<0.001) compared to the non-vaccinated group D (27/60-45.0%). No statistical differences were found between vaccinated groups with different number of doses and timing. All piglets were RT-PCR negative.

## **Discussion and Conclusion**

Sow vaccination during pregnancy with Progressis® increased significantly the maternal immunity transferred by colostrum against PRRSv in piglets at weaning. Thus, it could represent an effective strategy for the protection of newborn piglets against this pathogen.

COMPARISON OF THE EFFECT OF TWO COMMERCIAL ONE-SHOT VACCINES AGAINST MYCOPLASMA HYOPNEUMONIAE ON PRODUCTIVE PARAMETERS IN A SPANISH TWO-SITE COMMERCIAL FARM

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#### **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 performance parameters in farms. Thus, the aim of this study was to compare the effect of two different commercial vaccines against Mhyo in terms of performance parameters in a two site farm.

#### Material and Methods

Two consecutive batches (BI and B2) were selected from a Mhyo positive two-site farm with 1000 sows in Spain. Three-weeks-old piglets were vaccinated with vaccine A (n=1000) in BI and with Hyogen® (n=1000) in B2, following the manufacturer's instructions. Piglets from these consecutive batches were located in the same nursery and fattening unit under equal housing conditions. During the fattening period, average daily weigh gain (ADWG), feed conversion rate (FRC), mortality rate and medication cost were recorded. Total production cost per batch ( $\varepsilon/kg$ ) at the end of fattening period was also calculated.

#### Results

Piglets vaccinated with Hyogen® (B2) showed higher ADWG (723.0 g) than those vaccinated with the other vaccine (717.8 g). Likewise, B2 showed lower FCR (2.533) compared to B1 (2.595). Mortality rate and medication cost were also lower in B2 (3.02% and 1.39€) than B1 (3.27% and 1.69€), respectively. Finally, production cost of group B2 (1.17€) was lower than B1 (1.19 €/kg).

## **Discussion and Conclusion**

Vaccination of 3-weeks-old piglets with Hyogen® significantly improved all measured performance parameters compared to the previous batch vaccinated with 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.

## EVALUATION OF PPRSV-1 IMMUNOMODULATION: IN VIVO AND IN VITRO MODELS

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## **Background and Objectives**

Porcine reproductive and respiratory syndrome (PRRS) still causes severe economic losses in farmed pigs. A The clinical outcome of PRRS virus infection may be quite different and the interaction between PRRS viruses and the immune system (IS) is still unclear. Aim of the present study was to evaluate the innate immune response to virulent or attenuated PRRSV strains.

## **Material and Methods**

Two groups of six pigs each were experimentally infected with a virulent and an attenuated PRRSV strain, respectively; one group was maintained as negative control. Blood samples were collected within 21 days after infection (DAI) to measure innate and adaptive immune responses to PRRSV. In the second part of the study, the same PRRSV strains plus four field strains were analysed in vitro on peripheral blood mononuclear cells (PBMCs) from PRRS-naive pigs, not permissive for PRRSV replication.

## Results

Pigs infected with a virulent PRRSV strain showed visible clinical symptoms, and an interferon-⊠ response in serum until 14 DPI (p< 0.05). No differences were detected instead between controls and attenuated strain-infected groups. The in vitro assays showed that the majority of PRRSV strains caused an IL-8 response, whereas TNF-⊠ and IL-10 release was induced by fewer strains. Only the attenuated strain induced an IL-1∞ response.

# **Discussion and Conclusion**

In conclusion, PRRSV virulent strains causing disease induced suppression of the inflammatory response in vitro to a different extent. In particular, only an attenuated strain sustained a strong IL-12 response. This is likely to reduce the subsequent permissiveness for PRRSV replication in mature macrophages. Owing to the above, the lack of a robust primary inflammatory response may underlie an escape strategy of some virulent PRRSV strains.

# LOCAL IMMUNE RESPONSE AT THE MATERNAL-FETAL INTERFACE OF PREGNANT GILTS AFTER INFECTION WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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# **Background and Objectives**

Porcine reproductive and respiratory syndrome virus (PRRSV) is a small enveloped RNA virus with a huge economic impact on global swine industry. Infection during late gestation causes reproductive complications but local immunity in utero remains to be elucidated. With an experimental PRRSV-infection model, we investigated lymphocyte phenotypes at the maternal-fetal-interface during late gestation.

# Material and Methods

Three groups of pregnant gilts (n= 4/group) were infected with either one of two different PRRSV-1 field isolates (720789 or AUTI5-33) or sham-inoculated at day 84 of gestation. 21 days post infection gilts were euthanized, leukocytes from the maternal-fetal-interface were isolated and phenotypes were assessed by flow cytometry. Fetal preservation and viral loads were evaluated by visual appearance and qRT-PCR, respectively.

# Results

The fetal preservation status in AUTI5-33 infected gilts was impaired in 44% of the fetuses and 62% of them tested positive for PRRSV RNA in the fetal thymus and/or serum. Within the 720789 infected gilts, only 4% of the fetuses had an impaired fetal preservation. This indicates that the PRRSV strain AUTI5-33 is of high virulence. Sham-inoculated gilts showed a clear dominance of T-cells with a naive phenotype in the fetal placenta, whereas in the endometrium phenotypes of antigen-experienced T-cells prevailed. In AUTI5-33 infected gilts, we observed an increase in CD4 T-cells with an early (CD8@+CD27+) and late (CD8@+CD27-) effector phenotype in the fetal placenta. Similarly, an increase of putative early (CD8@+CD27<sup>dim</sup>) effector CD8 T-cells was found in the fetal placenta of AUTI5-33-infected gilts. Furthermore, we observed an increase of CD3<sup>-</sup>CD172a<sup>-</sup>CD16+CD8@+</sup> putative NK-cells in AUT15-33 infected tissues.

## **Discussion and Conclusion**

These results suggest that fetal NK- and T-cells respond to the PRRSV-infection but may rather cause immune-pathogenesis instead of protection. However, further functional and in situ investigations are required to corroborate this rationale.

#### EFFICACY OF THREE COMMERCIAL PORCINE PARVOVIRUS 1 VACCINES IN PREGNANT GILTS

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## **Background and Objectives**

The objective of this study was to evaluate the efficacy of a new porcine parvovirus 1 (PPV1) vaccine, named ReproCyc®ParvoFLEX (vaccine A), when administered to gilts before mating. Efficacy was compared to a negative control group and two other commercially available PPV1 vaccines (vaccine B and C).

## Material and Methods

Seventy-seven gilts were randomly assigned to four study groups. Animals were vaccinated following manufacturers' recommendations and were artificially inseminated three weeks after completion of vaccination. Pregnant gilts were challenged around 40 days of gestation with a heterologous PPVI strain. Viremia was evaluated in gilts by PCR. Fetuses were harvested at around day 90 of gestation and evaluated for the presence of PPVI by PCR as well as for their condition, size and weight. Thus, the percentage (%) of healthy piglets per gilt was annotated.

## Results

All three treatment groups showed statistical differences in comparison to the control group for: % of PPVI positive fetuses, % of mummified fetuses, mean number of healthy piglets and mean number of abnormal piglets. In addition, vaccines A and B showed statistically differences in comparison to the control group regarding the mean number of fetuses per gilt. Vaccine A was the only group showing statistical differences regarding the % of gilts with more than 11 healthy piglets and the only that prevented viremia in the challenged gilts. Indeed, vaccine A showed statistically better results in the mean number of healthy piglets per gilt compared to vaccine B. No differences were detected between vaccinated groups regarding % of PPVI infected fetuses at necropsy.

## **Discussion and Conclusion**

The present results place ReproCyc®ParvoFLEX as an effective alternative to control PPVI infection in breeding herds. Immunization with this novel PPVI vaccine resulted in a significant improvement of performance parameters after a challenge with PPVI.

