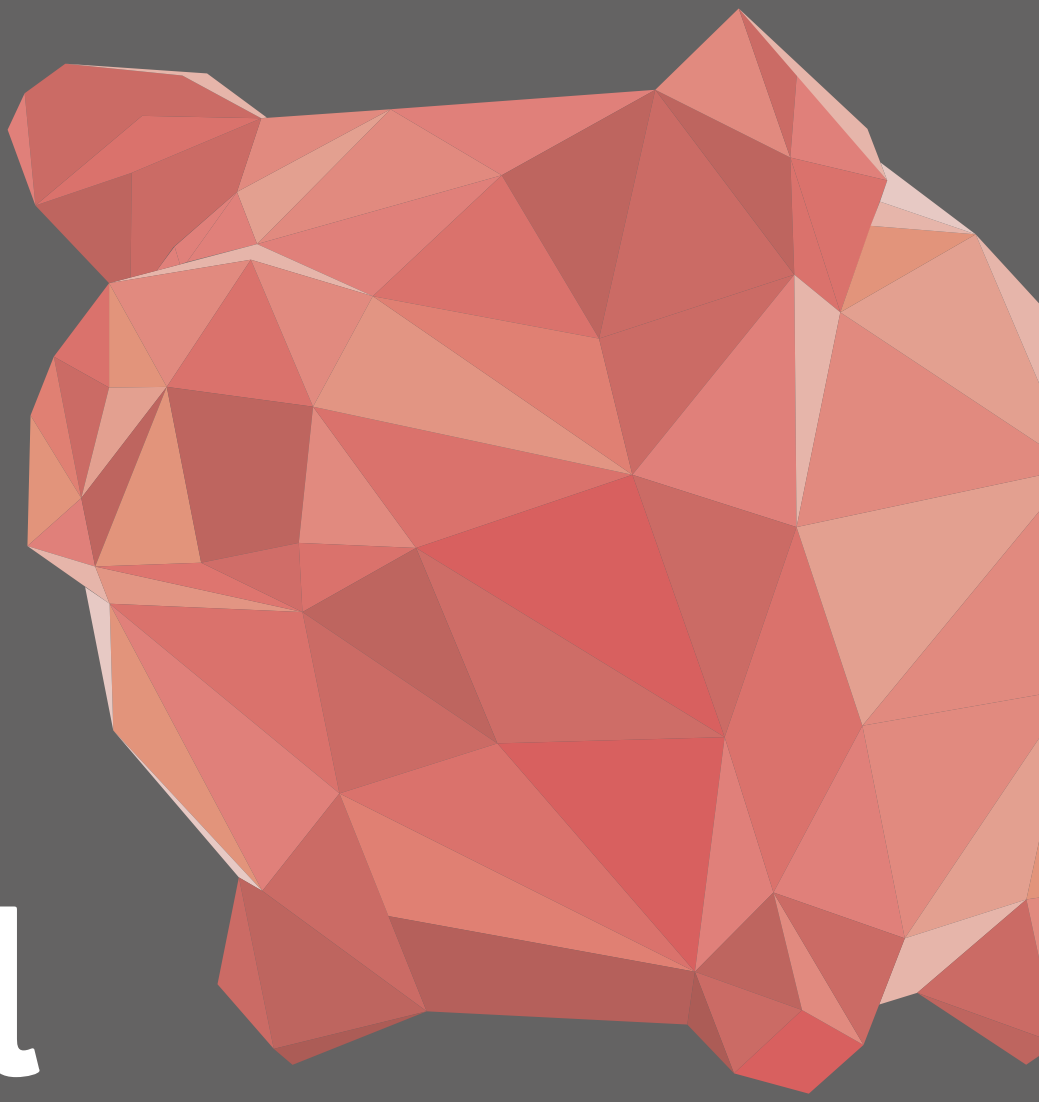
esphm 2015

7TH EUROPEAN SYMPOSIUM OF PORCINE HEALTH MANAGEMENT



22·24 april 2015

nantes, FRANCE

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esphm2015.org

PROCEEDINGS

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esphm 2015
EUROPEAN SYMPOSIUM OF PORCINE HEALTH MANAGEMENT

welcome
FROM THE PRESIDENT

On behalf of the organising committee, I am pleased to welcome all delegates, partners, supporters and guests to the 7th European Symposium of Porcine Health Management (ESPHM) in Nantes, France. Over the past years, this meeting has been jointly organised between the European College of Porcine Health Management and the European Association of Porcine Health Management. Consequently, the ESPHM is now recognized as a major event in pig health in Europe and this increasing attractiveness is confirming this year (more than 1200 delegates are expected).

The scientific program will mix keynote lectures and presentations of submitted short papers including ECPHM residents and industrial partners. The keynote lectures will focus on porcine health management issues such as group-housed sows, porcine epidemic diarrhea, mycotoxines and Actinobacillus pleuropneumoniae. Round table discussions will allow fruitful exchanges between researchers and practitioners. Among the 351 abstracts submitted, the international scientific committee has selected oral communications and posters. This will give the opportunity to discuss on new scientific findings both during sessions and in poster areas that will be located near the commercial exhibition.
The success of such an event is highly dependent on the contribution of all the participants: the delegates for their scientific input and the industrial partners for their essential financial support. We would like to highlight the key-roles of the boards of the College and Association for their constant help. We sincerely thank the members of the international scientific committee for having dedicated time to the evaluation of submitted papers.

The symposium takes place in Nantes, a very attractive city located in the North West of France, close to one of the main pig producing areas in Europe. It is organised in the dedicated conference facilities (La Cité Nantes Events Center) in the centre of Nantes.

We hope you will enjoy the symposium, the city and its surroundings, and of course the French Cuisine.

Catherine Belloc
President of the 7th ESPHM
Chair of the Local Organising Committee

committees

Local organizing and scientific committees

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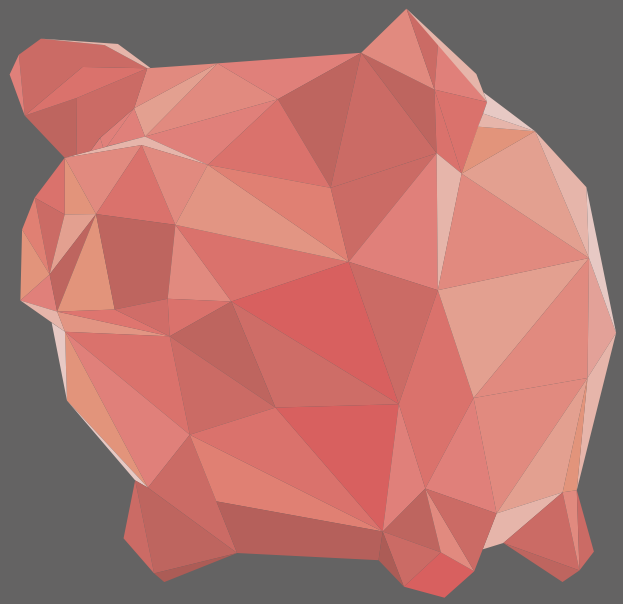

EAPHM

Full name: The European Association of Porcine Health Management

Abbreviation: EAPHM
Website: www.eaphm.org

Description: The EAPHM is a non-profit organization and aims to join all veterinarians dedicated to swine health and production, but is also open to other professionals and industry.
The EAPHM is representing all veterinary professionals in the field of porcine health management and, therefore, is gathering automatically all members of the European College of Porcine Health Management (Diplomates and Residents of the ECPHM) and is open to practitioners and other veterinarians working in the pig industry. The EAPHM is also closely connected to the Federation of Veterinarians in Europe (FVE) as it is offering the secretariat services and physical accommodation in Brussels; the heart of Europe.

The association was founded in 2010. So, the EAPHM is quite young and is very active, even though the association is still far away from what is aimed in terms of numbers of members. The EAPHM would like to accommodate all practitioners that work with pig health and/or production in Europe, but also all related academics, whether or not they are members of the ECPHM, as well as veterinarians in the industry, as the bylaws of the association allow them to be welcomed as members.

The association was created as a meeting point for industry professionals, promoting and facilitating contacts between them, regardless of country of origin and also collaborate with other organizations, both veterinary and agricultural, when it is necessary. Among the objectives of the EAPHM, the most important is to represent veterinarians and other professionals working in the pig sector in the various legislative committees and executives in Europe. Moreover, EAPHM plays an important role in the continuous postgraduate education of swine practitioners. Therefore, the association is publishing clinical cases written by Residents of the ECPHM. In addition, the association is responsible for publishing all the material presented at the annual European Symposium of Porcine Health Management (ESPHM) on its website. By doing so, the members of the EAPHM have continuous access to the posters and oral presentations of every ESPHM.

Becoming a member is easy, just visit the website www.eaphm.org and sign up for a membership. The annual fee is worth every Euro, when you look to the benefits of being an “EAPHM member”.

Join us today!

ECPHM

Full name: The European College of Porcine Health Management Ltd.

Abbreviation: ECPHM
Website: www.ecphm.org

Description: The ECPHM is a non-profit organization within the European Board of Veterinary Specialization (EBVS). EBVS recognizes new speciality Colleges and maintain a register of European veterinary specialists (Diplomates); moreover, EBVS encourage and promote the enhanced utilization and availability of speciality services to the public and the veterinary profession. Therefore, the ECPHM is the College that works for the advance of health oriented porcine production management in the herd context in Europe and the increase of the competency of those who practice in this field. Major objectives of the ECPHM include:

- Establishing guidelines and standards of training for postgraduate education and experience prerequisite to become a specialist in the speciality 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 products 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:

- Board: represents the College and it is its main government body
- Education Committee: educational issues for the residents, including organization of the pre-symposium workshop and the summer school
- Examination Committee: exam preparation and examination of residents
- Credentials Committee: review and approve the candidacy of applicants to sit the exam, to set a new residency program, and re-accreditation of the diplomates
- Nominations Committee: receive and manage the proposals for nominates at the different committees and board

ESPHM

Full name: European Symposium of Porcine Health Management

Abbreviation: ESPHM
Website: changing from year to year; this time www.esphm2015.org

Description: The first ESPHM was organized by the ECPHM in 2009 in Copenhagen (Denmark). Subsequent meetings were organized in Hannover (Germany) in 2010 and Helsinki (Finland) in 2011. After the creation of the European Association of Porcine Health Management (EAPHM) in 2010, the following symposia were organized in a three-party fashion, involving the EAPHM, the ECPHM and the local organizers. By means of this organizational formula, the ESPHM has been held so far in Bruges (Belgium), Edinburgh (United Kingdom) and Sorrento (Italy), in 2012, 2103 and 2014, respectively. Therefore, the symposium is being consolidated, and the 7th edition will be held in 2015 in Nantes (France). Moreover, consolidation is not just a matter of a yearly organization but also on an increasing number of delegates, reaching the maximum number of around 1500 attendants last year.

The ESPHM is the expression of a long-standing need at a European level. The lack of a continental swine veterinarian congress like in North-America (American Association of Swine Veterinarians Annual Meeting) and Asia (Asian Pig Veterinary Society), prompted first the ECPHM and then the EAPHM to organize a yearly meeting devoted to all subjects of porcine health management. The symposium philosophy consist of mounting a sound program, with cutting-edge scientific-technical knowledge, practically oriented, which is able to catch the attention of swine veterinarians all over Europe, but with full international vocation. The symposium's content includes invited lectures, initiating always with the state-of-the-art swine production in the organizing country, as well as oral communications and posters.

Importantly, the ESPHM is an excellent platform for introducing the ECPHM Residents into the scientific world, by presenting their studies (Resident oral communication session) and participating in the College activities organized around the symposium (i.e., Resident workshop, examination). In addition, the ESPHM must serve as a vehicle for potentiating networking among pig veterinary professionals all around Europe, and emphasize the global character of a borderless profession.
Also, Annual General Meetings of both EAPHM and ECPHM are organized within the program of the symposium, and facilitate that the critical masses of both organizations can joint together once a year.

PHM

Full name: Journal of Porcine Health Management

Abbreviation: PHM
Website: www.porcinehealthmanagement.com

Description: 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 and -studies; commentaries.

The Editors-in-Chief are Guy-Pierre Martineau (National Veterinary School, Toulouse) and Maurice Pensaert (Ghent University). PHM is the official journal of the European Association for Porcine Health Management (EAPHM) and European College for Porcine Health Management (ECPHM). The EAPHM and ECPHM will cover all article charges on behalf of their members, which means they can publish manuscripts in an open access journal free of charge! Members should contact the PHM editorial office for more information.

PHM is accepting submissions now. Please use the online submission system to submit your manuscript. For all enquiries about the journal, technical issues, payment of APC, etc. please contact: porcinehealthmanagement@biomedcentral.com.

Reasons to publish in PHM:

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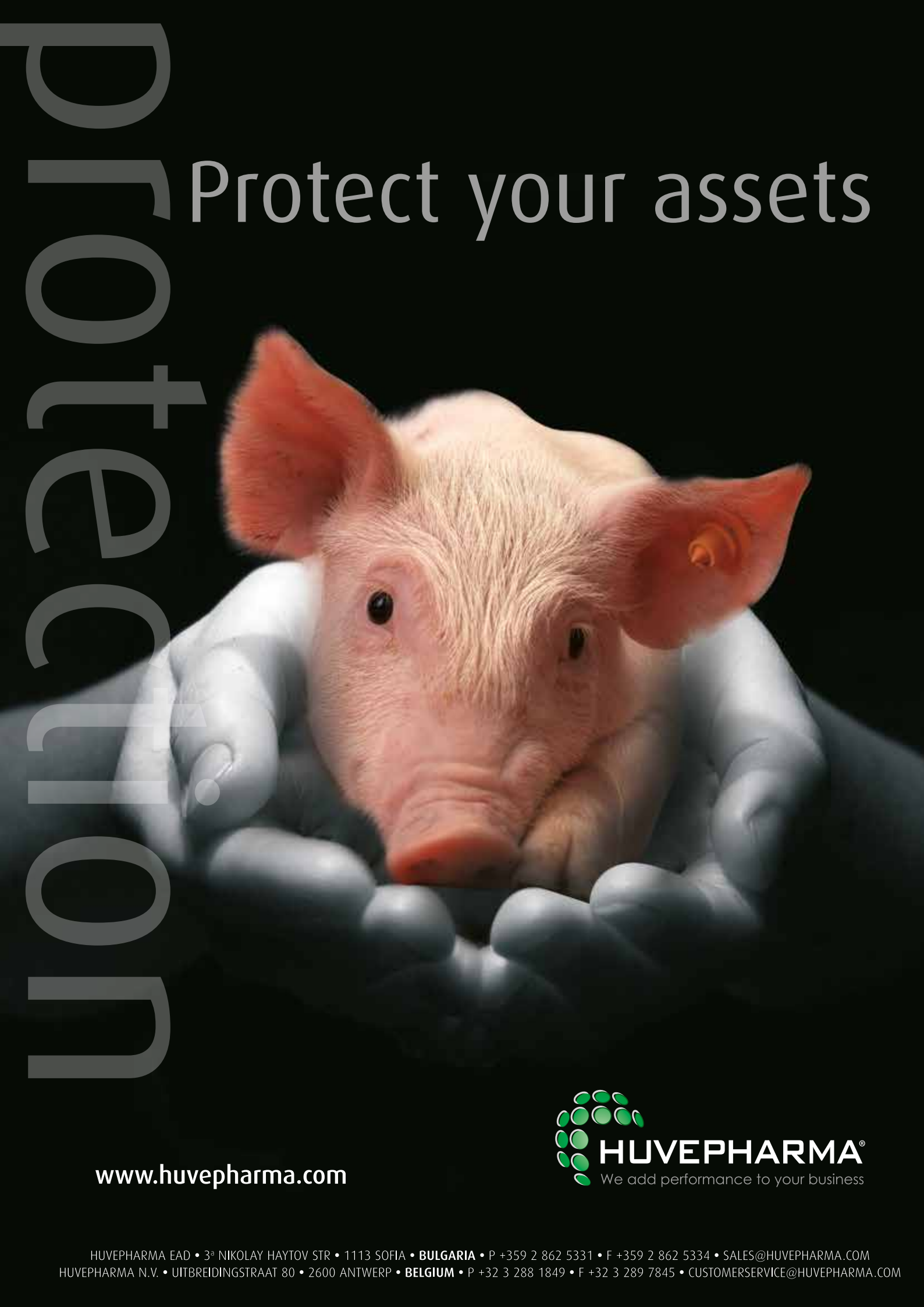

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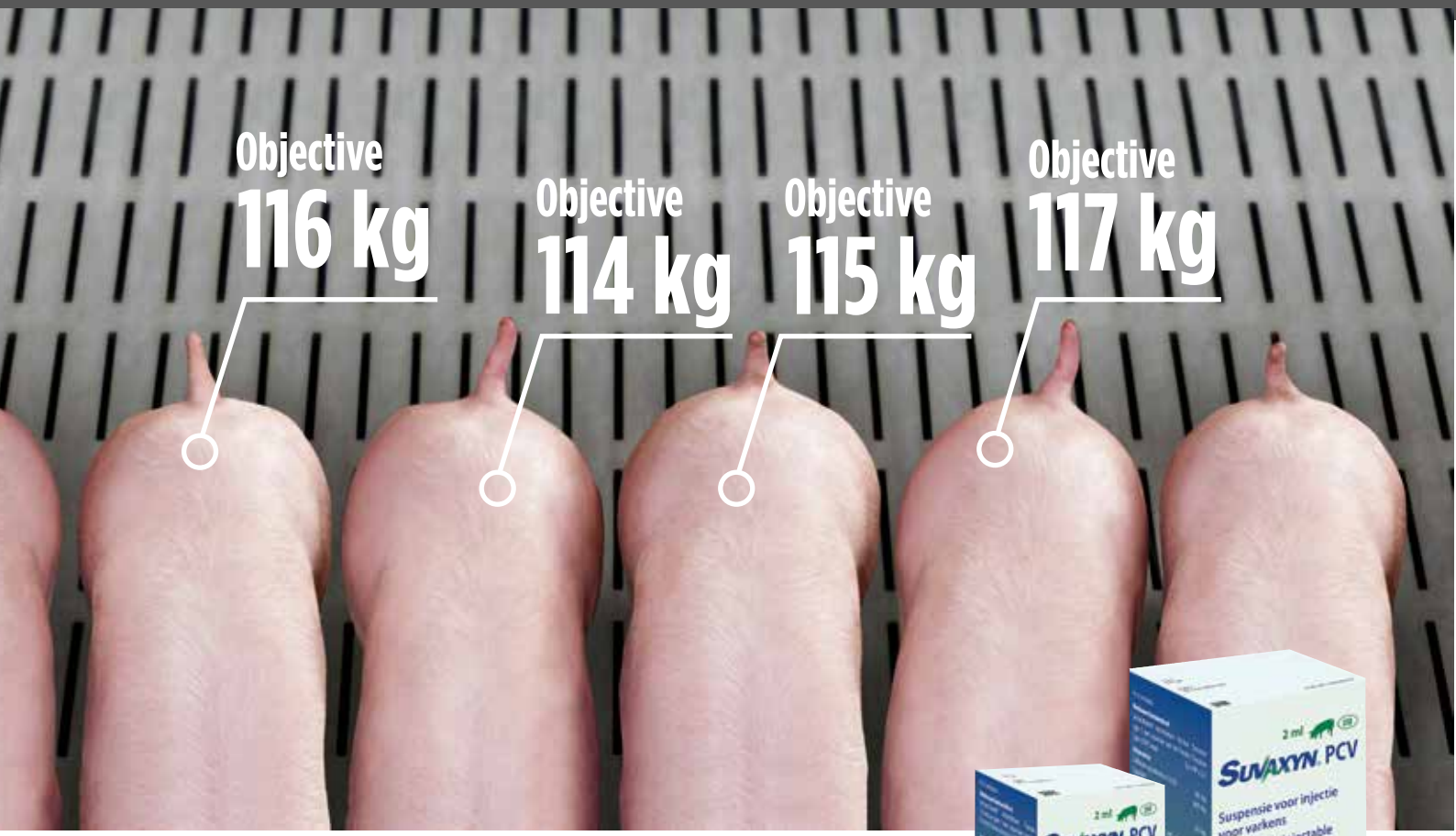

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13.00|13.10 **Welcome & opening**
GRAND AUDITORIUM

13.10|13.30 **Pig and pork production in France**
Christine Roguet, France
GRAND AUDITORIUM

13.30|14.40 KEYNOTE ADDRESSES **Group housed sows**
Chair persons: Guy-Pierre Martineau, Enric Marco
GRAND AUDITORIUM

13.30 > 14.00 **Reproductive issues in group-housed sows**
Olli Peltoniemi, Finland

14.00 > 14.30 **Impact of group housing of pregnant sows on health**
Dominiek Maes, Belgium

14.30|15.00 **Round table discussion**
GRAND AUDITORIUM

15.00|15.30 **Coffee break & posters viewing**
EXHIBITION AREA

15.30|17.30 ORAL PRESENTATIONS **Residents' session**
GRAND AUDITORIUM

15.30 > 15.50 **Longitudinal co-evolution of streptococcus suis serotype and antimicrobial resistance in UK pig farms**
Juan Hernández-García, Geng Zou, Jinhong Wang, Olu Oshota, Hui Jin, Sarah Peters, Thomas Wileman, Ian Dennis, Frankie Alcock, Jill Thomson, Duncan Maskell, Alexander Tucker

15.50 > 16.10 **IgG concentration of new born piglets in Western Europe**
Rutger Jansen, Leon Marchal

16.10 > 16.30 **Acute outbreak of porcine epidemic diarrhea in a piglet producing farm in southwestern Germany**
Andrea Ladinig, Julia Stadler, Susanne Zöls, Robert Fux, Sandra Blome, Dennis Hanke, Herbert Weissenböck, Mathias Ritzmann

16.30 > 16.50 **Use of buserelin in nulliparous and multiparous sows: Effect on the reproductive system and weight variability of the resulting offspring**
Antonio Vela Bello, Victoria Falceto, Joaquim Segalés, Enric Mateu

16.50 > 17.10 **Salmonella environmental contamination in slaughter pigs: a real risk ?**
Mily Leblanc-Maridor, Catherine Belloc, Françoise Le Gall, Martine Denis, Frederic Paboeuf, Brice Minvielle

17.10 > 17.30 **Effects of biosecurity measures on the antimicrobial usage in German farrow-to-finish farms**
Svenja Loesken, Merel Postma, Elisabeth Grosse Beilage

15.30|17.30 **ORAL PRESENTATIONS** **Miscellaneous / Welfare & nutrition**
AUDITORIUM 800

15.30 > 15.50 **Are tail biters more anxious? A study based on morphological, behavioral and physiological characteristics**
Noémie Ory, Anne Lacoste, Jean-Noël Sialleli

15.50 > 16.10 **Decreasing the new neonatal porcine diarrhea (NNPD) by limiting the muscle losses of the gilts during the last month of gestation**
Thierry Solignac, Anthony Jupin

16.10 > 16.30 **Effects of various phosphorus levels in diets for piglets on growth, intestinal fermentation, mineralisation of bones, molecular features and metabolic routes – first results**
Kerstin Büsing, Michael Oster, Franziska Just, Christian Polley, Brigitte Vollmar, Petra Wolf, Klaus Wimmers

16.30 > 16.50 **The effects of hemoglobin levels on growth rates in pigs**
Sarah Dunnigan, Tom Gillespie, Glen Almond, Randy Jones

16.50 > 17.10 **The UK Voluntary Monitoring Systems for Pig Health and Welfare: comparison of disease prevalence from 2005 to 2012**
C. Correia-Gomes, J.I. Eze, J. Borobia-Belsué, A.W. Tucker, D. Sparrow, D.W. Strachan, G.J. Gunn

17.10 > 17.30 **Comparison of different methods to measure IgG in sow colostrum**
Rutger Jansen, Rene Bonekamp, Leon Marchal

17.30|18.30 **ANNUAL GENERAL MEETING** **ECPHM**
Only for ECPHM members
AUDITORIUM 800

18.00|19.30 **Welcome cocktail**
EXHIBITION AREA

THURSDAY APRIL 23RD

08.00 **Registration & visit of the exhibition**
EXHIBITION AREA

08.00|08.30 ANNUAL GENERAL MEETING **EAPHM**
Only for EAPHM members
AUDITORIUM 800

08.30|10.00 KEYNOTE ADDRESSES **Mycotoxines**
Chair persons: Joaquim Segales & Jean-Luc Sevin
GRAND AUDITORIUM

08.30 > 09.00 **Impact of mycotoxines on pig health**
Isabelle Oswald, France

09.00 > 09.30 **Do mycotoxins really affect the health of pigs?**
Sven Daenicke, Germany

09.30 > 10.00 **Round table discussion**
GRAND AUDITORIUM

10.00|10.30 **Coffee break & posters viewing**
EXHIBITION AREA

10.30|12.10 ORAL PRESENTATIONS **Viral diseases**
GRAND AUDITORIUM

10.30 > 10.50 **First case of Porcine Epidemic Diarrhea (PED) caused by a new variant of PED virus in The Netherlands.**
P.J. Van der Wolf, A. Van Walderveen, M.N. Meertens, A.J. Van Hout, T.F. Duinhof, M.J. Geudeke, P.J.M. Franssen, J.C.F.M. Dortmans, R. Dikman

10.50 > 11.10 **Swine influenza viruses in circulation in European pigs exhibit an increasing genetic diversity since last pandemic in 2009.**
Gaëlle Simon, Lars E. Larsen, Ralf Dürrwald, Emanuela Foni, Timm Harder, Kristien Van Reeth, Iwona Markowska-Daniel, Adam Dan, Jaime Maldonaldo, Anita Huovilainen, Charalambos Billinis, Irit Davidson, Scott M. Reid, Ian H. Brown, Willie Loeffen

11.10 > 11.30 **Birth weight, intrauterine growth retardation and fetal susceptibility to porcine reproductive and respiratory syndrome virus.**
Andrea Ladinig, George Foxcroft, Carolyn Ashley, Joan K. Lunney, Graham Plastow, John C.S. Harding

11.30 > 11.50 **Modelling Porcine Epidemic Diarrhoea Virus (PEDV) spread in a pig densely populated area in France without population immunity.**
Nicolas Rose, Mathieu Andraud, Yannick Blanchard, Béatrice Grasland

11.50 > 12.10 **Early-life Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection: role of maternally derived antibodies and link with the immunity in the breeding herd.**
Mathieu Andraud, Christelle Fablet, Patricia Renson, Sophie Mahé, Olivier Bourry, Nicolas Rose

SCIENTIFIC programme

10.30|12.10 **ORAL PRESENTATIONS** **Herd Health Management**
AUDITORIUM 800

10.30 > 10.50 **A multi-centre study of haemoglobin concentration and weight gain in piglets at weaning.**
Jens Peter Nielsen, Sheeva Bhattarai, Erika Busch, Robert Friendship, Tom Gilesbye, Tore Framstad

10.50 > 11.10 **Impact of inadequate colostrum intake in piglets.**
Rutger Jansen, Rene Bonekamp, Leon Marchal

11.10 > 11.30 **Factors associated with still born piglets in Danish herds.**
Markku Johansen, Seth Dunipace, Flemming Thorup, Birgitta Svensmark, Svend Haugegaard, Mai Britt Nielsen, Hanne Kongsted, Jan Dahl, Poul Baekbo

11.30 > 11.50 **Development of a Salmonella Typhimurium challenge model in weaned pigs: evaluation of known interventions.**
P.J. Van der Wolf, H.M.J. Van Hees, A.E. Heuvelink, W. Swart, J.G.M. Wientjes, P.J. Roubos – Van den Hil

11.50 > 12.10 **Epidemiology of porcine reproductive and respiratory syndrome virus: a risk factor study in 109 French farrow-to-finish herds.**
Christelle Fablet, Corinne Marois-Créhan, Béatrice Grasland, Gaëlle Simon, Nicolas Rose

12.10|13.30 **Lunch break & posters viewing**
EXHIBITION AREA

13.30|15.00 **KEYNOTE ADDRESSES** **Porcine Epidemic Diarrhea**
Chairpersons: Paolo Martelli & Olivier Duran
GRAND AUDITORIUM

13.30 > 14.00 **Porcine Epidemic Diarrhea (PED) a constant threat.**
Julie Ménard, Canada

14.00 > 14.30 **Diagnostic and surveillance of PED.**
Ana Carvajal, Spain

14.30 > 14.50 **Round table discussion**
GRAND AUDITORIUM

14.50 > 15.00 **EAPHM Presentation & PHM promotion**

15.00|16.00 **ORAL PRESENTATIONS** **Industrial partners**
GRAND AUDITORIUM

15.00 > 15.15 **Post weaning diarrhoea in Europe: veterinarian and producer insights.**
Jorge Trindade, Alvaro Hidalgo - **ELANCO**

15.15 > 15.30 **Constitution of a European network on the detection of pathogens in oral fluid.**
Luc Mieli, Elvis Le Bon, Marie-Agnès Baudouard, Catherine Charreyre, François Joisel, Olivier Merdy, Guillaume Perreul, Benoît Boivent, Tthe European Network of Diagnostic Laboratories on Swine Oral Fluids - France - **MERIAL**

15.30 > 15.45 **Assessing sow herd PCV2 stability and vertical transmission utilizing colostrum and placental umbilical cord serum.**
Jessica Seate, Brian Payne, Darin Madson, Al Scheidt- **Boehringer Ingelheim Vetmedica Inc**

15.45 > 16.00 **Meta-analysis of the effects of Draxxin® injectable on Swine Respiratory Disease.**
Joaquim Morales, Paolo Doncecchi, André Dereu, Carlos Pineiro, Edgar G. Manzanilla - **ZOETIS**

THURSDAY APRIL 23RD

15.00|16.00 **ORAL PRESENTATIONS** **Veterinary Public Health**
AUDITORIUM 800

15.00 > 15.20 **Isolation of Salmonella enterica in pigs at slaughter and genetic identity between isolates of porcine and human origin in Northern Italy.**
Silvia Bonardi, Franco Brindani, Marina Morganti, Irene Alpigiani, Pierugo Cavallini, Elena Barilli, Luca Bolzoni, Stefano Pongolini

15.20 > 15.40 **A comparison between lesions found during meat inspection of finishing pigs raised under organic/ free-range conditions and conventional, indoor conditions.**
Marie Erika Busch, Lis Alban, Jesper V Petersen

15.40 > 16.00 **Meat juice serology, PCR and genotype patterns of Toxoplasma gondii in free-ranging, organic pigs in Italy**
Laura Helen Kramer, Alice Vismarra, Cristina Bacci, Carlo Mangia, Marco Genchi, Ilaria Bruini

16.00|16.30 **Coffee break & posters viewing**
EXHIBITION AREA

16.30|18.00 **ORAL PRESENTATIONS** **Industrial partners**
GRAND AUDITORIUM

16.30 > 16.45 **Swine enteric coronavirus disease (SECD) elimination and prevention in a genetic multiplication system in North America.**
Jean-Paul Cano, Anna Romagosa, Angela Baysinger, Tom Riek, Robert Thompson, Jerome Geiger, Wesley Lyons - **Pig Improvement Company**

16.45 > 17.00 **A practical approach to improve efficacy of antimicrobial treatment.**
Wouter Depondt, Alain Kanora - **Huvepharma NV**

17.00 > 17.15 **Financial calculator of the damages od edema disease and return on investment of the vaccine Ecoporc Shiga.**
Paul Crea'ch, Friedericke Schmelz - **IDT Biologika**

17.15 > 17.30 **The effects of altrenogest treatment in early gestation on disruption of pregnancy.**
Nicoline Soede, Roman Krejci, Bas Kemp, B. Laurenssen - **Ceva Sante Animale**

17.30 > 17.45 **Efficacy against a porcine parvovirus infection in gilts vaccinated with the mixed administration of ERYSENG® PARVO and UNISTRAIN® PRRS**
Augusti Camprodon, Ainhoa Puig, Mireia Fontseca, Joel Miranda, Ricard March - **HIPRA**

17.45 > 18.00 **Impact on immunity parameters of piglets fed mycotoxin contaminated diets**
Simone Schaumberger, Sabine Masching, Ursula Hofstetter - **BIOMIN**

18.00 > 18:15 **Efficacy of a new PCV2 and M. hyopneumoniae combination vaccine is independent of maternally derived immunity.**
Rika Jolie, Vicky Fachinger, Mathieu Hoeijmakers, Hans Holtslag, Tom Nell, Maarten Witvliet, Divine Dufe - **Merck Animal Health**

SCIENTIFIC programme

16.30|18.00 **ORAL PRESENTATIONS** **Bacterial Diseases**
AUDITORIUM 800

16.30 > 16.50 **Mycoplasma hyopneumoniae detection in tracheobronchial swabs, oral fluid and lung**
Maria Beatrice Boniotti, Enrico Giacomini, Alessandra Pitozzi, Sara Gasparrini, Michela Remistani, Giovanni Loris Alborali

16.50 > 17.10 **Post-weaning diarrhoea in France: antimicrobial susceptibility of enterotoxigenic Escherichia coli (ETEC) isolates**
Thomas Gin, Bernard Fily, Marc Henninger, Alvaro Hidalgo

17.10 > 17.30 **Streptococcus suis epidemiology (using serotyping and presence of virulence factors) and AMR (Antimicrobial Resistance) in a large pig production system in Italy.**
Giampietro Sandri, Davide Giovanardi, Patrizia Pesente, Giulia Rossi

17.30 > 17.50 **Immunoreactive proteins of Brachyspira hyodysenteriae in pigs.**
Nele De Paw, Katleen Van Steendam, Lien Vande Maele, Maxime Mahu, Filip Boyen, An Martel, Freddy Haesebrouck, Dieter Deforce, Frank Pasmans

17.50 > 18.10 **Oral fluid detection and dynamics of Bordetella bronchiseptica infection in pig herds.**
Ana Alba, Miquel Nofrarias, Laura Valls, Mireia Blanch, Lucia Acal, Jaime Madonaldo

18.10 > 18.30 **Characterization of Brachyspira hyodysenteriae from Italy by multilocus sequence typing and multiple locus variable number tandem repeat analysis.**
Loris Alborali, Sara Gasparrini, Alessandra Pitozzi, Jessica Ruggeri, Arrigo Daniele Nigrelli, Maria Beatrice Boniotti

19.00 **CONGRESS DINNER** **at Les Machines de L'Île**

FRIDAY APRIL 24TH

08.00 **Registration & visit of the exhibition**
EXHIBITION AREA

08.30|10.00 KEYNOTE ADDRESSES **Actinobacillus pleuropneumoniae**
Chairpersons: Andreas Palzer & Philippe Leneveu
GRAND AUDITORIUM

08.30 > 09.00 **Actinobacillus pleuropneumoniae heterogeneity of pathogenicity**
Marcelo Gottschalk, Canada

09.00 > 09.30 **How to cope with App in pig herds?**
Arnaud Lebret, France

09.30 – 10.00 **Round table discussion**
GRAND AUDITORIUM

10.00|10.30 **Coffee break & posters viewing**
EXHIBITION AREA

10.30|12.30 ORAL PRESENTATIONS **Reproduction**
GRAND AUDITORIUM

10.30 > 10.50 **Impact of co-culturing cumulus-enclosed porcine oocytes with denuded oocytes during in vitro maturation in a defined medium on cumulus expansion and oocyte maturation.**
Ruth Appeltant, Tamas Somfai, Michiko Nakai, Szilard Bodo, Dominiek Maes, Kazuhiro Kikuchi, Ann Van Soom

10.50 > 11.10 **Abortions in sows in the Netherlands: results of post mortem investigation and significance of leptospiral and chlamydial infections.**
Mattheus Geudeke, Karin Junker, Wouter Baas

11.10 > 11.30 **Multiple risk factors for return to oestrus in group housed sows; a case study.**
Tobias Tijs, Roxani Van Eijndhoven, Fokko Klip, Elise Wessels, Arie Van Nes

11.30 > 11.50 **Prevalence of bacteriuria and pathological changes in the urinary tract of culled sows.**
Lola Tolstrup Leihardt, Ken Steen Pedersen, Jens Peter Nielsen

11.50 > 12.10 **Distribution of regular and irregular inter-oestrus interval in sow during different period of the year in northern Italy**
Fabio RDe Rensis, Caludio Mazzoni, Annalisa Scollo, Paolo Bonilauri

12.10 > 12.30 **Post-partum ultrasound examination of the uterus as diagnostic tool for birth complications.**
Stefan Björkman, Claudio Oliveiro, Olli Peltoniemi

10.30|12.30 **ORAL PRESENTATIONS** **Immunology & Vaccinology**
AUDITORIUM 800

10.30 > 10.50 **Effect of maternally derived antibodies on porcine circovirus type 2 (PCV2) infection dynamics on average daily weight gain (ADWG) in PCV2 vaccinated pigs.**
Hua Feng, Marina Sibila, Lorenzo Fraile, Joaquim Segales

10.50 > 11.10 **Immunomodulating mechanism of oral ß-glucans in pigs could be hampered by the epithelial mucosal barrier.**
Eric Cox, Kim Baert, Bruno Goddeeris, Bert Devriendt

11.10 > 11.30 **Dynamic change in lung macrophages and cytokines environment during infection of pigs with a high or low virulent genotype 1 PRRSV strain.**
Patricia Renson, Nicolas Rose, Mireille Le Dimna, André Keranflech, Frédéric Paboeuf, Catherine Belloc, Marie-Frederique Le Potier, Olivier Bourry

11.30 > 11.50 **Effects of single or combined vaccinations against Mycoplasma hyopneumoniae and/or porcine reproductive and respiratory syndrome virus in dually infected pigs.**
Olivier Bourry, Corinne Marois-Créhan, Christelle Fablet, Gaëlle Simon, François Madec, Marylène Kobisch

11.50 > 12.10 **Sulfated polysaccharides-rich extract of Ulva armoricana green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells.**
Mustapha Berri, Cindy Slugocki, Michel Olivier, Emmanuelle Helloin, Henri Salmon, Isabelle Jacques, Sébastien Holbert, Pi Nyvall Collen, Hervé Demais, Matthieu Le Goff

12.10 > 12.30 **First efficacy evaluation of a novel combination vaccine against Enzootic Pneumonia (Mycoplasma hyopneumoniae) and Porcine Circovirus type 2 (PCV2) in the presence of strong maternally derived PCV2 immunity in pigs.**
Eleni Tzika, Panagiotis Tassis, Ioannis Tsakmakidis, Vasileios Papatsiros, Dimitrios Koulialis, Tom Nell, Georgia Brellou

12.30|12.45 **8th ESPHM / IPVS DUBLIN** **Presentation**
GRAND AUDITORIUM

12.45 / 12.50 **ISERPD 2015 KYOTO** **Presentation**
GRAND AUDITORIUM

12.50|13.00 **Closing address**
Catherine Belloc
GRAND AUDITORIUM

Ana Carvajal, *Department of Animal Health, University of León, Spain*

Born in León, Spain, Ana Carvajal completed a degree in Veterinary Medicine in 1990. Her PhD thesis addressed a research on porcine epidemic diarrhea virus in Spain. She completed her training through research stays at the Virolology Department of the Veterinary Faculty in Zürich, Switzerland. At the moment, she is involved in research on enteric disorders in pigs.