# SAFETY PROFILE OF REPROCYC® PARVOFLEX IN BRED PIGS AT DIFFERENT STAGES OF THE REPRODUCTION CYCLE AND IN OFFSPRING

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# **Background and Objectives**

To demonstrate safety of vaccines in the target species is crucial for regulatory approval and public acceptance. The objective of this study was to assess the safety of repeated doses of ReproCyc® ParvoFLEX, a recently licensed monovalent subunit vaccine based on the viral protein 2 of porcine parvovirus 1 (PPVI). Safety was evaluated in bred pigs and in offspring under experimental settings.

## Material and Methods

Safety at all breeding stages was assessed in four independent studies involving: pre-breeding gilts (study A), breeding-age gilts and boars (study B), early and late gestating sows and offspring (study C) and lactating sows and offspring (study D). All studies comprised one or two vaccinated groups that received the PPVI subunit vaccine and a negative control group. Safety was established by lack of significant differences between the vaccinated groups and the corresponding unvaccinated (negative control) groups. Gilts, sows and boars were evaluated for local and systemic reactions after vaccination as well as for reproductive performance. The survival rate and average daily weight gain (ADWG) from birth to weaning in offspring was evaluated in studies C and D.

## Results

No relevant differences were detected between groups for abnormal clinical findings, body temperatures and injection site reactions. In addition, repeated doses of ReproCyc® ParvoFLEX did not result in differences in the mean percentages of live piglets at birth neither at weaning compared to non-vaccinated controls. In parallel, during the suckling period, no significant differences between the vaccinated and non-vaccinated groups for the ADWG of piglets were observed.

# **Discussion and Conclusion**

No relevant significant differences between vaccinated and unvaccinated groups were observed in any experiment. Therefore, repeated doses of ReproCyc® ParvoFLEX were safe in target animals at different stages of the reproductive cycle and in offspring, placing this vaccine as a suitable candidate for mass vaccination programs in breeding herds.
# REPROCYC® PARVOFLEX ELICITS A 6-MONTH DURATION OF IMMUNITY THAT PROTECTS FETUSES AGAINST HETEROLOGOUS CHALLENGE WITH PORCINE PARVOVIRUS 1

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#### **Background and Objectives**

Long-lasting immunity achieved by means of vaccination is the main tool to prevent porcine parvovirus 1 (PPVI) infection and its associated clinical signs. Here it is reported the evaluation of the duration of immunity (DOI) conferred by a novel subunit vaccine based on the viral protein 2 of PPVI, named ReproCyc® ParvoFLEX.

#### Material and Methods

The DOI was assessed at six months post-vaccination. Phase I evaluated the DOI after the basic vaccination scheme, which consisted of two-doses given three weeks apart. Phase II evaluated the DOI after revaccination with a single injection administered 24 weeks after the basic scheme. Forty-six PPVI-negative gilts were randomly assigned to 6 groups (three in each phase): negative control, vaccinated and strict control groups. The negative control groups were injected with sodium chloride when their vaccinated counterparts were immunized. The strict controls were neither treated nor challenged. The negative control and vaccinated groups were challenged with a heterologous PPVI strain at approximately 40 days of gestation. Animals were necropsied at 90 days of gestation. Infection of fetuses assessed by PCR was the primary outcome for efficacy. Other supportive parameters were PPVI viremia and serological status of the gilts and the macroscopic appearance of their fetuses.

#### Results

All gilts vaccinated against PPVI tested seropositive at challenge and viremia after challenge was detectable only in the non-vaccinated animals. Fetuses positive to PPVI were only found in litters from non-vaccinated sows: 96.4% to 100% of fetuses in the negative control groups were positive. Contrarily, all fetuses in the PPVI vaccine groups were negative.

#### **Discussion and Conclusion**

This PPVI subunit vaccine is effective in terms of preventing viremia, transplacental infection of fetuses and fetal death caused by PPVI infection with a DOI of 6 months after vaccination. Re-vaccination at regular intervals every 6 month is recommended when using ReproCyc® ParvoFLEX.

## FIELD EVALUATION OF THE COMBINED ADMINISTRATION OF REPROCYC® PARVOFLEX AND REPROCYC® PRRS EU IN BREEDING PIGS

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#### **Background and Objectives**

Combined application of vaccines provides advantages regarding animal welfare, human resources and production costs. ReproCyc® ParvoFLEX is a novel subunit vaccine based on the protective viral protein 2 of porcine parvovirus 1 (PPV1), whereas ReproCyc® PRRS EU is a porcine reproductive and respiratory syndrome (PRRS) modified live virus vaccine. This work sought to evaluate the safety and compatibility of the combined administration of the abovementioned vaccines in two field contexts: "wild-type" PRRSV (experiment A) and PPV1 (experiment B) circulation in breeding herds.

#### Material and Methods

Safety and vaccines' compliance were established according to the absence of significant differences between combined vaccinated animals (PPRSV+PPVI) and single vaccinated animals against PRRSV (experiment A) or PPVI (experiment B). In both experiments, gilts and sows were evaluated for local and systemic reactions after vaccination, as well as for reproductive and productive performance. Tissues from abortions, mummified fetuses and stillborn piglets were analyzed for the presence of PRRSV and PPVI.

#### Results

In both experiments, combined PRRSV and PPVI vaccinated animals exhibited neither a relevant increased incidence of local nor systemic reactions after vaccination when compared to their single vaccinated counterparts. Similarly, no differences were devised in terms of conception and abortion rates, farrowing performance and number of weaned piglets between treatment groups. In addition, most organ tissues from abortions, mummies and/or stillborn piglets at farrowing were PRRSV and PPVI negative in both combined and mono-vaccinated groups.

#### **Discussion and Conclusion**

ReproCyc® PRRS EU mixed with ReproCyc® ParvoFLEX can be used as a safe method of protection against the detrimental effects of PRRSV and PPVI infections in breeding female pigs in one single injection. The present results bring up opportunities to tackle reproductive problems as a whole by combining control programs against swine reproductive pathogens.

# PRRS STABILIZATION AND RECOVERY OF PRODUCTIVE PARAMETERS WITH MASS VACCINATION IN SOWS AND PIGLET AT FIRST WEEK OF AGE

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#### **Background and Objectives**

It is estimated that the Porcine Reproductive and Respiratory Syndrome (PRRS) costs the swine industry close to 1,500 million euros a year in Europe and it has been shown that vaccination plus the management and biosecurity measures can reduce these losses. The following study attempted to assess the effectiveness of the vaccination protocol with Suvaxyn® (Zoetis PRRS MLV) on a farm of 1,200 sows after suffering an acute outbreak of PRRS, performing a mass vaccination of all sows of the farm and newborn piglets.

#### **Material and Methods**

The Site 1 of the farm is outdoor and the PRRS outbreak was confirmed by PCR in processing fluids. Nursery and fattening units are separated.During the outbreak there was a sudden increase from 4 to 75 abortions/month, decline in fertility (91,2% to 80,8%), and a decline in live newborns (12,59 to 9,69) and weaned piglets (11,64 to 6,86) per farrow. There was also a noted increase in cases of arthritis and meningitis in nursery pigs, in addition to respiratory problems in fattening pigs.

#### Results

The recovery of fertility and abortions is reduced in the following month to mass vaccination. Besides, a gradual recovery was observed in the number of animals born alive and dead, and the litter size at weaning. Results from serum analyzed by PRRS PCR in animals of 3–6–9 weeks of age became negative, indicating that apparently the virus was no longer circulating through the farm. Another observation was a reduction in secondary bacterial infections, helping to decrease the use of antibiotics.

#### **Discussion and Conclusion**

It is concluded that vaccination of sows and piglets is a great help for the stabilization and recovery of zootechnical parameters on affected farms, but it must always be carried out together with correct management measures and respecting the control of flows.