Sven Daenicke, *Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Germany*

Prof Dr Sven Dänicke is graduated from the University Halle-Wittenberg. In 1992-1998 he was Scientific Co-worker at the Agricultural Faculty of the Martin-Luther-University Halle-Wittenberg. In 1998-2009 he was Scientific Co-worker at the Institute of Animal Nutrition, German Federal Agricultural Research Centre Braunschweig (FAL); since 2008 he works for Federal Research Institute of Animal health-Friedrich-Loeffler-Institute and since 2009 he is Director of the Institute of Animal Nutrition of the Federal Research Institute of Animal health-Friedrich-Loeffler-Institute.

Marcelo Gottschalk, *Faculté de médecine vétérinaire/Faculty of Veterinary Medicine, Université de Montréal, Canada*

Full Professor, Department of Pathology and Microbiology of the Faculty of Veterinary Medicine, University of Montreal, Director of the reference laboratory for the serology of swine pleuropneumonia, Director of the reference laboratory of Streptococcus suis, Author of 2 chapters of the Diseases of Swine (Actinobacillus pleuropneumoniae and Streptococcus suis), Invited speaker of more than 200 conferences in 34 different countries and more than 200 publications in refereed international journals.

Arnaud Lebret *Porc. Spective, Chene Vert Conseil Vet grup, Noyal-Pontivy, Brittany, France*

Arnaud Lebret, DVM, is graduated from the Nantes Faculty of Veterinary Medicine (France) in 1995 and since 1998, works in pig production for 100% of his work time. In 2013, he passed with success the examination and since then he is diplomate of the European College of Porcine Health and Management (ECPHM). He works in a practice dedicated to swine consulting named Porc.Spective. This practice belongs to Chene Vert Conseil group.

Dominiek Maes, *Liesbet Pluym - Faculty of Veterinary Medicine - Ghent University, Belgium*

Dominiek Maes obtained his DVM at the faculty of veterinary medicine at Ghent University in 1993. In the next years, he completed a MSc program Herd Health and Epidemiology at Utrecht University, obtained his PhD at Ghent University in 1998 and worked as post-doc at the University of Minnesota. He is currently full professor and head of the unit of porcine health management at the faculty of veterinary medicine at Ghent University. He is currently president of the Belgian Pig Veterinary Society, past-president of the European College of Porcine Health Management (ECPHM), vice-president of the European Board of Veterinary Specialisation (EBVS) and section editor of Livestock Science.

Julie Ménard, *F. Ménard inc., Ange-Gardien, Québec, Canada*

Dr Julie Ménard obtained her DVM in 1987 from Montreal University and practice swine medicine since then at F. Menard in Quebec. In 2013, she has been promoted as the breeding herd director at F. Menard supervising more than 110 employees. Her main responsibilities and focus are sow herd health and management. In 2012, she received the prestigious award "Allen D Leman Science to practice" from the University of Minnesota for her achievements.

Isabelle Oswald, *INRA, UMR 1331, ToxAlim Research Centre in Food Toxicology, Toulouse, France*

Isabelle Oswald is the deputy head of the INRA research Center in Food Toxicology located in Toulouse. She qualified as an engineer in agricultural sciences in Rennes, France, get her Ph.D. at INRA in Tours and a post-doctorate at the NIH, USA. She is currently is leading the reaserch team "Biosynthesis and toxicity of Mycotoxicology". This team investigate the toxic effect of mycotoxins on animals especially pigs as well as the biosynthtic pathway of these toxins. Dr. I Oswald has been or is currently involved in several European funded projects. She has more than 120 peer-reviewed international publications and was appointed in the in the editorial board of several veterinary journals. She serves as expert for the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the European Food Safety Agengy (EFSA)

Olli Peltoniemi, *Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland*

Pr Olli Peltoniemi graduated as a veterinarian from Helsinki in 1992. Thereafter, he went to University of Sydney for a MVetSc and continued for a PhD in pig reproduction with a graduation from the University of Helsinki in 1999. He was appointed as a full professor in domestic animal reproduction in 2010 in the Faculty of Veterinary Medicine, University of Helsinki. He is double boarded with the ECPHM and ECAR with a quite a bit of activities in both of them – currently he is the president of ECAR and vice dean for research in his home faculty in Helsinki.

Christine Roguet, *IFIP-Institut du porc, Pôle Economie, 35651 Le Rheu, France*

Dr. Christine ROGUET is an agronomist (AgroParisTech, 1993) and has a PhD in Life and Environmental Science (INRA, 1997). She has been working at IFIP-Institut du porc, the French institute for pig industry, in the economic team, since 2003. Her research is focused on the economics of pig production, the structures of pig farms, their technical and economic performances in France and abroad, competitive factors and, since 2012, livestock and society subjects (perception of livestock farming, social acceptance).

PIG AND PORK PRODUCTION IN FRANCE

Christine Roguet
IFIP-Institut du porc, Pôle Economie, Le Rheu, France

France is the largest egg, poultry and beef meat producer of EU, the second largest producer of milk and the third largest producer of pork.

I. PORK BALANCE SHEET: CURRENT SITUATION AND EVOLUTION

I.1. PRODUCTION AND CONSUMPTION

In 2013, France slaughtered 23.5 million pigs and produced 2.199 million tonnes of pork carcass weight equivalent (Table 1). It is the third largest producer behind Germany (5.035 million tonnes) and Spain (3.521 million tonnes) and followed by Denmark and the Netherlands. France's self-sufficiency rate is 107%.

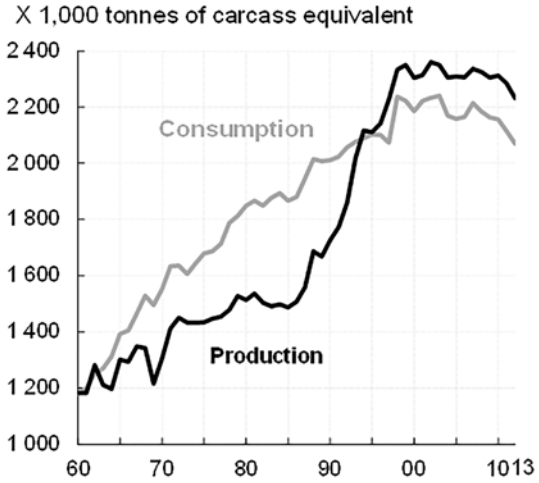
Table 1. Pork balance sheet in France in 2013 and comparison with 2000 and 1990

Year	1990	2000	2013	00/90	13/00
Slaughter (x 1,000 pigs)	20,374	25,868	23,553	+27.0%	-8.9%
1,000 tonnes of carcass equivalent					
Production (GDP)	1,726	2,304	2,199	+33.5%	-4.6%
Imports	508	533	580	+4.9%	+8.8%
Exports	224	652	726	+191%	+11.3%
Consumption (GDC)	2,011	2,185	2,053	+8.7%	-6.0%
Self supply (%)	86	105	107		
Consumption per capita (kg)	35.4	36.1	31.3	+2.0%	-13.3%

GDP Gross Domestic Production, GDC: Gross Domestic Consumption, including own consumption
Metropolitan France and overseas departments, Carcasses with head excluding flare fat, kidneys, diaphragm.
Source: IFIP from Agreste-SSP

Pig production in France sharply increased between 1985 and 2000 (+55%) which filled the large deficit in pork of the country. Consumption grew more slowly (+17%). Then, in the late 90s, production growth was suddenly stopped by environmental regulations. Since then, it tends to decrease, as well as consumption levels (Figure 1). In France, pork is mainly consumed in the form of processed products (75%), a quarter only of total consumption as fresh meat.

Figure 1. Development of pork production and consumption in France since 1960



Three periods of development

➢ **1970-1985 :**
the domestic deficit deepens to reach 400,000 tce in 1985 ➔ *general mobilization*

➢ **1985-2000 :**
strong growth ➔ *success*

➢ **Since2000 :**
decrease

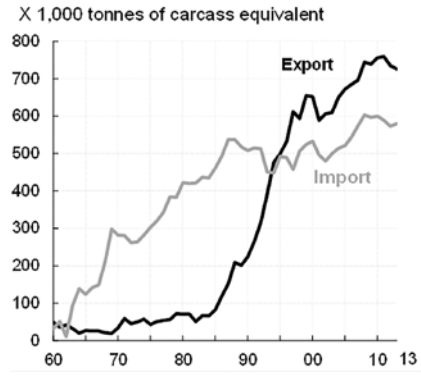
Time period	Production	Consumption
1970-1985	+13.9 %	+20.3 %
1985-2000	+54.9 %	+17.0 %
2000-2013	-4.6 %	-6.0 %

Source: IFIP from Agreste-SSP

I.2. IMPORTS AND EXPORTS

The very dynamic growth in production between 1985 and 2000 was accompanied by a boom of exports (+700% over the period), while imports slowed down (+15%). In 1994, exports exceeded imports (Figure 2). Exports and imports of live animals by France are minor (2.6% and 0.2% of production respectively) (Table 2). Trade mainly consists in cuts (58% of the imports and 51% of the exports). But France also exports carcasses (11% of trade) whereas it imports processed products which contributes to deteriorate its trade balance. In 2013, the French pork trade reached a record negative balance of 210 million euros while it was largely positive in 2000 (EUR 80 million).

Figure 2. Development of pork imports and exports of France since 1960



Source: IFIP from Agreste-SSP

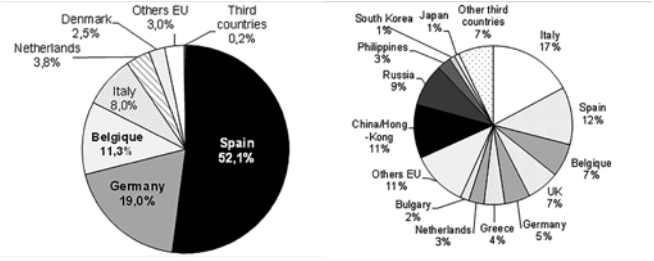
Table 2. French pork imports and exports by products in 2013

		Total (x 1000 tonnes)	**Total without live pigs** (x 1000 tonnes)	**among which**				
				Carcasses	Cuts	Processed products	Lard, fats	Offal
IMPORTS	Total from UE-27	**622**	**617**	0.4%	58.1%	23.0%	9.7%	8.8%
		100%	99.8%	99.7%	100.0%	99.8%	99.9%	98.5%
EXPORTS	Total to UE-27	**808**	**749**	11.4%	50.9%	10.3%	12.7%	14.7%
		71%	68.3%	99.9%	73.6%	68.8%	63.6%	29.0%

Source: IFIP from Eurostat

Spain is France's largest pork supplier, with 320,000 tonnes in 2013 (52% of the total) (Figure 3). France's customer portfolio is much more diverse, as the first one, Italy, absorbs only 17% of the French exports. Russia and China are two important markets with respectively 9% and 11% of the exported volume in 2013.

Figure 3. Suppliers and customers of France pork imports and exports in 2013



Source: IFIP from Eurostat and customs

II. PIG FARMS: LOCALIZATION, STRUCTURES, PERFORMANCES AND RESULTS

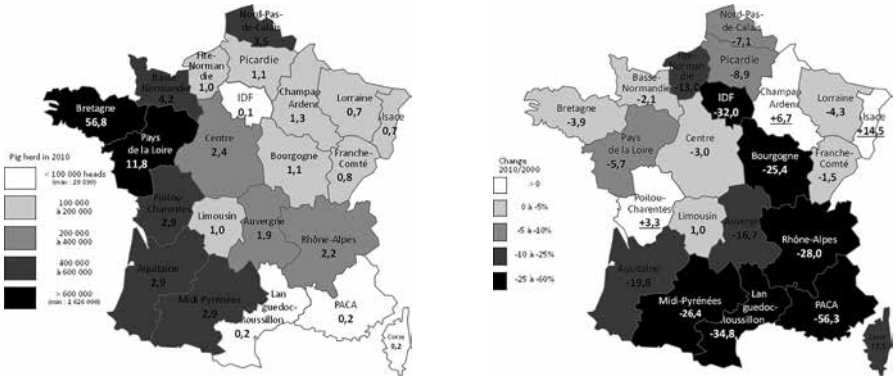
II.1. BRITTANY CONCENTRATES 57% OF PIGS POPULATION

According to the last agricultural census, France had 13.8 million pigs in 2010, of which 1.1 million sows and 8.4 million fattening

pigs. The Great West (Brittany, Pays de la Loire and Lower Normandy) concentrates 73% of the pig population. It decreased just a little whereas pig population collapsed in the rest of France (Figure 4).

Figure 4. Distribution of pig population by region in 2010 and change between 2000 and 2010

Distribution of pig population by region (%) Change in pig population between 2000 and 2010 (%)



Source: IFIP from SSP-Agreste, Agricultural Census 2000 and 2010

The agglomeration of production has been accompanied by an industrial and spatial concentration of the rest of the industry: 75% of slaughtering and 85% of pig feed production are located in Brittany, Pays de la Loire, Normandy and Poitou-Charentes. Three slaughter groups concentrate about half of the slaughtering in France: Cooperl Arc Atlantique (4.86 M. heads, 20.4%), Bigard (4.17 M., 17.5%) and Kermené (1.94 M., 8.2%).

II.2. STRUCTURAL CHARACTERISTICS OF FRENCH PIG FARMS

In 2010, metropolitan France had 22,300 farms with at least one pig, two-thirds less than 10 years earlier. But 99% of the pig herd was held by 11,500 farms with more than 100 pigs or 20 sows. 41% of them are specialized in pig production and do not have any herbivores and only a limited utilised agricultural area (UAA), 40% associate pig and cows (often milk production) and 19% combine pig farming and polyculture. On average, a French pig farm has 83 ha UAA in 2010.
The dominant model of pig farming in France combines farrowing and fattening. Farrow-to-finish farms hold 85% of the sows but only 66% of the fattening pigs (Table 3). A part of the piglets they produce are fattened by finishers under integration contracts.

Table 3. Distribution of pig operations, sows and fattening pigs by activity in 2013

2013, national	Pig operations	Sows	Fattening pigs
Farrow-to-finish	50.1%	85.1%	65.8%
Farrow-to-wean	2.9%	8.5%	0.2%
Farrow-to-feeder	2.0%	5.8%	0.2%
Feeder-to-finish	44.1%	0.5%	33.7%
Wean-to-feeder	0.9%	0.0%	0.1%
Total	100%	100%	100%

Source: IFIP from SSP-Agreste, pig survey of November 2013

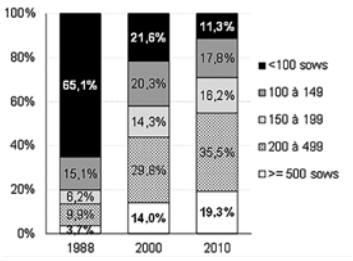
The French sow herd is concentrated in 5,700 farms of 50 sows or more, with an average size of 190 sows in 2010. The number of farms with less than 100 sows has greatly decreased but they remain very numerous (45% of the farms in 2010). However they hold only 11% of the sows herd (Figure 5).

II.1. TECHNICAL PERFORMANCES AND ECONOMIC RESULTS

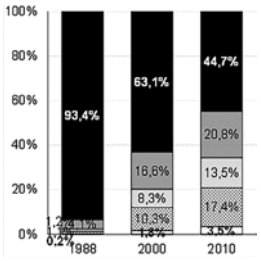
For 40 years, IFIP has been collecting and managing a national database with technical and economic data on pig farms to provide references. In 2013, a productive sow weaned 28.7 piglets on average, 3.5 more than in 2000 (Table 4). The feed conversion ratio between 30 and 115 kg live weight is 2.77 and the average daily gain 800 g. Slaughter weight has also increased, reaching 117 kg in 2013. With a fattening pig feed price of 275 € per tonne in 2013 (159 € in 2000), the cost of production reached 1.70 € per kg carcass vs 1.26 € in 2000.

Figure 5. Distribution of sows herd and farms by size class in 1988, 2000 and 2010

Distribution of sows by size class



Distribution of farms by size class



Source: IFIP from SSP-Agreste, Agricultural Census 1988, 2000 and 2010

Table 4. Technical and economic results of farms with sows in France in 2013 and 2000

Year	2000	2013
Technical management of breeding herds (GTTT device)		
Number of farms	3 745	1 918
Number of present sows	146	243
Piglets weaned per productive sow per year	25.2	28.7
Piglets weaned per litter	10.4	11.5
Technical-economic management (GTE device)		
Number of farms	1 968	1 711
Number of present sows	148	212
Pigs produced per present sow per year	19.4	22.6
Feed conversion ratio between 30-115 kg (kg/kg)	2.97	2.77
Daily gain between 30-115 kg (g)	758	800
Slaughter weight (kg live)	110.4	117.0
Fattening pig feed price (€/tonne)	159	275
Slaughter pig price (€/kg carcass weight)	1.35	1.64
Cost of production (€/kg carcass weight)	1.26	1.70

Source: IFIP, GTTT and GTE

Large White and Landrace are the first and second breeds used in France, based on sow numbers. They have benefited from the creation of a highly prolific line leading to major progresses in the litter size. They are crossbred for the production of LWxLR sows. Pietrain is commonly used for terminal boars.

III. FRENCH PIG INDUSTRY IS ORGANIZED BY PRODUCER GROUPS

In 2012, 91% of pig production is carried out by farmers, members of one of the 42 producer groups of the country. Their primary function is dual: (1) disseminating technical and genetic progress to improve the competitiveness of farms and better meet the needs of the downstream industry and (2) ensure the marketing of the pigs of their members. In addition, they have enriched their horizontal supply with various services (animal health, buildings and equipment, manure management) and expanded vertically their sphere of activity to downstream (slaughter-cutting and even processing to ensure outlets) and / or upstream activities (feed, genetics).

CONCLUSION

France has many strengths for producing pig: plenty of space, the biggest grains production in the EU, a pig sector structured around producer groups, pig farmers among the best in the world. These strengths have enabled the country to address the challenge of self-sufficiency reached in 1994. But now the French pig industry faces the challenge of modernization and restructuring of its tools, impeded by a narrow regulatory framework and insufficient profitability in recent years. From the upstream to the downstream of the pig sector, industrial and commercial investments are needed. The question is: how to invest in the absence of growth?

REPRODUCTIVE ISSUES IN GROUP-HOUSED SOWS

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INTRODUCTION

The female pig tends to be social in her behavior throughout the reproductive cycle. There is only one exception to her preference for being a part of a social group – when farrowing is approaching, she separates from her group and seeks for isolation in order to build a nest, in which she spends the first week or two with her piglets. In modern intensive pig production, the sow is often restricted to an individual cage for lactation and, in many European countries, she may still spend additional periods in stalls during pregnancy. While grouping of sows during lactation is an interesting option, presently this is encountered mostly in organic or otherwise extensive farming systems, such as outdoor farming. However, the present society is asking for more animal friendly models of production and there appears to be more need for studies of group housing issues during lactation. Grouping of sows after weaning causes stress, which imposes risks for fertility Thus, timing of grouping is probably very critical. It is well documented that the embryonic period of the pregnancy, lasting up to Day 35, is more vulnerable for loss of embryos and pregnancy than the subsequent fetal period. There are indications to suggest that stress of grouping can cause some harm to parameters indicating the vitality of blastocysts already while at the site of fertilization in the oviduct. Later on, during the critical periods of maternal recognition of pregnancy, endocrinological models testing maintenance of pregnancy suggest that chronic stress lasting for more than 2 days may cause abortion and loss of the whole litter. However, the sow may be resistant, in terms of her reproductive function, to acute stress lasting for hours or up to a day. In conclusion, grouping of sows during lactation may be of interest in the future. At present, issues of group housed sows after weaning and early pregnancy seem to be of most practical relevance. Chronic stress of sows lasting for more than 2 days during the embryonic phase of pregnancy may lead to cessation of luteal function and loss of the whole litter.

GROUP HOUSING OF SOWS DURING LACTATION

There appears to be a growing need for animal friendly models of grouping sows in Europe (Kemp and Soede, 2012; Nieuwamerongen et al., 2014). One phase of production where such a development appears possible is keeping sows in groups during lactation (Einarsson et al., 2014). Continuous exposure of the sow to her piglets may be stressful for the sow in a traditional lactation setup with individual housing. In group lactation systems (Hulten et al., 2006), sows can get away from the piglets and the burden of lactation may be somewhat alleviated. Reproduction performance might, however, be influenced by group-housing. In a Swedish study, where sows were housed in groups of four for weeks 3-7 of lactation, approximately every second sow came into oestrus during lactation (Hulten et al., 2006). Of those that came into oestrus, only 50% were showing signs of standing reflex. Moreover, lactational oestrus seems to be more common in older sows, which may be connected to more intensive mothering behavior observed in younger sows. While in groups during lactation, sows may not be subject to an efficient boar contact and heat detection may not be optimalFurther information is needed to help farmers to set up a production environment where group housing of lactating sows may become a practical option in the future. In principle, by having sows in groups during lactation, stress related to grouping sows at whichever point after weaning may be avoided. However, lactation groups may be too small for forming reasonable groups after weaning for the pregnancy unit.

GROUPING SOWS AFTER WEANING

In order to avoid mixing of sows after insemination, one practical option is to form a group right after weaning. This practice has clear advantages; 1) establishing a rank in the group is supposed to take about 24 hours – if the rank is established already before fertilization, the risk related to aggression during the embryonic period is absent, 2) social contacts between females are known to advance onset of oestrus, and 3) it has been stated that social interaction between females are considered as “positive stress”, which is supposed to stimulate induction of oestrus (Einarsson et al., 2014). However, counting disadvantages, there is more labor related to moving of sows to the boar or vice versa for oestrus detection and artificial insemination (AI). In addition, there is some evidence to suggest that social interactions may suppress oestrus signs in subordinate sows. Proper grouping of sows according to size and age may overcome this problem.

MANAGEMENT OF SOWS IN GROUPS AT AI

At AI, having sows in groups instead of stalls may improve the possibilities for observing symptoms of oestrus as sows show signs of oestrus freely, without restrictions by the stall structures. Moreover, the risk of inseminating a sow not in standing oestrus is considerably reduced in a group situation where sows are moving around freely. Yet another benefit related to group housing around the time of AI is that in such a situation, sows may be exposed to several boars instead of one only, which may potentiate the effect of boar stimulation, triggering the standing reflex better. It should be noted that fighting for dominance and mating and / or exhibition of oestrus behavior are temporal behaviours of sows that may require more space per sow as compared to a stable group situation during pregnancy.

GROUP HOUSING OF PREGNANT SOWS

Group housing of pregnant sows has been compulsory since year 2013 in Europe and it has been widely adopted in other parts of the world such as the US, New Zealand and Australia (Spooldler and Vermeer, 2015). However, transition to group housing has been somewhat slow with farmers being either reluctant to invest in new systems requiring more space or lacking confidence towards group housing. In management of group housing, producers are facing new challenges. Inter-sow confrontations mostly relate to feeding time. The confrontations may present a risk factor to fertility with an apparent tendency towards more early loss of embryos and increased rate of repeat breeding after a prolonged oestrus to oestrus interval (Munsterhjelm et al. 2008). Housing and management needs to be considered carefully to reduce this risk. The housing system should be designed in a way that allows sows to show proper social behaviour, and that gives subordinate sows a possibility to avoid harassment. It is also important to make sure that all sows can feed undisturbed. Regarding space allowance, a number of studies suggest that as a general rule sows should be provided with 2.5-3.5 m2 per sow. In addition, feeding enough bulk and fibre and provide the pen with areas to escape helps to alleviate aggressive behaviour problems of housing. Furthermore, it is regularly reported that having a teaser boar in the pregnant sow group calms the group down and reduces aggression among sows.. For the group size, small groups may be more natural for sows. However, large dynamic groups of up to 300 sows may provide the farmer with satisfactory fertility results given that there is enough space in the group and if they are otherwise well managed. There is some evidence to suggest that during 2-3 weeks after fertilization, when implantation and establishment of pregnancy occurs is the most vulnerable period subject. However, to the knowledge of the authors, the first 3 weeks of pregnancy, based on the studies discussed below, need all to be included as a vulnerable period where loss of embryos and pregnancies may occur more easily than at other times. Therefore, forming a stable group already at weaning seems like an advisable practice as opposed to mixing sows at any time during the first 3 weeks of pregnancy.

STRESS AND MAINTENANCE OF PREGNANCY – ENDOCRINOLOGICAL STUDIES

We have tried to evaluate the effects of stress on CL function and maintenance of pregnancy in group housed gilts and sows in a series of studies. In this line of research, stress effects are thought to be mediated via the hypothalamo-pituitary axis. It has been well documented that LH secretion is subject to stress effects, mainly through cortisol mediated stress responses. LH is likely to carry over the stress effects on to the CL function, which in turn is a basis for the endocrine milieu within the uterine lumen for successful establishment and maintenance of pregnancy. In pigs, development of the corpora lutea after ovulation and the secretion of progesterone occur independently of LH input from the pituitary, at least until 10–12 days after ovulation (Peltoniemi et al., 1995). Hypophysectomy on the day after oestrus or mating does not prevent the development of normal-sized, progesterone-secreting corpora lutea by day 12 after oestrus (Anderson et al., 1967), but corpora lutea do regress between days 16 and 20 in pregnant, hypophysectomised sows. Meduri et al. (1996) showed that at 48 h after follicle rupture, there is a marked decrease in the density of LH receptors in luteal cells, and 6 days after ovulation the receptor density seems to increase again. These findings indicate an LH-independent and LH-insensitive window during early development of the corpus luteum. More recent studies by our group have approached the role of LH on the maintenance of luteal tissue using three different models. First, pregnant gilts received GnRH agonist implants to down regulate GnRH receptors and suppress LH pulsatility (Peltoniemi et al., 1995). Second, active and passive immunisation against GnRH was used to reduce LH pulsatility in the early pregnant gilt (Tast et al., 2000). Third, a GnRH antagonist was used to directly down regulate LH pulses (Virolainen, 2004). Based on these models, beyond days 10 and 12 of pregnancy, support of the corpora lutea by LH does become important, although in some studies it seems that reduction in gonadotrophic support has to be severe and chronic to result in luteal regression and pregnancy failure. LH secretion during the luteal phase of the oestrous cycle and during early pregnancy is characterized by a lesser frequency of greater amplitude LH pulses. Chronic treatment with a GnRH agonist from days 14 to 21 of pregnancy abolished LH secretion and resulted in a decrease in progesterone secretion and loss of pregnancy in all sows at around 15 days after the start of treatment (Peltoniemi et al., 1995). Similarly, Easton et al. (1993) observed a decline in progesterone between 13 and 21 days after implantation with a slow-release agonist of GnRH (at oestrus), which is 3 d to 11 d after corpora lutea have started to become sensitive to LH. The use of a GnRH agonist by its nature initially increases LH release before down-regulating LH pulses, and may also cause some extra luteal tissue to be formed, delaying the suppression of LH and effects on luteal function. In contrast, single injection with a GnRH antagonist between days 14 and 19 after ovulation resulted in a more immediate disruption of LH secretion for a period of 2.7 days, on average, and loss of pregnancy in three of 15 sows (Virolainen et al., 2003). Active or passive immunisation against GnRH (Tast et al., 2000) had a more immediate effect with a reduction in progesterone within 2 to 4 days, and luteal failure evident within 7-10 days from immunization. In the immunisation model none of the sows maintained pregnancy. Interestingly, immunization at day 10 of pregnancy seemed to cause a reduction in progesterone and failure to establish pregnancy before total luteal failure occurred, whereas immunization at day 20 of pregnancy resulted in total luteal failure before abortion occurred. The models described above indicate that a strong suppression of LH that lasts 3 to 5 days will result in luteal failure and as a consequence result in no pregnancy being established or abortion, depending on the stage of pregnancy. As a conclusion, there is enough evidence to state that LH secretion or a minimum of LH pulses is important to support CL and maintenance of early pregnancy in the pig, beyond day 12 after fertilization. Under group housing, if a stress factor is suppressing LH secretion and thereby CL function for longer than 2 days, regression of CL may occur causing disruption of pregnancy and loss of the whole litter.

FEEDING ISSUES OF GROUP HOUSED PREGNANT SOWS

Sows may show aggressive behaviour either at the establishment of rank or when fighting for resources such as feed. Under normal production circumstances, feed restriction of up to 50% is applied to group housed pregnant sows. Earlier, it has been indicated that feed restriction of pregnant sows can be considered as a cause for stereotypic behaviour in the pig (Terlouw et al. 1991; Holt et al., 2006). A competitive feeding system, such as floor or trough feeding may present sows with a chronic stress situation, where they are subjected to stress for more than the threshold time of 2 days discussed above. If ad libitum feed is given, sows may have up to 13 occasions of feeding within a 24 hr period, which may approach the feeding behaviour seen in the nature. Ad libitum feeding therefore seems as an animal friendly way of feeding, reducing stereotypic behaviour related to feeding times (Spoolder and Vermeer, 2015). Common systems, such as free access stalls and electronic sow feeders, have been developed to control excessive competition for food – these systems may work satisfactorily if feeding and water supply management is otherwise well managed. However, sows prefer to synchronise their feeding, and individual feeders always introduce more competition, and thus a risk for increased aggression. It is a generally accepted as principle in the pig industry, that sows should remain in as stable as possible condition throughout the production cycle (Einarsson and Rojkittikhun 1993). This principle stems from the idea that sows should lose weight as little as possible during lactation and, on the other hand, gain weight only moderately during pregnancy. With the increase in litter size and in the number of fetuses, pushing the uterine capacity of contemporary sows to the limit, the challenge of not losing too much weight during lactation is becoming increasingly difficult (Foxcroft et al. 2007). The energy equation appears especially challenging for the low parity sows, as they are supposed to gain weight and grow during the first two parities. It has been shown that abundant feeding of low parity group housed sows may reduce the risk of interruption of pregnancy (Virolainen et al. 2004; 2005). Motivation for food is highest during the first 7 weeks of pregnancy as shown by an ad libitum feed intake profile along the duration of pregnancy (van der Peet Schwering et al. 2004). Furthermore, studies on seasonal infertility have indicated that an abundant feeding during early pregnancy may alleviate seasonal effects on pregnancy rates in the sow (Love et al. 1993; Love et al. 1995; Virolainen et al. 2004). It has been reported that adding large quantities of fibre in the diet of the pregnant sow decrease stereotypies and prolong resting time (Bergeron et al. 2000). In group housed sows, a good way of reducing stereotypies is actually to increase the fibre content and overall volume of feed and decrease the energy content. This seems like a solution to many practical problems related to group management of pregnant sows.

CONCLUSIONS

Grouping issues during lactation maybe of increasing interest in the future. At present, improving systems for group housed sows after weaning and early pregnancy seem to be of most practical relevance. Endocrinological models to manipulate LH secretion suggest that chronic stress of sows lasting for more than 2 days during the embryonic phase of pregnancy may lead to cessation of luteal function and loss of the whole litter. Management issues such as correct grouping of sows according to age and size right after weaning, adequate space allowance of more than 2,5 m2 per sow, use of teaser boar in pregnant sow groups, using feed stuff of higher volume with more fibre and avoiding competition at feeding and avoiding stressors such as mixing sows during the first 3 weeks of pregnancy may all improve fertility of group housed sows.

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IMPACT OF GROUP HOUSING OF PREGNANT SOWS ON HEALTH

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INTRODUCTION

Directive 91/630/EEC sets the minimum standards for the protection of pigs in the EU and has been amended several times. Directive 2008/120/EC collates the existing legislation into one document. The most important items in the legislation related to pregnant sows are shown in table 1.

Parameter	Requirement
Min. unobstructed floor space	Gilts >1.64 m² Sows >2.25 m² <6 animals: +10% >40 animals: -10%
Continuous solid floor for pregnant gilts and sows Drainage opening	>0.95 m² per gilt; >1.3m² per sow Max. 15%
Slats for pregnant gilts and sows	Gap width: max. 20 mm Slat width: min. 80 mm
Group housing	Mandatory from 4 weeks after service to one week before expected farrowing
Manipulable material	Permanent access for sows and gilts
Food	Sufficient bulky or high-fibre food as well as high-energy food for each individual
Feeding	At least once per day
Drinking water	Permanent access to fresh water
Diseased / injured pigs in group housing	May be housed individually in sick bay; should be able to turn around
Continuous noise levels	<85 dB
Amount of light	>40 lux for min. 8h / day
Lying area	Comfortable, all animals should be able to use it simultaneously

Member states are allowed to be more strict than the EU legislation. The present paper discusses the impact of group housing of pregnant sows on health, with emphasis on lameness, aggression and possible spread of infectious diseases. Comparison will be made mainly with housing sows in individual crates.

EFFECTS ON LAMENESS

Housing may affect the risk of lameness development either directly through its impact on the components of the locomotor system, or indirectly by influencing the number and type of movements sows can make. Floor space per sow, group size, pen design and flooring are the main components of group-housing that may be involved in lameness development.

FLOOR SPACE, GROUP SIZE AND PEN DESIGN

In a study by Salak-Johnson et al. (2007), three static social groups of five sows per group were assigned to 1.4, 2.3 and 3.3 m² of floor space per sow, respectively. Sows were fed using floor feeding. No space effect on lameness could be found within the first 13 days of group-housing but in the weeks thereafter, a high space allowance was related to an increased risk of lameness. Animals allocated to a high floor space may have had greater opportunity for activity which may have increased the risk of injuries leading to lameness. The effect of space allowance on lameness development may also be dependent on group size. In finishing pigs, restricted floor space resulted in greater lameness scores in large groups (n = 108) but reduced scores in small groups (n = 18) (Street and Gonyou, 2008). The group size used by Salak-Johnson et al. (2007) was rather small and may explain why the higher space allowance increased the risk of sow lameness in this study.

Furthermore, studies on aggressive and social behaviour of sows revealed that space requirements not only differ according to group size but also to group stability, pen design and feeding system (Weng et al., 1998). This may also apply to lameness development. Therefore, care must be taken when attempting to generalize the results found by Salak-Johnsosn et al. (2007). Differences in herd-related factors could have been the reason why Gjein and Larssen (1995b) and Heinonen et al. (2006) did not find a significant association between space allowance and lameness in group-housed sows. The minimal space according to EU legislation applies to all group-housing systems regardless of feeding system, group stability or use of bedding though adjustments are made with regard to

group size. For sows housed in dynamic social groups and fed by an electronic sow feeder, 33% more area than the EU legal minimum was demonstrated to induce better welfare considering agonistic behaviour and consecutive wounds (Remience et al., 2008). The impact on productivity and social physiological stress was not yet determined. Nonetheless, these results may indicate that the legal minimum requirements may be insufficient for sows housed in dynamic groups and fed by electronic sow feeders.

FLOOR CHARACTERISTICS

The type, building material and quality of the floor, hygiene and the use of bedding may all influence the risk of lameness.

Type

Three main floor types are used for pigs namely solid, partially slatted and fully slatted floors. Use of slatted, unbedded floors increases the risk for lameness in group-housed gestating sows. The odds of being lame on slatted floors is twice as high compared to non-slatted floors (Heinonen et al., 2006), 4.6 times higher compared to solid concrete floors with deep bedding and 4.8 times higher than in sows housed outdoors (KilBride et al., 2009a). The association between floor type and lameness might occur as a result of an increased prevalence of locomotor disorders or because locomotor disorders are more painful or cause more discomfort. On slatted floors, pigs may face some challenges such as chipped, sharp or worn slat edges (KilBride et al., 2009b), slipping and wedging the claws into the voids (Mouttotou et al., 1999), and an increased, uneven distribution of pressure on the claws (Mouttotou et al., 1999; KilBride et al., 2009b). These effects suggest that slatted floors cause lameness more likely by affecting claw health than by affecting joint soundness.

Building material

Concrete is the most commonly used floor material for housing pregnant sows. Gjein and Larssen (1995b) showed that the risk for lame sows on concrete slats was 2.4 times higher than on plastic slats. Claw lesions did not differ between both materials indicating that the difference in lameness may have been due to other locomotor disorders or that claw lesions may have been less painful when walking on plastic slats (Gjein and Larssen, 1995c). Plastic slats are softer and may result in less biomechanical stress to the claws and limbs compared with concrete slats (Gjein and Larssen, 1995b; Gjein and Larssen, 1995c). Hence, plastic slats may be more comfortable to walk on and mildly lame sows may be missed when scoring sows on plastic slats. However, plastic slats can be slippery (Gjein and Larssen, 1995c). So far, no clear-cut advice on alternatives to concrete slats can be given to reduce the risk of lameness development in group-housed sows.

QUALITY

Slip-resistance, abrasiveness, surface profile and hardness are the four main quality factors contributing to a floor's total injury potential (McKee and Dumelow, 1995). Floors with a low slip-resistance may result in slipping and falling causing skin abrasions, bruising, bursitis, torn dewclaws and even broken bones (Dewey, 2006; KilBride et al., 2009b). Floors with a high abrasion level predispose to calluses (Leeb et al., 2001) as well as skin wounds and claw lesions (Geyer and Troxler, 1988). Especially hoof cracks have been associated with abrasive floors. A minimum of abrasiveness however, is necessary to prevent hoof overgrowth (McKee and Dumelow, 1995). Using slatted floors, also the void ratio (i.e. area of holes per unit area of floor), slat width and edge design should be taken into account. Hard surfaces e.g. concrete may lead to pressure injuries. On soft surfaces, e.g. soil or rubber mats, the area of contact between the claw and the floor is higher. This may reduce the overloading of claws (Carvalho et al., 2009). Finally, durability of the floor is important. A worn surface can be injurious and is difficult to clean and disinfect. De Belie (1997) found that concrete slats could already show degradation within five years leading to increased surface roughness, enlarged gaps and animal injuries. These claw or limb lesions may result in clinical lameness (Webb and Nilsson, 1983), but studies directly associating clinical lameness with floor quality have not yet been published. Pigs may adapt their gait to potentially slippery surfaces by reducing walking speed, shortening stride length, prolonging stance time and by employing more three-limb support phases. However, when slip-resistance becomes too low, adaption might no longer be sufficient.

HYGIENE

Floors should be dry and clean. In pens with dirty, wet slatted floors, the risk of lame sows is 2.8 times higher than in pens with good floor hygiene (Gjein and Larssen, 1995b). Cador et al. (2014) showed a relation between dirtiness of sows and lameness. The increased risk is assumed to be mainly claw related. An increased risk of, particularly, heel/sole erosions on dirty and wet floors has been reported for finishing pigs (Penny et al., 1963) and lactating sows (Kilbride et al., 2010). In group-housed sows, heel overgrowth seemed to be more common in herds with poor floor hygiene (Gjein and Larssen, 1995d). Softening of the hoof horn, a higher slipperiness of the floor and the acidic caustic nature of slurry may contribute to the increased incidence of claw lesions on wet, slurry covered floors. Also, dirty floors may cause infection of claw lesions enhancing lameness. Gjein and Larssen (1995c) reported a 4.2 times higher risk of claw infections for group-housed sows in herds with poor floor hygiene.

USE OF BEDDING

Use of solid concrete covered with deep straw bedding has been associated with a lower prevalence of lameness compared to bare solid and slatted floors (Kilbride et al., 2010). This might be explained by a reduced prevalence of locomotor disorders or because deep bedding is more comfortable to sows. Housing sows during gestation on solid concrete with straw bedding decreased the risk of heel flaps but increased the risk of toe erosions during the subsequent lactation (Kilbride et al., 2010). Toe erosions may be more prevalent

on straw bedding, likely due to the lack of natural wearing. When straw bedding is used, hygiene is important. Damp straw, soiled with feces and urine softens the hooves, makes them more prone to abrasion and pressure-induced lesions and increases the risk of claw infections and hence lameness. Provision of straw has not been universally adopted as it is not compatible with slatted floors and liquid manure handling systems. In addition, it can be costly and labour intensive (Whittaker et al., 1999). Rubber mats have been suggested to be a useful and inexpensive alternative for straw (Elmore et al., 2010; Díaz et al., 2013). Elmore et al. (2010) could not find a difference in lameness scores between sows housed on rubber mats versus concrete floors. However, in that study, rubber mats were only added to the feeding stalls and not to the area with concrete slatted flooring between the stalls. By contrast, Díaz et al. (2013) covered the concrete slatted flooring in both the feeding stalls and the group area with rubber slat mats. Sows housed on rubber slat mats had a significantly reduced risk of becoming lame. On rubber mats, the contact area between the claw and the floor is larger improving the claw pressure distribution and reducing overloading of claws and joints (Carvalho et al., 2009). This cushioning effect may also improve circulation in the foot (Singh et al., 1993). Finally, rubber slat mats may provide more traction and hence reduce lameness due to slipping (Boyle et al., 2000). Besides the potential to reduce the risk of lameness, rubber slat mats have also been associated with an increased risk of toe overgrowth, heel-sole cracks, white line lesions and wall cracks (Díaz et al., 2013).

Effects on aggression

Aggression occurs predominantly because of competition for access to a limited resource, or to establish a social hierarchy. Aggression related to competition for feed is generally short in duration, but very frequent. Aggression related to establishing a social hierarchy is less frequent, but can be far more intense. Most of the lesions caused by aggression are scratches or cuts on the skin. Vulva biting is also possible, but this is more regarded as a sign of frustration instead of aggression. Aggression may also result in lameness. Persistent aggression can decrease welfare as indicated by increased stress hormone concentrations (Otten et al. 1999), increased heart rates (Marchant et al. 1995), increased injuries, and restricted access to resources (O'Connell et al. 2003) in animals that are aggressive or ones that are being attacked. Many of the aspects discussed above (see lameness) also apply to prevent aggression. Key factors to prevent aggression include a gradual familiarization of unfamiliar animals, stage of gestation at mixing, sufficient space and pen structure during initial mixing, minimizing opportunities for dominant sows to steal food from subordinates, the provision of a good quality floor, environmental enrichment and the use of straw bedding (Knox et al., 2014; Stevens et al., 2015). Some reports suggest that aggression may also be managed through selection of pigs (Erhard et al. 1997).

EFFECTS ON DISEASE TRANSMISSION

No or very scarce evidence based information is available on the relationship between group housing and disease. Animals that are stressed are more susceptible to disease, or may shed more pathogens e.g. Salmonella. In general, cortisol levels in sows that are housed in groups are similar or lower than in sows housed individually (McGlone et al., 2004). Von Borell et al. (1992), McGlone et al. (1994) and Broom et al. (1995) found that antibody production and neutrophil-to-lymphocyte ratio were not different between sows kept individually compared with those kept in groups.

Compared to housing sows in individual crates, sows in group housing have more nose-to-nose contact, and they have more oral contact with feces and urine. These factors could contribute to a higher or faster transmission of pathogens. However, also other aspects should be considered. Regarding respiratory disease, one could expect slightly better air quality in group housing systems because of a potential better ventilation of the entire stable. However, if hygiene is poor, ammonia levels can also be high. If straw is used, there will be more dust, but likely lower stress levels. Because of more contact with feces, one can expect a higher risk for infections with intestinal pathogens such as Brachyspira spp., Lawsonia intracellularis, Salmonella, Ascaris suum. Reports (Chouet et al. 2003 and Stege et al. 2000) showed higher seroconversion rates for L. intracellularis in group housing systems with straw bedding. The hygiene levels are largely dependent on the housing conditions and the management. The fact that the lying and defecation area are separated improves hygiene and decreases oral contact with feces. Sows in group housing can move around and generally suffer less from chronic stress, potentially leading to less problems with gastro-intestinal torsions and gastric ulcers. Movement is also beneficial to avoid constipation and urine stasis. The transition to the farrowing house may be more critical in group housing, but the type of stable, the feeding system and the management are important. Effects of group housing on body condition, production and reproduction are discussed in the accompanying paper of this workshop.

CONCLUSION

In contrast with housing sows in individual crates, group housing allows the animals to express normal activity and behavior. However, group housing as such does not automatically imply better animal welfare. Particular attention should be paid to prevention of lameness and aggression as these may severely compromise welfare. Optimal functioning of group housing systems depends on the combined effect of different factors. Management is a crucial factor and it should be animal oriented. But also feeding strategies, flooring and bedding, design of housing are very important as relatively minor adjustments may have major effects on the animals.

IMPACT OF MYCOTOXIN ON PIG HEALTH

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INTRODUCTION

Food safety is a major issue throughout the world. In this respect, much attention needs to be paid to the possible contamination of food and feed by fungi and the risk of mycotoxin production. Mycotoxins are secondary metabolites produced by filamentous fungi, mainly by species from the genus Aspergillus, Fusarium and Penicillium. They are produced on a wide variety of raw materials before, during and after harvest. Very resistant to technological treatments, mycotoxins can be present in human food and animal feed. The main mycotoxins considered to be important from an animal health perspective are aflatoxins, ochratoxins, fumonisins, zearalenone and trichothecenes, specifically deoxynivalenol (CAST, 2003; Oswald & Taranu, 2008). These toxins have multiply effect on pigs (Table 1), they all affect the immune system and recent data show that the intestine is a target for these contaminants.

EFFECT OF THE MAJOR MYCOTOXINS ON PIG INTESTINE AND CONSEQUENCES FOR PIG HEATH

The intestinal epithelium is a single layer of cells lining the gut lumen that acts as a selective filter, allowing the translocation of essential dietary nutrients, electrolytes, and water from the intestinal lumen into the circulation. It also constitutes the largest and most important barrier to prevent the passage from the external environment into the organism of harmful intraluminal substances, including foreign antigens, microorganisms, and their toxins. Following the ingestion of mycotoxin-contaminated feed, intestinal epithelial cells may be exposed to high concentrations of toxicants, potentially affecting intestinal functions (Oswald, 2006).

Mycotoxins can affect several intestinal functions as the nutrient uptake, the barrier function and the intestinal immune response (Grenier & Applegate, 2013; Maresca 2013; Pinton & Oswald, 2014). The alteration of intestinal integrity as demonstrated by the altered expression of tight junction molecules, the decreased trans-electrical resistance and the increased paracellular permeability could lead to the entry of luminal antigens, feed contaminant and bacteria that are normally restricted to the gut lumen by the intestinal barrier function. Such increase in the bacterial passage through intestinal epithelial cells after mycotoxin exposure could have major implications for pig health in term of sepsis, inflammation and susceptibility to enteric infection. In pigs, the effects of deoxynivalenol, and fumonisins, have been studied in detail, by contrast only few papers concern the effects of ochratoxin or aflatoxin on the pig intestine (Bouhet & Oswald, 2007; Pinton & Oswald., 2012).

Porcine ileal loops were used to reproduce S. typhimurium induced intestinal inflammation. Co-exposure to bacteria and DON dramatically enhances the inflammatory response to S. typhimurium in the ileal loops, with a clear potentiation of the expression of IL-1β, IL-8 or IL-6. It was suggested that this potentiation coincided with a significantly enhanced Salmonella invasion in and translocation over intestinal epithelial cells. A higher susceptibility of the intestine to bacteria other than Salmonella was also reported in pigs treated with FB1. Two separate studies analyzed the effect of low to moderate doses of FB1 on intestinal colonization and mucosal response to pathogenic strains of Escherichia coli. Besides, intestinal colonisation, translocation of bacteria to the mesenteric lymph nodes a dissemination of bacteria to the lungs, and to a lesser extent to liver and spleen was observed in FB1-treated pigs (Oswald & Taranu, 2008).

EFFECT OF MAJOR MYCOTOXINS ON THE PIG IMMUNE RESPONSE AND CONSEQUENCE FOR PIG HEALTH

Within the large panel of toxic effects that mycotoxins display, immuno-modulatory properties have been described for most of these fungal food contaminants (Table 1). Mycotoxin-induced immunomodulation is significant for several reasons. From an agricultural standpoint, mycotoxin exposure might predispose livestock to infectious diseases and reduce productivity. From a public health perspective, increased infections in food animals may result in increased animal-to-human transmission of pathogens and/or increased antibiotic concentrations in meat or milk, as a consequence of animal treatment. The sensitivity of the immune system to almost all mycotoxins can be attributed to the vulnerability of the immune cells that are constantly dividing and differentiating to insure their functions in the complex network of the innate and acquired immune response. Immuno-modulatory effects of mycotoxins are often observed at low exposure levels when compare to other toxic effects Mycotoxin may act on both innate and acquired immune responses and this may have consequence for animal health (Oswald et al., 2005; Meissonnier et al., 2006).

Susceptibility to infectious diseases. The broad immunosuppressive effect of mycotoxins may decrease host resistance to infectious diseases. In pigs, consumption of feed contaminated with AF increased the severity of the Erysipelothrix rhusiopathiae infection. Similarly, during an experimental infection with Brachyspira hydysenteria, the consumption of AF reduced the incubation time and increases the severity of the diarrhea. Ingestion of OTA contaminated feed also increased susceptibility to natural infectious disease. Indeed, salmonellosis arose spontaneously in all piglets receiving an OTA contaminated diet and when the animals were vaccinated against salmonellosis, the consumption of contaminated feed lead to spontaneous Brachyspira hyodysenteria and Campylobacter coli infections.

In pigs, the ingestion of FB1-contaminated feed was associated with an increased susceptibility to pulmonary infection with bacterial or viral pathogens.

Reactivation of chronic infection. The effect of mycotoxin intoxication on the reactivation of chronic infection was also investigated. However, the experiment was not performed with pigs but with rodents. In the immunocompetent host, Toxoplasma gondii infection progresses to a chronic phase characterized by the presence of encysted parasites. Cyst rupture may occur, but infection remains latent and reactivation is prevented. In immunosupressed animals and human subjects, such as HIV infected patients, rupture is associated with the formation of new cysts and disease. Low and repeated doses of either AFB1 or T-2 toxin are able to accelerate Toxoplasma cyst rupture in previously infected mice.

Table 1: Multiple effect of mycotoxins in pigs

Symptoms/organ targeted	Mycotoxins				
	AFB1	OTA	DON	FB1	ZEA
					
Anorexia	+	+	+++	+	
Growth	+++	+	+++	+	
Liver damage	+++	+		++	
Kidney damage		+++		+	
Abortion				+	++
Infertility					+++
Vulvovaginitis					+++
Pulmonary oedema				+++	
Immunomodulation	+++		++	+++	
intestine	?	+++	?	++	?

Vaccination efficacy. Immunity acquired through vaccination is also impaired by mycotoxin ingestion. For example, AFB1 interferes with the development of acquired immunity in swine following erysipelas vaccination with bacterin preparation of Erysipelothrix rhusiopathiae. Ingestion of feed contaminated with AFB1 or T-2 Toxin reduced the vaccinal response to the model antigen, ovalbumin, acting on the cellular and the humoral response respectively. Ingestion of low doses of another mycotoxin, FB1, decreases the specific antibody response mounted during Mycoplasma vaccination in pigs.
It should also been mentioned that the vaccinal immune response is altered at mycotoxin doses that do not alter the global immune response (Oswald et al. 2005). The breakdown in vaccinal immunity may lead to the occurrence of disease even in properly vaccinated flocks. These reactions are of considerable consequence in animals for which we rely on an effective vaccination program for disease prevention.

THE PROBLEM OF CO-CONTAMINATION

Most fungi are able to produce several mycotoxins simultaneously; moreover food and feed can be contaminated by several fungi species at the same time; complete diet is made from various different commodities. Thus, pigs are generally not exposed to one mycotoxin but to several toxins at the same time. This is supported by global surveys underlying the multicontamination (Streit et al., 2012).

The toxicity of combinations of mycotoxins cannot always be predicted based upon their individual toxicities. The data on the combined toxic effects of mycotoxins are limited and therefore, the health risk from exposure to a combination of mycotoxins is incomplete. Most of the studies concerning the toxicological effect of mycotoxins have been carried out taking into account only one mycotoxin. Interactions between concomitantly occurring mycotoxins can be antagonistic, additive, or synergistic. Three main methodological approaches have been used to determine the interaction between mycotoxins; the arithmetic model of additivity, factorial designs and the theoretical biology-based models of additivity. These latter models are the most advanced. In this respect, the Chou-Talalay method, that is not linked to mechanistic considerations, appears more reliable and present the advantage to allow a quantitative assessment of the interaction. Using this model in several cellular systems, we have observed a synergistic interaction for trichothecenes on pig intestinal epithelial cells, especially when used at low concentration (Alassane-Kpembi et al., 2015). The synergistic effects observed after cell exposure to a mixture of low concentrations of mycotoxins could pose a significant threat.

CONCLUSIONS

Mycotoxin contamination can occur in all agricultural commodities in the field and/or during storage, if conditions are favorable to fungal growth. The intestine and the immune systems are two targets for these toxins and as illutrated in this paper this may have some consequences for pig heath. At the intestinal level, ingestion of mycotoxin contaminated feed can alter gut integrity lead inflammation and/or susceptibility to enteric infection. Suppressed immune function by mycotoxins can decrease resistance to infectious diseases, reactivate chronic infections and/or decrease vaccine and drug efficacy.
Co-occurrence of mycotoxins is a reality and more research are needed to understand the impact of mycotoxin combinations and to determine when synergistic interactions occurs. These data are needed to asses the health risk due to exposure of multi- mycotoxins contaminated feed (Grenier & Oswald, 2011).

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DO MYCOTOXINS REALLY AFFECT THE HEALTH OF PIGS?

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INTRODUCTION

Molds belong to the natural microflora which commonly infests feedstuffs. Under certain gross-environmental conditions, such as temperature, moisture content, air humidity and water availability, oxygen availability and micro-environmental conditions such as microbes competing for ecological niches the molds are stimulated to form secondary metabolites. If these metabolites exert toxic effects against the living environment, they are collectively termed as mycotoxins. Due to the ubiquitous occurrence of molds it is not surprising that mycotoxins belong to natural contaminants often detected in feedstuffs.

PIGS RESPOND ESPECIALLY SENSITIVE TO A NUMBER OF MYCOTOXINS

Owing to different chemical structures of mycotoxins, their toxicodynamics also varies considerably (Table 1). Mycotoxins might exert toxic effects in farm animals when feedstuffs are contaminated at levels that exceed the tolerance level by the animals. This tolerance is species specific, but is not unique among the mycotoxins. In addition, the clinical manifestation of a particular mycotoxin might also differ between species (e.g., fumonisins). By comparing the species-specific sensitivity together with the relevance for practical feeding conditions it becomes obvious that the pig is on special risk in spite of different chemical natures and toxicodynamics of different mycotoxins (Table 1).

Table 1. Overview on selected mycotoxins, their mechanisms and toxic effects and their relevance for practical feeding (literature compilation)

Mycotoxins	Mechanisms and toxic effects	Predisposed feedstuffs	Concerned livestock and relative sensitivity1	Relevance 2
Zearalenone (ZEN)	Estrogen-like, endocrine disruptor / reproductive disorders	Maize, wheat	Pig > cattle > poultry	++
T-2 Toxin	Inhibition of protein synthesis, immune-modulating / anorectic, cytotoxic, dermatotoxic, hepatotoxic	Oats, barley, maize, wheat	Pig > cattle ~ poultry	+
Deoxynivalenol (DON)	Inhibition of protein synthesis, ribotoxic / immune-modulating, anorectic	Maize, wheat	Pig > cattle ~ poultry	++
Fumonisins (FUM)	Disruption of sphingolipid metabolism / carcinogenic, neural tube defects, hepatotoxic, pneumotoxic (Porcine pulmonary edema), neurotoxic (Equine leukoencephalomalacia)	Maize	Horse ~ pig > cattle ~ poultry	+
Aflatoxin B1 (AFB1)	DNA- and protein adduct formation / carcinogenic cytotoxic, hepatotoxic	Pea nuts, cotton seed, maize, sunflower seed, soy beans	Cattle, pig, poultry	++
Ergot alkaloids (EA)	Vessel-active, pharmacological effects, performance depressive	Rye, triticale	Poultry, Cattle, Pig	+

[1] Farm animals which are not considered are less sensitive or there is insufficient information on toxic effects
[2] Relevance for practical feeding (occurrence of the toxins in relation to sensitivity of livestock):»++ «highly important, «+» important,» -» less important, «?» relevance unclear.

CURRENT RISK MANAGEMENT

The practical relevance of a particular mycotoxin for a specific species results from its sensitivity to this toxin and from the occurrence of the toxin on feedstuffs commonly used for feeding of that species (Table 1). For risk management is it consequently necessary to consider both aspects, i.e., the most sensitive animal species and the most pre-disposed feedstuffs (Table 1).

Table 2. European Commission recommendation of 17 August 2006 on the presence of mycotoxins in products intended for animal feeding

Mycotoxin	Products intended for animal feed	Guidance value in mg/kg (ppm) relative to a feedstuff with a moisture content of 12 %
Deoxynivalenol	**Feed materials (*)** - Cereals and cereal products (**) with the exception of maize by-products - Maize by-products **Complementary and complete feedstuffs with the exception of:** - Complementary and complete feedstuffs for pigs - Complementary and complete feedstuffs for calves (< 4 months), lambs and kids	8 12 5 0.9 2
Zearalenone	**Feed materials (*)** - Cereals and cereal products (**) with the exception of maize by-products - Maize by-products **Complementary and complete feedstuffs** - Complementary and complete feedstuffs for piglets and gilts (young sows) - Complementary and complete feedstuffs for sows and fattening pigs - Complementary and complete feedstuffs for calves, dairy cattle, sheep (including lamb) and goats (including kids)	2 3 0.1 0.25 0.5
Ochratoxin A	**Feed materials (*)** - Cereals and cereal products (**) **Complementary and complete feedstuffs** - Complementary and complete feedstuffs for pigs - Complementary and complete feedstuffs for poultry	0.25 0.05 0.1
Fumonisin B1 + B2	**Feed materials (*)** - Maize and maize products (***) **Complementary and complete feedstuffs for:** - Pigs, horses (Equidae), rabbits and pet animals - Fish - Poultry, calves (< 4 months), lambs and kids - Adult ruminants (> 4 months) and mink	60 5 10 20 50

(*) Particular attention has to be paid to cereals and cereals products fed directly to the animals such that their use in a daily ration should not lead to the animal being exposed to a higher level of these mycotoxins than the corresponding levels of exposure where only the complete feedstuffs are used in a daily ration.
(**) The term 'Cereals and cereal products' includes not only the feed materials listed under heading 1 'Cereal grains, their products and by-products' of the non-exclusive list of main feed materials referred to in part B of the Annex to Council Directive 96/25/EC of 29 April 1996 on the circulation and use of feed materials (OJ L 125, 23.5.1996, p. 35) but also other feed materials derived from cereals in particular cereal forages and roughages.
(***) The term 'Maize and maize products' includes not only the feed materials derived from maize listed under heading 1 'Cereal grains, their products and by-products' of the non-exclusive list of main feed materials referred to in the Annex, part B of Directive 96/25/EC but also other feed materials derived from maize in particular maize forages and roughages.

The European Commission responded to the risk characterization and evaluation of the presence of DON, ZON, OTA and FUM in feedstuffs by the European Food Safety Authority (EFSA, 2004b, c, a, 2005) with the release of a recommendation for critical orientation (guidance) values for these toxins in feedstuffs (see Table 2) and for monitoring of the simultaneous occurrence of these mycotoxins (CEC, 2006).

A distinction must be made between guidance values for individual feedstuffs (feed materials) and for the total diet (complete feedstuffs) or parts of it (complementary feeding stuffs) (Table 2). From a toxicological viewpoint the guidance values for complete feedstuffs needs to be considered as total dietary toxin concentrations, together with the level of feed intake, determine the toxin exposure of the animal.

While the guidance values for critical feedstuff concentrations of DON, ZEN, OTA and FUM were derived based on animal health aspects, the upper limits for AFB1 consider mainly the high carry over into milk, but also animal health effects in young animals including pigs (Table 3).

Table 3. Upper limits for Aflatoxin B1 and for rye ergot (Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed)

Undesirable substance	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feedstuff with a moisture content of 12 %
Aflatoxin B1	All feed materials, Complementary and complete feedstuffs excepting: - Complete feedstuffs for dairy cows and calves, dairy sheep and lambs, dairy goats and kids, piglets and young poultry - Complete feedstuffs for cattle (excepting dairy cows and calves), sheep (excepting dairy sheep and lambs), goats (excepting dairy goats and kids), pigs (excepting piglets), and poultry (excepting young poultry)	0.02 0.01 0.005 0.02
Rye ergot (Claviceps purpurea)	All feedstuffs containing unground cereals	1000

INTERPRETATION OF GUIDANCE VALUES FOR CRITICAL MYCOTOXIN CONCENTRATIONS IN FEEDSTUFFS

The compliance with the guiding values for complete feedstuffs ensures that no adverse effects occur in farm animal feeding under normal production conditions. Deviations from "normality" might include the presence of stress factors such as latent or manifest infections of the animals by pathogenic agents, poor keeping conditions (too high stocking density, inadequate nutrition, too high concentration of corrosive gases and others) and can result in a decrease of mycotoxin concentrations in the diet to be considered as critical and thus lower than the guidance values.

It needs to be stressed that the current mycotoxin management is based on the free forms of the toxins in feed which are detectable by routine methods. However, in evaluating the overall toxicity of the free form of mycotoxins it needs to be further considered that mycotoxins often exist in feedstuffs in a modified form (Rychlik et al., 2014) which is usually not detectable by routine methods. Digestive processes in general, and species-specific differences in the site of microbial digestion in particular, also influence the sensitivity to mycotoxins.

Pre-ingestively modified forms of mycotoxins (e.g., matrix associated; thermally formed; conjugated by plants) might further be exposed to pre-absorptive (i.e., ingestive) structural alterations, as mediated by gastro-intestinal microbes (e.g., DON-3-O-glucoside β free DON, Nagl et al., 2012) or endogenous digestive enzymes (e.g., Fumonisins bound to starch β free Fumonisins, Humpf and Voss, 2004), which can result in a reverted toxicity of the originally free mycotoxin (i.e., DON and Fumonisins, respectively) or in a further modification giving rise to an altered toxicity (e.g., DON-3-O-glucoside β DON β de-epoxy-DON, Nagl et al., 2012).

Although de-epoxy-DON seems to be of a generally low toxicity both for bovine and porcine immune cells, the interspecies differences in pre-absorptive modification need to be considered in evaluating the overall toxicity of DON for cattle and pigs. While DON is nearly completely modified to de-epoxy-DON in cattle by rumen micro-organisms prior to systemic absorption, this modification occurs also in the hindgut of pigs, but only very small amounts of non-absorbed free DON reach this intestinal segment. Therefore, in DON exposed cattle mostly de-epoxy-DON is detectable in blood while in pigs the majority is in the form of free DON (e.g., Dänicke and Brezina, 2013).

CONCLUSIONS

Compliance with the guiding values for critical mycotoxin concentrations for complete feedstuffs for pigs ensures that no adverse effects occur under normal production conditions. Deviations from these assumptions might result in adverse health effects at mycotoxin concentrations lower than the corresponding guidance values. Furthermore, compliance with the guidance values for a particular mycotoxin such as DON assumes that all other modified (non-free) forms of the same toxin as well as other mycotoxins which might be present in the contaminated feedstuff at the same time, but for which no management exists, are controlled at the same time. However, under certain contamination scenarios this assumption might not apply with the result that adverse effects on pig health are observed at indicator toxin concentrations (e.g. DON) lower than the guidance value for its critical (free) concentration. These aspects and the occasional exceedance of critical concentrations of mycotoxins in feedstuffs for pigs lead to the conclusion that mycotoxins are relevant for health of pigs. Therefore, preventive measures should include both minimization strategies to decrease feedstuff contaminations according to the ALARA (as low as reasonably achievable) principle and all measures to optimize the housing conditions for pigs.

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PORCINE EPIDEMIC DIARRHEA (PED) – A CONSTANT THREAT

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INTRODUCTION

PED is a widespread disease found in many countries of the world. First identified in Asia over 10 years ago, it was first introduced into the United States in May 2013. Many different states were affected at the same time, in different systems and associated with different vet clinics. There was no known links between them. The PEDv strain that was found was genetically related to a Chinese strain identified in 2012. The initial agent for introducing PEDv in America has not been identified but feed ingredients are the most likely suspect.

A friend of mine, Dr Luc Dufresne from Seaboard Farm in the U.S., described the amazing transmission of PEDv between his farms. In May – June 2013, more than 125,000 sows suffered from a break-out of PEDv causing 100% piglet mortality over a six week period. He described the clinical case like "TGE on steroids." Nursing piglets had profuse watery diarrhea and vomiting; and within 24 to 48 hours, 100% of the piglets were affected.[2]

In December 2013, more than 20 different states had experienced PED, 40% of the U.S. sow herds were infected and more than 1,500 cases has been confirmed by the labs. In Canada, because of the high traffic for culled sows and weaned piglets between the two countries, contamination was a continuous fear.

A BIT OF HISTORY

The first 2 cases appeared in Canada in January 2014, both at same time: one in a farrow to finish barn in Ontario and the other at an abattoir loading dock in Quebec. The threat was real. Within 20 days, 11 farms became infected with PEDv, the majority of which were in the province of Ontario and the other one in PEI.

The links between these farms: plasma proteins sourced from the U.S. Investigations by consulting vets and the CFIA confirmed that plasma protein used in creep feed diets was PCR positive for PEDv and the use of this feed infected all the farms at the same time.[4,6]

At F. Menard, a swine integration company in Quebec producing more than 1 million pigs per year, preventing the introduction of PEDv was a priority. February 10, 2014 showed a first positive PCR detected at our abattoir loading dock. Through traceability measures, 17 different farms had delivered pigs to slaughter that morning. Samples collected from all these farms helped us to identify the grow-finish barn as the contaminated site. The PCR positive group of pigs never showed any clinical signs. The source of the virus came from the transporter who did not follow the biosecurity rules when coming from another contaminated slaughter plant, specifically not washing his truck adequately at night during very cold weather, and coming into our farm with the same dirty boots as the ones he used at slaughter the day before. Our producer also spread the virus to his second barn by using the same boots and clothes as the ones he used in the infected barn.

A NICE ENDING TO THE STORY

The F. Menard supervisor over-looking that specific client was also visiting more than 50 clients every two weeks. According to our records, the farm was contaminated for a month when we discovered that it was PEDv positive. The supervisor never contaminated any of the 50 other client barns because he simply followed the basic biosecurity rules: changing boots, coveralls and gloves between each farm and keeping his truck cab clean. The lesson learned: good biosecurity can prevent PEDv transmission.

We got rid of this first case through strict biosecurity procedures and extreme sanitation. We learned that PEDv was highly resistant in the environment since we found PCR positive samples in the entrance to the barn, in cracks in the pen walls, on producer's boots and even on the outside of the barns. We kept that barn empty for 3 months before putting a new group of pigs in, which remained negative after their introduction into the barn.

WHAT DID WE IMPROVE AT F. MÉNARD?

Even before the first case, the ultimate goal of F. Ménard was to prevent contamination of breeding herds. The economic impact of PEDv introduction into a breeding herd is dramatic with an average loss of 2,500 piglets per 1,000 sows and 7.4 weeks before going back to 80% of the original production level.[5] The integrated structure of F. Ménard, linking all the production units through common feed delivery, trucking, manure handling and slaughter plant, can have a devastating impact. Any one nursery or finishing farm infected with PEDv can have a cascade effect throughout our system leading to bigger losses through infected breeding herds. We had to preserve our markets.

ACTIONS TAKEN TO PREVENT PED

FEED

As of February 2014, we completely stopped using porcine protein in our two feed mills. No porcine plasma and no blood meal are used in our diets.

TRANSPORTS

We already had specialized trucks for each step of production. We split the risk by keeping the gilts and nucleus truck, the weaned piglet trucks and grow-finish pig trucks in 3 different heated garages. They are washed, disinfected and dried every night after the driver's shift.

FARMS

We are currently operating under multiple sites production. We then have a different crew taking care of the breeding herds, nurseries and grow-finish farms. Strict rules are respected for farm visits: shower in/out, boot and coverall change, registration for visitors. All introduced materials have to be disinfected and heated for 24 hours before being introduced into the barn. No cell phones and no lunches are allowed inside for visitors. Sow farms are equipped with loading docks preventing any introduction of a transporter into the barns and therefore limiting the risk of spreading the disease.

DIAGNOSTICS

We think that prevention through intensive diagnostics is essential to the control of PED. The quicker new infections are detected, the higher the success rate in eliminating the virus. Since January 2014, samples are collected at every high risk spot: abattoir loading dock, nursery post placements, pre-slaughter grow-finish pigs, gilts pre and post transport, washing bay and trucks. This intense monitoring gave us the opportunity to intervene very rapidly and prevent the spread of the disease.

NEW CASES IN 2015

Summer time gave some relief to new cases in Canada but with the very cold temperatures of the last few months, PEDv came back with force. Again within two weeks, three large nursery-finish sites were contaminated and the virus spread very rapidly between the different barns on each site. Introduction of the virus again came with the transport of incoming piglets and was then spread to more than 20,000 pigs through biosecurity rules not being respected. The producers and our crew responsible for vaccinations were associated with these new cases. These waves of disease spreading involving 22 different barns showed how PEDv can spread rapidly and highly contaminate the environment. Table 1 shows every spot where PCR positive results were found.

TABLE 1 – PED PCR POSITIVE ENVIRONMENTAL SAMPLINGS

Deep pit	+++	Farm loading dock	++
Floor	+++	Producer's truck	++
Pen wall	++	Pressure washer	++
Farm office	+	Service man truck	+
Entrance of barn	+	Abattoir loading dock	+
Outside barn	+	Truck washing bay	+
Attic	+		

THE LESSONS LEARNED

This virus is highly contagious, is very resistant in the environment and clean is never clean enough. The different risks factors for PEDv introduction are:

1. Feed: the U.S. and Ontario experience shows how porcine protein could have introduced PEDv into herds.[1,4]
2. Transport: Poor sanitation, cold weather, contaminated abattoir loading dock, and breaking biosecurity rules by transporters can all be sources of PEDv introduction.[3,4,7]
3. The producer: No respect for the rules such as changing boots, coveralls and gloves, can trigger transmission from one barn to another one.
4. Equipment/repairs/servicemen: Dirty equipment can easily transmit PEDv from one farm to another. Beware of service people!5

THE CANADIAN SITUATION

In March 2015, only 2% of our Canadian sow herds were contaminated as compared to 60% in United States. 90% of the Canadian primary cases have been successfully eliminated. In response to the growing number of cases, different groups have been created in Canada in order to help in the control of this new disease. OSHAB (Ontario Swine Health Advisory Board) and EQSP (Equipe Québécoise de Santé Porcine) are the two provincial entities. CSHB (Canadian Swine Health Board) is the Canadian link. All new cases are declared

through these organizations and locations as well as type of production are specified. These groups help producers with elimination planning and ensure follow up. They also supervise environmental swab samplings at abattoirs, assembly yards, truck washes and feed ingredient mixing, and compile results that they published regularly.
They also provide the producers with guidelines and protocols for PEDv elimination and biosecurity procedures. The goal is to control and eliminate PEDv in Canada.

IN CONCLUSION

PED has been and still is a very educating disease. It showed us how weak the application of our biosecurity rules were in some areas of our production system. Every detail is important with this disease and excellent sanitation is a key point in the control measures. This challenge will help us to improve and help to prevent other diseases such as PRRS. With more than 60% of the U.S. sow herd infected, it still represents an important threat for us. But a special thanks to our U.S. colleagues and our Ontario neighbours for all the information they shared with us. We learned a lot from them. We know what to do and what not to do. We are more prepared for the war. Better to be armed when the enemy does appear.

ACKNOWLEDGEMENTS

A special thanks to the F. Ménard production team. Together we are working every day to improve biosecurity measures and prevent the spread of this costly disease.