#### EVALUATION OF LACTOFERRIN IN SOW COLOSTRUM IN RELATION TO PIGLET DIARRHEA BEFORE WEANING

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#### **Background and Objectives**

Colostrum is regarded as a high-density source of different nutritional and immunological components, among them, Lactoferrin (LF) has been considered as one of the key players. LF can protect piglets from gastrointestinal infections, thus maintaining the homeostasis of gut microbiota and positively modulating immune responses. Previous studies found that LF concentration in sow colostrum and milk are higher on the first day of farrowing and gradually decrease towards late lactation. The objectives of the present study were to examine if LF concentration in sow colostrum at farrowing could be a predictor for better intestinal health of piglets before weaning.

#### Material and Methods

Twenty ml of colostrum from 31 sows, sampled from the first three anterior teats of the same side, within the first two hours after the first piglet birth. All the samples were stored at -20 °C until further analysis. Litters were checked for any signs of diarrhea till weaning. Pig LF level in sow colostrum was assessed using a commercial pig lactoferrin ELISA kit (Cusabio, China).

#### Results

We found that in litters with no signs of diarrhea the LF concentration of their mother colostrum was 1.87 ± 0.10 Mg/ml (mean ± SEM, n=22) while in litters showing diarrhea signs it was 1.37 ± 0.16 Mg/ml (n=9; P<0.05).

#### **Discussion and Conclusion**

Our study shows that litters from sows having a high level of LF in colostrum had experienced less diarrhea signs than litters from sows with lower LF levels in their colostrum. This effect might be due to better homeostasis and immune response at the intestinal level, modulated by higher levels of LF absorbed via colostrum. To extend these observations and to examine the changes of LF, further research is needed in a broader aspect in the future.

#### EFFICACY OF VACCINATION AGAINST EARLY MYCOPLASMA HYOPNEUMONIAE INFECTIONS

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#### **Background and Objectives**

Mycoplasma hyopneumoniae (M.h.) is a primary pathogen of lungs and one of major agents responsible for Porcine Respiratory Disease Complex (PRDC), which causes substantial losses to the swine industry. Pigs of mid-finishing to slaughter age are mainly affected by PRDC. In some cases the circulation of the pathogen starts already in nursery. The aim of this study was to compare Hyogen® with another vaccine in the control of such early M.hyo infection.

#### Material and Methods

1 week old piglets were vaccinated either with Hyogen® (inactivated M.hyo and Imuvant<sup>™</sup>), Ceva or with Vaccine A (inactivated M.hyo and Amphigen®), or not vaccinated. At 7 woa challenge groups were inoculated intratracheally with different M.hyo strains on three consecutive days. Four weeks later the pigs were slaughtered and the lung lesions scored according to the European Pharmacopoeia 9.0. In questionable cases lung samples were collected for PCR and histopathology to confirm the specificity of the lesion. Blood samples were collected for M. hyo serology (IDVet ELISA) before vaccination, before challenge and at slaughter

#### Results

Hyogen vaccination induced stronger humoral immune response than vaccine A before the challenge (100% vs 95% positives and 3,3 vs 2,6 log BC titers (p<0,05). Further, Hyogen® vaccination lead to numerically lower mean LLS (0.3) compared to Vaccine A (0.4) and significantly lower than the positive (non-vaccinated challenged) control (0,7; p<0,05). Vaccine A lead to numerically yet not significantly lower LLS compared to the positive control.

#### **Discussion and Conclusion**

Hyogen® vaccination, contrary to Vaccine A provided significant protection against the development of lung lesions compared to the non-vaccinated challenged group in the early and massive M.hyo infection model. Its use can substantially aid the control of even early M.hyo infections in swine farms.

EVALUATION OF LUNG LESIONS AT SLAUGHTER AND COMPARISON OF ANIMALS BATCHES VACCINATED WITH DIFFERENT MYCOPLASMA HYOPNEUMONIAE VACCINES IN ARGENTINA

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#### **Background and Objectives**

Mycoplasma hyopneumoniae infections have been detected in almost all countries with intensive production systems and are responsible for major economic losses in the pig industry. Different vaccination schemes can be implemented, depending on the type of herd, the production system, the infection pattern and the preference of the farmer. The aim of this study was to compare lung lesions at slaughter from pigs vaccinated with three different vaccines against Mycoplasma hyopneumoniae.

#### **Material and Methods**

From January 2018 to October 2019, 15.407 slaughter lungs were evaluated in Argentina with Ceva Lung Program Scoring Methodology.In total, 145 batches larger than 50 lungs per batch, were classified into 3 groups according to the vaccination protocol:animals vaccinated with Hyogen®; vaccinated with Mhyo One Shot or with Mhyo Two Shots vaccines.The prevalence of EP like lesions was recorded and statistically compared.

#### Results

The prevalence of lungs with EP-like lesions was 54%;60% and 53% in Hyogen®, 1 shot and 2 shots vaccinated animals respectively. Hyogen® vaccinated animals had lungs with a lower percentage of affected surface area (P<0.05) at 4.6% compared to 1-shot vaccinated animals (6,5%) and 2-shot vaccinated animals (5,7%). And the same ratio was obtained in relation to the affected lung surface with bronchopneumonia: Hyogen® vaccinated group presented 9.4% and 1 shot vaccinated group presented 9.9%

#### **Discussion and Conclusion**

The average percentage of affected lung surface provides information on the extent of the lesions. Thus, it is an important parameter to evaluate the severity of infection in animals. The lungs of Hyogen<sup>®</sup>-vaccinated animals had fewer pneumonic lesions compared to the Mhyo one- and two-shot vaccinated groups. As in this study, previous studies have also shown the superiority of Hyogen<sup>®</sup>, reducing the rate of lung injury in the slaughterhouse and contributing to the increase of animal performance.

#### EFFICACY OF SUVAXYN PRRS MLV VACCINE ADMINISTERED TO 3-5 DAY OLD PIGLETS IN PRESENCE OF HIGH LEVEL OF MATERNALLY DERIVED ANTIBODIES AT A POLISH FARM

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#### **Background and Objectives**

Newborn piglets get already infected with PRRSv in the farrowing room. One strategy to prevent the economic impact is vaccination as early as possible. The objective was to prove the efficacy of newborn piglets vaccination with high MDAs under field conditions.

#### **Material and Methods**

A 300-sow farm in Poland, PRRSv positive/stable. Sows had been periodically mass vaccinated previously 4 times/year. PRRS MDA levels were measured in serum samples from 3-day-old piglets. Processing fluids (collected during castration from 2-day-old piglets) from each group were tested for presence of PRRSv. Pigs from two consecutive farrowing batches with 3-week interval (650 piglets each) were divided in two groups: UV-unvaccinated and V-vaccinated. Group V was vaccinated against PRRS at 3-5 days of age (2ml, IM). Both groups were vaccinated at 3 weeks of age against M.hyo/PCV. 20 pigs form each group were randomly selected and ear tagged for follow-up, and blood sampled at 3 days, 3, 6, 9 and 12 weeks of age. Serum samples were mixed in pools of 5. At 10 weeks of age, pig were transferred to finishing facilities until marketed. Pigs were sampled for PRRS PCR testing during the fattening period.

#### Results

Processing fluids were PRRS negative for both groups. Piglets from vaccinated group showed high MDA titers before vaccination (average S/P rate1.5). Comparison between both groups shows: PCR PRRSv positive animals in UV-group till 19-weeks-of-age, PCR PRRSv positive animals in V-group till 6-weeks-of-age – vaccine virus was confirmed by qPCR DIVA PRRS, PCR PRRSv negative animals in V-group from 6 to 19-weeks-of-age.

#### **Discussion and Conclusion**

Vaccination with Suvaxyn PRRS MLV 3-5-day-old piglets is effective in the presence of high levels of MDAs. This was demonstrated by the reduction of PRRS viremia during the finishing period.