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DIAGNOSTIC AND SURVEILLANCE OF PORCINE EPIDEMIC DIARRHEA

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Porcine epidemic diarrhea (PED) is a highly contagious enteric disease of swine caused by a coronavirus, porcine epidemic diarrhea virus (PEDV), and characterized by severe diarrhea that affects pigs of all ages. It was described for the first time in Europe in 1971 and in Asia during the 1980s. In both regions, the disease spread after the first reports leading to outbreaks of importance on pig farms and causing variable mortality rates in sucking piglets (0%-100%). However, the disease has evolved differently over time. While in Europe the frequency and severity of PED outbreaks decreased during the 1980s and 1990s, in Asia the disease has remained as a relevant cause of diarrhea outbreaks on swine farms for more than 30 years.

PEDV was reported for the first time in the USA in May 2013 (Stevenson et al., 2013). In the subsequent months the virus spread rapidly across the country and also to other countries in the Americas. Up to now, PED outbreaks have been reported in Mexico, Peru, Dominican Republic, Canada, Colombia and Ecuador. In all these countries as well as in the USA, PEDV have caused major economic losses for many producers by killing millions of piglets.

The main and usually the only obvious clinical sign of PED is a profuse watery diarrhea caused by villous atrophy in the small intestine (Song & Park, 2012; Saif et al., 2012). Clinical signs and histopathological lesions associated with PEDV infection are shared with many other diseases and thus the laboratory detection of the virus is always required. Among the assays that can be used for direct detection of PEDV, we should mention those based in the detection of viral RNA as well as techniques based in the detection of specific PEDV antigens such as enzyme-linked immunosorbent assays (ELISA) and immunohistochemistry techniques. At present, ELISA methods are being abandoned in favor of molecular diagnostic techniques including both conventional reverse transcription polymerase chain reaction (PCR) protocols and real-time reverse transcription PCR for viral detection in fecal samples.

PEDV is a coronavirus, an enveloped virus, that possess a positive-sense, single-stranded RNA genome of about 28.000 nucleotides (Song & Park, 2012;). This genome includes seven known open reading frames (ORF) encoding both structural and non-structural proteins. Approximately two-thirds of the viral genome is composed by two ORF, ORF1a and ORF1b, that encode two non-structural polyproteins that direct genome replication and transcription. The remaining part of the genome includes other five ORF that encoded for four structural proteins and one non-structural protein. Among the structural proteins there are three membrane proteins: S protein or spike protein codified in ORF2, the E protein codified in ORF4 and the M protein codified in ORF5. The fourth structural protein is the nucleocapsid protein or protein N that encapsidates the genomic RNA and is codified in ORF6. On the other hand, ORF3 encodes a non-structural protein that has been reported to be an ion channel protein.

In general, it has been described that genetic diversity is higher among PEDV S, E and ORF3 genes than in M and N genes. According to this, the first targets used for developing diagnostic reverse-transcription PCR assays for the direct detection of PEDV were M and N genes, although recently a number of protocols have also been focused on S gene. High values of sensitivity and specificity for these reverse-transcription assays have been reported, showing their ability to detect isolates from different regions and avoiding false negative results by similar enteric viruses such as transmissible gastroenteritis virus or rotavirus. A very low detection limit has also been described, around 10 TCID50, a value significantly lower than the previously reported for ELISA techniques used to detect PEDV. There are also some immunochromatographic rapid commercial tests that can be used on the farms and provide a rapid result in field conditions. Although their detection limit is clearly lower than in molecular diagnostic methods, the high viral load that is usually shed by affected animals in epidemic outbreaks of PED, allow their use in field conditions. The sensitivity of the assays is more critical when carrying out the diagnosis on farms suffering from endemic PEDV infections in which the virus titer shed in the stool is much lower (Song & Park, 2012).

Direct detection PEDV antigens in sections of the small intestine of piglets by immunohistochemistry assays is also a useful tool (Kim & Chae, 2002). It is usually based on the use of monoclonal antibodies directed against different structural proteins of PEDV and allows characterization of the degree of lesion in the small intestine. It is important to note that these methods require pigs slaughtered in early stages of the infection.

Serology is very useful to determine if an animal or a herd has been previously infected by PEDV, thus a reliable tool for surveillance. However, the number of tests for the detection of specific antibodies of PEDV is limited. There are ELISA or immunofluorescence tests but most of them are in-house assays and information regarding their sensitivity and specificity is scarce. Purified viral antigens have been traditionally used for the coating of the ELISA plates while recent protocols have included recombinant PEDV proteins as target . On the other hand, indirect immunoflorescence tests for PEDV usually used monolayers of VERO cells infected with PEDV. Although agreement between these techniques varies, usually, most of the ELISA techniques have demonstrated to be able to detect specific antibodies against PEDV after 7 days post-inoculation. Sensitivity appears to be somewhat lower for immunofluorescence assays, detecting specific antibodies around 10-12 days post-inoculation (Carvajal et al., 1995a).

The lack of well-established tests for serological diagnosis of PEDV infections have hampered the monitoring of PEDV infection. Although antibody seroprevalence data are scarce, there are a few examples of studies performed in European countries. In Spain, there is no recent data although a cross-sectional study carried out in 1992-1993 reported almost 30% of PEDV seropositive sows and more than 50% of seropositive herds (Carvajal et al., 1995b). In Belgium, specific antibodies against PEDV were detected in 41% of the fattening farms in the first nineties but no seropositive animal was found in 1997 (Pensaert, 2007) nor in 2014 (EFSA, 2014). In UK, a serological survey including 640 blood samples collected at slaughter between 1996-1997 reported a very low seroprevalence, lower than 2% (Pritchard et al., 1999), although a more recent research estimated 9.0% of PEDV-seroprevalence in slaughter pigs (EFSA, 2014). In Denmark, specific antibodies were not detected in any of the serum samples tested in the period 2000-2006 (EFSA, 2014). Recently, a serosurvey in three Italian provinces detected PEDV-specific antibodies in 52% of the pig farms (Alborali et al., 2014).

In most of the countries, only passive surveillance is performed and only those samples collected on farms suffering outbreaks of diarrhea and submitted to diagnostic laboratories or services are processed for the detection of PEDV. Besides, in most of the countries, private laboratories are not required to report the diagnoses performed since the disease is not notifiable. At the moment, PED is not included in the OIE List of Diseases and only France has declare PEDV as a notifiable infection in Europe.

References regarding recent PEDV outbreaks in Europe are scarce. The only well-documented epidemic of PEDV in Europe during the last 10 years was reported in the Po valley, Italy, in an area densely populated of pigs (Martelli et al., 2008). It started in May 2005 and the disease spread through the winter 2005-2006 affecting more than 60 farms and causing diarrhea in pigs of different ages and an increase in pre-weaning mortality (from 8.31% to 11.9%).

On the other hand, sporadic outbreaks have been described in several European countries during the last year. During 2014 and 2015, PEDV infected farms have been reported in Germany (Henninger & Schwarz, 2014; EFSA, 2014), Italy (Alboralli et al., 2014) or the Netherlands although only mild clinical signs have been reported in the affected farms. Sequence analysis of some of the recent European PEDV isolates have demonstrated that these virus have high sequence identity to a new variant PEDV strain, also identified as the S INDEL strain. It has been pointed out that the virulence of this strain is lower than original PEDV isolates from the USA. In Spain, we have monitored several PEDV outbreaks in order to characterize the clinical disease and its economic impact. According to our results, recent PED outbreaks in Spanish swine farms have not been associated with a severe disease and the economic impact has been significantly lower than that reported in PED outbreaks in USA.

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ACTINOBACILLUS PLEUROPNEUMONIAE HETEROGENEITY OF PATHOGENICITY

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INTRODUCTION

Porcine pleuropneumonia caused by Actinobacillus pleuropneumoniae (App) is a contagious disease still reported to cause economic losses worldwide. Main clinical signs of the acute disease are anorexia, depression, fever, coughing, dyspnea, and/or polypnea1. Disease can progress very rapidly and death can occur within a few hours. The disease can also take a chronic form where clinical signs are not as evident but production losses can still suffer and lesions at slaughter (such as adherence, pleuritis and lung abscesses) are usually observed. Finally, in many herds, App is present in a sub-clinical form. This often happens in conventional herds, which may be simultaneously infected not only with different low pathogen serotypes, but also with more virulent serotypes1. In the latter case, outbreaks may suddenly appear in the presence of concomitant diseases or as a consequence of management procedures. Hence, early identification of sub-clinically infected herds is important for the overall control of the disease as carrier animals are one of the main sources of transmission between herds. Clinical disease in its outbreak presentation is relatively well controlled in the USA and Canada, but is still an important problem in many Latin American, Caribbean, Asian and Western/Eastern European countries. In North America, efforts have been mainly directed towards monitoring sub-clinical infections in breeding herds and keeping these free of either the most common virulent App serotypes or all serotypes of App. Occasional clinical cases are observed in high health status herds infected with serotypes not typically associated with disease in the past. The standardization and application of new diagnostic tools have had a tremendous impact on the diagnosis and control/elimination of these sub-clinical infections. Situations where experienced practitioners have had to make difficult decisions on the App status of farms, particularly working with multiplier and nucleus herds, may occur. Exceptionally, some of these situations have ended up in courts of law.

App can be classified based on its in vitro growth characteristics and requirement for factor V (NAD) in biotype I (NAD-dependent) or biotype II (NAD-independent)1. Swine practitioners should be aware that biotype II isolates, sometimes referred to as "atypical" App, may cause clinical problems and they may not be detected in carrier animals by isolation procedures that have been standardized to detect typical biotype I isolates. Presently, there are 15 recognized serotypes (based on the composition of the capsular polysaccharide) of App. Of these, serotypes 1 to 12 and 15 have been described as biotype I App, and serotypes 13 and 14 as the atypical biotype II. However, some European strains of serotypes 2, 4, 7 or 9 can also be atypical biotype II and North American isolates of serotype 13 are biotype I. Some serotypes share common antigens (at the LPS level); these antigens are currently used in serological tests (see below) and are classified together in serological groups: serotypes 1, 9 and 11; serotypes 3, 6, 8, and 15; serotypes 4 and 71.

DISTRIBUTION OF SEROTYPES: THE PROBLEM OF SEROTYPING

Serotyping is still of major interest for App since different serotypes have different virulence potential depending on the geographical origin (see Table 1 for NOT UP-TO-DATE data). Complete serotyping using antibodies with a variety of classical serological tests is provided by very few (if any) specialized reference laboratories in different countries. In addition, cross-reactions observed with antibodies often prevent correct identification of some serotypes. To overcome these problems, several molecular techniques (mainly PCR) have been recently developed2: they can easily be adapted and must be used from now on by first-line diagnostic laboratories. In fact, since the serological surveillance to detect sub-clinically infected animals in conventional herds must be directed towards the most important serotypes in a given country or continent (see below), serotyping data are indispensable. In addition, whole-cell vaccines (bacterins: commercial or autogenous) provide serotype-specific protection1. The prevalence of a serotype should be closely associated to serotypes recovered from diseased animals, which would be an indication of more virulent serotypes in a specific region/country. For App, serotypes that are highly prevalent by serology or by detection of sub-clinically infected carrier animals are often different from those frequently recovered from diseased animals, as recently shown in Canada3. In addition, some serotypes (such as serotypes 6, 8, 12, and 15) present a high degree of transmission, even in the absence of clinical signs, so herds infected by these serotypes are more easily detected by serology, especially when a low number of animals/herd is tested.

Table 1: App serotypes most commonly associated with disease in different areas:
PLEASE NOTE THAT FOR MOST COUNTRIES, NO DATA ARE AVAILABLE FOR THE LAST 20 YEARS

Area	Serotypes
North America	5, 7, 8[a,b]
Latin America[c]/Caribbean[c]	1, 5, 7
Western Europe[c]	2, 9, 11, 8[b], 5, 4[d]
Eastern Europe[c,e]	1, 9, 2, 5, 7, 8
Asia[c]	5, 2, 9, 1
Australia	15, 7, 5

a High health status herds
b PCR-confirmed: United Kingdom
c Data available for very few countries (many: unpublished data)
d Spain only, rare in other countries
e Very few data available: Czech Republic, Croatia, Poland

Historically, serotypes 1 and 5 were considered highly virulent in North America and most clinical cases were caused by these serotypes. This has changed in the last 15 years: App serotype 1 is presently extremely rare, probably as a consequence of the absence of this serotype from animals in most breeding companies. In Canada, 80% of isolates recovered from clinical cases in conventional herds belong to either serotype 5 or serotype 7, in similar proportions. These two serotypes should be considered as the most important serotypes in commercial herds in North America since, usually, clinical cases caused by these two serotypes result in high mortality. High health status herds are sometimes infected by App; however, in general, clinical cases observed in such herds are sporadic cases with relatively low mortality but high morbidity, and caused mostly by serotypes 8, 12 or 15 (unpublished data).

STRAINS BELONGING TO THE SAME SEROTYPE…BUT WITH DIFFERENT VIRULENCE PROPERTIES

As can be observed in Table 2, isolates belonging to a same serotype may vary in their virulence, usually depending on the geographical origin. Animals experimentally infected with French strains of App serotype 2 showed clinical signs a few hours post-inoculation and presented high mortality. Animals infected with Canadian strains of the same serotype did not present any clinical signs, nor fever (unpublished observations). There is a clear difference between the two groups of serotype 2 strains: European strains produce toxins ApxII and ApxIII, whereas Canadian (and US) strains produce ApxII only. However, it is not completely clear if such a difference can explain the lack of virulence. On the other hand, serotype 4 strains are described as highly virulent in Spain, but not so in most other countries. When compared to two strains isolated from clinically healthy animals in Canada (unique isolates recovered in North America so far), no difference concerning toxin production or antigenic characteristics could be detected (unpublished observations). However, simultaneous experimental infections with both types of strains have not yet been done. Finally, serotype 15 strains are clinically important in Australia, but less frequently isolated in other countries, such as Canada, the USA, Mexico, Japan, and Brazil.

Table 2: Examples of variability in virulence of App strains belonging to the same serotype isolated from different geographical locations

Serotype	Origin	Virulence
2	Europe/Canada	High/low
4	Spain/Canada	High/low
15	Australia/others	High/moderate
8	UK/Canada	High/moderate

There are serotypes that are considered to be of low or moderate virulence in most countries (serotypes 3, 10, 14). Serotypes 10 and 14 are almost never recovered from diseased animals. Serotype 3 should be considered a low virulent serotype as it was misidentified with serotype 8 in the past4,5. Finally, although most strains of a given serotype in a specific geographical area would have a similar virulence potential, some exceptions may occur. Genetically confirmed App serotype 1 strains isolated from healthy animals in Canada were shown to be unable to reproduce disease after experimental infection of susceptible animals (unpublished observations).

A SINGLE STRAIN MAY CAUSE CLINICAL PROBLEMS IN ONE HERD…BUT ONLY SUB-CLINICAL INFECTION IN ANOTHER HERD

App still remains a mystery in some aspects. The problem is to predict when clinical problems may develop in a sub-clinically infected herd, which is also one of the reasons why there are still problems with App today. Isolation of a strain belonging to a virulent serotype in healthy carrier animal DOES NOT mean that the strain is non-virulent. Let's use an example: a multiplier serologically positive for a virulent serotype (sub-clinical infection with no disease and no lesions) that sells animals to a conventional herd known to be free of such serotype. If clinical signs appear in the conventional herd…can we suspect the strain to be present in the multiplier? It is not really easy to prove, but it is possible to have a strain not causing any clinical signs or lesions in one herd that is creating problems in other herds with a different health status and/or using a different management system.

As such, App disease may be directly linked to the herd conditions. We mentioned before that North American serotype 2 strains are, in general, non-virulent. We do have, once/twice a year, clinical disease in conventional herds caused by serotype 2 strains in Canada…these strains also produce only one toxin, similar to those described as "non-virulent" after experimental infection!!! (see above). On the other hand, serotypes of relatively low virulence (such as serotype 12) often cause disease in high health status herds free of most important infections, but much less frequently in conventional herds already infected by other pathogens, including other App serotypes and A. suis. It can be hypothesized that in such herds, animals have relatively high levels of antibodies against toxins (produced by other App or A. suis) that perhaps protect animals against low virulence strains. So, in conventional herds, virulent serotypes may use concomitant infections to induce disease…but in high health status herds, the absence of other microorganisms may predispose animals, which are more susceptible to less virulent serotypes. However, this is only a hypothesis and not all is clear and understood as far as this pathogen is concerned.

DETECTION OF SUB-CLINICALLY INFECTED HERDS

Serology is the most cost-effective diagnostic approach to identify herds sub-clinically infected by App1. Originally, the complement fixation was largely used but it has been abandoned in most countries due to its low sensitivity and specificity. This test is still required, oddly, by The People's Republic of China to import live animals into their territory6. Presently, ELISAs are widely used and there are two types of serological tests: a) those that are serotype/serogroup-specific based O-chain LPS7, b) those that detect all

serotypes of App (without discrimination among serotypes) based mainly on ApxIV toxin8. The LPS-ELISA is the most popular and well-studied test to measure antibodies against specific serotypes or serogroups of App: 1/9/11; 2, 3/6/8/15; 4/7; 5, 10, 12, 13, and 14 and it has been widely used in North America, Denmark and France. At least two commercial kits are available. On the other hand, ApxIV, a toxin specific to App, has been used in ELISA test to detect antibodies against all serotypes. A commercial kit is also available. It has been suggested that animals sub-clinically infected by App (in the absence of clinical signs) may induce low levels of antibodies against toxins9. In fact, a relatively low sensitivity was recently reported for this test6.The new fluorescent microbead-based immunoassay test using the same antigen was shown to possess higher sensitivity than the ELISA test10.

LPS- and ApxIV-based ELISA-detecting antibodies against specific serotype/serogroups and all serotypes of App, respectively, are both useful, but they should be used appropriately. Although false-positive results have been exceptionally described11, high health status herds already known to be App-free may decide to use the ApxIV-based test as a surveillance tool. It may be hypothesized that even though this antigen seems to present lower sensitivity than the LPS-ELISA6, an App strain that would have been introduced in a completely naïve herd would induce seroconversion, and, depending on the strain, a detectable seroprevalence. Under these circumstances, the ApxIV-ELISA test should increase its sensitivity. If positive reactions are observed in herds, strong serological data using antigens of different serotypes of App should be available. Otherwise, it would be extremely difficult to evaluate the level of risk of disease if positive reactions are only available by the ApxIV-ELISA, especially for breeders.

More conventional herds are usually infected with one or more serotypes. In some multi-site herds, sows may be infected by two serotypes, but only one of them is transmitted to offspring piglets and found in grower-finisher animals11. The use of the ApxIV test is not useful in these cases, since it is not able to evaluate the risk. As mentioned above, it is important for a country to have reliable data regarding the most important serotypes clinically affecting animals in order to determine which serotypes (or serogroups) should, as a priority, be serologically tested. For example, in France, serotypes 2 and 9 are responsible for more than 80% of clinical cases of pleuropneumonia. Serological surveillance is mostly directed towards those two serotypes in that country (LPS-ELISA), since it would be unnecessary at this point to test for all serotypes. In fact, one study showed that 15 French herds tested positive with the ApxIV-test but were negative with the LPS-ELISA for the serotypes 2 and 1/9/11; in fact, they were shown to be infected by serotypes 3/6/8/15, 4/7, 5, 10, and/or 12, most of them considered as being of relatively low risk for conventional herds11. The recent development of a microsphere-based multiplexed fluorometric immunoassay, which would perform serology for all serotypes of App but identifying the serotype involved, would be a highly interesting diagnostic tool12,13. Further testing and a complete validation of this technology should still be done.

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HOW TO COPE WITH APP IN PIG HERDS?

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INTRODUCTION

Actinobacillus pleuropneumoniae (App) is the causative agent of pleuropneumonia in pigs. This disease is a major concern in most pig-producing areas in the world.

Most of the time, infection doesn't induce any clinical sign. The severity of the disease depends on several factors: the pathogenicity of the bacteria (and we know that pathogenicity of different serotypes is "country-dependent"), the co-infections with other swine pathogens, the quality of the ventilation system, the batch management compliance …

In acute cases, this pathology is really stressful for the farmer because of its contagiosity and the high level of mortality.

This presentation will briefly review my field experience of prevention and treatment of App in our French context.

FRENCH SITUATION WITH APP

In France, the most virulent serotypes of App are serotype 2 and serotype 9/11. In the biggest French laboratories of vet diagnostic, they are the most frequently isolated in pathologic lungs.

Like in other countries, clinical signs in positive herds are present either recursively or occasionally when co-infection (especially with swine influenza or Porcine Reproductive and Respiratory Syndrome virus (PRRS)) occurs. Acute cases are observed mainly in finishers with sudden deaths, fever, rapid and abnormal breath. Sometimes, we observe only sudden death in the farm just before leaving to slaughterhouse or total seizures for acute pleuritis after slaughter.

Subclinical cases can be seen with serotypes 2 and 9/11 but also with "minor virulent" serotypes (especially 3-6-8 and 4-7). In these cases, only cough in the farm and partial seizures for pleuritis and/or abscesses are common.

DIAGNOSTIC

Several diagnostic tools are available in France.

Bacteriology is the diagnostic of certainty in pleuropneumonia affected lungs. French isolates of App are sensitive to most of the antibiotics currently used even if we observe differences in sensitivity between serotypes. Isolation of the bacteria and determination of its serotype (minimum in two different animals in two different clinical outbreaks) are essential before the implementation of an autogenous vaccination program.

Histology is not a common diagnostic tool for this infection. It can be useful in parallel of bacteriology if it is not possible to isolate the bacteria. Histological lesions of App are quite suggestive.

Serology is used for epidemiologic survey especially in nucleus herds. Depending on the known status of the farm, serological kits detecting all serotypes could be used in App-free herds. In France, all nucleus herds are regularly controlled at a minimum for serotypes 2 and group 1-9-11.

Serologic tests specific of serotype (mainly Biovet and IDVET in my experience) are also useful in order to determine the moment of the infection of the pigs in a batch, to screen the decrease of the level of maternal antibodies or to state about the serologic response after vaccination. Sometimes, we have to be prudent in the interpretation of serological tests, especially in sow herds.

PCR is useful in affected lungs in case of failure of bacteria isolation and also in tonsils biopsy when, for example, serologic results are doubtful in nucleus herds.

PREVENTION

Only one commercial toxin-based vaccine is available in France and licensed for piglets. Different French labs can produce autogenous vaccines for sows (two shots vaccines / with alumina or oiled adjuvant) or for piglets (one or two shots vaccines / with alumina or oiled adjuvant). French authorities give permission to practitioners to use these vaccines because of the absence of commercial bacterin vaccines.
In table 1, I give my opinion on the advantages and disadvantages of commercial and autogenous vaccines.

Table 1: Advantages and disadvantages of commercial and autogenous vaccines in my experience.

	Advantages	Disadvantages
Commercial vaccine	Effective against all serotypes (reduction of clinical signs). Possibility to screen the infection by serology in vaccinated animals (but be careful with the interpretation especially for some serotypes).	No effect on bacterial circulation in the herd (neither vertical nor horizontal transmission). Adverse reactions in some animal in some farms. Only licensed for piglets.
Autogenous vaccines	Effect on vertical and horizontal transmission. Possible use in sows and piglets.	Effective against only one serotype. Sometimes adverse reactions especially with oiled adjuvant (local and general). Not possible to screen the infection in vaccinated animals.

In acute cases, if necessary, I recommend to vaccinate the piglets with the commercial vaccine during the time needed to ensure the diagnostic (determination of the serotype involved in the clinical problems) and to produce an autogenous vaccine. After that, in chronically infected herds, I implement the vaccination of sows and piglets with an autogenous vaccine. In my opinion, vaccination of the sows is necessary in order to control vertical transmission with the objective to stop the vaccination of piglets.

Of course, measures like audit of the internal biosecurity (especially human and pig flows in the farm) and management of co-infections are essential for a long term effect in the herd.

When clinical problems are under control (no clinical signs in the herd, no seizures at the slaughterhouse), it is useful to make a serological screening in sentinels gilts (not vaccinated and introduced for nose to nose contact in the sow herd) and piglets (not vaccinated pigs in the finishing unit) before stopping the vaccination.

Last but not least, we are strongly involved in our practice in the management and the control of the air quality in swine herds. As everybody knows, air flow and air renewal in pig rooms are considered as major triggers of clinical outbreaks of App.

TREATMENT

Swine vets of the different French technical associations promoted in 2014 a national good practice guide for antibiotics prescription in pig production. The stated objective of these guidelines is to limit the use of antimicrobials, targeting in particular systematic/collective treatments or treatments with critical antibiotics. Treatment of App is described in one specific form.
It is detailed in Table 2.

Table 2: Recommendations for App treatment (part of the National Guidelines for antibiotic prescription in swine)

Selection of active ingredients	**Treated animals category**
First -line treatments (amoxicillin, phenicols, peni-strep combination, cyclins or sulfamids associated with trimethoprim) can be applied without prior antibiotic susceptibility testing. In case of failure or relapse, macrolides may be used without analysis. Except if the withdrawal time before slaughter makes necessary the use of fluoroquinolones or cephalosporins, a preliminary analysis will be required before their prescription.	When the morbidity exceeds 5 % and/or the disease is recurrent in a herd, a collective treatment is necessary. An individual treatment will be sufficient in other cases. The transition to a second-line treatment is recommended when the cure rate is under 75 %. A systematic oral treatment or the use of a critical antibiotic will need to be re-evaluated twice a year.

CONCLUSION

Many questions about App infection have no answer at this time especially regarding infection dynamic at the herd level and effectiveness of passive and active immunity. Despite this, most of the time, in the field and to be optimistic, we have good experiences in management of App outbreaks. To do it, we have to combine different management practices (internal and external biosecurities, enhancement of immunity, ventilation system audit ..) to limit vertical and horizontal transmission of the agent and to reduce clinical and economical impacts of the disease. What a challenge for swine vets !

oral
PRESENTATIONS

oral
PRESENTATIONS

RESIDENTS' SESSION

Wednesday, April 22nd > 15:30-17:30

01 **Longitudinal co-evolution of streptococcus suis serotype and antimicrobial resistance in uk pig farms**
J. Hernández-García, G. Zou, J. Wang, O. Oshota, H. Jin, S. Peters, T. Wileman, I.Dennis, F. Alcock, J. Thomson, D. Maskell, A. Tucker

02 **IgG concentration of new born piglets in Western Europe**
R. Jansen, L. Marchal

03 **Acute outbreak of porcine epidemic diarrhea in a piglet producing farm in southwestern Germany**
A. Ladinig, J. Stadler, S. Zöls, R. Fux, S. Blome, D. Hanke, H. Weissenböck, M. Ritzmann

04 **Use of buserelin in nulliparous and multiparous sows: Effect on the reproductive system and weight variability of the resulting offspring**
A. Vela Bello, V. Falceto, J. Segalés, E. Mateu

05 **Salmonella environmental contamination in slaughter pigs: a real risk ?**
M. Leblanc-Maridor, C. Belloc, F. Le Gall, M. Denis, F. Paboeuf, B. Minvielle

06 **Effects of biosecurity measures on the antimicrobial usage in German farrow-to-finish farms**
S. Loesken, M. Postma, E.Grosse Beilage

01

LONGITUDINAL CO-EVOLUTION OF STREPTOCOCCUS SUIS SEROTYPE AND ANTIMICROBIAL RESISTANCE IN UK PIG FARMS.

J. Hernández-García (1), G. Zou (2), J. Wang (1), O. Oshota (1), H. Jin (2), S. Peters (1), T. Wileman (1), I.Dennis (3), F. Alcock (4), J. Thomson (4), D. Maskell (1), A. Tucker (1)

(1) Department of Veterinary Medicine, University of Cambridge, Cambridge, UK; (2) Huazhong Agricultural University, Wuhan, China; (3) BQP, Stradbroke, UK; (4) SAC (Consulting) Veterinary Services, Bush State, Penicuik, Midlothian, Scotland, UK

Introduction. A longitudinal survey was done to study the diversity of serotypes (ST) and antimicrobial resistance (AMR) in Streptococcus suis (SS) isolates from healthy pigs on UK farms with a history of SS disease.
Methods: Tonsil scrapes were collected twice from 5 all-in all-out wean-to-finish farms at five weeks old (W5) and at 20 weeks old (W20), from 25 individuals randomly selected. Swabs were cultured on Columbia Sheep Blood Agar and Edwards medium for 24 hours aerobically at 37°C. Suspect (β/β haemolytic) colonies were sub-cultured and biochemically profiled using API20Strep kits. Antibiotic disc sensitivity testing was done using ISO-Sensitest agar against a profile of 11 antibiotics. Serotyping was performed in silico by analysing the serotype-specific capsule biosynthesis genes in the whole genome sequence of isolates.

Results: At W5, SS was isolated in 59% (74/125) of samples and at W20 in 28% (35/125) of samples. A total of 232 isolates were obtained and tested for antibiotic disc sensitivity. The percentage of resistant isolates at W5 and W20 respectively, taking all farms together, was: tetracycline 90% and 97%, neomycin 37% and 48%, trimethoprim + sulphamethoxazol 33% and 56%, lincomycin 41% and 61%, penicillin 19% and 23%, ceftiofur 2% and 5% and tylosine 47% and 65%, minimal variation for ampicillin 1% and 3%, amoxicillin+clavulanic acid 2% and 0% and florfenicol 1% and 0%, No resistance was detected for enrofloxacin. Over all, strains at W20 presented more resistances (mean 4.0/isolate, n=61 isolates) than at W5 (mean 3.1/isolate, n=171) ($p<0.01$).
Sixteen different SS STs were identified and there was frequent detection of multiple STs in an individual pig. A greater diversity of ST was found at W5 (average 6.6 ST/farm) than W20 (4.6 ST/farm). On thirteen occasions the same ST was found present at W20 on the same farm as at W5; for this sub-population, the number of antibiotics against which they were shown to be resistant increased from an average of 2.6 resistances/isolate at W5 to 4.7 at W20 ($p<0.01$). On ten occasions, STs were detected at W20 that were not detected at W5 in the same farm, the mean number of AMR for these W20 STs was 3.7 resistances/isolate.

Conclusions: Even allowing for limitations due to sample size, the prevalence of SS isolates declined between W5 and W20, accompanied by a decline in ST diversity. Conversely, the prevalence of AMR increased between W5 and W20 and included antibiotics that had not been used on the farm, indicating co-selection of AMR. These data emphasise the value of on-going monitoring for AMR, and of emerging methods for typing SS that could result in improved preventive programs.

NOTES

02

IGG CONCENTRATION OF NEW BORN PIGLETS IN WESTERN EUROPE.

R. Jansen (1), L. Marchal (1)
(1) ForFarmers, Lochem, Netherlands

Introduction. Early and adequate colostrum uptake of young born piglets is important for a good transfer of maternal immunity since maternal antibodies can't cross the placenta. One way to check the quality of colostrum management is to measure the serum antibodies of new-born piglets. A successful transfer of maternal antibodies is important for the immune status of weaned piglets and plays a vital role in the reduction of preweaning mortality and the use of antibiotics.

Material and methods. Starting September 2013, Farmers in the Netherlands and in Belgium were given the opportunity to measure the blood IgG concentration of new born piglets. A sample protocol was provided. Briefly: six litters (gilts together with older parity sows) in the age between minimum 24 hours and maximum 5 days were selected (cross fostered litters to be excluded). Six piglets were selected estimated on visible weight differences: 2 light weight piglets, 2 middle weight piglets and 2 heavy weight piglets. The weights was determined at sampling. A blood sample was obtained by jugular venepuncture. Blood samples were analysed for IgG using the immunocrit assay which was validated by our own ForFarmers lab with an increased centrifugation time of 10 minutes.

Results. In total 220 farms and 7828 blood samples were analysed with an average IgG concentration of 37.2 mg/ml. From 71 farms (2271 piglets) growth was followed until weaning (227 gram/day). Piglets born out of gilts (n=1785) had a significant ($p<0.001$) lower IgG concentration (34.3 mg/ml) compared to elder parity sows (n=6043; 38.0 mg/ml). Litter size was negative correlated with piglets IgG for gilts and fourth parity sows ($p<0.005$) but with a very poor Rsq of 0.01. In total 6.5% of the piglets had an IgG concentrations below 15 mg/ml which is associated with increased preweaning mortality. The 20% lightest piglets (<1120 gram) had significant ($p<0.005$) odds ratio's (OR) of 4.03 for having a too low IgG concentration (<10 mg/ml) and an OR of 3.00 for having a low concentration of IgG (<15 mg/ml) compared to the other piglets. Growth of the piglets was positively correlated with weight at bloodsampling (Rsq of 0.16; n=2271). Piglets with a low IgG (<20mg/ml) had a significant lower daily growth until weaning compared to the piglets with a high IgG concentration (>=20 mg/ml). There was no seasonal or outside temperature influence on the IgG concentration.

Conclusion.This is the first large scale report of neonatal piglets IgG concentration under practical circumstances. Our findings clearly indicate that offspring of gilts and lightweight piglets (<1120 gram) are at risk for low IgG concentrations.

NOTES

03

ACUTE OUTBREAK OF PORCINE EPIDEMIC DIARRHEA IN A PIGLET PRODUCING FARM IN SOUTHWESTERN GERMANY.

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Introduction. An acute outbreak of porcine epidemic diarrhea (PED) is described for a farrow-nursery farm in southwestern Germany.

Case Presentation. Clinical signs started around October 30 2014, when a group of sows was anorectic for the first seven days after weaning and showed signs of yellowish, watery diarrhea. Two to 3 days after the clinical signs were observed in the breeding area, diarrhea and reduced to absent feed intake occurred in the farrowing unit in sows which were nursing piglets of about 2.5 weeks of age. Suckling piglets started with mild pasty, yellowish diarrhea but were not severely affected and only 3 piglets died before weaning. Piglets from the next farrowing batch, which were born between November 7 and 9, were acutely affected and started to show yellowish, pasty to watery diarrhea about 2 days after birth. Individual piglets also showed vomiting and severe dehydration. First piglet losses occurred on November 10 and 67.6% (291/431) of piglets from this farrowing group died or had to be euthanized before weaning. In total, piglets from 30 out of 35 litters were affected. In addition to signs in piglets, nursing sows had diarrhea and severely reduced feed intake. Pasty to watery diarrhea also occurred in boars and sows in the gestation unit which are housed in small groups of 6 to 8 animals on slatted floor. Clinical signs in the nursery mainly consisted of severely reduced feed intake and diarrhea in individual pigs. During the acute outbreak, mortality increased to 7.1 % in the nursery from <5% before the outbreak. At necropsy, suckling piglets showed typical, thin intestinal walls in the small intestines but partially also in the colon region. Intestinal contents were yellow, foamy to watery or were completely absent. Histologic lesions were described as shortened, blunted and fused villi with vacuolation of enterocytes. Laboratory investigations confirmed the presence of PED virus (PEDV) in fecal samples from suckling piglets and sows by RT-qPCR. Full length genome sequencing showed high similarity to recently described S INDEL PEDV strains (99.4% identity with the S INDEL strain OH 851).

Conclusions. This case report indicates that PEDV strains similar to S INDEL PEDV, which was reported to circulate in the US and induce milder clinical disease compared to highly pathogenic PEDV, can be found in Germany and that those strains can induce severe clinical signs and high mortality rates in suckling piglets.

NOTES

04

USE OF BUSERELIN IN NULLIPAROUS AND MULTIPAROUS SOWS: EFFECT ON THE REPRODUCTIVE SYSTEM AND WEIGHT VARIABILITY OF THE RESULTING OFFSPRING.

A. Vela Bello(1), V. Falceto (2), J. Segalés (3), E. Mateu (3)

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Introduction. Increasing prolificacy in pig farms is a challenge for swine production, because selecting for increased litter size has a direct impact on the variability of the piglet´s weight.
The present study includes two objectives: to determine how buserelin affects the reproductive tract and to evaluate the relationship between the administration of buserelin and piglet weight and litter uniformity.

Material and Methods. In the first experiment, reproductive structures were compared between two gilt groups: Treated group (n=19) was administered a dose of 10µg of buserelin in gilts 120 hours after the last dose of altrenogest «Regumate®» and compared with non-treated Control (n=18).
In the second, treated sows were administered 10µg of buserelin (2.5 ml «Porceptal®»), 85 hours after weaning (n=30) vs non-treated Control group (n=30). Treated sows were inseminated with a single fixed time AI, compared to the untreated group that was inseminated twice. In both cases the semen was from the same ejaculate. Birth weight and homogeneity of the resulting litter was evaluated.

Results. Progestogens were found to be an effective method of oestrus synchronization in gilts. An injection of was effective in reducing variability in follicle size and significantly increased the number of follicles above 6mm (P=0,05). Furthermore, the size (Buserelin=306cm Vs Control=238cm) and weight (Buserelin=685g Vs Control=238g) of the uterine horns was significantly greater in the treated group than the control (Size-P=0,001 & weight-P=0,026). Similarly, the buserelin sows had a significantly greater average piglet weight at birth (1,63g), regardless of size, (P=0,001) than the control group (1,49g). This difference remained in favor of buserelin group throughout lactation and nursery with an average daily weight gain in lactation and nursery that was higher in the buserelin group (Lac=215g/Nurs= 311g) than the control group (Lac=198g/Nurs= 288g).