#### INFLUENCE OF AGE AND ADMINISTRATION ROUTE OF A MYCOPLASMA HYOPNEUMONIAE VACCINE ON IGA+ AND IGG+ B LYMPHOCYTES AND TOLL-LIKE RECEPTOR (TLR) RESPONSES IN CONVENTIONAL PIGS SUBSEQUENTLY INFECTED IN FIELD CONDITIONS

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#### **Background and Objectives**

In order to improve the efficacy of conventional vaccines, the timing and administration routes are important issues. For Mycoplasma hyopneumoniae (M.hyo.), intradermal (ID) vaccination was demonstrated to be efficient in priming the immune response and sustaining good but not complete clinical protection. The aim of this study was to compare intramuscular (IM) and ID routes for M.hyo. vaccination at different age in inducing B cells and toll-like receptors (TLR).

#### Material and Methods

Five groups of pigs (N=10, each) were enrolled. IM- and ID-vaccinated groups were inoculated at 1 week or 4 weeks of age while controls were non-vaccinated (NV). Blood was collected at vaccination and until 20 weeks post-vaccination (PV). M.hyo-specific CD79+IgA+ and CD79+IgG+ B cells in PBMC were investigated by flow cytometry. Modulation of pathogen-recognition receptors TLR-2 and TLR-7, mainly produced in vivo by conventional (cDC) and plasmacytoid (pDC) dendritic cells, was investigated by rt-PCR.

#### Results

At vaccination, 1-week-vaccinated groups had high IgA+ and IgG+ B cells, comparable to controls which significantly declined at 4 weeks PV. Higher B cell levels were detected upon infection at 16 weeks PV in both vaccinated groups. Four week-vaccinated pigs showed very low levels of IgA+ and IgG+ B cells at vaccination and significant responses after vaccination and upon infection, being comparable between IM and ID routes. Furthermore, IM and ID vaccination groups showed positively modulated TLR-2 gene expression both after vaccination and infection.

#### **Discussion and Conclusion**

Vaccination at 4 weeks was suitable for inducing IgA+ and IgG+ B cells and a significant anamnestic response upon M.hyo field infection. ID vaccination was efficacious in sustaining mucosal and systemic B cells, with levels comparable with the IM route, as well as to induce conventional dendritic cell activation for a potentially more efficient innate antigen recognition.

Immunology and vaccinology

SURVEY OF ENZOOTIC PNEUMONIA (EP)-LIKE AND PLEUROPNEUMONIA (APP)-LIKE LESIONS IN HEAVY PIGS AT SLAUGHTERHOUSE USING THE CEVA LUNG PROGRAM METHODOLOGY: IMPACT OF THE VACCINATION SCHEDULE AGAINST MYCOPLASMA HYOPNEUMONIAE AND ACTINOBACILLUS PLEUROPNEUMO

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#### **Background and Objectives**

Examination of lung and pleural lesions at slaughterhouse is essential for monitoring Mycoplasma Hyopneumoniae (Mhyo) and Actinobacillus Pleuropneumoniae (App) infections. Objective of this survey is to investigate the prevalence and severity of such lesions in Italian heavy pigs (>160 Kg) at abattoir and evaluate the impact of different vaccination schedules against Mhyo and App.

#### Material and Methods

Ninety-one farms (21,575 pigs) were involved in the study. Lungs were examined at slaughterhouse using the Ceva Lung Program (CLP) Scoring Methodology. In the EP evaluation, animals were classified in 4 groups. Pigs in GI (n=4,815) were vaccinated with Hyogen® (Ceva Animal Health) while in G2 (n=8,782), G3 (n=3,799) and G4 (n=4,197) were vaccinated with other commercial one-shot, two-shots or bivalent vaccines, respectively. Prevalence of lesions, average EP-index, Scar-Score and APPI index with corresponding 95%-CIs were calculated for all groups. In the App investigation, pigs not vaccinated (n=18,144) were compared with these vaccinated with Coglapix® (Ceva Animal Health) (n=2,432) or competitors (n=999).

#### Results

Pigs vaccinated with Hyogen<sup>®</sup> showed the lowest prevalence of lung lesions (38.13%) and scars (6.19%). Average EP-index in GI (0.998 [0.979; 1.017]) was significantly lower (p<0.05) than in G2 (1.054 [1.041; 1.068]), G3 (1.119 [1.097; 1.141]) and G4 (1.303 [1.357; 1.410]). The average Scar-score in GI (0.062 [0.060; 0.064]) was significantly lower (p<0.05) compared to G2 (0.098 [0.096; 0.100]), G3 (0.103 [0.100; 0.107]) and G4 (0.082 [0.079; 0.085]). Prevalence of App-like lesions was 22.00% and 37.14% in pigs vaccinated with Coglapix<sup>®</sup> and other vaccines, respectively. Coglapix<sup>®</sup> vaccinated pigs showed significantly lower (p<0.05) APPI Index (0.511 [0.472; 0.551]) than pigs vaccinated with competitors (0.668 [0.566; 0.769]).

#### **Discussion and Conclusion**

CLP is a valuable tool for monitoring vaccination programs against Mhyo and App infections. Vaccination with Hyogen<sup>®</sup> and Coglapix<sup>®</sup> significantly reduced prevalence and severity of lung lesions compared to competitors.

#### RETROSPECTIVE STUDY TO EVALUATE THE EFFECTIVENESS OF A BACTERIN AGAINST PYOGENIC BACTERIA TO REDUCE CONDEMNATION ASSOCIATED TO SWINE LYMPHADENITIS IN A MYCOBACTERIUM TUBERCULOSIS COMPLEX POSITIVE FREE-RANGE FARM.

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#### **Background and Objectives**

Swine lymphadenitis is a complex disease caused by a variety of pathogens, including M. tuberculosis (MTC) and M. avium complex, Trueperella pyogenes and Streptococcus spp. The multiple etiology of this process and its subclinical character makes its control difficult, being responsible for important economic losses associated to condemnation at the slaughterhouse. In this study, a retrospective analysis from 2015 to 2018 is accomplished to evaluate the effectiveness of vaccination against pyogenic bacteria to reduce condemnation in a MTC positive free-range pig farm.

#### **Material and Methods**

This study was accomplished in one free-range pig farm from southwest Spain with an average of 5775 slaughtered pigs per year and a percentage of condemnation due to lymphadenitis ranging from 2.64% to 4.4%. MTC positive status in sows was also evidenced by serological analysis. Samples from condemned animals were obtained at slaughterhouse and analysed during 2015-2016. The resulting bacterin was administered since 2016 without any adjuvant, according to the following protocol: two immunizations of sows before delivery and immunizations of piglets each five months. Data and microbiological analysis of condemned animals from the period of study were evaluated.

#### Results

After the application of the bacterin a progressive reduction of T. pyogenes and Streptococcus positive samples from condemned animals were obtained: 57.14% to 19.35% and 42.9% to 22.58% respectively; however condemnation was increased from 2.64% (2015) to 4.4% (2018) during the period of study with a percentage of MTC positive condemned animals increased from 14.30% to 82.76%.

#### **Discussion and Conclusion**

A significative reduction of condemned animals and economic losses associated to T. pyogenes and Streptococcus was obtained after vaccination with the evaluated bacterin. However other biosecurity measured need to be implemented to eliminate the economic impact of lymphadenitis in MTC positive freerange systems, including the serological testing and selective sacrifice of positive breeders and limiting the contact among pigs and wildlife reservoirs.

#### SEROLOGICAL EVALUATION OF ANTIBODIES AGAINST INFLUENZA A VIRUS IN AUSTRIAN SOW HERDS

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#### **Background and Objectives**

Influenza viruses are members of the Orthomyxoviridae family, out of which Influenza A virus (IAV) is the main genus of respiratory disease in pigs. An involvement of reproductive disorders in sows has long been assumed but just recently demonstrated, especially involving pandemic subtypes. In this study, we present a recent evaluation of antibodies against IAV in Austrian sow farms.