Conclusion.
1. The use of progestogens is an effective synchronization of oestrus in gilts method.
2. The size and weight of the uterine horns was statistically higher in the group treated with buserelin group than in the control group.
3. The application of buserelin in gilts was effective in reducing variability follicle size and to increase the number of follicles with above 6mm.
4. The application of buserelin in weaned sows had significant differences with the control group on the average birth weight of piglets and Buserelin group had higher average weight at 21 and 60 days after birth.

NOTES

05

SALMONELLA ENVIRONMENTAL CONTAMINATION IN SLAUGHTER PIGS: A REAL RISK?

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Introduction. Salmonella is a leading cause of foodborne illness worldwide and the consumption of pork meat is a major source for human infection. Pigs get infected through oral intake of Salmonella and they can carry this bacterium asymptomatically in their tonsils, gut and gut-associated lymphoid tissue for months resulting in so called Salmonella carriers. During periods of stress, re-excretion may occur. In this way, carriers are a permanent potential source of infection for other animals, including humans. Stress factors can occur during the fattening period, but also prior to slaughter, for instance during transport to the slaughterhouse or during the stay in the lairage. This study aims at investigating Salmonella environmental contamination of trucks and lairages in two French pig slaughterhouses. In parallel, we evaluate the efficiency of an improved cleaning and disinfection protocol to reduce Salmonella environmental contamination.

Materials and Methods. During 7 sampling days, the lairages of two pig slaughterhouses were sampled twice when pigs were present and once at the end of the week after the cleaning protocols. Samples were collected by swabbing floor surfaces of 1m2. In parallel, in each slaughterhouse, six trucks per day were randomly selected and environmental samples were collected at their arrival at the slaughterhouse (transport duration between 30 and 180 min) and after the cleaning procedures. Salmonella occurrence, the level of contamination (semi-quantitative bacteriological method) and the variety of serotypes were determined. The efficiency of the cleaning and disinfection procedures on the presence of Salmonella was also estimated.

Results. Salmonella was isolated in 90 to 100% of the lairages samples when pigs were present (contamination levels >104 UFC/m2) and in 30 to 60% of the trucks samples (contamination levels from 10 to >104 UFC/m2). An improved cleaning and disinfection procedure reduced efficiently the occurrence and the level of contamination in the trucks (almost 100%) compared to a simple wash with cold water (no effect), more partially in the lairages.

Conclusion. The high levels of contamination in holding pens point them out as the major risk sources of Salmonella spreading within the slaughterhouses. The waiting period in the lairage of at least two hours contains a substantial risk for slaughter pigs to become infected with Salmonella as the cleaning and disinfection procedures still was not satisfactory. Nevertheless, this study showed the importance of a good cleaning and disinfection protocol to decrease the level of contamination or eliminate the bacteria in the trucks used for the transport even if it is not always sufficient to eliminate the substantial risk for slaughter pigs in the lairage.

NOTES

06

EFFECTS OF BIOSECURITY MEASURES ON THE ANTIMICROBIAL USAGE IN GERMAN FARROW–TO–FINISH FARMS

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Introduction. The antimicrobial usage in food producing animals is of major concern. Thus the evaluation of strategies that will reduce the antimicrobial usage require profound knowledge of factors that are consistently and strongly correlated with antimicrobial usage. Data on biosecurity and herd management practices are crucial factors to investigate the antimicrobial consumption. However, there is limited quantitative data available which describe the effects of biosecurity and management factors that might have an impact on the amount of antimicrobial treatments. The aim of this study, conducted within the MINAPIG (www.minapig.eu) research project, was to describe in detail the biosecurity and compare it with the antimicrobial usage in German farrow-to-finish farms.

Materials and Methods. To determine the relationship between herd characteristics and antimicrobial consumption data was collected on 66 German farrow-to-finish herds. Only herds with 100 sows and 500 fatteners at minimum were included. The selected herds were visited by one investigator. Data on biosecurity, management practices and preventive treatments such as vaccination, deworming and the use of anti-inflammatory drugs were collected. A risk based scoring system (www.biocheck.ugent.be), was used to quantify the level of biosecurity on pig farms. It calculates a score for external and internal biosecurity. As for the biosecurity, all treatment data was collected in the single herds 12 months retrospectively. This data was then used as input for a web based tool (www.abcheck.ugent.be) that enables the quantification of antimicrobial usage by calculating the treatment incidence. The treatment incidence expresses the percentage of animals which are treated with one daily dose of antimicrobials. Moreover, it is based on a standardized life expectancy of pigs from birth till slaughter. This period was set to 200 days.

Results. The external biosecurity score was in mean 70% (min 50%, max 98%) whereas the internal biosecurity score was on average 55% (min 37%, max 82%). The overall biosecurity score was on average 64% (min 47%, max 82%). Farms with a high biosecurity score had more weaned piglets per sow and year. Moreover, a higher overall biosecurity score was associated with a lower treatment incidence.

Conclusion. These results indicate that the biosecurity level is generally an important indicator to evaluate and subsequently improve performance and health status in pig herds. Moreover this study implies associations between treatment incidence and level of external and internal biosecurity.

NOTES

WELFARE AND NUTRITION

Wednesday, April 22nd > 15:30-17:30

07 Are tail biters more anxious? A study based on morphological, behavioral and physiological characteristics

08 Decreasing the new neonatal porcine diarrhea (NNPD) by limiting the muscle losses of the gilts during the last month of gestation

09 Effects of various phosphorus levels in diets for piglets on growth, intestinal fermentation, mineralisation of bones, molecular features and metabolic routes – first results

MISCELLANEOUS

Wednesday, April 22nd > 15:30-17:30

010 The effects of hemoglobin levels on growth rates in pigs

011 The UK Voluntary Monitoring Systems for Pig Health and Welfare: comparison of disease prevalence from 2005 to 2012.

012 Comparison of different methods to measure IgG in sow colostrum

07

ARE TAIL BITERS MORE ANXIOUS? A STUDY BASED ON MORPHOLOGICAL, BEHAVIORAL AND PHYSIOLOGICAL CHARACTERISTICS

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(1) Cooperl Arc Atlantique, Lamballe, France; (2) Selas Hunaudaye, Plestan, France

Introduction. Tail biting is an abnormal behavior characterized by repeated biting of one pig towards a pen mate. This behavioral disorder can cause poor animal welfare and economic losses (additional work, reduced weight gain, condemnations at slaughter). If the ban of tail docking could be the next step to improve the welfare of pigs, mastering of tail biting is essential. Even if pigs can be in the same pen and in the same environmental conditions, only some of them develop tail biting. In this study, pig's individual characteristics were considered in order to clarify if the most anxious pigs, because of their own life experience, are more likely to become biters.

Materials and Methods. In three nucleus pig-breeding farms, 3698 pigs were characterized (sex, birth and weaning weights, litter size, cross-fostering, teat fidelity, fights at the udder, age at weaning, ADG, social behaviors in farrowing, growing and finishing pens). These elements have been linked with biters involved in tail biting outbreaks. In the slaughterhouse, 54 adrenal glands were extracted from carcasses and were weighed. 19 adrenal glands were removed from pigs determined previously as biter and 35 from control pigs (neither biter nor bitten). The carcass weight was also collected. Student's t-test, analyses of variance, chi-square test and generalized linear model were used.

Results. Results of the present study revealed that biters are lighter at birth ($P=0.06$) and at weaning ($P<0.05$), they weigh an average of 100g less at birth and 200g less at weaning than control pigs. They show less suckling stability (72.5%; $P<0.05$) compared to control pigs (87.5%), moreover, biters fight more at the udder than control pigs (5.9% vs 0.9; $P<0.05$). Results from slaughterhouse show that the status of biter affects the weight of adrenal glands ($P<0.01$) and the relative adrenal weight to carcass weight ($P<0.001$). The biters had significantly heavier adrenal glands (2.94 ± 0,4g) compared to control pigs (2.55 ± 0,4g). The relative adrenal weight to carcass weight is also significantly higher for biters (0.033 ± 0,005g vs 0.028 ± 0,005g).

Conclusion.This study gives some news insights on characteristics of biters and a level for breeders in maternity. The neonatal period appears to play a significant role in the development of tail biter behavior. Because of their small size, lighter piglets may have difficulties to access to the sow's udder and to keep a teat which generate stress. Through measurement of adrenals' weight, the findings confirm that pigs which initiate bites seem more anxious than control pigs. Stressful experiences may predispose pigs to develop anxiety that manifests, in discomfort situation, by a behavioral disorder like tail biting.

NOTES

08

DECREASING THE NEW NEONATAL PORCINE DIARRHEA (NNPD) BY LIMITING THE MUSCLE LOSSES OF THE GILTS DURING THE LAST MONTH OF GESTATION

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This study consisted in investigating the influence of the growth dynamic of gilts from conception to farrowing on reproductive performances and quality of piglets. This study was performed in a conventional farm and included 279 gilts from a three-way cross Sino-European line delivered in one farm at the same age of 159 days, inseminated at 243 days, fed and housed similarly. Weighing was performed at AI and at farrowing. Backfat (BF) and loin muscle (LM) were measured at the following stages: AI, 28 days after AI, 21 days before farrowing and at farrowing. Duration of gestation, litter size (total born) and the presence of NNPD from birth to day 15 were recorded. High prolific gilts (TB $\geq$ 15) (n=114) had higher LM losses during the last month of gestation compared to low prolific gilts (TB $\leq$ 12) (n=54) (ΔLM = -0.9 $\pm$ 1.9 vs -0.1 $\pm$0.5 mm, respectively, P< 0.05). Among high prolific gilts (15 $\leq$ TB $\leq$ 17), those having higher LM losses during the last month (ΔLM < -2 mm) (n= 21) were more affected by NNPD compared to gilts having no LM losses (ΔLM > 0 mm) (n=34) (57% vs 32% of the litters were affected by NNPD, respectively, P < 0.05). These results show the interest of limiting excessive muscle losses during the last month of gestation to reduce NNPD.

NOTES

09

EFFECTS OF VARIOUS PHOSPHORUS LEVELS IN DIETS FOR PIGLETS ON GROWTH, INTESTINAL FERMENTATION, MINERALISATION OF BONES, MOLECULAR FEATURES AND METABOLIC ROUTES – FIRST RESULTS

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Introduction: Phosphorus (P) is an essential element in pig nutrition, which influences endogenous metabolic pathways. Therefore, the aim of this pilot study was to investigate the impact of various P-levels on performance, metabolism, and bone characteristics as well as molecular features and metabolic routes responsible for the dietary P utilisation.
Material and Method: 18 weaned 28d old, castrated male and female piglets were divided into 3 groups. The piglets with were individually caged on a flat deck and fed a pelleted diet ad libitum for 5 weeks. The calculated digestible P content was set at 0.29% (P-low), 0.48% (P-medium), and 0.66% (P-high), respectively, and the calcium (C) content at 1.05% in each group. Parameters like growth, intestinal fermentation and bone metabolism were determined. Gene expression was assessed in blood samples.

Results: The analyzed P content corresponded to the calculated values, whereas the analysed calcium content in group P-low and P-medium amounted 0.9% and was slightly lower than calculated. Despite of the nutritional insults, neither differences in body weight gain nor feed intake were observed. Only in the 2nd week feed conversion was temporarily elevated due to P-deficiency and acetic acid content in faeces was significantly reduced ($p < 0.05$). In P-low the levels of serum phosphate and parathyroid hormone were lowered, but calcium and vitamin D were increased. The P-supplementation above requirements persistently affected animals as shown by microstructural parameters, including increased trabecular bone mineral density, trabecular number and Structure Model Index. The reorganization of osseous tissue was reflected by altered abundances of transcripts associated with bone morphology.

Conclusion: The study revealed considerable organismal plasticity of the animals in response to modulated P-supplementation and functional biodiversity regarding coping with dietary challenges. Genes found to be differentially expressed due to variable P-supply are involved in pathways relevant to P-utilisation and are potential candidate genes for improved P-efficiency.
The results are supported by “ScienceCampus Phosphorous Research Rostock”.

NOTES

010

THE EFFECTS OF HEMOGLOBIN LEVELS ON GROWTH RATES IN PIGS

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Introduction: Iron deficiency in piglets has continually been an issue in the swine industry. Piglet's low iron stores at birth (40-50 mg) and low amounts of iron absorbed from sow's milk (1 mg/day), increase piglet's risk of developing an iron deficiency during lactation. Confounding these factors is the rapid growth rate of pigs, which quickly depletes their low stores. Iron deficiency leads to an anemia characterized by a lack of iron-containing hemoglobin (Hg) in red blood cells. Without Hg there is a decreased oxygen carrying capacity of blood. Hg levels less than 9 g/dl characterize a severe anemia; levels between 9 g/dl and 11 g/dl typify subclinical anemia. Prior to weaning, the heaviest pigs have lower Hg levels due to faster depletion of stores. Once in a negative iron balance, growth is stunted. Studies show that iron requirements during lactation are up to 67 mg/kg weight gain. Thus, the 200 mg iron injection at birth will theoretically yield only 4 kg of growth during lactation. The objective of this study was to survey anemia through the nursery phase to determine if Hg levels have an impact on weight gain during this stage.
Materials/Methods: Three piglets (one large, one small, one random) were selected from 34 different litters on one sow farm (102 piglets total). The piglets used were given 100 mg iron injections at 1 and 4 days of age (200 mg total). Weight and hemoglobin levels were recorded at three different times; day 20 (prior to weaning), day 41 and day 64 (prior to leaving nursery). Blood was collected in EDTA tubes. Hg levels were measured using a HemoCue analyzer. Weights were measured using calibrated scales. Data was plotted visually and statistical analysis was done using SAS.

Results: At weaning, 78% of pigs were subclinically anemic with 35.6% being severely anemic. The mean Hg level was 9.5 g/dl with a SD of 1.7. Large pigs had lower Hg levels than those of average and smaller size. At day 41, 92% of pigs were subclinically anemic with 16% being severely anemic. The mean Hg was 9.75 g/dl with a SD of 1. At day 64, 74.2% of pigs were subclinically anemic with 21.5% being severely anemic. The mean Hg was 9.5 g/dl with a SD of 0.9. Using PROC REG, a SAS statistical analysis program, we found that total weight gain was higher for pigs with higher Hg levels ($p=0.014$). For each 1 g/dl increase in Hg, the average total weight gain increased by 1206.9 grams. However, the adjusted r-squared value is quite low (0.0537), meaning this model does not explain most of the variability in the data. The Hg levels at times 2 and 3 had the most significant linear relationship to total weight gain ($p=0.007$ and $p<0.0001$). For each 1 g/dl increase in Hg at time 3, the average total weight gain increased by 2070.9 grams.

Conclusion: This data reveals that anemia in piglets is an area of concern for modern swine production. Though the data has variability, the research shows that preventing anemia during lactation allows for improved gain through the nursery phase. Continued anemia, despite the industry's modern genetics and production techniques, creates a need to understand if changes in iron administration protocols are needed to better meet piglets' growth needs

NOTES

011

THE UK VOLUNTARY MONITORING SYSTEMS FOR PIG HEALTH AND WELFARE: COMPARISON OF DISEASE PREVALENCE FROM 2005 TO 2012.

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Introduction. Surveillance of animal diseases provides information essential for the protection of animal health. The voluntary pig health schemes, implemented in the United Kingdom, are integrated systems which capture information on different macroscopic conditions detected in slaughter pigs. Those schemes are the Wholesome Pigs Scotland in Scotland, the BPEX Pig Health Scheme in England and Wales (E&W) and the Pig Regen health and welfare checks done in Northern Ireland (NI). This study compares and evaluates the prevalence, seasonal variations and year trends of eight conditions assessed by these Pig Health Schemes.

Material and Methods. Data collected by these schemes between July 2005 and December 2012, from 4,420 pig units in 46,321 batches of pigs supplied to 28 abattoirs spread across the UK, were used in this investigation. In total 2,061,779 pigs were examined. The eight conditions assessed were enzootic pneumonia-like (EP-like) lesions, pleurisy, pleuropneumonia (PP) lesions, abscesses in the lung, pericarditis, milk spots (MS), papular dermatitis (PD) and tail biting (TB).
Two approaches were used in this study. The semi-parametric generalised additive model was used to obtain the smooth effects of season and trend for each condition in order to visualise the shape of these effects and compare overall prevalence estimates among schemes. The generalised linear mixed model was then used to quantify these effects and compare differences between annual trend and seasonal effects across schemes.

Results. An increase in the prevalence of EP-like lesions was observed in Scotland and E&W since 2009, while a decrease was observed in NI over the years of the scheme. Pleurisy prevalence has increased since 2010 in all three schemes, whist PP has been decreasing. Prevalence of abscesses in the lung has decreased in E&W and NI but has increased in Scotland. An increase in the prevalence of pericarditis was observed in all three schemes, while a decrease was observed for MS and PD. TB has been on the increase in Scotland and NI while decreasing in E&W. Seasonal effects were observed for all schemes and conditions although were more evident for MS and PD.

Conclusion. Similarity of schemes allows for trend comparisons to be made between countries over time in gross pathology, especially between countries with a similar profile in terms of pig production. This enables early detection of prevalence increases which allows the industry and researchers to investigate the reasons behind them. These schemes are, therefore, valuable assets for endemic disease surveillance, emerging disease early warning and for welfare outcomes monitoring.

NOTES

012

COMPARISON OF DIFFERENT METHODS TO MEASURE IGG IN SOW COLOSTRUM

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Introduction. There is increasing interest in the production and the carry over of maternal antibodies towards the sows offspring. A good reliable techniques to measure the IgG content in colostrum is therefore important. In the current scientific literature several methods are used to measure colostrum IgG resulting in different analysed IgG concentrations. In our own research we were confronted with difficulties in using an ELISA to measure IgG concentrations in colostrum. In bovine colostrum Radial immunodiffusion (RID) is the current golden standard. However, as far a we know, there is no published data of a comparison of methods to measure IgG in porcine colostrum.

Material and methods. In an earlier study in 2013 we obtained colostrum from 70 sows (Topigs 20) directly after birth of the first piglet. Samples were measured for IgG with a commercial available ELISA (Bethyl laboratories) and protein (spectrophotometric determination). Colostrum samples were stored at -32 °C and were thawed again for the determination of IgG with the other methods using a refractometer and radial immunodiffusion (RID). To homogenize fat, colostrum was warmed up for 30 minutes at 36˚C and cooled back towards 20˚C. The Breaking index percentage (BRIX) was measured using a calibrated refractometer (VWR, 0-50% brix). Colostrum samples were diluted 50% with demineralized water to measure IgG with a RID kit (Triple J farms swine IgG kit). Calibrated wiretrol pippets (Drummond) were used to add 5µl of sample to the wells. After 24 hours incubation at 22˚ C end point reading was done using a digital calliper. Samples exceeding the reference sample concentrations were diluted to 25% colostrum solution and measured again. Data between methods were compared using linear regression models (SPSS).

Results. Total protein of the colostrum was 174 g/kg (min 131 g/kg; max 225 g/kg; sd 24). Colostrum IgG, measured with RID, had an average concentration of 60.2 mg/ml (min 32.3 mg/ml; max 77.1 mg/ml sd 8.56). Elisa showed a higher variation with a mean IgG of 993 mg/ml (min 88 mg/ml, max 2650 mg/ml; sd 777) and was only weakly correlated with RID IgG (Rsq 0.29; $p<0.001$) Brix percentage was positive correlated with RID IgG (Rsq 0.68; $p<0.001$) and total protein (Rsq 0.90; $p<0.001$).

Conclusion. The results clearly showed different values for Elisa and RID measured IgG. Elisa IgG was only weakly correlated with RID IgG. On the other hand a very simple brix percentage measurement showed a much higher correlation with RID. For research the RID will remain the golden standard. However the refractometer proved to be a quick and robust method to measure colostrum IgG under field conditions.

NOTES

oral
PRESENTATIONS

VIRAL DISEASES

Thursday, April 23rd > 10:30-12:10

013 First case of Porcine Epidemic Diarrhea (PED) caused by a new variant of PED virus in The Netherlands.

014 Swine influenza viruses in circulation in European pigs exhibit an increasing genetic diversity since last pandemic in 2009

015 Birth weight, intrauterine growth retardation and fetal susceptibility to porcine reproductive and respiratory syndrome virus

016 Modelling Porcine Epidemic Diarrhoea Virus (PEDV) spread in a pig densely populated area in France without population immunity

017 Early-life Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection: role of maternally derived antibodies and link with the immunity in the breeding herd

013

FIRST CASE OF PORCINE EPIDEMIC DIARRHEA (PED) CAUSED BY A NEW VARIANT OF PED VIRUS IN THE NETHERLANDS.

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Introduction. Porcine Epidemic Diarrhea (PED) is a devastating disease caused by an alpha-coronavirus. Pigs of all ages can show symptoms of lethargy, inappetence and diarrhea with high morbidity, but especially in suckling piglets mortality can be high as a result of dehydration. Within the monitoring system for pig health in The Netherlands no report or diagnoses of PED had been made for many years. Here we report the first case of PED caused by a new variant of PED virus in The Netherlands.

Material and Methods. The monitoring system for pig health aims to identify outbreaks of OIE listed and reportable diseases, identify new diseases and to follow trends of known diseases. This is achieved by a help desk for vets and herd owners and offering a wide range of diagnostic facilities including necropsy facilities, bacteriology, and qPCR's for e.g. Lawsonia, Brachyspira spp, PED and PDCoV. Partial sequences of the orf1b and S1 gene of the Dutch variant of PED virus were obtained and compared to PEDV sequences available in GenBank.

Results. GD Animal Health was notified of a case of pigs showing lethargy and inappetence for up to 24 hours after which profuse diarrhea occurred in almost all pigs in 9 compartments within a finishing barn. The finishing barn consists of 19 compartments with 104 pigs each divided over 8 pens, compartment 1 is a sick bay. First symptoms occurred on October 26th in compartment 2, subsequently followed by adjoining compartments to compartment 13 on 21th of November. Initial diagnostics for E. coli, Salmonella, Lawsonia and Brachyspira were negative. Six fecal samples were found to be positive for PED, but negative for PDCoV on November 14th. On the same day 3 pigs were submitted for necropsy. All animals showed severe villus atrophy typical of PED virus infection on histopathology and were found to be positive by qPCR for PED virus performed on small intestinal content. Sequencing of the PCR material showed that the S-gene had a 99% homology with OH851, a mildly virulent PED strain found in the USA and in Germany. Strict in- and external biosecurity measures have been taken to stop the spread of the virus. Up till now (Dec. 1st) not all compartments are infected and no other farms have been reported infected so far. The sow farm at 150 metres distance is still free from infection. So far no pigs have died as a result of the infection and pigs recover spontaneously after about 2 weeks.

Conclusions. Here we report the first known case of PED in The Netherlands caused by this new mildly virulent strain with signs of lethargy and inappetence quickly followed by profuse diarrhea with high morbidity but no mortality so far.

NOTES

014

SWINE INFLUENZA VIRUSES IN CIRCULATION IN EUROPEAN PIGS EXHIBIT AN INCREASING GENETIC DIVERSITY SINCE LAST PANDEMIC IN 2009.

G. Simon (1), L. E. Larsen (2), R. Dürrwald (3), E. Foni (4), T. Harder (5), K.Van Reeth (6), I. Markowska-Daniel (7), A. Dan (8), J. Maldonaldo (9), A. Huovilainen (10), C. Billinis (11), I. Davidson (12), S. M. Reid (13), I. H. Brown (13), W. Loeffen (14)

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Swine influenza causes concern for veterinary and public health. Thus, ongoing knowledge of influenza A viruses circulating in pigs is necessary for the prevention and control of the disease in pigs, but also to detect reassortant viruses that may pose a threat to humans, as was the case for the H1N1 virus responsible for the last pandemic in 2009 (H1N1pdm).
In continuation to two previous coordination actions (2001-2008) that initiated the surveillance for swine influenza viruses (SIVs) circulating in European pigs, a third phase of the European Surveillance Network for Influenza in Pigs (ESNIP3) aimed to expand the knowledge of European SIVs from 2010 to 2013. ESNIP3 stimulated surveillance in European countries and supported the coordination of appropriate diagnostic tools and subtyping methods. Thus, in an extensive virological monitoring, mainly conducted through passive surveillance programs, more than 9 000 herds in 17 countries were examined over a period of three years.
Influenza A viruses were detected in 31% of herds, from which 1887 viruses were subtyped preliminary. Viruses of the 3 subtypes enzootic in swine (H1N1, H3N2, H1N2) were identified but several genetic lineages were distinguished depending on the origin of genomic segments. Thus, the dominating lineages were identified to represent the 3 previously known European enzootic SIVs, i.e. the avian-like swine H1N1 (53.6%), the human-like reassortant swine H1N2 (13%) and the human-like reassortant swine H3N2 (9.1%), but also the H1N1pdm lineage (10.3%). Viruses from these four lineages co-circulated in several countries, but with very different relative levels of incidence. The H3N2 lineage was missing from some areas whereas it was still prevalent in other parts of Europe. Interestingly, regions free of H3N2 were those that exhibited the highest frequencies of H1N2 viruses in circulation. H1N1pdm viruses were detected at an increasing rate in some countries throughout the three year period, indicating that this subtype has become established in the European pig population. Finally, based on HA and NA identification, 13.9% of the viruses represented reassortants between these four lineages. Among them, reassortants between older enzootic SIVs and H1N1pdm emerged in several countries and were detected at an increasing frequency in 2012-2013.
Surveillance of SIV infections on a European scale revealed striking differences between countries participating in the ESNIP3 network regarding the prevalence of ancient and newly emerged SIV lineages. Some novel reassortants might become established in pig herds in the near future and potentially provide implications for zoonotic infections.

NOTES

015

BIRTH WEIGHT, INTRAUTERINE GROWTH RETARDATION AND FETAL SUSCEPTIBILITY TO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Introduction. Selection for increased litter size in pigs can increase the risk of intrauterine growth retardation (IUGR), which is defined as impaired growth and development of embryos and fetuses and their organs characterized by disproportionate growth of brain compared to other fetal organs (relative brain sparing). The postnatal consequences of IUGR are diverse and include increased neonatal morbidity and mortality, abnormal gastrointestinal morphologies and dysfunction, altered carcass composition and development of muscle fibers, and decreased reproductive performance. In contrast to human medicine, the characterization of immune responses and disease susceptibility in low birth weight or IUGR pigs is incomplete.

Materials and Methods. We compared the severity of porcine reproductive and respiratory syndrome (PRRS) in pregnant gilts originating from high and low birth weight litters and their fetuses. One-hundred and eleven pregnant gilts experimentally infected with PRRS virus (PRRSV) on gestation day 85 (±1) were necropsied along with their fetuses 21 days later.

Results. Ovulation rates and litter sizes did not differ between groups, but fetuses from low birth weight gilts were shorter, lighter and demonstrated evidence of asymmetric growth with large brain:organ weight ratios (i.e. brain sparing). The number of IUGR fetuses, defined by brain:organ weight ratios greater than 1 standard deviation from the mean, was significantly greater in low compared to high birth weight gilts. Viral load in serum and tissues, gilt serum cytokine levels, and litter outcome, including the percent dead fetuses per litter, did not differ by birth weight group. Thus, this study provided no substantive evidence that the severity of PRRS is affected by dam birth weight. However, IUGR fetuses had lower viral loads in both fetal thymus and in endometrium/fetal placenta adjacent to the umbilical stump. Crown rump length did not significantly differ between fetuses that survived and those that died at least one week prior to termination.

Conclusions. Taken together, this study demonstrates that birth weight is a transgenerational trait in pigs, and provides evidence that larger fetuses are more susceptible to transplacental PRRSV infection.

NOTES

016

MODELLING PORCINE EPIDEMIC DIARRHOEA VIRUS (PEDV) SPREAD IN A PIG DENSELY POPULATED AREA IN FRANCE WITHOUT POPULATION IMMUNITY

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(1) Anses, Ploufragan, France

Introduction. A devastating epizooty of porcine epidemic diarrhoea (PED) has been striking the US since April 2013. More than 8500 herds have been affected with up to 90-95% mortality in suckling piglets. In Europe, PED has been described since the 1970's till the end of the 1990's except a limited epizooty reported in Italy in 2006. Recently, sporadic cases have been reported in Germany, Italy and the Netherlands. However, seroprevalence studies carried out in different European countries (UK, Belgium, France, Denmark) suggest that the immunity of the population in these countries against PEDV is low. The objective of the study was to assess the impact of an introduction of PEDV in a pig densely populated area in Europe using a modelling approach. The example of Brittany, one of the main pig production areas in France, is taken.

Material and method. A Spatio-temporal individual-based, stochastic model has been built to simulate the spread of the virus in the area. Data reporting geographical location of the herds, herd type and size, annual movements of animals between herds and frequency of contacts with vehicles and other mechanic vectors have been used for parameterisation. The model was developed using the North American Animal Disease Spread Model. The model structure is based on a compartmental SEIRS structure with state transitions governed by parameters derived from the literature and reports from the current epizooty in the USA and Canada. Some control measures were also evaluated in the model (limitation of animal movements in a high risk zone around the identified case, increased biosecurity, stamping-out). Different durations of the 'high-risk' period (time between infection and notification) were also evaluated.

Results. Simulation results showed that a massive epizooty would be expected with similar characteristics as observed in the US. The most efficient control measure was stamping out infected herds with a notification delay shorter than 10 days and with increased biosecurity. Limitations of animal movements in less than 10 days after infection for herds located in a 2 km radius area around the infected herd reduced also significantly the epidemic size. When animal movements were not suppressed and only biosecurity was enhanced, the epizooty tended to move to an enzootic form.

Conclusion. In the absence of immunity, the introduction of PEDV strains currently circulating in the US would have an important impact on the French swine population. Rapid intervention would be required to limit the propagation of the virus.

NOTES

017

EARLY-LIFE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS INFECTION: ROLE OF MATERNALLY DERIVED ANTIBODIES AND LINK WITH THE IMMUNITY IN THE BREEDING HERD

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(1) Anses, Ploufragan, France

Introduction. Passive immunity is an essential factor influencing early-life infections for a wide variety of infectious agents and hosts. However, maternally derived antibodies (MDA) only provide a partial short-term protection to new-born individuals. Vaccination of breeding animals to provide MDA protecting their offspring in early-life is a common practice to control PRRS virus infection. The aim of this work was to study the characteristics of passive immunity in terms of protection and duration, which might be pivotal to optimize husbandry management policies and vaccination schedule for diseases control.

Material and Method. Two separate datasets were analysed: (i) longitudinal serological data in gilts after vaccination from a PRRSv free herd; (ii) longitudinal serological data from 120 pigs monitored from birth to slaughter in three pig herds chronically infected by PRRSv. A mathematical model describing the vaccine-induced antibody kinetics in gilts was developed to estimate the duration of immunity and the delay to the peak of immunity. Piglets MDA kinetics was similarly analyzed, to derive distribution of the duration of passive immunity. An individual-based markovian model was developed, accounting for passive immunity duration, and differential forces of infection in piglets with and without MDAs. Markov-chain Monte-Carlo methodology was employed to estimate the model parameters.

Results. Three profiles were highlighted in gilts: individuals with low immune response, those with high but relatively short immunity, and those with intermediate and sustained immunity. Half of vaccinated animals lost vaccine-induced immunity within 30 weeks. In piglets, the level of MDAs was found correlated with the dam's antibody titre at birth. Moreover, the intensity and duration of protection conferred by passive immunity was strongly linked with the initial MDAs titres in piglets. Forces of infection were on average 18 times lower in presence than in absence of MDAs but varied widely among the observed pig batches [7.7; 49].

Conclusion. An indirect relationship, based on two distinct datasets, was established between early life PRRSv infection and the immunity in breeding animals. However, this study was carried out in PRRS-free gilts and a similar analysis would be necessary to evaluate vaccine effect in pre-immune sows. Nevertheless, the results showed the importance of vaccination schedule in sows, to optimize the delivery of antibodies to suckling piglets, as well as in piglets to avoid possible antagonistic effects between vaccine and MDAs.

NOTES

oral
PRESENTATIONS

HERD HEALTH MANAGEMENT

Thursday, April 23rd > 10:30-12:10

018

A MULTI–CENTRE STUDY OF HAEMOGLOBIN CONCENTRATION AND WEIGHT GAIN IN PIGLETS AT WEANING

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Introduction; Haemoglobin values in piglets at weaning are highly variable and often indicative of anaemia due to inadequate iron supplementation in the suckling period. Recently, a Danish study showed that the haemoglobin levels in piglets at weaning were positively correlated with average daily gain (ADG) post-weaning. The purpose of the present study was to determine if similar effects could be observed in countries with different production systems and genetics.

Materials and Methods. A total of 13 sow herds were recruited from four countries on two continents: Canada (CA) (5), Denmark (DK) (5), Norway (NO) (2) and USA (US) (1). Piglets in all herds received an iron injection during the first days of life for prevention of iron deficiency anaemia. Within each herd 20 litters close to weaning were selected. From each litter a piglet was chosen at random by a counting procedure. Piglets were weighed and blood sampled for Hb testing at weaning and weighed again after three weeks to determine average daily gain (ADG). The effect of Hb on ADG was tested using a general linear mixed model with weight at weaning as a fixed effect and herd as random effect. Hb values were categorised as: Low <90, Moderate 90-110 and High >110, g/l. Statistical significance was set at 0.05.

Results. One piglet from each of 262 litters was included for analysis. The mean weaning weights in CA, DK, NO, and US were 6.8, 6.2, 11.4 and 6.7 kg respectively. Mean weaning ages in days were: CA=22.6, DK=25.7, NO=33.2, US=21. The mean Hb concentrations in g/L were: CA=111.2, DK=121.5, NO=119.8 and US=93.4. The mean ADG in grams/day were: CA= 305.6, DK=202.9, NO=411.4 and US=196.5. The percentages of piglets with Low Hb were: CA=7.2, DK=3.5, NO=2.5 and US=38.2. The percentages of piglets with Moderate Hb levels were: 35.0 (CA), 15.4 (DK), 12.8 (NO) and 47.0 (US). Based on the statistical model, ADG was significantly associated with Hb concentration at weaning (P=0.001) and weaning weight (P<0.0001). An increase in 10 g Hb/L blood corresponded to a weight gain improvement of 10 grams daily weight gain in the 3 week post weaning period.

Discussion and conclusion. Evidence of general biological principles in pig herds is strengthened when observed in a diversity of production systems. In this study a strong association between Hb-level at weaning and growth rate in piglets in the next three weeks was observed in spite of herd variations in anaemia prevalence, weaning age and weight and country effects within genetics, feed, health status and management. Although the exact mechanism of this effect is unknown, it seems reasonable that high iron stores and corresponding high Hb values improve potential for growth. Hence, strategies for improving the haemoglobin level of weaning piglets are important tools for herd health and production management.

NOTES

019

IMPACT OF INADEQUATE COLOSTRUM INTAKE IN PIGLETS

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(1) ForFarmers, Lochem, Netherlands

Introduction. The increasing number of live born piglets reduces the average birth weight and subsequent increases the risk of mortality. Colostrum intake is crucial for survival in the first days of life. However, studies on the uptake of colostrum by piglets are scarce.

Material and methods. In December 2012 and January 2013 the farrowing process of in total 70 litters (Topigs 20 sows x Tempo boar) was followed over a period of four weeks time. Piglets were weighted (directly after birth and at 24 hours after the birth of the first born piglet; n=1040) to estimate the colostrum intake. After birth of the first piglet a colostrum sample was taken. IgG was determined in this colostrum by radial immunodiffusion. Twice a week blood was collected by jugular venapuncture from 3 piglets per litter: first live born (FB), middle live born (MB) and last live born piglet (LB). Piglets were sampled between the age of 24 hours and 4 days. Serum IgG was determined using the immunocrit assay with an increased centrifugation time of 10 minutes. IgG concentrations were corrected for differences between the weight at 24 hours and the weight at blood collection, assuming that the concentration of antibodies are linear diluted by weight increase between 24 hours of age and the time of blood sampling.

Results. The mean birth weight (BW) was 1.266 kg (n=1087). In the first 24 hours (after the birth of the first piglet) the average weight increase (WI) of the piglets was 56 gram (3,9% of BW; n=1004) with a calculated colostrum intake of 270 gram. BW was positive correlated with the relative weight increase (Rsq 0,064; p<0,001). Piglets that died during the suckling period had a significant lower calculated colostrum intake and a lower percentage of weight increase compared to the piglets that survived (166 gram, -4.9% vs 281 gram, 5.1%; p<0.001). The 25% lightest piglets at birth had a significant higher mortality compared to the other 3 quartiles (<1.08 kg 36.8%a; 1.01-1.26 kg 10%b; 1.28-1.45 kg 9.7%b; >1.46 kg 8.0%b;p<0.001).Mean colostrum IgG was 60.2 mg/ml (min 32.3 mg/ml; max 77.1 mg/ml). Colostrum IgG was positive correlated with the serum IgG of the piglets (Rsq 0.073; p<0.001). Mean piglets serum IgG was 37.3 mg/ml (min 2.5 mg/ml; max 85.4 mg/ml). Serum IgG significantly lowered with birth order (FB 41.4a mg/ml; MB 37.0ab mg/ml; LB 33.4b mg/ml; p<0.001).