#### **Material and Methods**

From October 2019 – October 2020, blood samples from 445 sows/ gilts and 36 farms were sent for IAV analysis. Serology was performed by a hemagglutination-inhibition assay (HI) with three porcine and six pandemic strains. IAV-unvaccinated farms were included with a minimum of  $\ge$  10 samples per farm. A sample was evaluated as positive if the seroconversion value was  $\ge$  1:80 for at least one strain whereas farm positivity was determined in case of  $\ge$  2 positive samples.

#### Results

In total, 89% of all farms were positive according to evaluation criteria. On average, 80% of these farms seroconverted to porcine subtype HINI, 54% to HIN2, and 46% to H3N2. The average seroconversion rate for pandemic strains was 49%. Five positive farms showed reactions to porcine strains only (14%), whereas four farms solely reacted to pandemic ones (11%).

#### **Discussion and Conclusion**

The results of present assessment indicate that IAV is widely spread among Austrian unvaccinated sow herds. Regarding the size of the entire sow population (approx. 234.000 head) and low vaccination rates (approx. 15%), IAV is probably an undiagnosed issue. Further studies involving direct virus detection are needed to monitor the situation, especially when having in mind the challenges posed by HI. Involved IAV might remain undetected because of antibody specificity for one specific strain within a subtype. On the other hand, cross-reactivity might mask one specifically involved subtype/strain of IAV.

#### AN INVESTIGATION INTO THE EFFECT OF BODY CONDITION AND PUBERTY STATUS OF GILTS ON THEIR ESTRUS RESPONSES POST ALTRENOGEST TREATMENT AND ON REPRODUCTIVE PERFORMANCE

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#### **Background and Objectives**

This study was conducted to investigate the effect of body condition and puberty status of gilts on their estrus responses post altrenogest treatment and on reproductive performance on a 2.200 farrow-wean sow farm with Danish genetic.

#### Material and Methods

A total of 161 gilts (255 days of age, approximately 150 kg of weight) were treated with 20 mg altrenogest (Altresyn ®) orally for 18 consecutive days, boar exposure done twice daily, estruses checked and gilts bred by artificial insemination on AM/PM basis. Ultrasound (RTU) was applied to determine the following at the beginning and least treatment, respectively: puberty and ovary status; backfat (BFT) and muscle thickness (MT).The interval last altrenogest-onset of heat was recorded. RTU was done to test for pregnancy, and conception (CR) and pregnancy (PR) rates as well as litter sizes determined. Individual piglet birth weight was also recorded (n = 70 litters).

#### Results

147 gilts were tested pubertal (P) and 14 prepubertal (PP). All gilts had small-midsize follicles at the end of altrenogest and 160/161 came into estrus (the majority within 6.5 days). BFT and MT were similar at the two measurements. Overall CR was 100%, PR 95.6%, and total born 18.1  $\pm$  2.6, life born 17.4  $\pm$  2.5 and still born piglets 0.7  $\pm$  1.0. Average piglet birth weight was 1.3  $\pm$  0.2 kg. There were very little effects of body condition, age and weight on the responsiveness to altrenogest and reproductive performance. Also, P and PP gilts did almost not differ in this regard.

#### **Discussion and Conclusion**

In conclusion, altrenogest has shown to properly block follicle growth and synchronize estruses in gilts, and that there was no effect of body condition and puberty and also not on reproductive performance providing that, gilts are in optimum age and condition, and proper estrus stimulation management is applied.

#### EFFECT OF VITALITY SCORE AND BIRTH ORDER ON PREWEANING PIGLET PERFORMANCE AND MORTALITY

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#### **Background and Objectives**

Pre-weaning piglet mortality is a major problem, especially within the first three days after birth. The greatest problem is seen in low birth weight piglets. In addition, the higher farrowing duration increases the risk of asphyxiation in those piglets expulsed late in the birth order. This makes piglets more hypothermic, alters the blood energy parameters, and reduces colostrum intake. Together these alterations reduce the growth performance and survival. We aimed to evaluate the effect of vitality score and birth order on growth performance and preweaning mortality.

#### Material and Methods

We included forty sows (Yorkshire × Landrace) balanced for body condition and parity (2-4). Immediately after birth, the vitality (V) of the piglet was visually assessed and scored using a four categorical scale 0-3. Piglets were categorized into four birth order (BO) groups. Blood was collected from the umbilical cord of piglets for testing blood glucose, lactate, and butyrate. Observed piglet parameters were colostrum intake (CI), blood glucose, lactate, and butyrate, body weight at birth, and weaning, and pre-weaning mortality.

#### Results

Piglets with high vitality score had higher body weight at birth and weaning, higher blood glucose, and butyrate, lower blood lactate, higher colostrum intake, and lower mortality rate before weaning (p<0.05). We found no significant difference among the four birth order groups, except for CI that was significantly higher (p<0.05) in BO1 and BO2 compared to BO3 & BO4.

#### **Discussion and Conclusion**

High vital and early born piglets had higher CI. Moreover, the mortality rate was significantly higher in the low vital piglets. Our results show that improving piglet vitality and the farrowing process can reduce piglet mortality.

# FIELD AND PATHOLOGICAL EVIDENCE FOR A CONTRIBUTION OF DEOXYNIVALENOL (DON) ON REPRODUCTIVE FAILURES

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#### **Background and Objectives**

DON is a mycotoxin built by molds of Fusarium spp. It is known to cause vomiting, but has also immunosuppressive effects. Those effects may be generalized but also at local levels including the genital tract. This report summarizes pathological findings of 19 genital tracts (cervix, vagina, uterus, oviducts) from different parity sows and gilts including the urinary bladder submitted from 8 farms in 2019/20.

#### Material and Methods

DON and zearalenone (ZEA) were determined by using HPLC in 13 individual and 2 pooled (2 & 4, resp.) bile samples. Microbiology of uterine (n = 17) and bladder (n = 8) samples were also done. Fertility problems reported were increased returns and low/fluctuating pregnancy/ farrowing rates, occasionally in conjunction with vaginal discharge.

#### Results

A total of 8 individual and both pooled bile samples were found DON "positive" with concentrations ranging between 75,5 to >200,0 mg/l. ZEA was detected in 5 samples (only individual; range 6,57 to 21,20 mg/l). The genital tract of 14 animals were found to have chronic inflammations simultaneously in all or more than one organs investigated, with 8 individual animals tested DON positive and 3 in pooled bile samples. Lymphocytes, plasma cells and occasionally macrophages were the predominating immune cells (mostly diffusely distributed within the endometrial stroma and underneath the epithelium). Microbiology usually revealed a mix of different several gram-positive and –negative bacterial species including potentially genitopathogenic species such as E. coli (n = 10), Streptococcus spp. and Enterococcus spp. (n = 6, respectively). In a majority of cases, the same or a similar bacterial flora was also found in the urinary bladder.

#### **Discussion and Conclusion**

In conclusion, based on the similarity of the pathological findings (chronic inflammation in many organs) in DON-positive animals, a contribution of DON on the clinical as well as pathological picture as observed is strongly suggested.

# CASES OF REPRODUCTIVE FAILURE IN SOWS CHARACTERIZED BY AN INCREASE IN STILL BORN AND MUMMIFIED FOETUSES IN DANISH SWINE HERDS

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#### **Background and Objectives**

Porcine Parvovirus (PPV), more correctly named Ungulate protoparvovirus I species, is a single-stranded DNA virus classically leading to stillbirths, mumification, embryonic death and infertility (SMEDI). PPV viruses are prone to constant mutations and are classified into several strains of variable pathogenicity, some of them with very high virulence. Here we report two recent, thoroughly investigated case of SMEDI in PPV vaccinated sow herds out of several similar clinical cases in Denmark.

#### Material and Methods

During months of the Spring and Summer of 2020, these farms demonstrated severe increase in mummified and still-born of, primarily, 1st-parity farrowing's.Farm-1: in average 2.0 mummies per gilt; 2-5 per affected litter; no abortions. Submission to lab: 12 foetuses of 8-23cm (gestational age approximately 50-90 days) from 4 litters.Farm-2: some 1st-parity litters almost all mummified – few live-born; no abortions. Submission to lab: 18 mummies of 6-13cm (gestational age approximately 45-60 days) from 2 litters.The farms were checked for and demonstrated excellent vaccine management and application. For repro-prophylaxis, the gilts were vaccinated twice pre-mating and boosted prior to each subsequent mating by a commercial NADL2-like PPV plus Erysipelas rhusiopathiae plus hexa-valent Leptospira spp. combo-vaccine.

#### Results

Both farms demonstrated strongly and only PPV-PCR positive findings in the foetal material. Negative PCV2-IHC was demonstrated when investigated; here Farm-1. Application of an alum adjuvated PPV-K22 Ery combo-vaccine reversed the reproductive disorders.

#### **Discussion and Conclusion**

Vaccination against PPV is the one most important repro-prophylactic protocol for breeding stock worldwide. However, one must consider that PPV consists of several strains with various pathogenicity and antigenicity. When vaccinating against PPV there are indications that the Kresse-like K22-strain as an antigen confers a wide and efficient clinical PPV protection particularly against virulent strains; wider and stronger than NADL-2 and NADL2-like PPV strains.

#### THE USE OF A PMSG/HCG COMBINATION TO SUPPORT HERD REPOPULATION AFTER AFRICAN SWINE FEVER INFECTION

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#### **Background and Objectives**

Many Chinese producers are using commercial fattening females as replacement animals due to difficulty sourcing African Swine Fever free replacement stock. These animals demonstrate poorer reproductive performance, commonly showing issues such as a delayed first estrus. In order to improve their estrus rate, a hormone combination with HCG/PMSG and improved husbandry was evaluated.

#### **Material and Methods**

206 fattening females of age 160 days or more were randomly selected from 2 farms, 101 from farm A, 105 from farm B.

The average boar exposure start age of the 2 groups were 170 days and 165 days respectively. All the females followed a 28 – day gilt development protocol with gilts given direct fenceline contact with boars from day 1-13, remixing and re-penning of all gilts on day 14, followed by continued stimulus till day 23. Those not on heat by 23 days were given a combination of 400IU PMSG/200IU HCG. Females that achieved estrus within 13, 23 and 28 days were recorded.

#### Results

The % estrus in day 1-13 was 57% for Farm A and 52% in Farm B.

The % estrus in day 1-23 was 68% and 60% respectively.

The % estrus in day 1-28 was 87% and 85% respectively.

19 gilts from A and 26 from B experienced estrus in day 23-28 after treatment.

#### **Discussion and Conclusion**

The result shows that a combination of proper gilt development protocol and the application of 400IU PMSG/ 200IU HCG combinations can assist producers in getting good gilt heat rates, even when using fattening females as replacements.

A 28-day gilt development protocol with 400IU PMSG/ and 200IU HCG combinations could be an efficient solution for estrus induction in prepubertal gilts selected from fatteners.

Unfortunately in this situation it was not possible to follow the reproductive performance further due to operating constraints of the farms surveyed.

#### BACILLUS-BASED PROBIOTIC STRAINS IMPROVE SOW PERFORMANCE

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#### **Background and Objectives**

Good sow reproduction and piglet vitality are important for obtaining high profitability in the sow unit. Bacillus-based probiotics have long been recognized as a feed additive for weaned piglets to support performance but probiotics for sows is still new to many. Therefore, the main objective of this study was to evaluate the benefits of two Bacillus-based probiotic strains on the performance of sows and sucking piglets.

#### Material and Methods

At a commercial farm, three trials were conducted testing the effect of two Bacillus-based probiotic strains on days from weaning – to – service, pre-weaning mortality and piglets weaned. The trial setup was the same in all 3 trials: control (TI), Bacillus subtilis (DSM 25841) (T2) and Bacillus amyloliquefaciens (DSM 25840) (T3). 41 sows were allocated to each treatment. Dam parity and body weight were the same across treatments at study start. The probiotic strains were added to the feed for sows for two consecutive reproduction cycles throughout gestation and lactation and to the creep feed for sucking piglets in T2 and T3. Sows were individually fed and piglets were fed ad libitum. The results from the three trials were pooled and analyzed. Each individual dam/litter was considered an experimental unit in the statistical analysis. R was the software used for statistical analysis where T2 and T3 was compared to TI in separate analysis.

#### Results

Sows fed either T2 or T3 had significantly fewer days from weaning - to - service (P=0.015 (T2) and P=0.011 (T3)), significantly lower pre-weaning mortality among piglets (P=0.018) and significantly more piglets were weaned (P=0.020 (T2) and P=0.001 (T3) compared to sows not fed Bacillus strains.

#### **Discussion and Conclusion**

The findings in this study indicate that the Bacillus-based probiotic strains used in this study could be an approach to improve sow performance and vitality of their off-spring which can result in a more profitable sow unit.

#### CLOPROSTENOL ADMINISTRATION 24 HOURS POST-PARTUM INCREASED FARROWING RATE IN SOWS

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#### **Background and Objectives**

The prostaglandins and their analogues present principal effects in the reproductive performance in sows by luteolytic induction, smooth muscle and hormonal stimulation (Dial,1984). The porpouse in this study was to analyse the action of the injection of a synthetic prostaglandin (PGF2  $\alpha$ ) analogue on subsequent reproductive performance.

#### Material and Methods

A total of 448 sows of different cycles (parity 1-7) were randomized into two groups: experimental (PGF, n = 233) and control (C, n = 215), with homogeneous values of parity and litter size (p> 0.05). The animals in the experimental group received a 2ml im injection of Cloprostenol sodium (Planate, MSD Animal Health) 24 h postpartum, compared to the control group that was not injected, and each animal was monitored to study the interval of weaning to oestrus (days), pregnancy and farrowing rate (%) and subsequent litter size.

#### Results

Although the weaning to oestrus interval was similar in both groups (C:  $6.20 \pm 0.22$  vs PGF:  $5.72 \pm 0.23$ , p = 0.13). The percentage of sows bred by 6 days was higher in the experimental than the control group (C: 62.33% vs, 73.39%; p = 0.01). The farrowing rate increased in the PGF group (from 86.51 to 93.99%; p <0.01). The pregnancy length was also increased in PGF group (115.33 ± 0.14 vs. 115.76 ± 0.16; p = 0.04). This increase in the farrowing rate was especially marked in old-parity sows, for parity 6 and 7 the birth rate increased from 69.57 to 96% (p <0.01).

#### **Discussion and Conclusion**

Administration of PGF2 $\alpha$  after farrowing can facilitate uterine contractions, elimination of postpartum lochia, stimulating the uterine involution and reducing the incidence of endometritis (Koketsu et Dial 2002). On the other hand, induction of luteolysis could solve the cases of persistent luteal body existence has been observed in 8% of postpartum sows (Lopez et al, 2009).

## ASSOCIATION BETWEEN UTERINE INVOLUTION IN SOWS AND REPRODUCTIVE PERFORMANCE IN THEIR NEXT GESTATION

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#### **Background and Objectives**

A physiological uterine involution during the puerperium is essential for the reproductive health of sows. The aim of this study was to describe the continuous regression of the uterine diameter from day 2 until day 14 after parturition and on the day before weaning (DBW) by means of ultrasound, and to compare these findings with the reproductive performance of the subsequent gestation. Furthermore, it was hypothesized that the reproductive performance of the next litter can be predicted by measuring the diameter of the uterus on single days.

#### Material and Methods

The diameter of three uterus cross-sections was determined by ultrasound in 46 sows housed in a free farrowing system (FFS) and in 49 sows housed in farrowing crates (FC) belonging to two different herds. For the evaluation of the reproductive performance of the next litter the weaning to oestrus interval in days, number of total-born and live-born piglets, and the gestation length were recorded.