Conclusion. Mortality was correlated to a low colostrum intake and a low birth weight. This study (70 litters, 1040 piglets) clearly showed a decrease in piglet serum IgG with increasing birth order. In our opinion this stresses the importance of a quick birth process and early access to the udder.

NOTES

020

FACTORS ASSOCIATED WITH STILL BORN PIGLETS IN DANISH HERDS

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Introduction; Still born piglets are a major contributor to piglet mortality. The objective of this study was to analyse factors associated with still born piglets in herds with large litter sizes.

Materials and Methods. In nine herds 926 to1261 piglets from 54 to 74 consecutive farrowings were ear tagged and weighed at birth. All still born piglets were necropsied either in the herd or at the Laboratory for Swine Diseases in Kjellerup. The following factors were tested for a possible association with still birth: gestation length, time in pen before farrowing, parity, litter size, assisted farrowing, night and weekend farrowing, still born in previous litter, back fat, MMA or oxytocin treatment, gender and birth weight of the piglets. The association between risk factors and still born was analysed in a generalized linear mixed model with herd and sow as random effects. For significant risk factors Population Attributable Risk (PAR) was calculated. PAR is the proportion of still born that is explained by the risk factor and therefore also the potential for reduction in still born rate.

Results. Average litter size was 17.6 total born and 1.6 still born (9,1 %). Assisted farrowings, still born in previous litter, sex, and birth weight of piglets and interaction between parity and back fat were statistically associated with being still born. Assisted farrowing is not responsible for still born piglet but an indicator for weakness in the sow. Litter size was not significant when birth weight, an intermediary variable, was included in the analysis. No associations were found to gestation length, time in pen before farrowing, night or weekend farrowing, MMA or oxytocin treatments. If assisted farrowing was not needed, the still born could be reduced to 6.8%. If sows with parity > 2 had a back fat above 13 mm then the still birth rate could be 8.2%. If the risk from sow with still born piglets in the previous litter could be avoided the still birth rate would be 7.3% Excluding the combined effect of the three management associated risk factors would result in a stillborn rate of 6.3%. Without male pigs and pigs < 1 kg then still born rate could have been further reduced to 2.5 %.

Conclusions.This study indicates that the rate of still born potentially can be reduced from 9.1% to 6.3% when avoiding factors leading to assisted farrowing, culling/monitoring sows with a previous history of still born and asserting older sows to have at back fat level > 13 mm.

Acknowledgment: This study was supported by the Danish Pig Levy Fund and the European Union and the Rural District Program under the Danish Ministry of Food, Agriculture and Fisheries j.nr. 32101-U-12-00229.

NOTES

021

DEVELOPMENT OF A SALMONELLA TYPHIMURIUM CHALLENGE MODEL IN WEANED PIGS: EVALUATION OF KNOWN INTERVENTIONS

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Introduction. Our aim is to develop a Salmonella Typhimurium (S. Typh) challenge model in weaned pigs, suitable for the evaluation of effects of dietary interventions on fecal shedding. In a previous study we successfully infected weaned pigs with a S. Typh field strain via an oral inoculation for 7 consecutive days, resulting in quantifiable fecal shedding. In this study we validate whether our model is able to show significant differences in fecal shedding between (proven effective) dietary interventions and a positive control group.

Materials and methods. Four groups of 8 individually housed weaned male piglets were used. Treatments were: NC (negative control; no inoculation); PC (positive control; inoculation); WI (inoculation + water intervention: 2L/m3 Selko pH in drinking water); FI (inoculation + feed intervention: 4.6L/ton Selko pH in feed). At 7 days, piglets of treatments PC, WI and FI were orally inoculated with a S. Typh field strain (1 ml of 1.09*109 cfu/ml) for 7 consecutive days (days 1-7). Rectal fecal samples were taken before (day -7, -4 and 1) and after inoculation (day 2, 3, 7, 9 and 21) for Salmonella quantification. Diarrhea incidence and body temperature were recorded daily, and body weight was measured weekly.

Results. No fecal shedding of Salmonella was detected before inoculation, nor in the NC group during the trial. Fecal shedding peaked at day 3 in the other treatments. This peak fecal shedding tended to be lower for WI (4.1 log10 cfu/g) compared to PC (5.5 log10 cfu/g; P = 0.06), and was intermediate in FI (5.4 log10 cfu/g). After day 3, fecal Salmonella shedding gradually declined to 1.4 log10 cfu/ml at day 21, for all treatments. Diarrhea incidence was lower in NC (53%) compared to other treatments (67-71%; P<0.05). After inoculation, a short (2 days) 0.5°C increase in body temperature was detected in the inoculated groups, but body temperatures returned to base levels before day 3 in all groups. Average daily growth during day 1-7 was higher in NC (382 g/d) than in PC (182 g/d; P<0.05), whereas WI (307 g/d) and FI (225 g/d) were intermediate.

Conclusions. We successfully infected weaned pigs with a S. Typh field strain, resulting in significant fecal shedding. Although differences in fecal shedding between the groups were not significant, the water intervention resulted in a 1.4 log10 cfu/ml lower peak shedding at day 3 after inoculation (P=0.06), which may be relevant in practice. We conclude that the model may be suitable for the evaluation of effects of dietary interventions on peak fecal shedding, although some further fine-tuning (e.g. more frequent fecal sampling during peak shedding) is required.

NOTES

022

EPIDEMIOLOGY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS: A RISK FACTOR STUDY IN 109 FRENCH FARROW-TO-FINISH HERDS

C. Fablet (1), C. Marois-Créhan (1), B. Grasland (1), G. Simon (1), N.Rose (1)
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Introduction. Porcine reproductive and respiratory syndrome virus (PRRSV) infection is responsible for huge economic losses for the swine industry worldwide. PRRS control only based on the use of vaccination has often provided limited results, complicating disease management. It is therefore of paramount importance to thoroughly understand the epidemiology of PRRS to implement efficient control strategies and elimination programs. Studies investigating risk factors for PRRSV infection at the herd level have mainly been carried out in North American multisite production systems but few dealt with farrow-to-finish systems, a common herd type in European pig producing countries. Hence, the aim of this study was to identify factors associated with PRRSV infection in farrow-to-finish herds.

Materials and Methods.The study was carried out in 109 French herds. Samples (tracheo-bronchial mucus and blood) were taken from a random sample of 4, 10, 16 and at least 22 week-old pigs (45 pigs/herd). Serum samples were tested by ELISA for PRRSV antibodies. Infection by Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, swine influenza viruses (SIV) H1N1 and H1N2 and porcine circovirus of type 2 were detected by specific serological assays or PCR tests. Data related to herd characteristics, biosecurity, management and housing conditions were collected by a questionnaire during a herd visit. Climatic conditions in the nursery and fattening rooms, where the oldest sampled pigs were housed, were measured over 20 hours. A herd was deemed to be PRRSV positive when a minimum of one pig of 10, 16 or at least 22 weeks old tested positive by ELISA. Factors associated with PRRSV seropositive status of the herd were identified by logistic regression.

Results. Large herd size (≥200 sows/herd; Odds-ratio [OR]=5.5; IC95%[1.8-16.4]), the lack of disinsection in the gestation facilities (OR=3.8; IC95%[1.2-11.5]), on-farm semen collection (OR=5.9; IC95%[1.4-25.9]), a short time-period for gilt quarantine (≤49 days; OR=4.9; IC95%[1.4-17.9]) and a low temperature setpoint for the ventilation controller in the fattening room (OR=3.4; IC95%[1.0-11.3]) significantly increased the odds of a herd being seropositive for PRRSV. Infection by M. hyopneumoniae (OR=5.5; IC95%[1.8-16.6]) and H1N2 SIV (OR=3.1; IC95%[1.1-8.5]) were associated with a PRRSV positive status.

Conclusion.Recommended measures aimed at a better control of PRRSV infection would include proper biosecurity measures to minimize the risk of virus introduction, management practices minimizing direct and indirect virus transmission within the herd whilst providing the pigs with favorable climatic conditions.

NOTES

INDUSTRIAL PARTNERS' SESSION

Thursday, April 23rd > 15:00-18:15

023 Post weaning diarrhoea in Europe: veterinarian and producer insights.

024 Constitution of a European network on the detection of pathogens in oral fluid

025 Assessing sow herd PCV2 stability and vertical transmission utilizing colostrum and placental umbilical cord serum

026 Meta-analysis of the effects of Draxxin® injectable on Swine Respiratory Disease

027 Swine enteric coronavirus disease (SECD) elimination and prevention in a genetic multiplication system in North America

028 A practical approach to improve efficacy of antimicrobial treatment

029 Financial calculator of the damages od edema disease and return on investment of the vaccine Ecoporc Shiga

030 The effects of altrenogest treatment in early gestation on disruption of pregnancy

031 Efficacy against a porcine parvovirus infection in gilts vaccinated with the mixed administration of ERYSENG® PARVO and UNISTRAIN® PRRS

032 Impact on immunity parameters of piglets fed mycotoxin contaminated diets

033 Efficacy of a new PCV2 and M. hyopneumoniae combination vaccine is independent of maternally derived immunity

023

POST WEANING DIARRHOEA IN EUROPE: VETERINARIAN AND PRODUCER INSIGHTS.

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Introduction. Post-weaning diarrhoea (PWD) is a major cause of economic losses to the pig industry from both mortality and reduced growth rates (Fairbrother et al., 2005). PWD is caused by some strains of E. coli, known as enterotoxigenic E. coli (ETEC), with the ability to produce one or several enterotoxins and attach to intestinal cells. The objective of the present study was to investigate the importance of PWD, its economic impact and control strategies across Europe as reported by pig professionals.

Materials and Methods. A survey was conducted in six European countries (Belgium, Denmark, France, Germany, Poland and Spain) among veterinarians (n=160) and pig producers (n=240). Inclusion criteria included being a dedicated pig veterinarian (≥50% of their time spent on pig production) or being responsible for at least the nursery section in their respective farms for producers. The geographic spread of the interviews was in line with the national distribution of pig production in each country. A set of questions was designed in order to quantify the prevalence of E. coli as a cause of PWD, the economical and emotional impact of the disease and to explore the level of satisfaction with current solutions to prevent and control PWD. Questions were validated, interviews conducted and results analysed by a third party (iQube Marketing Ltd, UK).

Results. Veterinarians affirmed that 40% of the herds under their care have experienced outbreaks of PWD. A 15% of the piglets suffer PWD with a mean increase in mortality due to the disease of 2% according to producers. When questioned about the impact of PWD, (1) slow growth of pigs/lowered daily weight gain and (2) mortality were the most prevalent answers, 77% and 49% of producers and 83% and 74% of veterinarians respectively. As a result, a delay of 10 days on average to reach target weight is reported in both groups, needing a 7-9% of extra feed. The overall satisfaction with efficacy of antibiotics for controlling PWD was about 80%. Around a third of veterinarians in France, Germany, Poland and Spain recommend a higher antibiotic dose rate to control PWD than in the past. Additionally, PWD was reported by veterinarians and swine producers as being the cause of important stress, hassle and frustration, especially when facing recurrent clinical cases of PWD, despite the preventive use of antibacterials such as colistin and zinc oxide.

Conclusion. As reported by veterinarians and producers across Europe, PWD is an important and prevalent disease resulting in lost productivity and increased costs. On average, PWD delays the time to reach target weight by 10 days, resulting in 7-9% of additional feed costs.

NOTES

024

CONSTITUTION OF A EUROPEAN NETWORK ON THE DETECTION OF PATHOGENS IN ORAL FLUID.

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Studies on the use of swine oral fluid (OF) in veterinary diagnostics emphasize the interest of this type of sample for animal health monitoring. While several publications have validated the interest of a given analytical test (serology or PCR) performed on OF, few studies have compared the performances of such tests between laboratories.

A network linking 17 diagnostic laboratories
After a first meeting, in 2012, where members of 18 European diagnostic laboratories expressed their interest in comparing the reliability of their routine test results, they agreed to create an informal working group. This group, later named the 'European network of diagnostic laboratories on swine oral fluid', gathered diagnostic laboratories from 13 EU countries. Namely: the DGZ Vlaanderen (Belgium), the State Veterinary Institute Jihlava and the University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic), the DTU (Denmark), Labocea (France), IVD (Germany), the Aristotle University of Thessaloniki (Greece), the National Food Chain Safety Office, Veterinary Diagnostic Directorate (Hungary), the IZLER (Italy), the Pulawy Institute and the Warsaw University of Life Sciences (Poland), Vetdiagnos (Portugal), the CReSA (Spain), the GD Animal Health service and Thermofisher (the Netherlands) and the AHVLA and Newcastle University (UK).

Two ring trials in two years
The supervision of the network was awarded to Labocea and Merial, while Merial funded the activities of the network. It was agreed that these activities would first aim, through a common and homogeneous approach, to develop, validate and provide detailed information to pig health professionals in Europe on what can be expected from OF as a monitoring/diagnostic tool. A first ring trial was organised in 2012/2013, to compare the capacity of each network member to perform common and separate analytical methods for the detection of PRRSV, PCV2 or SIV in shared samples of native (i.e. unspiked, field-collected) OF. In 2013/2014, the network organised a second ring trial, focusing on the detection of PRRSV by the RT-PCR methods and using sera and OF collected in a Specific Pathogen Free farm, spiked with two different EU-PRRSV strains. Results of both ring trials are detailed in a further communication at this meeting. They demonstrate that a coordinated and multidisciplinary work is a realistic aim for diagnostic laboratories from 13 different countries, and that swine professionals can rely on the laboratory results provided by members of the network.

NOTES

025

ASSESSING SOW HERD PCV2 STABILITY AND VERTICAL TRANSMISSION UTILIZING COLOSTRUM AND PLACENTAL UMBILICAL CORD SERUM

B. Payne (1), J. Seate (2), D. Madson (3), A. Scheidt

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Introduction. PCV2 vaccination in wean age pigs is necessary to protect pigs against PCV2. Vertical transmission of PCV2 has previously been described using techniques such as placental umbilical cord serum (PUCS), colostrum, presuckle serum and fetal tissues. However, the most sensitive, practical and economically feasible measurement has not been identified in the literature to determine the number of PCV2 positive litters at the time of piglet birth. Although PUCS and presuckle serum have, PUCS and colostrum samples have not been compared.

Materials/Methods. Paired PUCS and colostrum (n=659) sample results from eight sow herds (A-H) were compared as a litter evaluation. To collect PUCS, expelled placenta was inverted and 3-4 umbilical cords (attached to the placenta and not visibly contaminated) were milked into a single serum tube. Colostrum (1-3ml) was manually milked into a snap cap tube. Both PUCS and colostrum samples were kept on ice to transport and tested individually using TaqMan real-time PCR reagents (Life Technologies Corp., NY, USA at HMC, IA, USA) with a detection limit of below 3.5 genomic equivalents/reaction.

Results. There were four outcomes for paired samples: 1) PUCS negative/colostrum negative (PN/CN, n=561 sows, 85%), 2) PUCS positive/colostrum negative (PP/CN, n=65 sows, 10%), 3) PUCS negative/colostrum positive (PN/CP, n=11 sows, 2%), and 4) PUCS positive/colostrum positive (PP/CP, 22 sows, 3%). Utilizing PUCS as the gold standard, colostrum had 67% sensitivity (95% CI, 48-92). For unpaired samples (n=692 PUCS and 669 colostrum) herds G and H (% positive PUCS/colostrum was 63/16% and 51/19%, respectively) PUCS had significantly more positives than colostrum ($p<0.0001$). There were no significant differences in low positive sow herds (0-4% PUCS PCR positive).

Conclusions. Although there was 88% agreement in the sample types (PN/CN+PP/CP), there were fewer positive samples than expected. The large number of negative sows is likely due to the management and PCV2 vaccination of incoming gilts in six of the herds. The diagnostic comparisons indicate PUCS is more sensitive than colostrum in high prevalence sow herds but not in low prevalence sow herds. In an unknown herd, PUCS is recommended over colostrum. Research, including pooling of samples to decrease cost while minimizing the impact of pooling, is ongoing.

NOTES

026

META-ANALYSIS OF THE EFFECTS OF DRAXXIN® INJECTABLE ON SWINE RESPIRATORY DISEASE

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Objective: To evaluate the effectiveness of Draxxin® (tulathromycin) injectable solution administered intramuscular once for the treatment of Swine Respiratory Disease (SRD) in nursery and fattening pigs.

Material and Methods: A total of 31 publications of trials using Draxxin® injectable in SRD compared to both negative and positive (animals treated with a different antibiotic; PC) controls were reviewed to be included in a meta-analysis. The data included studies on both nursery and growing/finishing pigs. After the review, a total of 27 studies (20 with negative and 7 with positive controls) were used to assess the effect on mortality and 23 studies (16 with negative and 7 with positive controls) to assess the effect on average daily gain (ADG).Meta-analysis was carried out using R metaphor package (Viechtbauer, 2010) for R 3.1.0. (R core team, 2014). For mortality, the effect was measured as Relative Risk (RR) of death for a pig. For ADG, the measure of variability was always transformed to SD and the effect was measured as standardized mean difference (SMD) of the ADG. In both cases the meta-analysis included also the type of control used (negative or positive control). The growing stage of the animal was not included due to some studies comprising both at the same time. No other covariates were provided in all the selected papers with enough detail to be included in the model.

Results: The overall result for mortality was that the RR of casualties in animals treated with Draxxin® compared to control animals was found to be 0.58 (with 95% CI: 0.46 to 0.73; $P < 0.01$). Therefore, the risk of mortality in Draxxin® treated animals is reduced by 42% as compared with the control group risk. For the ADG, the SMD between those animals treated with Draxxin® and those in the control group was 0.60 (with 95% CI: 0.34 to 0.86; $P < 0.001$) meaning that ADG is improved by 0.60 × SD g/d. For example, for a population with a growth rate of 500 g/d and a SD of 20% (100 g) Draxxin® could improve growth rate by 60g/d. In both mortality and ADG these results were not affected by whether Draxxin® was compared to a negative or a positive control.

Conclusions: The results of the meta-analyses conducted suggest that the risk of death in Draxxin® treated animals is reduced by 42% as compared with the control group risk, while the growth rate in Draxxin® injectable treated animals is for a given population 0.60 × SD g/d better than the control group.

NOTES

027

SWINE ENTERIC CORONAVIRUS DISEASE (SECD) ELIMINATION AND PREVENTION IN A GENETIC MULTIPLICATION SYSTEM IN NORTH AMERICA

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Introduction. PEDV, PDCoV and TGEV are causal agents of SECD. Diarrhea, vomiting and 100% mortality for 3-5 weeks are observed in suckling pigs infected with PEDV in NA. It has been estimated that about 60% of the USA breeding herd has been impacted by PEDV since its detection in May 2013 causing a shortfall of 12.500.000 market hogs (11%) for 2014. PDCoV was also recently reported in NA with a similar but milder clinical presentation. The route of introduction to the USA has not been confirmed but genetic analyses indicate relationship with isolates previously detected in Asia. This abstract describes the elimination experience and prevention program in a 92.000 sow genetic multiplication system in NA.

Materials and Methods. Between November 2013 and April 2014, 47,4% of the breeding herds in the system became infected. Specifically, 26,4% were infected with PEDV only, 10,5% with PDCoV only and 10,5% with both viruses. Likely because their remote location and intensive biosecurity none of the genetic nucleus farms were affected. The system has accumulated a record of only one or no PRRS or M. hyopneumoniae outbreaks per year in the past several years. As soon as the presence of PEDV is confirmed, piglets are weaned early and moved to an offsite nursery for better survival, the gilt development unit is loaded and controlled oral live virus exposure is initiated to all sows and gilts aiming to homogenize immunity to minimize the duration of shedding. In order to interrupt the introduction of susceptible individuals, every piglet is weaned off site and the herd is closed to replacement pigs until the third consecutive PCR-negative biweekly test. Negative sentinels are brought in at this time, and if no clinical or diagnostic evidence of infection is detected after five weeks, negative replacements can enter the herd. Keeping litter integrity, minimizing piglet handling and intensifying cleaning and disinfection in the farrowing rooms are key for the success of the project.

Results. By August of 2014 all breeding herds were consistently weaning negative pigs. By the end of October 2014 the lack of shedding had been verified through the use of sentinel pigs in all herds. It took 20,1 (7,1 – 28,1) weeks for the herds infected with PEDV and 14,6 (11,9 – 17) weeks for the herds infected with PDCoV to consistently wean PCR-negative pigs. No new or "reactivated" infections have been detected and 100% of the growing sites are negative at this time. Likely as the result of the surveillance program, the transportation biosecurity protocols and the quarantine at destination, no SECD has been transmitted through pigs or semen.

Conclusions. The successful elimination of PEDV and PDCoV from all herds is encouraging but reconstituting a susceptible population is concerning. Therefore, a SECD prevention initiative has been initiated across the system focused on five main risk areas: (1) feed ingredients, reception, manufacturing and delivery, (2) transportation decontamination and inspections, (3) people training and engagement, (4) manure management and (5) mortality disposal.

NOTES

028

A PRACTICAL APPROACH TO IMPROVE EFFICACY OF ANTIMICROBIAL TREATMENT

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As pig practitioners, we constantly aim to improve and safeguard the health status of our farms through good management and biosecurity. Despite these preventive measures, curative intervention with antimicrobials is sometimes required. Choosing the right antimicrobial for the diagnosed causative pathogen, and a correct application, is of major importance to ensure effectiveness of the treatment. The choice of the right antimicrobial depends on the known or suspected sensitivity of the infectious agent. Susceptibility testing can only give an indication of what the clinical outcome will be. The antimicrobial should also reach the site of infection and is determined by the pharmacokinetic parameters of the active, such as bioavailability, tissue distribution, half-life and tissue kinetics. These parameters depend on characteristics of the active ingredient, such as the pKa value, but also on formulation, the route of administration and dosage regimen. Sometimes the use of more than one antibiotic may be justified, based upon synergistic effect in vitro and their pharmacokinetic behavior. After choosing the antimicrobial, correct administration is also of importance. Dosing should be done in grams per kilogram live bodyweight, independently of the application form. By doing so, underdosing will be avoided by taking account of changing ratio bodyweight/water intake. Also the dosage regimen is of importance. The daily dose can be administered continuously or as a pulse. For concentration-dependent antibiotics, such as aminoglycosides, a high concentration will result in a faster and better effect on bacteria. The most important parameter for these antimicrobials is the Cmax/MIC. Consequently pulse medication will work better for these types of antimicrobials. For the time-dependent antimicrobials, such as macrolides, their efficacy depends on the period during which the bacteria are exposed to the antimicrobial. The most important parameter is the time period in which the concentration is higher than the MIC (T>MIC). Better efficacy can be expected if these antimicrobials are provided continuously. Next to pharmacokinetic behavior the formulation determines the stability and solubility of a veterinary medicine. For water soluble veterinary medicines for example, the formulation should condition the water and improve the stability and solubility of an antimicrobial. Often loss of activity and precipitation of the active occurs after dissolving in the drinking water and will affect the effectiveness of the therapy. Loss of activity can also occur during storage after opening the primary packaging. Proper storage conditions and re-closable packaging will preserve the active for a longer time.

To conclude - we need a holistic approach to all medicine use and by that we mean responsible promotion, responsible prescribing and responsible use of all medicines including antimicrobials. The prescribers and users of veterinary medicines should operate to the principle of “as little as possible but as much as necessary”.

NOTES

029

FINANCIAL CALCULATOR OF THE DAMAGES OD EDEMA DISEASE AND RETURN ON INVESTMENT OF THE VACCINE ECOPORC SHIGA

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Introduction: Edema disease (ED) causes serious economic losses in a farm and psychological distress in farmers. Although the primary economic cost is due to the increase in mortality, other expenses due to therapy and prophylaxis (antimicrobials, acids, special feed) as well as the decrease in daily weight gain (DWG) should not be underestimated. The goal of this study is to establish an objective method to calculate the economic impact of ED in order to offer a tool to veterinarians to help them to decide whether or not to implement the vaccine Ecoporc Shiga.

Material and methods: the calculator is an excel file that is divided into 4 parts. It takes into account the individual situation of a farm and is based on the results obtained since the launch of the vaccine.

Results
1. presentation of farm data: performances, costs of feed, price of the products sold (pigs or piglets) and information about ED (% losses, weight at death, costs of control strategies)
2. economic impact ED: the economic impact of a mortality is based on the value that it would have brought if the piglet did not die minus the costs not yet consumed (feed etc.) The costs of the control strategies must be filled as the costs per piglet
3. technical improvement in order to recover the cost of the vaccination:
a: criteria (separately): what is the decrease of the % mortality needed OR by how much does the g/day of DWG have to increase in order to pay for the vaccine
b: criteria (combined in cascade): % of the investment in the vaccine recovered with the decrease in mortality and then the rest of the investment to be covered with the DWG.
c: real gain of the additional DWG is calculated: it is figured into additional weight if the ideal market weight is higher than the current one OR in an shorter fattening period (fewer days needed to the market) if the current weight is already ideal
4. economical synthesis of the vaccination

Summary of the net investment in the vaccination(cost of the vaccine minus the control strategies removed), the financial gain due to the reduction of mortality and the increase in DWG, leading to the net gain of the vaccination (per farm, per sow and per piglet). In the end, the percentage of return on investment (ROI) is calculated (100 % meaning that for 1 € invested, the return is 2 €)

Conclusion: This calculator has been designed in a comprehensive and objective tool in order to help the veterinarian to make decision whether to vaccinate or not. Tablet and smartphone versions are also available.

NOTES

030

THE EFFECTS OF ALTRENOGEST TREATMENT IN EARLY GESTATION ON DISRUPTION OF PREGNANCY

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Introduction. Early embryonic death is considered to be the cause of autumn repeat breeding. In this season an increase is seen in sows that return to oestrus, typically 25-30 days after insemination The exact mechanism of autumn repeat breeding is unclear. It seems lower progesterone levels around week 3 of pregnancy are involved in repeat breeding.The aim of this study was to evaluate the possibility to use altrenogest (Altresyn®, Ceva) to compensate possible drop of progesterone in early gestation and thus reduce the risk for irregular repeat breeding. Furthermore, the effect of altrenogest treatment on litter size was studied.

Materials and methods. The study was carried in the autumn-winter season at a 600 sow farm with a recent record of a higher irregular repeat breeding rate. All barns have 16 hours of artificial light daily. Sows are inseminated once or twice with 24 hours interval after the first standing reflex. On day 22 after insemination they are transferred to a competitive group housing system with pens for 8 sows, which can be connected to create variable group sizes. Sows randomized according to the parity, previous back-fat loss and WOI were allocated into two groups. In total 29 sows in G1 received Altresyn (20mg altrenogest daily) from 21 days post-insemination for 5 consecutive days and 26 non-treated sows served as a control (G2). Blood progesterone was measured on day 21 of gestation. Sows were scanned for pregnancy through on day 23, 28, 37, 42 and 56 of gestation.

Results. Farrowing rate in the control group was 87.5%, while in the altrenogest group only 63%. Irregular rebreeding was significantly higher (p=0.04) in G1 sows than in G2, which was 37.0% and 12.5% respectively. For altrenogest and control sows the number of total born piglets (16.1 ± 3.3 and 16.5 ± 2.7, respectively) did not differ significantly (p=0.73). In high parity sows the number of piglets born alive for altrenogest (16.4 ± 2.7) was higher (p=0.08, one-sided Ha) than for control sows (14.5 ± 1.7). Sows that became irregular repeat breeders tended to have a lower serum progesteron (p=0.07). The weaning to insemination interval for irregular repeat breeding sows was longer (p=0.01). Total fresh skin lesion score one day after moving to competitive group housing was higher (p=0.05) for sows that would irregular repeat breed.

Conclusions. In this experiment multiple factors contributed to irregular returns. A 5 days treatment with altrenogest increased the risk for irregular repeat breeding by 4.1 times. In the high parity sows it seemed that altrenogest treated sows had more live born piglets. Possibly, a negative effect of altrenogest treatment on pregnancy maintenance is related to suppression of LH release by altrenogest and, consequently, lower endogenous progesterone levels which resulted in disruption of pregnancy in more vulnerable (low pre-treatment progesterone and more fighting after moving) sows. To gain more insight into the effects of altrenogest on hormone levels and early pregnancy, additional research needs to be done.

NOTES

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EFFICACY AGAINST A PORCINE PARVOVIRUS INFECTION IN GILTS VACCINATED WITH THE MIXED ADMINISTRATION OF ERYSENG® PARVO AND UNISTRAIN® PRRS

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Objective. The aim of this study was to evaluate the efficacy against PPV of the mixed administration of ERYSENG® PARVO and UNISTRAIN® PRRS in gilts after a challenge with a pathogenic PPV strain.

Materials and methods. Twenty six-month-old gilts, clinically healthy and free from antibodies against PPV, E. rhusiopathiae and PRRSV, were randomly assigned to group 1 (n=12) and group 2 (n=8). Animals in group 1 were vaccinated following the recommended administration plan; they were immunised intramuscularly with ERYSENG® PARVO (2 ml/dose) and revaccinated three weeks apart with the combination of ERYSENG® PARVO and UNISTRAIN® PRRS (2 ml/dose, the freeze-dried tablet of UNISTRAIN® PRRS was reconstituted with ERYSENG® PARVO). Revaccination was done four weeks before mating. Animals in group 2 (placebo) received PBS following the same schedule as group 1. Blood samples were obtained on days 0, 21, 76 and 90 and antibody titres against PPV in serum were determined by the haemagglutination inhibition (HI) assay. Animals in both groups were challenged intravenously and intranasally on day 40 of gestation (day 90 of the study) with a 4 ml 106.1 CCID50 of a pathogenic PPV strain. All animals were humanely sacrificed to perform necropsy on day 90 gestation (day 140 of the study). The appearance of the foetuses was evaluated and blood samples as well as lung, liver and intestinal tissue were collected for virus detection (by haemagglutination, HA) and antibody detection (by HI). The differences between groups in antibody titres and reproductive parameters were assessed using the T-test ($p<0.05$).

Results. From day 76 until the end of the trial, the titres of PPV-specific HI antibodies were statistically significantly different between vaccinated and placebo-injected gilts. Regarding the appearance of the foetuses, the percentage of normal foetuses per litter and the number of piglets per litter were 99.25%/12 in the vaccinated group and 26.51%/3.14 in the placebo group, with significant differences ($p<0.05$). While 92.77% of the foetuses in the placebo group were infected by PPV, no infection was detected in any of the foetuses from the vaccinated group ($p<0.05$).

Conclusions: Vaccination with the mixed administration of ERYSENG® PARVO and UNISTRAIN® PRRS effectively protects animals from transplacental infection caused by PPV.

NOTES

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IMPACT ON IMMUNITY PARAMETERS OF PIGLETS FED MYCOTOXIN CONTAMINATED DIETS

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The negative effect of mycotoxins on the immunity status of animals is discussed regularly. An important aspect are the synergistic and additive effects for diets contaminated with more than one mycotoxin. The aim of this feeding trial was to investigate the negative effects of a zearalenone (ZEN), deoxynivalenol (DON) and fumonisin (FUM) contaminated diet on immune parameters and liver health in weaning piglets.

Twenty four female weaning piglets were randomly assigned to 2 groups with 12 piglets each. The trial lasted for 42 days. Piglets were assigned to a vaccination program including hog cholera, pseudorabies and food and mouth disease. Naturally mycotoxin contaminated corn was used to prepare the feed for the trial groups. Groups were as following: negative control group (A) and a mycotoxin contaminated (ZEN 1183 ppb, DON 1740 ppb, FUM 988 ppb) feed group (B). Parameters evaluated included antibody titers for pseudorabies referred to as sample to negatives ratio (S/N 0.6 was defined as positive), plasma CD4+/CD8+, IL-2, TNF-β, IgA, IgG, IgM, total plasma protein (TP) and liver enzymes (ALT, AST).
The ratio of S/N in group B was significant higher compared to group A at day 42 ($P<0.05$). There were no significant differences of plasma CD4+/CD8+ among the two groups. The plasma IL-2 levels of group B were significant lower than group A at days 14, 28 and 42 ($P<0.05$). The plasma TNF-β level of group B was significant lower than group A at day 28 and 42 ($P<0.05$). The IgA, IgG levels of group B were significant lower than group A at days 28 and 42 ($P<0.05$). There was no significant difference of IgM among the two groups. Plasma TP levels of group B were significantly lower than group A at days 14, 28 and 42 ($P<0.05$). Liver enzymes AST and ALT of group B were significantly higher compared to group A at day 14, 28 and 42 ($P<0.05$).

To conclude, the combination of mycotoxins can damage the liver and impair immune response in weaner piglets. The decreased antibody titer against pseudorabies and the increase of S/N ratio in the group fed with mycotoxin contaminated diet, suggests animal's decreased protection against pseudorabies when mycotoxins are present. This points to the need for greater awareness of a multiple mycotoxin contamination in feed and the protection of animals against said toxins.

NOTES

033

EFFICACY OF A NEW PCV2 AND M. HYOPNEUMONIAE COMBINATION VACCINE IS INDEPENDENT OF MATERNALLY DERIVED IMMUNITY

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Introduction. Under field conditions, the majority of pigs are vaccinated for PCV and M. hyopneumoniae (M hyo) in the face of maternally derived antibodies. Accordingly, PCV2 and M hyo vaccines need to induce immunity in the presence of MDA. Recently, a new ready-to-use PCV M hyo combination vaccine, Porcilis® PCV M Hyo, was registered. The impact of MDA on vaccine efficacy under field conditions was studied in a meta-analysis.

Material and Methods. To demonstrate safety and efficacy of Porcilis® PCV M Hyo, 10 field studies were performed on 17 farms across Europe between 2010 and 2013. Blood samples derived from 1118 three week old animals were analyzed for the level of PCV2 and M hyo MDA titer by ELISA. From 669 animals (333 vaccinates, 336 controls) from 9 field studies blood samples were taken at 3-4 wk intervals over the whole study period for determination of the viral load by quantitative PCR. In 7 of the studies, M hyo lung lesions were evaluated at slaughter. The serological result at 3 wks of age was correlated with a positive PCV qPCR at least once during the observation period and M hyo lung lesions at slaughter.

Results. For PCV, the antibody titers were first classified as low (<4 log2), moderate (≥4 log2 to 10 log2) and high (≥10 log2), followed by a comparison of overall % of animals with distinct MDA titers (from 1 log2 to 16 log2). According to the analysis, the majority of 3 wk old pigs had moderate antibody titers (65%, range 23%-94%) and the variability in frequency of animals with low, moderate or high antibody titers between the individual study sites as well as between individual animals of the same herd was considerable. The % vaccinated pigs PCV positive at least once was generally low (9% low MDA, 13% high MDA) compared to the % control animals (81% low MDA and 78% high MDA). The preventive fraction was greater than 80% in the 3 levels of MDA. The Spearman Rank correlation coefficient between viral load in blood and MDA was not significant (p=0.79).
For M hyo, the % serologically positive pigs at vaccination ranged between 2% and 48%. Lung lesions were significantly reduced after vaccination in farms with either low or high % of MDA positive pigs. There was no relation between the % MDA positive pigs at vaccination and reduction of lung lesion scores of vaccinated pigs compared to control pigs. A correlation analysis between % of MDA positive pigs at 3 weeks of age revealed no significant correlation with lung lesions at slaughter (p=0.48).

Conclusion. Timing of vaccination with Porcilis® PCV M Hyo is not impacted by the level of PCV or M hyo MDA as the vaccine is efficacious even in the presence of high MDA at 3 weeks of age.

NOTES

oral
PRESENTATIONS

VETERINARY PUBLIC HEALTH

Thursday, April 23rd > 15:00-16:00

034 **Isolation of Salmonella enterica in pigs at slaughter and genetic identity between isolates of porcine and human origin in Northern Italy**

035 **A comparison between lesions found during meat inspection of finishing pigs raised under organic/free-range conditions and conventional, indoor conditions**

036 **Meat juice serology, PCR and genotype patterns of Toxoplasma gondii in free-ranging, organic pigs in Italy**

034

ISOLATION OF SALMONELLA ENTERICA IN PIGS AT SLAUGHTER AND GENETIC IDENTITY BETWEEN ISOLATES OF PORCINE AND HUMAN ORIGIN IN NORTHERN ITALY

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Introduction: Salmonella spp. is a zoonotic microorganism responsible for food-borne disease worldwide. Even if the EU-trend is decreasing, salmonellosis is still the second most common food-borne disease in the European Union and swine are considered an important reservoir of microorganisms for humans.