#### Results

The median diameter of the uterus decreased from 32.4 mm (min: 18.6 mm, max: 52.3 mm) on day 2 to 9.0 mm (min: 7.6 mm, max: 12.7 mm) on DBW in sows in FFS. The median diameter of the uterus of sows in FC decreased from 38.5 mm on day 2 (min: 21.6 mm, max: 56.3 mm) to 10.1 mm (min: 8.8 mm, max: 13.6 mm) on the DBW. On day 11 a weak negative correlation between uterine diameter and total-born piglets (p= 0.022) as well as live-born piglet (p=0.002) in the next litter was detected.

#### **Discussion and Conclusion**

A continuous regression of the uterine diameter was observed until day 14 after parturition. The ultrasound examination was a suitable method to describe intra vitam the uterine involution. However, current data do not support the identification of a clear effect of the uterine involution on the reproductive performance in the subsequent gestation.

#### INFLUENCE OF TWO DIFFERENT ARTIFICIAL INSEMINATION TECHNIQUES ON REPRODUCTIVE PERFORMANCE IN SOWS.

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#### **Background and Objectives**

Artificial insemination (AI) of sows is commonly used in intensive swine production. The intracervical insemination (ICAI) technique, where the semen is deposited in the cervix, is the most commonly used worldwide. The intrauterine insemination (IUAI), where semen dose is deposited directly into the uterus, makes better use of ejaculate due to lower spermatic concentration and volume per dose. The objective of this study was to evaluate the impact of different insemination techniques on reproductive performance of sows.

#### **Material and Methods**

The study was conducted in high performing Polish farm (3200 sows) managed in accordance with Danish production standards. Genetic line (DanBred), health status (PRRSV-negative), farm staff, vaccinations protocol, feeding and housing system remained unchanged during the experiment. In boar station, animals and semen quality were under strict veterinary control. All sows in the trial were artificially inseminated after natural return to heat using ICAI or IUAI protocol. The parameters, i.e. farrowing rate and number of total born piglets, were observed during 75 (ICAI) and 47 (IUAI) consecutive weeks.

#### Results

Mean number of total born piglets was higher in IUAI group (ICAI=17.6 vs. IUAI=18.3). Improvement in mean (ICAI=86.9% vs. IUAI=90.7%) was also observed. Both differences were statistically significant (p<0.05).

#### **Discussion and Conclusion**

The results of the study showed the IUAI method to be significantly more effective than ICAI technique, in terms of mean number of piglets born per sow and the mean farrowing rate. However, IUAI technique is considered to be more invasive and requires additional training of the personnel.

# EVALUATION OF THE EFFECT OF REGUMATE PORCINE (MSD ANIMAL HEALTH) ON REPLACEMENT GILTS SYNCHRONISATION IN A LARGE PRODUCTION SYSTEM.

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#### **Background and Objectives**

Gilts management is one of the key factors influencing economy and efficiency of pigs reproduction. Modern batch production requires proper amount of replacement gilts to reach breeding targets and avoid financial losses due to farm over- or understocking. Anticipated introduction of gilts can also provide economic benefits by stability of replacement rate. Preparing adequate number of gilts using pharmacological agents allows to mate them in very precise weight and age recommended by genetic companies. Altrenogest is an orally active synthetic progestogen which can be used to control pig oestrus cycle trough suppression of follicular phase. After the cessation of such inhibition in group of animals, procedure results in synchronised onset of oestrus. The purpose of this study was to assess efficiency of altrenogest (Regumate Porcine, MSD Animal Health) for DanBred gilts synchronisation in a large production system.

#### **Material and Methods**

The analysis was performed in a high productive 8000 sow farm with weekly farrowing cycle. The impact of altrenogest was compared in two groups of 2740 replacement gilts. The control group was artificially inseminated at the second natural heat. In the treatment group cycling gilts were individually fed by Regumate Porcine solution for 18 consecutive days, at the same time, via drencher at the dose of 5 ml (20 mg of altrenogest/day). In both groups, the age of the first service was compared.

#### Results

Percentage of gilts inseminated in ≤238, 239-259 or ≥260 days of live varied between examined groups. In the control group it was 40%, 50.5% and 9.5%, respectively. In the treatment group it was 80.3%, 14.3% and 5.4%.

#### **Discussion and Conclusion**

Comparison of the results obtained from synchronised and non-synchronised group indicated that pharmacological synchronisation is an effective tool contributing to significantly improved gilt management by optimising their age at first service.

# EVALUATION OF INTRAVAGINAL AND INTRAMUSCULAR PROSTAGLANDIN E2 APPLICATION IN FREE FARROWING SOWS DURING PARTURITION

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#### **Background and Objectives**

The duration of parturition has a significant impact on the survival of piglets and the health of the sow. Hence, oxytocin is widely used to speed up the birth process. However, oxytocin comes along with negative side effects requesting alternatives as already used in human medicine. The aim of this study was to evaluate the efficacy of Prostaglandin E2 (PGE2) applied intravaginal (PGE2-V) (1.0 mg) and intramuscular (PGE2-M) (2.5 mg) to improve the birth process after expulsion of the fourth piglet compared to intramuscular oxytocin (OXY-M) (20 IU) and a placebo group (P-V), which received gel intravaginal.

#### **Material and Methods**

In total, 201 sows stratified by parity were examined. The sows were randomly allocated in the following groups: 50 (PGE2-V), 49 (PGE2-M), 48 (OXY-M), 54 (P-V). Farrowing duration (time between first piglet and last piglet), piglet interval and placenta expulsion duration (time between first and last placenta) were recorded, and each piglet was scored for meconium staining and vitality. Furthermore, stillborn piglets were categorized into ante-partum and intra-partum deaths.

#### Results

The farrowing duration varied between the different groups (PGE2-V 188 min, PGE2-M 238 min, OXY-M 156 min & P-V 219 min). A significant difference was detected between group OXY-M and P-V (z-value: 2.5) as well as OXY-M and PGE2-M (z-value:2.7). No significant difference were observed considering the other parameters when comparing treatments to P-V.

#### **Discussion and Conclusion**

In this study, the different treatments had a limited influence on the birth process in free farrowing sows compared to the P-V. This observation might be influence by the fact that only few parturitions were prolonged, i.e. needed pharmaceutical intervention. Based on presented results, a routine treatment with uterotonic agents in sow farms with free farrowing system cannot be recommended. The effect of PGE2 in actually prolonged farrowing still need to be proven.

# EVALUATION OF VARIOUS POINT-OF-CARE TESTS TO CHARACTERIZE THE LOCHIA OF SOWS AFTER PARTURITION AND PREDICTION OF FURTHER REPRODUCTIVE PERFORMANCE

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#### **Background and Objectives**

The lochia is the physiological uterine discharge postpartum and pathological changes are risk factors for puerperal disorders in sows, which negatively influence the reproductive performance. Therefore, the aim of the study was to characterize the lochia in sows using simple and practicable test methods and correlate the evaluated parameters with the next reproductive performance of the sows.

#### Material and Methods

The birth process of 48 clinically healthy free farrowing sows was monitored and several parameters characterizing the vaginal discharge such as total amount, colour, amount of cells (somatic cell count test) and cell characteristics (cytology) were collected daily the first five days after parturition. Finally, the reproductive performance of the following gestation was evaluated and compared to the characteristics of the lochia.

#### Results

The amount of vaginal discharge was significantly increased on the second (p = 0.0005), third (p = 0.019) and fourth (p = 0.011) day post partum compared to day one. Furthermore, a decrease in the percentage of neutrophilic granulocytes from day one to the third (P = 0.038), fourth (p = 0.038) and fifth (p = 0.048) day post partum was observed. The percentage of leukocytes in the yellowish vaginal discharge was increased compared to whitish (p = 0.02) or clear (p = 0.027) vaginal discharge. In addition, obstetrics (p = 0.003) and an increased farrowing duration (p = 0.017) significantly increased the amount of vaginal discharge. No correlation between the evaluated parameters of the lochia and the following reproductive performance was detected.