Materials and Methods: From June 2013 to October 2014, 201 pigs at slaughter were tested for Salmonella spp. in mesenteric lymph nodes. Pigs were reared in 61 farms of four Italian regions (Lombardy, Emilia-Romagna, Veneto and Piedmont) and slaughtered in one abattoir in Emilia-Romagna region. A total of 67 batches of pigs were tested, and 3 animals per batch were randomly selected along the slaughter line. Prior to slaughter, 66 faecal samples were collected at lairage (two per sampling day). Salmonella spp. was detected following the ISO 6579:2002 method for lymph nodes and the ISO 6579:2002/Amd.1:2007 for faecal samples. Serotyping of isolates was performed following the Kauffmann White - Le Minoir scheme. Genotyping was carried out by PFGE after digestion of DNA with the restriction enzyme XbaI according to the Pulse Net protocol. A total of around 3.000 Salmonella isolates from hospitalized human patients of Emilia-Romagna region were typed from October 2011 to October 2014. They were linked to several salmonellosis outbreaks or single cases.

Results: Overall, 39 lymph nodes (19.4%) and 33 faecal samples (50.0%) were found positive for Salmonella. The following Salmonella serovars were detected, listed in order of frequency: S. Derby, S. enterica 1, 4, [5], 12:i:-, S. Rissen, S. Brandenburg, S. Manhattan, S. London, S. Livingstone, S. Muenchen, S. Stanley, S. Give.
All serovars except S. Stanley were isolated from human cases of infection. Comparing PFGE profiles between the porcine and human compartments, the largest proportion of pulsotypes that where present in both compartments was observed among the isolates of S. enterica 1, 4, [5], 12:i:- (7 out of the 10 porcine pulsotypes were present in the human isolates) whereas the smallest proportion of shared pulsotypes was observed among the isolates of S. Derby (6 out of the 15 porcine pulsotypes were present in the human isolates). Interestingly, pulsotypes of S. enterica 1, 4, [5], 12:i:- isolated from pigs did not always correspond to S. enterica 1, 4, [5], 12:i:- isolates in the human compartment, instead, in some cases they corresponded to the biphasic S. Typhimurium.

Conclusion: This study confirms the role of pigs as reservoir of pathogenic Salmonella strains for humans. Future studies will be necessary to acquire better knowledge on the ecology of S. Typhimurim and its monophasic variant between the porcine and human compartments, considering the importance of these serotypes in human salmonellosis.

NOTES

035

A COMPARISON BETWEEN LESIONS FOUND DURING MEAT INSPECTION OF FINISHING PIGS RAISED UNDER ORGANIC/FREE-RANGE CONDITIONS AND CONVENTIONAL, INDOOR CONDITIONS

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Introduction. It is often argued that pigs raised under less intensive production conditions – such as organic or free-range – have a higher level of animal welfare compared with conventionally raised pigs. This may be used to advocate organic/free range pig production.

Materials and Methods. To look into whether organic or free-range pigs have a higher level of welfare, an analysis of data from a large Danish abattoir slaughtering both organic, free-range, and conventionally raised finishing pigs was undertaken. Meat inspection data from a period of 1 year were collected. These covered 201,160 organic/free-range pigs and 1,173,213 conventionally raised pigs. The prevalence of each individual type of lesion was calculated, followed by a statistical comparison between the prevalences in organic/free-range and conventional pigs. Because of the large number of data, the P-value for significance was lowered to P=0.001, and only biological associations reflecting Odds Ratios above 1.2 or below 0.8 were considered to be of significance.

Results. The majority of the lesion types were recorded infrequently (<4%). Only chronic pleuritis was a common finding. A total of 13 lesion types were more frequent among organic/free-range pigs than among conventional pigs - among others healed rib fracture (OR=3.8), tail lesion (OR=3.2), chronic infectious arthritis (OR=3.2), old fracture (OR=2.2), osteomyelitis (OR=2.1), chronic peritonitis (OR=1.5), and chronic pericarditis (OR=1.3). Four lesion types were equally frequent in the two groups: chronic pneumonia, chronic pleuritis, fresh fracture, and abscess in head/ear. Four lesion types were recorded less frequently among organic/free-range pigs compared with conventionally raised pigs. These included abscess in leg/toe (OR=0.7), hernia (OR=0.7) and scar/hock lesion (OR=0.4).

Conclusion. We suggest that the higher occurrence of a number of lesions observed during meat inspection of organic/free-range pigs can be linked to the following factors: 1) A higher level of tail biting, 2) Limited all-in all-out management (batch management) and poorer hygiene, 3) A humid/wet floor in parts of the pen, and 4) Squeezing by the sow during the suckling period.
The results emphasize the importance of using direct animal based parameters when evaluating animal welfare in different types of production systems. Several of the lesions that occurred more often in organic/free-range production compared with conventional production were associated with infections that could have been treated with antimicrobials. This could suggest that the consumption of antimicrobials in organic and free range pig production is in fact too low - seen from an animal welfare point of view. Projects to elucidate this will be undertaken in Denmark in 2015.

NOTES

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MEAT JUICE SEROLOGY, PCR AND GENOTYPE PATTERNS OF TOXOPLASMA GONDII IN FREE-RANGING, ORGANIC PIGS IN ITALY

A. Vismarra (1), C. Bacci (1), C. Mangia (1), M. Genchi (2), I. Bruini (1), L. H. Kramer (1)
(1) Università di Parma, Parma, Italy; (2) IZS Lombardia ed Emilia Romagna, Pavia, Italy

Introduction. Toxoplasmosis is considered one of the most important food-borne parasitic zoonoses globally that could affect humans through the ingestion of tissue cysts present in raw or undercooked meat of the many intermediate hosts of the parasite, including pigs. Several recent studies have reported that free-ranging pigs are at a higher risk of acquiring infection compared to pigs raised in confinement. Pigs raised in non-confinement systems on organic farms are increasing in Italy, due to an upsurge in consumer demand for "organically raised", and "free range" pork products. The pig breed "Suino Nero" is indigenous to a small region in northern Italy (Emilia Romagna) and is bred for the production of both fresh meat and typical, salted/aged meat products, such as sausage and Parma ham. The aim of the study is to evaluate infection seroprevalence and genotype of T. gondii infection in this organically raised breed.

Materials and methods. Meat juice serology. From all the 21 pigs analyzed, meat juice (used for the serology) was obtained from 50 g of heart muscle, taken from the apex, frozen at -20°C and defrosted at room temperature after 24 h. ELISA test was carried out according to the manufacturer's instructions (Id Screen® toxoplasmosis indirect multi-species, Idvet, Switzerland). DNA extraction and identification of T. gondii. Fifty grams of heart tissue were sampled and blended with PBS and two-hundred µl of this omogenate were used for the DNA extraction carried out using a commercial kit (PureLink™ Genomic DNA Mini Kit-Invitrogen). T. gondii infection was confirmed by a nested-PCR targeting a 529 bp region, using the primers TOX4 and TOX5. Genotyping. Samples positive for the 529 bp region were genotyped by a multiplex multilocus nested PCR-RFLP to identify the major genotypes present in swine.

Results. Meat juice ELISA was positive in 20/21 (95.2%) pigs. Twelve out of the 21 (57.1%) pigs tested were positive on PCR. PCR-RFLP analysis resulted in a predominance of Type II or potential virulent types (Type I).

Conclusion. The high level of T. gondii infection highlighted in these pigs suggests that food risk associated with the ingestion of meat and undercooked meat products derived from the pig breed "Suino Nero" is very serious and further studies would be needed. Different genotypes of T. gondii were identified, maybe because organically bred swine have access to potentially new strains of T. gondii that may circulate in wildlife. Recombination events could potentially generate more T. gondii strains that may possess new biological properties, such as increased virulence for humans and this potential risk should not be ignored.

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oral
PRESENTATIONS

BACTERIAL DISEASES

Thursday, April 23rd > 16:30-18:00

037

MYCOPLASMA HYOPNEUMONIAE DETECTION IN TRACHEOBRONCHIAL SWABS, ORAL FLUID AND LUNG

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Introduction. Detection of M. hyopneumoniae (M.hyo) by quantitative polymerase chain reaction (qPCR) can be used to identify the beginning of an infection and its variations according to the age of the animal. The collection of OF showed advantages over the collection of blood samples and tracheobronchial swabs (TBS) regarding animal welfare, as it is less intrusive and pigs are more cooperative. The aim of this study was to compare the M. hyo titers in TBS and OF specimens by quantitative Real Time PCR (qPCR) in vaccinated and unvaccinated animals.

Materials and Methods. The study was carried out in a three-site herd in the North of Italy with endemic M. hyo infection. The animals were bred in the first site until a month of life, in the second site from one month to three months and in the third site from three months until slaughterhouse. Different M. hyo vaccination schemes were applied in three groups: 1) 30 pigs were vaccinated at 7 days of life, 2) 30 pigs were vaccinated at 26 days old and 3) 30 pigs were used as controls. Blood, TBS and OF samples were taken from each pig at the first week of life (T1) and once a month until 9 months old (T2-T10). Blood samples were tested by HerdChek® M. hyo antibody test kit while TBS and OF were analyzed by qPCR directed against the p102 gene of M. hyo. Samples were considered positive when the n° of genomic copies was equal or above 1,9 * 10^3 copies/ml.

Results. Both in TBS and OF of all groups, M. hyo infection was detected by qPCR when the animals were moved to the third site (T5) till the last sampling (T10). In TBS the percentage of positive animals at T5 was 30, 35, and 42% in group 1, 2 and 3, respectively. The higher number of positive pigs occurred at T6 in group 1 and T9 in group 2 and 3. Overall, TBS were positive for 52% out of 90 while OF for 8% samples. Copies/ml values were in the range of 1,9 * 10^3-1,7* 10^8 in TBS samples and 1,9 * 10^3-6,4* 10^5 in OF samples. ELISA results showed a maternal immunity at T1 in all groups. At T3, vaccinated animals developed antibodies against M. hyo while unvaccinated animals seroconverted when infection started (T5).

Conclusion. TBS showed to be a more sensitive specimen compared to OF in both vaccinated and unvaccinated animals. In TBS samples the M. hyo titers were meanly 3 logs higher than OF. Moreover, TBS analysis by qPCR allows the detection of a higher number of infected pigs. QPCR is a suitable method to detect M.hyo in all of the sampling times.

NOTES

038

POST-WEANING DIARRHOEA IN FRANCE: ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROTOXIGENIC ESCHERICHIA COLI (ETEC) ISOLATES

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Introduction. Post weaning diarrhoea (PWD) is characterized by decreased feed consumption, dehydration, sudden death and mild to severe watery diarrhoea occurring typically in the first weeks after weaning. Antimicrobials are usually required for treatment of PWD. However, due to the occurrence of resistance to one or more classes of antimicrobials (Luppi et al., 2013), monitoring antimicrobial susceptibility of clinical isolates of E. coli is recommended. The objective of this study was to investigate the antimicrobial susceptibility of recent isolates of E. coli from cases of PWD in French farms.

Materials and Methods. A total of 156 haemolytic E. coli isolates were included in this study. Isolates originated from 56 farms sampled across France in 2014 with a confirmed diagnosis of enterotoxigenic E. coli (ETEC) by PCR and had been obtained from diarrheic pigs within the first 48 hours of a PWD outbreak. Antimicrobial susceptibility testing was performed by disc diffusion method following the recommendations of the Antibiogram Committee of the French Microbiology Society and as listed in the French AFNOR Standard NF U47-107 (Labocea, France). The following antimicrobials were tested: neomycin (NEO), gentamycin (GEN), apramycin (APR), amoxicillin (AMX), amoxicillin-clavulanic acid (AMC), ceftiofur (CFT), enrofloxacin (ENR) and spectinomycin (SPT). Colistin (CST) was tested by E-test.

Results. The highest percentage of E. coli resistant isolates was found for AMX (58.97%), followed by SPT (48.08%). 38.46%, 37.18% and 14.74% of isolates were resistant for APR, NEO and GEN respectively. Only 1.92% of the isolates were resistant to CFT and none to AMC and ENR. In addition, 15.38%, 10.90%, 4.49%, 3.85% and 0.64% of the isolates had intermediate susceptibility to GEN, AMC, AMX, NEO and ENR respectively. Regarding colistin, 50% of the isolates had decreased susceptibility (MIC>2mg/L). Using an MIC breakpoint of ≥8 mg/L, 17% of the isolates were considered resistant to CST. For CST, a MIC90=8 mg/L and a MIC50=4 mg/L (range: 0.062-16 mg/L) was shown. Sixty-three isolates (40.38%) presented resistance to 4 or more of the antimicrobials tested. Twenty-six isolates were resistant to 5 or more antimicrobials, including decreased susceptibility to CST.

Conclusions. This study shows that antimicrobial resistance is a common occurrence among E. coli isolates from PWD cases in France. Decreased susceptibility to colistin was commonly found. Antimicrobial susceptibility testing is needed to assist in the selection of the antimicrobials. The high number of resistant isolates suggests the implementation of other measures to prevent and control PWD.

NOTES

039

STREPTOCOCCUS SUIS EPIDEMIOLOGY (USING SEROTYPING AND PRESENCE OF VIRULENCE FACTORS) AND AMR (ANTIMICROBIAL RESISTANCE) IN A LARGE PIG PRODUCTION SYSTEM IN ITALY

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Introduction. Streptococcus suis may induce clinical problems throughout the entire life of pigs and it is the second most frequently isolated pathogen in weaned piglets after ETEC E. coli. As many as 30 different capsular types of Strep. suis are described worldwide but only a limited number are causing clinical problems.The aim of this study is to describe the results of a four year long epidemiological study in a large, three-site production system showing the vertical distribution of the most frequently isolated serotypes and pathotypes (based on presence of capsular types and virulence factors) and the evolution of AMR (Antimicrobial Resistance) pattern of Streptococcus suis in time.

Materials and methods. Fifty-seven strains, from pathological samples (milking and/or weaned piglets, organs like liver, spleen and lungs and brain swabs) were isolated on Blood Agar Base and then further analyzed using five multiplex PCR to detect virulence associated-genes for cps1(capsular type 1), cps2 (capsul. type 2), cps7 (capsul. type 7), cps9 (capsul. type 9), epf (110 kDA extracellular factor), sly (suilysine), mrp (muramidase-relased protein) and arc(arginine deiminase). Serogrouping was also performed using commercial antisera (Statens Serum Institute, Denmark).All the strains, together with several hundreds more collected starting from year 2002, underwent an AMR test (Kirby Bauer) to monitor the sensitivity in time of the most commonly used antibiotics.

Results. Nine out of the fifty-seven submitted isolates originated from sow herds (site 1) while all others, except one, were originating from nursery pigs (site 2) thus reflecting the production period most affected by S. suis problems. In 80,7% of submitted cases S. suis was isolated from multiple organs (septicemic) while in 19,3% it was cultured exclusively from brain (meningitis). Serotype/capsular type 9 was the most frequently isolated (31%) and 50% of the serotype 9 isolates belonged to Arc/mrp/Sly + profile. The second most common isolate was type 1 / 2 (22%) while the third was serotype 4. Eight strains (14%) were non-typable both with PCR and the available antisera. Twenty-two strains (38,6%) had a complete Arc/mrp/Sly + profile. S. suis showed an extremely limited resistance to Betalactamic antibiotics like ampicillin (86-98% sensitive) and amoxicillin (95-100% sensitive) while showing a very high degree of resistance toward sulfonamides + trimethoprim (>90% resistent) and lincomycin (85-92% resistent).

Conclusions. Although the most commonly reported clinical symptom was meningitis, the large majority of the isolated strains proved to be highly invasive and could be isolated from different organs even when no virulence factors were present. It was impossible to find a one-way correlation between the sow-herd of origin and the respective sites 2 in term of isolated strains. Different serotypes could be isolated in time from the same flow of pigs. It is crucial to keep monitoring antimicrobial sensitivity of S. suis in time to be able to effectively treat affected pigs, but betalactamic antibiotics can still be considered first choice in case of treatment.

NOTES

040

IMMUNOREACTIVE PROTEINS OF BRACHYSPIRA HYODYSENTERIAE IN PIGS

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Introduction. Swine dysentery (SD), caused by the anaerobic spirochete Brachyspira hyodysenteriae, is a major problem in swine industry worldwide. Few antimicrobial drugs are active against B. hyodysenteriae and acquired antimicrobial resistance is regularly reported. The introduction of asymptomatic carrier animals represents an important threat for negative herds. Diagnosis is based on clinical signs, culture and PCR. However, no reliable diagnostic tests are commercially available to verify the SD status of a herd. By use of two-dimensional gel electrophoresis and western blotting (2DWB) followed by mass spectrometry (MS) we aimed to identify immunoreactive proteins of B. hyodysenteriae that could be useful for the development of a serological diagnostic test.

Materials and Methods. A total protein extract of B. hyodysenteriae was separated by a 2DWB protocol. Immunoreactive proteins were visualized by incubation of the blot with sera from pigs that showed clinical signs of SD after experimental inoculation with B. hyodysenteriae. Spots of interest were excised from the protein gel and digested with trypsin prior to MS. In a modified 2DWB protocol, serum of an infected pig was first incubated with a pool of total protein extracts of B. innocens, B. intermedia, B. pilosicoli, B. murdochii and "B. hampsonii" to capture antibodies that also reacted with proteins from these species, thus selecting for antigens specific to B. hyodysenteriae.

Results. The 2DWB, with sera from experimentally infected pigs, followed by MS resulted in a comprehensive list of potentially immunoreactive proteins of B. hyodysenteriae. However, total protein extracts of other Brachyspira species showed important similarities to that of B. hyodysenteriae. Preabsorption of the primary serum with total protein extracts of these Brachyspira species resulted in a less extensive pattern on the blots compared to the regular 2DWB protocol offering a solution to this problem.

Conclusion. Asymptomatic carrier animals are an important risk factor for the spread of SD between herds. A serological test could detect these dormant sources of SD and facilitate diagnosis and prevention. A drawback in the search of proteins unique for B. hyodysenteriae is the similarity between Brachyspira species. Using our adapted 2DWB protocol followed by MS we were able to compensate for this problem and identify proteins of B. hyodysenteriae that are potential candidates for the development of diagnostics. Further research is needed to confirm the importance of these proteins of B. hyodysenteriae by recombinant expression followed by one-dimensional gel electrophoresis.

NOTES

041

ORAL FLUID DETECTION AND DYNAMICS OF BORDETELLA BRONCHISEPTICA INFECTION IN PIG HERDS

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Bordetella bronchiseptica (Bb) is a recognized secondary opportunist invader in swine pneumonia. Some Bb strains cause a mild non progressive rhinitis. If co-infection with toxigenic Pasteurella multocida (PMT) occurs, then the combination can produce severe atrophic rhinitis (AR), causing production losses and trade limitations. Although Bb has large worldwide presence, little knowledge is available regarding its epidemiology. A cross-sectional study was designed to monitor Bb dynamics in porcine commercial herds, and to investigate the associations that may exist between Bb infection, presence of respiratory clinical signs, and associated risk factors within farms.

This study was conducted in 20 commercial farms housing pigs unvaccinated against AR, and testing negative to PMT by qRT-PCR. Farm selection was balanced according to the type of production and the presence/absence of respiratory clinical signs. Between 25 and 30 ropes per herd were placed across pig production life: pigs aged 3-4, 8-10, 14-15, 18-20 and sows. Bb in oral fluid (OF) was detected by qRT-PCR. A survey was carried out to gather data about demographical, management, genetics and environmental traits in each farm. A descriptive analysis was conducted to describe the frequency of Bb infection detected at herd level and within herds. Diverse logistic regression models with aggregated and disaggregated data by age group, were proposed to assess the association between Bb infection and different factors.

A total of 557 ropes were placed from which 494 OF were obtained (89%). While sampling effectiveness in pigs was almost 100%, in sows it was only 65%. All herds studied were infected by Bb with a prevalence ranging 16 to 71% (RT-qPCR-positive OF; median 55%). The frequency of positive OF samples did not differ significantly either among farms with different production systems, nor in farms with or without presence of clinical signs compatible with AR. Similar, though decreasing, positive proportions of Bb in OF were detected in pigs from 3 (70%) to 20 (34%) weeks of age, whereas the percentage of OF positive to Bb in sows was low (6%).
Higher percentages of within-herd Bb infection could be associated with inefficient ventilation, poor environmental conditions, and big farms and large pen size, with differences between age groups. Moreover, additional statistical analyses showed that the onset of clinical signs was linked to the detection of infection in weaning pigs. However, this association could not be demonstrated in growers or finishers, fact that could be explained by the colonization by Bb in the early ages.

NOTES

042

CHARACTERIZATION OF BRACHYSPIRA HYODYSENTERIAE FROM ITALY BY MULTILOCUS SEQUENCE TYPING AND MULTIPLE LOCUS VARIABLE NUMBER TANDEM REPEAT ANALYSIS

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Introduction: Brachyspira hyodysenteriae is an anaerobic intestinal spirochaete and the aetiological agent of swine dysentery (SD). The disease is characterized by severe muchoemorragic diarrhoeal and affects pigs primarily during the growing–finishing period. The increased resistance to antimicrobials observed in many countries induces to understand the dynamics of SD. The development of more effective measures to counter its spread depends also on the ability to characterize B. hyodysenteriae variants and trace relationships of epidemic strains through reliable strain typing methods.

Materials and method: One hundred and four B. hyodisenteriae isolates were collected during a period of three years (2012–2014) from 75 Italian farms placed in 13 different administrative areas. The typing of every single strain was conducted using both multilocus sequence typing (MLST) and multiple locus variable number tandem repeat analysis (MLVA) to understand the better tool for their characterization. In particular, the MLST approach analyzes the allelic variation in nucleotide sequences of seven housekeeping gene loci (adh, alp, est, gdh, glpk, pgm and thi). Whereas, MLVA technique is based on the detection of the number of tandem repeats at seven multiple variable number tandem repeat (VNTR) loci within the genome (Bhyo6, Bhyo7, Bhyo12, Bhyo17, Bhyo21, Bhyo22 and Bhyo23).

Results: The study conducted with the MLST method described 19 different sequence type (ST), 8 clusters and 11 unique. One predominant ST (ST83) was observed in 54 strains isolated from 36 different farms located in 9 areas. In addition, the amino acid analysis recognized the presence of 12 amino acid type (AAT) parted in 2 clonal complex e 1 singleton. Phylogenetic analysis of the concatenated nucleotide sequences showed the presence of three major groups. The MLVA approach allowed to characterize completely 86 strains on a total of 104. The isolates were then gathered into 13 different VNTR profiles, 6 clusters and 7 unique. The VNTR profiles included all the STs previously recognized. Only in one case the analysis of VNTR gave a different and more specific characterization. The calculation of Diversity Index (DI) showed that MLST (DI=0,720) is a more discriminatory method than MLVA (DI=0,663).

Conclusion: In conclusion, MLST approach is a reliable and discriminant method to characterize Brachyspira hyodisenteriae strains and to establish epidemiological correlation among different outbreaks.

NOTES

REPRODUCTION

Friday, April 24th > 10:30-12:30

043

IMPACT OF CO-CULTURING CUMULUS-ENCLOSED PORCINE OOCYTES WITH DENUDED OOCYTES DURING IN VITRO MATURATION IN A DEFINED MEDIUM ON CUMULUS EXPANSION AND OOCYTE MATURATION

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Introduction. Recent research has revealed that oocyte secreted factors (OSF) affect cumulus expansion and play important roles during in vitro maturation (IVM) and embryo development of mammalian oocytes. The use of denuded oocytes (DOs) as supplements during IVM in a non-defined medium improved developmental competence of cumulus-enclosed oocytes (COCs). We investigated the effect of DOs on cumulus expansion and nuclear maturation of COCs during IVM using a defined medium. If the DOs exert a positive influence on IVM, the defined medium can then be analyzed for the presence of OSF.

Materials and Methods. Immature COCs were collected in the slaughterhouse from prepubertal gilts. To obtain DOs, COCs were completely denuded by pipetting through a narrow-bore glass pipette. The COCs used as a source for DOs fulfilled the same morphological criteria as the COCs used for IVM. The IVM medium was porcine oocyte medium (POM) with hormones applied only during the first 20 h of IVM. The COCs were fixed to the bottom of 35 mm plastic petri dishes in 3x3 grids by Cell-Tak (BD Bioscience, Bedford, MA, USA) in 100 µl droplets POM covered by paraffin oil. Culture droplets were supplemented with (DO+, n=179) or without 16 DOs (DO-, n=143). After 20 h of IVM, the medium was replaced with a hormone free POM and oocytes were cultured for an additional 28 h. At 0 h, 20 h and 48 h of IVM, images of each grid were taken at the same magnification. The size of each COC was measured as a 2 dimensional area in pixels by analyzing images with ImageJ software. Relative cumulus expansion was calculated at 20 h and 48 h of IVM on the basis of the initial COC size at 0 h which was assigned as 1. At 48 h of IVM, the COCs were denuded and examined for oocyte maturation by orcein staining. Five replicates were performed. Cumulus expansion ratios at 20 h and 48 h of IVM were compared between DO+ and DO- by ANOVA. Maturation rates were compared between DO+ and DO- by binary logistic regression.

Results. No difference in cumulus expansion between DO- and DO+ could be observed at 20 h (1.83 and 1.75, respectively) and 48 h (1.41 and 1.47, respectively) of IVM. Nuclear maturation rates of COCs in DO- and DO+ groups did not differ significantly (39.0% and 32.9%, respectively).

Conclusion. Addition of DOs to the defined IVM medium did not affect the cumulus expansion and oocyte maturation of follicular porcine COCs. Further research is needed to assess the effects of DOs during IVM on subsequent fertilization. If DOs prove to be beneficial for fertilization, the nature of the OSF will be investigated.
This study was supported by FCWO of UGent and by FWO-Flanders (grant number FWO11/ASP/276).

NOTES

044

ABORTIONS IN SOWS IN THE NETHERLANDS: RESULTS OF POST MORTEM INVESTIGATION AND SIGNIFICANCE OF LEPTOSPIRAL AND CHLAMYDIAL INFECTIONS.

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(1) GD Animal Health, Deventer, Netherlands

Introduction. The histopathology department of GD Animal Health in the Netherlands receives about 3000 submissions of pigs for post mortem examination (PME) every year. About 200 of these involve aborted litters. Unfortunately in only 20 - 30% of these cases a diagnosis can be made. In an attempt to improve this figure, farmers and veterinarians were asked to submit aborted placentas along with the piglets

Material & Method. Piglets from 100 aborted litters as well as 50 aborted placentas were meticulously examined (macroscopy, histology, bacteriology, virology). In addition, to establish the significance of pathogenic Leptospira and Chlamydia species, in 25 placentas a general PCR on Chlamydia spp. was performed, from 80 aborted litters pooled tissue samples (lung, kidney) were tested for pathogenic leptospira by PCR at IVD Hannover, and from another 25 aborted piglets liver and lung samples were tested specifically for Chlamydia suis by PCR at Ghent University.

Results. In 17 submissions PRRSv was found, 14 times in late abortions and 3 earlier in gestation. US strain PRRSv was found twice. In 16 submissions PPV was detected, 8 of which were actually born (almost) a terme. In 6 cases there was a co-infection with PRRSv (4) or Staphylococcus hyicus (2). Only in 2 cases there was no other indication for an infection besides PPV. In 9 submissions piglets appeared to have a myocarditis. This can be caused by a PCV2 infection. However, PCV2 was identified only once. In 17 piglets bacteria have been demonstrated, i.e. Staph. hyicus (7) E. coli (6), Str. dysgalactiae (3) and Enterococcus faecalis (1). In 4 of these cases also viruses were detected. Most of the cultured bacteria are environmental, opportunistic pathogens. Pathogenic Leptospira spp. and Chlamydia suis, however, were never detected in tissues of aborted piglets. Out of the 50 examined placentas, 11 showed alterations varying from hyperaemia (5) to placentitis (3). One placenta revealed calcification, the impact of which is unclear. Chlamydia spp. were never detected in aborted placentas.

Conclusion. The 'diagnosis rate' in PME of aborted piglets can be increased by 5 to 10 per cent by thorough investigation and involving the assessment of placentas, but still in many cases of abortion it is difficult to establish a diagnosis by performing PME plus extensive follow-up testing. Apparently, the significance of leptospirosis and chlamydiosis in cases of abortions in pigs in the Netherlands is low.

NOTES

045

MULTIPLE RISK FACTORS FOR RETURN TO OESTRUS IN GROUP HOUSED SOWS; A CASE STUDY

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Background: For three years in a row, a 600 head sow farm encountered many sows that irregularly returned to oestrus (15% average (range (0 – 50% per week group) at median 31 days after insemination. Sows are housed in small stable groups (30 sows) with one electronic sow feeder per group.

Methods: We analysed the computerised data, inspected the housing, feed(ing) and the sows. Additionally gestating sow behaviour was analysed from video recordings.

Results: Data analyses: For return to heat, there were no associations with season, sow parity and total born piglets in previous litter. Sows with >13 weaned pigs had higher odds to return to oestrus during next breeding. The number of total born piglets of the second parity sows was too low. Animal inspection: Lactating sows showed more than 4 mm loss in back fat during lactation, known to be associated with return to oestrus (1). Data analyses suggested that some sows are inseminated while not being in heat, which was confirmed during inspection of insemination. Housing analyses: Overcrowding (<143%) was observed in some groups (2). Video analyses showed that sows expressed a great deal of distress around feeding stations. The entry gates of some feeding stations malfunctioned, resulting in sows being chased out of the feeding box by the next sow before their portion was eaten. Finally, aerial ammonia levels between gestating sows were found up to 84 ppm (2).
Analyses of feed management: 84% of lactating sows were fed below the recommended energy intake levels. Too many sows were observed with mastitis post-partum and also ambient temperature of the farrowing unit was too high (>24°C). Environmental heat and low feed intake in early lactation are known to reduce embryonic survival after next breeding (3). Finally, gilt feeding was suboptimal, resulting in too low body weight and condition at first insemination (1).

Discussion and conclusion: Multiple management related risk factors for increased return to heat were detected on one farm. These factors may induce chronic stress which is known to be a major risk factor for loss of pregnancy in sows (2). When sows do not express clinical signs of disease during early gestation, these management factors should be thoroughly investigated first, before considering infectious pathogens as a cause. Whereas it is debated whether group housing is a risk for abortions and return to heat due to ingestion of placental material, this case shows that many other risk factors should be investigated and improved first. A follow-up on this case will be presented.

(1) Whittemore; Livestock Prod. Sci. 1996
(2) Spoolder et al.; Livestock Sci. 2009
(3) Kirkwood et al.; Can, Journ. An. Sci., 1987

NOTES

046

PREVALENCE OF BACTERIURIA AND PATHOLOGICAL CHANGES IN THE URINARY TRACT OF CULLED SOWS.

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Introduction. Treatment of urogenital infections, including urinary tract infections (UTI), constitutes the second most common indication for antibiotic treatment of sows in Denmark. However, the prevalence of cystitis in Danish sows is largely unknown. The target population for this study was culled sows in Denmark. The first objective was to estimate the prevalence, and bacterial causes, of bacteriuria in sows. The second objective was to investigate association between bacteriuria and macroscopic pathological changes in the bladder.

Material and methods. A total of 180 sows from 105 herds were sampled at one Danish slaughterhouse. From each sow a urine sample was taken by cystocentesis 0-3 hours after removal of the bladder at the slaughter line. The urine was cultured on standard blood agar immediately after urine collection and incubated for 24 hours at 37 degrees celcius. Semi quantitative bacterial count was performed and bacterial isolates were identified with the MALDI-TOF procedure. The bladders were stored at 0-10 degrees celcius for 18-26 hours. For each bladder pathological changes and drained weight were recorded. Sows with bacterial count of 1000 CFU per ml of urine or more were classified as having bacteriuria. Chi-square and ANOVA tests were used for unconditional testing of associations between bacteriuria, pathological changes and bladder weight.

Results. The prevalence of sows with bacteriuria was 32%. Preliminary results from the MALDI-TOF procedure showed that approximately 50% of the positive urine cultures were Escherichia coli. Prevalence of pathological changes observed was mucosal hyperaemia (63%), thickening of the bladder wall (1%), oedema (9%) and trabecular patterns of the serosa (4%) respectively. The prevalence of hyperaemia was higher in sows with bacteriuria (81%) compared to sows without bacteriuria (54%) (odds ratio = 3.6, p = 0.001). No other pathological changes were associated with bacteriuria. Mean bladder weight was 302 g and 241 g in sows with and without bacteriuria respectively (p = 0.0003).

Conclusion. Bacteriuria was demonstrated in a third of the culled sows which is comparable to results from studies in other countries. The finding of a higher bladder hyperaemia prevalence and bladder weight in sows with bacteriuria suggest that occurrence of more than 1000 CFU per ml urine may be associated with inflammation of the bladder mucosa. Histological examinations are needed to confirm this and implications with regards to welfare and production of the sow should be addressed in further studies.

NOTES

047

DISTRIBUTION OF REGULAR AND IRREGULAR INTER-OESTRUS INTERVAL IN SOW DURING DIFFERENT PERIOD OF THE YEAR IN NORTHERN ITALY

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INTRODUCTION. Seasonal infertility in sow includes reduced farrowing rate, prolonged wean-to-oestrus interval, delayed onset of puberty, and autumn abortion syndrome. Sows that experienced a conception failure or very early embryonic losses will return to oestrus at a regular interval (18-24 days), while animals that are pregnant but subsequently show a complete embryo loss return at irregular intervals (25-36 days). The aim of this study has been to determine the pattern of regular or irregular inter-oestrus interval during different months of the year to better understand the effect of temperature and/or photoperiodism on fertility.

MATERIALS AND METHODS Insemination data for 51,048 sows from 20 farms located in the northern Italy from January to December 2009 were extracted. During the year 2009 a significant increase in temperature were observed in June, July and August. The sows that returned-to-oestrus after weaning were divided into three groups: 1) Rint-1 (Regular inter-oestrus interval type 1): sows with inter-oestrus interval between 18 and 23 days. 2) Rint-2 (Regular inter-oestrus interval type 2): sows with inter-oestrus interval between 36 and 48 days. 3). IRint (Irregular inter-oestrus interval): inter-oestrus interval between 24 and 35 days.

RESULTS. The total number of successful insemination at first oestrus after weaning was 44,275 out of 51,048 inseminated animals (86,7%). The number of not pregnant sows at ultrasound pregnancy exam was 6,773 of which, 5,103 (75%) returned in oestrus. The rate of successful insemination was reduced (83,6%) during the month of June, July and August compared the rest of the year was (87.7%) ($P<0.05$). Between June and August the proportion of sows with Rint-1 was increased ($p <0.05$) respect to the rest of the year. Conversely an increase of IRint was observed between September and October. No differences were observed in the fraction of Rint-2 throughout the year.

DISCUSSION. The observation that a greater proportion of sows present regular inter-oestrus interval during the period of June-August suggest a failure of the insemination or very early embryos losses during this period of the year. The increased of irregular inter-oestrus interval during the period of September and October suggests that insemination was successful but then embryos were lost. To know these patterns of reproductive losses is useful from a clinical point of view because suggest that, during the high temperature months of the year, the focus should be on insemination management while during the period of September-October the focus should be mainly on pregnancy maintenance.

NOTES

048

POST–PARTUM ULTRASOUND EXAMINATION OF THE UTERUS AS DIAGNOSTIC TOOL FOR BIRTH COMPLICATIONS

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Birth complications can increase the risk of post-partum diseases, such as PDS, and decrease fertility after weaning. Specifically, prolonged farrowing duration can lead to decreased subsequent pregnancy rate. Aim of the study was to explore the association between post-partum (p.p.) ultrasound examination with uterine size determination and traits of the birth process.
At birth, we recorded 107 sows for the following traits: piglet expulsion duration (PiD, time interval between birth of first and last piglet), placenta expulsion duration (PlD, time interval between expulsion of first and last placenta), last piglet last placenta duration (LPLPD, time between expulsion of last placenta relative to the last piglet), total birth duration (TBD, time between birth of first piglet and expulsion of last placenta), primary placental retention (PPR, if no placenta was expelled at all or if LPLPD < 0 minutes), dystocia (DYS, how many times the time interval between two piglets was more than 60min), birth help (BH, how many times birth help was given), and oxytocin (OT, how many times oxytocin was given). The transabdominal ultrasound examination was performed once between second and seventh day after farrowing. The uterus was imaged with a 4,3 MHz convex array probe (MyLab™OneVet, Esaote, SC3421 Vet). The areas (cm2) of 5 cross-sections were measured cranially of the urine bladder and an average was calculated.