#### **Discussion and Conclusion**

It seems that the amount of vaginal discharge alone is not a predictor for the performance of sows during their next gestation, but might be an indicator for an acute endometritis. In conclusion, different parameters of the vaginal discharge determined by means of point-of-care tests might be useful to strengthen a presumptive diagnose of endometritis in sows after birth.

#### COMPARATIVE EXAMINATION OF REARING GILTS OVARIES - A FIELD STUDY

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#### **Background and Objectives**

In order to reach the maximum performance of a herd which works in batch system we must calculate the amount of replacement gilts for each batch. If there are not enough rearing gilts, we realize economical losses. The aim of the study was to check reproductive tract of living problematic gilts.

#### Material and Methods

A Hungarian large-scale farm (2.300 sows) buys 200 rearing gilts in every 10<sup>th</sup> week. The farm manager realized the increased ratio of anestrus (from 0.5-1% to 25%). No changes were made in the management of the gilts. We made ultrasonography (USG) examination on 25,10-12-month-old gilts to check the reproductive tract, especially ovaries. The gilts were culled and slaughtered 8 days later. We made post mortem examination on the reproductive tract and we sent samples to the laboratory for investigation.

#### Results

By USG image we measured the size of the structure of the ovaries: there were 10 ovaries with very small (<2mm diameter), 9 ovaries with small (2-3mm) follicles. In 9 cases we could not find any other structure and in 10 cases we saw corpus albicans-like structures beside the follicles in this two groups. In 6 ovaries there were medium size (4-6mm) follicles. The results of the post mortem examinations showed that there were 15 samples with signs of obvious or suspected ovarian inactivity and 7 out of 15 were juvenile only with growing follicles.

#### **Discussion and Conclusion**

USG is a good tool to identify abnormalities of the ovaries in live gilts. In this way we can make individual decisions to cull or treat the animals and we can support the prudent hormone use, as well.

# ISOQUINOLINE ALKALOIDS IN THE SOW'S DIET REDUCES BODY WEIGHT LOSS DURING LACTATION AND INCREASES IGG IN COLOSTRUM

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#### **Background and Objectives**

Plant extracts containing isoquinoline alkaloids (IQ) have shown beneficial effects on sows. We hypothesized that supplementing sow feed with IQ during gestation would decrease stress at farrowing and improve colostrum and milk quality, affecting piglets' gut development and growth performance.

#### **Material and Methods**

Twenty-four sows were allocated in three dietary groups: NC - basal diet without supplementation, IQI - 90 g/t IQ in the diet from gestation day (G) 80 to 110 and 150 g/t IQ from G110 (when entering maternity) until weaning, IQ - 150 g/t SE from G110 until weaning. Blood was taken from sows five days before, during and one week after farrowing to measure cortisol, glucose and insulin. Colostrum and milk were analysed for protein, fat, IgA and IgG. Sow body weight, feed intake, back-fat thickness and back-muscle thickness were monitored. Measurements of piglets included body weight (weekly), diarrhoea score, intestinal histomorphometry and gene expression (IL6, IL10 and TNF-a), on day 5 post-weaning.

#### Results

The IQ-fed sows lost less body weight. No differences were found in feed intake, back-fat thickness, and backmuscle thickness. Five-days before farrowing, sows' blood glucose and insulin levels were lower in the IQ groups (IQ2, p<0.05) compared to the NC group. There were no differences in cortisol between treatments. Colostrum of IQ groups had higher content in protein and IgG (p<0.05), but no differences were found in fat content. Piglets showed no effects in the zootechnical parameters nor in the physiological measurements.

#### **Discussion and Conclusion**

IQ seem to have their main effect on sow's metabolism, reducing body weight loss during lactation. Providing IQ to sows from entrance to the maternity barn might be sufficient to induce those effects. IQ improved colostrum quality increasing protein and IgG content, providing piglets with a better passive immunity.

#### THE IMPACT OF BIOSUIS PARVO L(6) VACCINE (BIOVETA) ON REPRODUCTIVE PARAMETERS IN GILTS AND SOWS

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#### **Background and Objectives**

Pigs are susceptible to many Leptospira serovars. Endemic infection of breeding herd may not produce clinical signs. However, during periods of immunodeficiencies it can result in reproductive failure. Porcine parvovirus (PPV) is one of the most important viral factors of reproductive failure, mainly in gilts. The objective of this study was to assess the impact of gilts and sows vaccination with Biosuis Parvo L(6) (Bioveta), the product containing antigens of six Leptospira serovars and PPV, on their reproductive parameters.

#### **Material and Methods**

The study was conducted in a high performing farm (3200 sows) managed in accordance with Danish standards. In total, 3393 gilts (group L+PE) were vaccinated with Biosuis Parvo L(6) (Bioveta) and Eryseng (HIPRA) and 4008 gilts (group PE) were vaccinated with Eryseng Parvo (HIPRA), so the potential impact of PPV infection was eliminated. The same protocol was applied in sows, in 13211 and 5296 animals, respectively. The farrowing rate (FR), number of liveborn, stillborn piglets and mummified foetuses were analysed.

#### Results

In gilts, the average numbers of liveborn, stillborn piglets and mummified foetuses were 14.5, 0.8 and 0.3 in PE, and 15.3, 1.0 and 0.4 in L+PE females, respectively. FR in PE gilts (88.7%) was lower than in L+PE group (91.2%). In sows, the average number of liveborn, stillborn piglets and mummified foetuses were 16.7, 1.6 and 0.4 in PE and 17.1, 2 and 0.5 in L+PE pigs, respectively. FR in PE and L+PE was 90.6% and 88.3%, respectively. All observations, except differences noticed in FR in sows, were statistically significant (p<0.05).

#### Discussion and Conclusion

The obtained results indicate that the vaccination with the product containing Leptospira antigens can be beneficial in a high performing farm and provide improvement of reproduction parameters. According to epidemiological data vaccination might be profitable in other herds.

#### BACTERIAL AGENTS DETECTED IN ABORTED AND DEAD BORN PIGLETS

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#### **Background and Objectives**

Information of gram-positive and gram-negative bacteria being involved in embryonic death and abortion are scarce and bacteria are usually considered as contaminants. The objective of our study was to retrospectively evaluate bacterial agents in 58 cases of aborted swine foetuses and dead born piglets submitted to the University Clinic for Swine of the Vetmeduni Vienna between 2017 and 2019.

#### Material and Methods

Pool samples of liver, kidney, lung and stomach content were collected and homogenized from each foetus. Bacteria were isolated and identified from all samples by culture or by PCR (Leptospira sp. and Chlamydia sp.).

#### Results

Bacteria were found with different frequencies: Streptococcus spp. (23%), Staphylococcus spp. (11%), Enterococcaceae (7%) as well as Enterobacteriales (54%) wherefrom E. coli was the most predominant (41%) amongst others (5%). DNA of Leptospira spp. and Chlamydia sp. could not be detected in any of the samples.

#### **Discussion and Conclusion**

The majority of identified bacteria are considered as commensals or facultative pathogens in the genital tract of sows and it cannot be distinguished if colonisation occurred prior or after death. Nevertheless, bacterial species such as E. coli and Streptococcus sp. are regularly detected in abortion material. While E. coli was frequently detected (41%), S. suis was isolated only in 1,7% of the submitted samples, although discussed as fertility disorders associated agent. Interpretation of the role of bacteria isolated from foetal tissues is complicated due to potential environmental contamination. Hence, significance of potentially pathogenic bacteria isolated from foetal tissues should be evaluated in all foetuses of multiple aborted litters of a herd before considering them as primary cause of abortion.

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