The following uterine sizes (mean ± SD) were determined: 2nd day p.p. (n=9) 4.3 (±1.2) cm2, 3rd day p.p. (n=28) 4.2 (±1.9) cm2, 4th day p.p. (n=25) 4.1 (±1.2) cm2, 5th day p.p. (n=21) 3.8 (±1.2) cm2, 6th day p.p. (n=14) 3.8 (±1.1) cm2, and 7th day p.p. (n=10) 3.4 (±0.9) cm2. According to the mean and day of examination, the uterine size was categorized into SMALL (n=61) and LARGE (n=46). The effects of PiD, PlD, LPLPD, TBD, DYS, and BH on the uterine size were analyzed with a logistic regression model and the effects of PPR and OT with a Chi-square test. The following traits had significant effects on the uterine size: PiD, TBD, BH, and PPR. PiD was 359min (SMALL) vs. 470min (LARGE, p=0,024). TBD was 652min (SMALL) vs. 769min (LARGE, p=0,027). BH was 0.11 (SMALL) vs 0.53 (LARGE, p=0,024). DYS was 1.42 (SMALL) vs. 2.00 (LARGE). All sows with PPR had LARGE uterine size (OR 19.5). OT use had a tendency to decrease the uterine size (p=0,072).
The results show, that post-partum ultrasound examination can give an idea if a sows might have had birth complications and provide a tool for diagnosis of post-partum diseases leading to decreased fertility after weaning.

NOTES

oral
PRESENTATIONS

IMMUNOLOGY & VACCINOLOGY

Friday, April 24th > 10:30-12:30

049 Effect of maternally derived antibodies on porcine circovirus type 2 (PCV2) infection dynamics on average daily weight gain (ADWG) in PCV2 vaccinated pigs

050 Immunomodulating mechanism of oral ß-glucans in pigs could be hampered by the epithelial mucosal barrier

051 Dynamic change in lung macrophages and cytokines environment during infection of pigs with a high or low virulent genotype 1 PRRSV strain

052 Effects of single or combined vaccinations against Mycoplasma hyopneumoniae and/or porcine reproductive and respiratory syndrome virus in dually infected pigs

053 Sulfated polysaccharides-rich extract of Ulva armoricana green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells

054 First efficacy evaluation of a novel combination vaccine against Enzootic Pneumonia (Mycoplasma hyopneumoniae) and Porcine Circovirus type 2 (PCV2) in the presence of strong maternally derived PCV2 immunity in pigs

049

EFFECT OF MATERNALLY DERIVED ANTIBODIES ON PORCINE CIRCOVIRUS TYPE 2 (PCV2) INFECTION DYNAMICS ON AVERAGE DAILY WEIGHT GAIN (ADWG) IN PCV2 VACCINATED PIGS

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Introduction. The present study aimed at comparing the efficacy of a porcine circovirus type 2 (PCV2) vaccine in terms of seroconversion, infection and average daily weight gain (ADWG) between animals with different maternally derived antibody (MDA) levels.
Materials and Methods. Six hundred 2 week-old piglets from vaccinated (n=33) and unvaccinated (n=31) sows were bled and weighted. From these 600 animals, piglets with high (ELISA S/P values >1.44; n=169) and with low (ELISA S/P, <0.96; n=168) ELISA S/P ratios were included in the study and distributed (based on weight and S/P ratios) in 4 groups: (high S/P ratios (H)-vaccinated (V), n=93; low S/P ratios (L)-V, n=78; H-NV, n=76; L-NV, n=90). At 3 weeks of age, V piglets (n=171) received 1 ml of Ingelvac Circoflex® and NV (n= 166) ones received 1 ml PBS. All piglets were subsequently bled at 7, 12, 18, 22 and 25 weeks of age and weighted again at 12 and 25 weeks of age. Mortality was registered and ADWG was calculated. Viraemia and PCV2 antibody dynamics were assessed by PCR plus real time quantitative PCR (QPCR) and ELISA commercial kits, respectively.
Results. The number of dead animals per group was 7 for H-V, 3 for L-V, 5 for H-NV and 5 for L-NV during the period of study. No significant differences between groups in terms of mortality were observed. Globally, V animals showed lower PCV2 infection rate ($p<0.05$ at 18 to 25 weeks of age), lower mean PCV2 viral load ($p<0.05$ at 18 to 25 weeks of age) and higher mean ELISA S/P values ($p<0.05$ from 7 to 18 weeks of age) than NV ones. Also, V pigs showed 17 and 33g higher ($p<0.05$) ADWG in the 2-25 and 12-25 week periods, respectively, than NV ones. Among the V piglets, L pigs showed lower PCV2 infection rate ($p<0.05$ only at 22 weeks of age) and an earlier seroconversion due to vaccination ($p<0.05$ at 12 weeks of age) than H ones. When comparing ADWG, 2 and 18 g higher ($p>0.05$) ADWG in the 2-25 and 12-25 week periods were observed, respectively, than H ones.
Conclusions. In conclusion, PCV2 vaccination was effective in reducing viral load as well as percentage of infected pigs in both L-V and H-V groups compared to control ones. However, L-V pigs showed numerically higher (but no statistically significant) ADWG compared to H-V animals. Acknowledgements: this study was supported by the PCV2 Boehringer Award 2012 and H. Feng is a recipient of a scholarship from the Chinese Scholarship Council.

NOTES

050

IMMUNOMODULATING MECHANISM OF ORAL ß3-GLUCANS IN PIGS COULD BE HAMPERED BY THE EPITHELIAL MUCOSAL BARRIER

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Introduction. The prophylactic use of antibiotics is prohibited in Europe since January 2006. Therefore, alternatives are searched for in order to prevent infection on problem farms. Certain ß-glucans, polymers of D-glucose, have the capacity to activate the innate immune system. In a number of experiments we aimed to determine if they could increase the resistance against infections with enterotoxigenic E. coli (ETEC), an important cause of economical losses in newly weaned piglets and we wanted to understand their mode of action.
Material and methods. Several experiments were performed. In a first experiments four groups of pigs, either receiving one of three different ß-glucans or no glucans were challenge infected with F4+ ETEC. In a second experiment, one ß-glucan preparation was given to 1-week-old gnotobiotic pigs during 35 days, whereafter these piglets were orally immunized with F4 fimbriae in order to see an adjuvant effect of the glucans. In a third experimental set up, the effect of seven different ß-glucans on lymphocyte proliferation, reactive oxygen species (ROS) production by neutrophils and monocytes and cytokine production was analyzed. In a fourth experiment, the role of the ß-glucan receptors dectin-1 and the complement-receptor 3 (CR3) in activation of monocytes and neutrophils by ß-glucans was examined.
Results. The challenge study in conventionally bred, weaned piglets showed that ß-glucans can protect against an ETEC infection. Macrogard from S. cerevisiae and scleroglucan significantly reduced faecal excretion of F4+ ETEC. No effect of ß-glucans was seen on an oral F4 immunization in gnotobiotic pigs, suggesting that the glucans did not exert their protective effect by modulating the humoral mucosal immune response. Incubating different leukocytes with different ß-glucans showed that these glucans exerted different effects on the leukocytes. Macrogard induced production of reactive oxygen species (ROS) by monocytes and neutrophils, while this was not the case for scleroglucan. The latter enhanced cytokine production, similar to the induction of cytokines by macrogard. This suggest that cytokine production rather than ROS production might be important for protection.
The fourth experiment demonstrated that the complement-receptor 3 (CR3) plays a cardinal role in ß-glucan receptor recognition and signaling by porcine neutrophils, while CR3 and dectin-1 were important for activation of macrophages, similar to what has been seen in man, but not in mice.
Conclusion. Protection of pigs against F4+ ETEC infection is ß-glucan dependent. One of the protective ß-glucans is macrogard. This ß-glucan does not exert its effect via an adjuvant effect on antibody production. It activates innate immunity via the CR3 in neutrophils and via the CR3 and dectin-1 in macrophages. Most likely crossing the intestinal mucosal barrier is crucial for ß-glucans to exert their effect on an ETEC infection

NOTES

051

DYNAMIC CHANGE IN LUNG MACROPHAGES AND CYTOKINES ENVIRONMENT DURING INFECTION OF PIGS WITH A HIGH OR LOW VIRULENT GENOTYPE 1 PRRSV STRAIN

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Introduction. Lung macrophages play an important role in host defense at both innate and adaptive immunity levels. During PRRSV infection, these key cells are the main target for virus replication and their functions are altered. In 2007, a new highly pathogenic PRRSV strain (Lena, genotype 1.3) was isolated in Eastern-Europe. To better understand the pathogenesis of Lena infection, we characterized the longitudinal dynamic of macrophages and their cytokines environment in the lung of infected pigs and compared them to that of pigs infected by a low virulent genotype 1.1 PRRSV strain (Finistere).
Materials and Methods. Six-weeks-old SPF pigs were inoculated with either Lena (n=8; L group), Finistere strain (n=5; F group) or media (n=5, Control group). The animals were monitored daily for clinical signs. Bronchoalveolar Lavage Fluids (BALF) and blood were collected regularly after infection to follow macrophages (flow cytometry), virus (RT-PCR) and cytokines (ELISA) dynamics.
Results. In L group, pigs exhibited high fever and clinical score leading to death of 3 animals whereas in F group, pigs displayed only mild clinical signs. Compared with F group, PRRS viral load in L group was 10 to 100-fold higher in serum and BALF, but decreased faster from 22 dpi in BALF. After infection, BALF cells displayed a drop in viability and phagocytosis activity matching with a decrease of macrophages population for both groups. In F group, the loss of lung macrophages was delayed compared to L group. From 8 to 15 dpi, animals of L group showed a simultaneous increase of monocytes in BALF and blood whereas it was only observed in BALF of F group. Regarding cytokines, low levels of IFNa and TNFa were measured in BALF for both strains but with a delayed response for F group. In serum, high levels of IFNa and TNFa were detected in L group but not in F group. Conversely, IL8 levels were 12-fold higher in L group than in F group in BALF whereas low levels were measured in serum for both strains. High levels of IFNg were also found in BALF in L group from 8 to 15 dpi whereas the IFNg level was low in BALF of F group and no IFNg were detected in serum of both groups.
Conclusion. We confirm that Lena strain induces high fever, high systemic viral load and inflammatory response. In the lung, high macrophages alteration linked to high viral load was associated with a rise of monocytes population and high IL8 levels. For Finistere strain, we showed a delayed and lower innate response as well as a prolonged viral replication possibly related to weak IFNg response. Better understanding the immune response to highly virulent PRRSV strain is a key point for a better control of these strains.

NOTES

052

EFFECTS OF SINGLE OR COMBINED VACCINATIONS AGAINST MYCOPLASMA HYOPNEUMONIAE AND/OR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN DUALLY INFECTED PIGS

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Introduction. Porcine Respiratory Disease Complex (PRDC) is a main cause of economic losses for swine producers. This complex is due to a combination of pathogens and their interactions. Two major pathogens involved in PRDC are Mycoplasma hyopneumoniae (Mhp) and Porcine Reproductive and Respiratory Syndrome virus (PRRSV). The objectives of this work were (i) to develop a model of experimental dual Mhp/PRRSV infection of specific pathogen free (SPF) pigs with European strains of Mhp and PRRSV (study 1) and (ii) to assess and compare the effects of single Mhp, single PRRSV or combined Mhp/PRRSV vaccination against this dual Mhp/PRRSV infection (study 2).

Materials and Methods. In study 1, 4 groups of 4 piglets each (7 weeks old - wo) were inoculated by Mhp, PRRSV, both pathogens simultaneously, and culture media, respectively. Clinical follow-up (daily rectal temperature, cough counts, weekly body weight measure) was performed during 4 weeks and pneumonia was scored at necropsy at 28 day post-inoculation (DPI).

In study 2, 4 groups of 8 piglets (1 wo) were either vaccinated with a Mhp killed vaccine (at 1 and 4w), VM group; with a PRRSV modified live vaccine of genotype 1 (MLV1, at 4w), VP group; with both Mhp and PRRSV vaccines (Mhp at 1 and 4w, MLV1 at 4w), VMP group; or were kept unvaccinated, UV group. Three weeks after the last vaccine, pigs were inoculated with Mhp/PRRSV, according to study 1. One control group of 8 unvaccinated and uninoculated animals was also included. Clinical signs were assessed daily and blood samples were collected weekly to evaluate serological responses. At 21 DPI, swabs were collected to detect PRRSV and Mhp. At necropsy (28 and 35 DPI), pneumonia was scored and samples collected for Mhp and PRRSV detection.

Results. In study 1, pigs dually infected with Mhp and PRRSV exhibited a combination of typical symptoms for each pathogen, i.e. PRRSV related hyperthermia and reduced average daily gain (ADG) as well as coughing and pneumonia typical to Mhp infection. However, no exacerbation of individual pathogenicity was observed.

In study 2, hyperthermia was reduced in PRRSV vaccinated animals (VP and VMP groups), whereas ADG was restored in dually vaccinated pigs only (VMP group). Regarding respiratory symptoms and pulmonary lesions, no vaccine induced a decrease of coughing. However, all vaccines induced decreases in pneumonia score but with a higher extent for VM and VMP groups. Those vaccines also induced a decrease in Mhp load in the respiratory tract.

Conclusion. Combined vaccination against Mhp and PRRSV efficiently joins the efficacy of each vaccine and could be an interesting tool to control PRDC in European swine production.

NOTES

053

SULFATED POLYSACCHARIDES–RICH EXTRACT OF ULVA ARMORICANA GREEN ALGAE EXHIBITS AN ANTIMICROBIAL ACTIVITY AND STIMULATES CYTOKINE EXPRESSION BY INTESTINAL EPITHELIAL CELLS

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Introduction. Antibiotics have been used for a long time in pig production to protect animals against pathogens. However, EU policy has been adopted to implement a sustainable production without adding antibiotics as growth promoters. Marine algae contain in their cell wall water-soluble sulfated polysaccharides with potential biological activities such as antiviral, antibacterial and immunomodulating that are explored to be used as an effective alternative to antibiotics. An extract of sulfated polysaccharides was prepared from the green algae Ulva armoricana harvested in Brittany region (France) and tested for its antibacterial activity as well as its capacity to modulate the immune response.

Material and Method. Green tide algae Ulva sp. were collected on the beach at Plestin les Grèves in Britany (France), and a sulfated polysaccharides-rich extract was prepared to be tested as an antibacterial compound against 40 strains of bacterial pathogens found in livestock animals. The antimicrobial activity of this extract and the MIC value were determined using mutispot inoculation assay on agar plates containing increased concentration of the extract. The stimulation of the host's intestinal immune response mediators was evaluated using an in vitro system of porcine intestinal epithelial cells (IPEC-1). To identify the involved receptor, this extract was tested on HEK293 cell line expressing TLR4, TLR2, TLR5, TLR9, NOD1 and NOD2 receptors.

Results. The incorporation of the sulphated polysaccharide extract exhibited selective inhibition of tested microorganisms' growth in a dose-response relationship compared to untreated control. Both gram-positive and gram-negative bacteria growths were affected. The most susceptible pathogens were Pasteurella, Mannheimia haemolytica, Erysipelothris, Streptococcus suis, Staphylococcus aureus and chromogenes, Bordetella brochiseptica, Corynebacterium and Actinomyces pyogenes. The treatment of polarised IPEC-1 cells with the polysaccharide extract induced an up-regulation of broad range of intestinal cytokines such as TNFalpha, IL1alpha, IL1beta, IL-6, IL-8, IL12p40 and CCL20 chemokine. This stimulation of cytokines expression involved a signalling pathway activated following the interaction of the polysaccharide extract with TLR4 receptor.

Conclusion. Our results showed that this extract could be used in animal diets to inhibit the pathogens growth and stimulate the immune response, thereby to reduce the occurrence of infections in animal herds and the subsequent use of antibiotics. This extract may also constitute a potential adjuvant in the design of mucosal vaccine approaches to improve both innate and adaptive immunity.

NOTES

054

FIRST EFFICACY EVALUATION OF A NOVEL COMBINATION VACCINE AGAINST ENZOOTIC PNEUMONIA (MYCOPLASMA HYOPNEUMONIAE) AND PORCINE CIRCOVIRUS TYPE 2 (PCV2) IN THE PRESENCE OF STRONG MATERNALLY DERIVED PCV2 IMMUNITY IN PIGS

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Introduction. The objective of the study was to assess the efficacy of a novel ready-to-use combination vaccine consisting of a PCV2 subunit and inactivated Mycoplasma hyopneumoniae (M. hyo) strain J [Porcilis PCV M Hyo (Intervet International BV, The Netherlands)] against M. hyo clinical and PCV2 subclinical infection, under field conditions. Based on serology results, maternally derived antibodies against PCV2 at 3 weeks of age were on average 11.08 log2, which can be considered high.
Material and Methods. The study was performed according to a controlled, randomised and blinded design in a Greek swine unit with Enzootic pneumonia (EP) and a mild PCV2 infection during late finishing. In total, 600 healthy three week old suckling piglets were allocated randomly, within litters, to one of two groups. First group piglets were vaccinated with the test product and the 2nd group animals (control) were injected with Unisol (sterile buffered saline). The primary efficacy parameters were lung lesions at slaughter (LLS) and average daily weight gain (ADWG) during finishing.
Results. Vaccinated pigs did not have any local or systemic safety reactions and had significantly reduced severity of lung lesions than controls. The overall mean lung lesion score was 9.6 in the Porcilis PCV M Hyo group and 12.2 in controls. The average daily weight gain was 25 grams higher during the finishing phase and 18 gram better overall in the vaccinated compared to the control pigs.
Conclusion. In conclusion, although maternally derived immunity was present at significant levels at the age of vaccination, this new PCV and M. hyo combination vaccine was safe and effective for vaccination of 3 week old piglets (reduction of LLS and improvement of ADWG) against concurrent clinical M. hyo and subclinical PCV2 infection under field conditions.

NOTES

posters
PRESENTATIONS

P001

DIABETES INSIPIDUS: DIAGNOSIS OF A COMPLEX DISEASE IN PIGS

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Introduction
Diabetes insipidus (DI) is a rare polyuric and polydipsic disorder in human and animals resulting from an inadequate secretion, release or activity of antidiuretic hormone (ADH). The most important effect of ADH is to increase water reabsorption in the renal system. Therefore, diseased animal are characterized by polyuria, a markedly decreased urine-specific gravity (USG) and compensatory polydipsia without other findings. In order to confirm the diagnosis of DI a thorough anamnesis, a clinical examination and further diagnostic tests such as water deprivation test and ADH stimulation test are required.
Case Description
Two purebred intact Duroc boars were referred to the clinic, because of pronounced polyuric and polydipsic symptoms. Physical examinations revealed no abnormalities. Results of concurrent blood examinations showed slight chronic inflammatory signs. Furthermore, urine was examined. USG and urinary osmolality, only measured in one boar, were markedly decreased (1.002/1.001; 32.5 mOsm/Kg), whereas all other parameters were within their physiological range. For further diagnostics a modified abrupt water deprivation test was conducted with one animal. The body weight was measured and a urine sample was taken at the beginning of the trial. The boar was withhold water and food for 6 hours. Afterwards a 6.7% loss of body weight and a slightly increased USG of 1.008 were measured. Based on these results, a 5 days lasting therapeutic attempt with Desmopressinacetat, an ADH analogue, was initiated, resulting in a slight increase of USG at the end of the period. For further diagnostics, a macroscopic examination and histopathology were performed on both boars. No distinctive lesions were found which could explain the disorder. Finally, three genes known to be involved in inherited DI in humans were analysed to explore a possible genetic background of the disease, because in previous years three other very similar cases were described in boars of identical breed in the same stud. However, disease-associated mutations could not be found.
Conclusion
Until today, DI in pigs has not been described in the literature. Although DI is rare in pigs, it should be contemplated in the differential diagnoses of diseases with polyuric and/or polydipsic symptoms. The present case report highlights the usefulness of a modified water deprivation test as a diagnostic tool, whereas an administration of ADH showed no effect and did not contribute to an exclusion of differentials. This is likely due to low efficiency of human ADH in pigs. Since hereditary forms of DI have been recorded in humans, manifestation in pigs should be considered in breeding programs.

P002

SIMULATION OF OUTBREAK MECHANISMS OF RESPIRATORY DISEASE IN FINISHING PIGS CAUSED BY ACTINOBACILLUS PLEUROPNEUMONIAE

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Infection with A. pleuropneumoniae may result in clinically diseased pigs as well as non-diseased carrier pigs. However, what actually happens during an acute outbreak episode of pleuropneumonia is unclear. The aim of this study is to test two potential underlying mechanisms for such outbreaks, by identifying conditions for occurrence of such outbreaks under these mechanisms, using mathematical modelling. The first mechanism assumes that a common risk factor causes all cases directly by changing the host pathogen interaction in already colonised pigs (trigger mechanism). The second mechanism assumes that a transmission chain is started by the first case, inducing disease in the infected contact pigs (transmission mechanism). First, a characteristic outbreak of pleuropneumonia was defined by conducting a review of the literature; defining an outbreak at 12 weeks of age and affecting 50% of animals and lasting 4 days. Simple mathematical models showed that a characteristic outbreak could be caused under both mechanisms. However, the transmission mechanism needed a 50 times higher transmission rate for diseased pigs than described in literature for colonised pigs. Furthermore, the trigger mechanism showed that outbreaks in young pigs must be rare, as these are not yet colonised, which is supported by literature. We conclude that outbreaks of A. pleuropneumoniae on endemic farms are most likely explained by exposure of already infected pigs to a trigger. This may imply that management on farms has to focus on reducing transmission by colonised pigs and by preventing the occurrence of a trigger.

P003

ADMINISTRATION OF CLOSTRIDIUM PERFRINGENS TYPE A AND E.COLI ISOLATES FROM HEALTHY PIGLETS TO LITTERS WITH A HIGH PREVALENCE OF DIARRHEA

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Introduction: Immediately after birth, next to ubiquitous microorganisms also pathogens are taken up by suckling piglets, often resulting in neonatal enteritis. The high incidence of suckling piglet diarrhea and the public and scientific demand for a reduction of the use of antimicrobials in veterinary medicine reveals the urgent need for alternatives in disease treatment. Probiotics promote the establishment of a beneficial gut flora and inhibit the growth of pathogenic bacteria in the intestine. This clinical field study was driven by the hypothetic principle of competitive exclusion of pathogenic bacteria due to prior colonization of the gut mucosal surface by non-pathogenic strains of the same bacterial species in order to prevent disease. The objective was to evaluate the effects of porcine derived specific bacterial strains, classified as non-pathogenic by molecular typing and by evidence of lack of production of known toxins in vitro, on growth performance and clinical gut health of newborn piglets.

Material and Method: Herd health status was impaired by a high incidence of postpartal dysgalactia syndrome in sows (70%) and a high incidence of neonatal diarrhea caused by enterotoxigenic E.coli and C. perfringens type A. Isolated pathogenic E. coli strains from diarrhoeic pigs were resistant to commonly used antimicrobials. The bacterial cocktail was administered to newborn piglets from 15 crossbred sows prior to first colostrum intake and on the two consecutive days. Results were compared to a control group (n=15), given sterile sodium chloride orally at the same time points. Weights at birth, day 2, 3, 7 and at weaning were recorded. A clinical examination of all piglets and sows was performed daily. Died or crushed piglets were necropsied and alterations of the intestine were macroscopically assessed.

Results: Overall, the health status of the sow was the most important influencing factor on piglet health superimposing most treatment effects. In the treatment group the piglet mortality rate was lower (17.9% treatment group vs. 28.5% control group, P=0.013), at the same time the average daily weight gains were lower on an individual level (P < 0.001). Pathological alterations of the small intestinal wall tended to be higher in the control group (P = 0.06). Necrotizing enteritis was only found in the intestine of dead pigs from the control group.

Conclusion: No adverse effect of the bacterial treatment occurred and little positive effects on health status and performance were found. Further basic research is needed to select potential bacterial candidates for competitive exclusion and to define preconditions for an effective treatment scheme.

P004

ANALGESIA DURING PIGLETS CASTRATION: EFFECT OF DICLOFENAC SODIUM ON BEHAVIOURAL AND PHYSIOLOGICAL INDICATORS OF PAIN

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Introduction
Surgical castration is a common management practice performed on male pigs to prevent the occurrence of boar taint. However, the procedure causes pain and induced distress, with increasing public concern on animal welfare. Therefore, it would be necessary to develop commercially viable ways to reduce pain during castration. Aim of this study was to evaluate, by behavioural and physiological indicators, the effectiveness to alleviate pain of diclofenac sodium administered before castration.
Material and Method
The study involved 132 male piglets of five days of age: 44 handled (H), 44 castrated without pain relief (C), and 44 treated with diclofenac sodium (Reuflogin® 2,3 mg/kg intramuscular, equal to 0,05 ml/kg) 30 min before castration (R). Blood samples were collected on twelve piglets for each treatment at 0h30 and 2h00 after castration for cortisol evaluation. Other 24 piglets per treatment were instead used for behavioural observation. Behaviour was observed for 30 min at four time point from castration (0h00, 1h00, 3h30, 24h00) using a scan-sampling technique (2-min interval). Ten different behaviours/postures were recorded. Dataset on cortisol was processed by a repeated mixed linear model (PROC MIXED of SAS 9.2, SAS Institute Inc.) using Bonferroni correction for post hoc pairwise contrasts. Behavioural data, expressed as percentage of events/time, were analysed by a generalized liner model (PROC GENMOD).
Results
Results showed significant differences for average cortisol level in H group compared to C group (17,1 and 31,6 µg/dl respectively; P<0,001), and no difference between H and R groups (23,9 µg/dl; P>0,05). Similar results were obtained for average behaviours/postures such as 'sternal lying' (H group=29,8% of the time vs. C group=37,1%; P=0,05; R group=35,9%), 'lateral lying' (H=24,5% vs. C=19,7%; P=0,05; R=19,2%), 'suckling synchronized with the litter' (H=26,4% vs. C=24,0%; P=0,019; R=25,0%) and 'suckling isolated from the litter' (H=3,9% vs. C=2,0%; P=0,001; R=4,7%), with no significant difference between H and R groups (P>0,05). No significant differences were showed on time of observation neither for cortisol nor for behaviour.
Conclusion
In conclusion, intramuscular administration of diclofenac sodium at 2,3 mg/kg before castration reduces some behavioural/postural and physiological indicators of pain in piglets. Finding suggests a decrease in pain, improving health and welfare around castration.

P005

SEROPREVALENCE AGAINST SELECTED BACTERIAL AND VIRAL PATHOGENS IN FREE-RANGE PIGS FROM SOUTH WEST SPAIN

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INTRODUCTION
Pigs in free-range systems can be exposed to diverse environmental factors which may favour the spread of different pathogens. However, the epidemiological situation of different swine diseases in pigs reared in free-range systems is limited to those diseases included in control and eradication programmes. Thus, a serosurvey against Porcine Circovirus type 2 (PCV2), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Influenza Virus (SIV), Mycoplasma hyopneumoniae, Aujeszky's Disease Virus (ADV) and Erysipelothrix rhusiopathiae in fattening free-range pigs was conducted.

MATERIAL AND METHOD
Serum samples from 915 animals belonging to 61 herds from the South West of Spain were collected from two different slaughterhouses and analysed by means of commercial ELISA kits.

RESULTS
Our results showed a wide spread of PCV2 (97.7%, CI95% 96.73-98.68%) and M. hyopneumoniae (85.25%, CI95% 76.35-94.15%) and a moderate circulation of PRRSV (36.07%, CI95% 24.02-48.12%) and SIV (40.98%, CI95% 28.64-53.33%) among pigs reared in free-range systems. Seroprevalences obtained against PCV2 and M. hyopneumoniae were similar to those previously observed in intensive systems (highlighting the elevated seroprevalence against PCV2), but a lower PRRSV and SIV seroprevalences were observed. The low seroprevalence against ADV demonstrates the success of the national control programme, which may serve as a mirror for the control of swine diseases and the design of eradication programmes against other pathogens of interest in pigs. E. rhusiopathiae seroprevalence (54.10%, CI95% 41.59-66.6%) was lower than initially expected considering the existence of regular vaccination, fact that could be attributed to an impairment of the host immune response associated to PCV2 infection.

CONCLUSION
The lack of vaccination schemes against the pathogens under study together with the combination of three or more infectious agents responsible of Porcine Respiratory Disease Complex (PCV2, PRRSV, SIV, M. hyopneumoniae) in more than 50% of herds, encourage the establishment of immunoprophylactic protocols against these pathogens, especially PCV2, in pigs reared in free-range systems.

P006

RELATION BETWEEN THE PIG FARM DENSITY AND THE CIRCULATION OF MYCOPLASMA HYOPNEUMONIAE MLVA-TYPES IN THE FARM

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Introduction
Previous studies have shown a large variety in Mycoplasma hyopneumoniae (Mhyo) MLVA-types circulating in a pig herd (Vranckx et al., 2011). It is also known that Mhyo can be transmitted between herds over long distances (4.7 km) Dee et al. (2009) and that the pig herd density in the area may influence the infection dynamics in a herd (Stärk, 2000). The influence of pig herd density on diversity of MLVA-types in a herd is however unknown. The present study investigated the relationship between the circulation of different Mhyo MLVA-types in pig herd and the pig herd density in the area.
Materials and methods
From 10 randomly selected closed pig herds (>100 sows, vaccination piglets against Mhyo) Lambert Coordinates were obtained to calculate the straight distance between the registered (n=6274) pig herds and the trial herds with the Pythagorean theorem. Next, the number of herds in a radius of <5 km towards the trial herds was calculated. From the 10 herds, BAL fluid was collected from 60 pigs in the slaughter house and tested by nPCR (Stärk et al., 1998). Positive samples were submitted to MLVA analysis, amplicating 4 variable number of tandem repeat regions (VNTR) in the genome of Mhyo and measuring the peak length (Vranckx et al., 2011). An MLVA-type differed at least one locus mutation from another type.
Results
The number of other pigs herds located within a 5 km perimeter of the 10 study herds were: A (102), B (65), C (154), D (141), E (133), F (49), G (139), H (26), I (144), and J (54). In total, 495/600 samples were positive by nPCR and 135 different MLVA-types were found. Two or three MLVA-types could be detected in some BAL fluid samples. The total number of MLVA-types and the number of different MLVA-types within a herd were as follows: herd A (67 / 16), B (11/6), C (66/10) , D (49/19), E (94/18), F (46/23), G (69/14), H (53/15), I (23/12) and J (18/7). Two separate linear regression models were performed with the number of herds in a radius of <5km as the independent variable in both models and number of different or total number of MLVA-types as the dependent variable in respectively model 1 and 2 In both models a positive association was present between the X and Y values, but none of the results were significant (model 1: ß0: 13,5; ß1: 0,005; SE: 0,04; P-value: 0,9 and model 2: ß0: 26,7; ß1: 0,228; SE: 0,176; P-value: 0,231).
Conclusion
The present study did not elucidate a significant association between pig herd density in the area and number of different MLVA-types in a herd. This may indicate that the diversity of MLVA-types in a pig herd is more influenced by other factors different from pig herd density.

P007

LUNG AND PLEURAL LESIONS: IMPACT ON LEAN MEAT PERCENTAGE IN SWINE CARCASSES

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Introduction
The porcine respiratory disease complex is one of the most challenging issues in the pig industry worldwide. In particular, enzootic pneumonia-like lesions and chronic pleural lesions are the most frequent findings in pig lungs at slaughter. Significant economic losses due to the reduction in growth performance and feed efficiency, and the requirement for antibiotic treatment are well known. However, the impact of lung and pleural lesions on carcass quality are poorly investigated, especially in heavy pig production. Aim of the present work was to evaluate a possible association between the presence of lung and pleural lesions and the judgement of the carcass quality in heavy pigs.
Material and Method
A total of 71 batches of pigs used for the Denomination of Protected Origin Ham production were randomly selected from those slaughtered from July to September 2014 in a commercial slaughterhouse in Northern Italy. The number of pigs per batch was 134,7±4,5 and the body weight was 168,4±6,5 kg. For each batch, lungs and pleura belonged at least to 100 pigs were inspected by palpation and visual appraisal, using the Madec grid on six lobes (0-24 scores) and the SPES grid (0-4 scores) respectively. Carcasses were evaluated using the EUROP grid that classifies the carcass regarding the percentage of lean meat. Batches were classified according to the level of lung and pleura lesions: 'low damage' batches were those with both scores lower than the 25th percentile while 'high damage' batches had lung and pleura lesions scores higher than the 75th percentile. Percentage distribution of carcasses in the EUROP grid were analysed in these two total lesions categories by z-test comparison.
Results
'Low damage' batches showed a higher percentage of carcasses in the U class (45,8%, P<0,001; kurtosis index=-0,89) whereas 'high damage' batches showed an equal proportion of carcasses in classes U and R (U=42,1%; R=38,7%; P>0,05; kurtosis index=-1,49).
Conclusion
In conclusion, the absence of severe enzootic pneumonia-like lesions and chronic pleural lesions in heavy pigs induces an increase in carcasses with highest percentage of lean meat.

P008

EFFECT OF A HIGH SOY DIET ON FAECAL CONSISTENCY AND DRY MATTER CONTENT

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Brachyspira (B.) hyodysenteriae causes swine dysentery and can be diagnosed in faecal samples. Feeding of soy has successfully been used in experimental B. hyodysenteriae infection models maintaining diarrhoea independent of the challenge. Hypotheses: Feeding soy extraction grist influences faecal consistency (FC). It decreases the faecal dry matter content (FDMC) which can be determined by a modified microwave drying method.
A preliminary test was performed with eight healthy pigs (two weaners, two growers, three fatteners, one sow). A short clinical examination with emphasis on feed intake and FC was performed daily. FC was described with a score of 1 (liquid with blood or mucus), 2 (liquid) to 5 (firm). Half of the daily feed ration was replaced by pure soy extraction grist and given in the evenings of seven consecutive days (D0 – D6). The pigs had free access to water and straw. Daily (D0 – D7) rectal faecal samples from every pig were placed in individual closed containers, stored at 4°C, and analysed the same day. FDMC was determined in triplets: petri dishes were weighed (W1) and subsequently loaded with 2-3 g of faeces before weighing again (W2). The loaded petri dishes were placed in a microwave (LIFETEC, Medion AG, Germany), and samples were dried at 119 W for 30 min, followed by 10 min at 385 W. The individual weights were determined before the samples were reheated at 385 W for 5 min. Samples were weighed and reheated until two consecutive heating and weighting cycles resulted in the same weight (W3). FDMC (%) was calculated using the equation FDMC = (((W3 – W1) / W2) x 100).
All pigs consumed the soy. No signs of illness were observed except loose stool in some pigs at some days. The mean faecal score at D0 (before soy) was 5 on average, lowest at D2 (3) and increased again the following days (D7: 5). An association with the median FDMC could be seen with the following results: D0: 28.8% (22.3 – 33.6); D2: 18.8 % (14.2 - 24.2); D7: 26.3% (16.5 - 31.7). Individual trends differed and were inconclusive. The median FDMCs tended to increase (19.6 / 22.6 / 30.4 / 24.2%) with age.
The soy feeding resulted in a consistent decrease in the faecal score and the FDMC in every pig until D2, which has not been described in literature. At D7, values reached almost the levels of D0, despite the high soy diet. This could be explained by an adaption of the intestinal milieu. Due to the small study population, influence of individual variation is high. The freely available straw could also have masked a further or lasting decrease of the FDMC. This was corroborated by the fact that the weaners ate nearly no straw in contrast to the sow and the fatteners.

P009

TAIL BITING : IN-FEED ALCOHOL AS A SUBSTITUTE OF FIXED OR FREE TOY

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Introduction
In a fattening unit where pigs are fed with liquid byproduct from the starch industry, preliminary clinical observation on a severe tail biting outbreak lead to the hypothesis that alcoholic fermentation and production of ethanol may be an alternative to control this abnormal behavior.

Material and method
During summer 2013, 2 contemporary groups of 120 pigs originating from one sow herd were assigned to 2 rooms for the fattening period. They were liquid fed (24% dry matter) according to a classical feeding pattern. The diet was based on a complementary feed (50% of the DM) and liquid byproduct coming from the starch industry delivered on the farm and stored in 2 different 40 m3 empty tanks. On arrival one tank was inoculated with a live yeast selected for its capacity to produce high levels of ethanol. The second tank was receiving 1 L of liquid propionic acid (80% concentration) to control the endogenous yeasts growth (control group). Both tanks were treated with 20 kg/m3 of phosphoric acid to avoid any bacterial fermentation (pH kept below 2.5). After a 7 day storage period delivery the liquid byproducts were used to balance the liquid feed delivered to the pigs just after they were moved to the fattening rooms. Both tanks were sampled every 24 hours for the first 7 days and then twice a week and the samples frozen at – 24°C for further chemicals analysis (HPLC essay for ethanol and methanol, lactic and acetic acids). Behavior (chain attractiveness, drunck behaviour), tail lesions, blood and air analysis were checked on a regular basis. Gross liver check at slaughter exam were also performed. 10 pigs/group were also selected at random at the slaughterhouse for histopathological liver examination.
Results and conclusion
Mean alcohol consumption is equivalent of daily 2,5 bottles of wine per pig day for 4 months in the experimental group. The score of hepatic macroscopic lesions, blood enzymes are higher in the experimental group but tail lesion score was also highly significantly reduced.

P010

THE EFFECT OF A SYNERGISTIC BUTYRATE BASED PRODUCT (BUTIFOUR® NF) ON THE PERFORMANCE AND DIARRHEA INCIDENCE IN WEANED PIGLETS

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Introduction

Butyrate is a substance with a biological role in mammals, as it is naturally produced by the gastrointestinal microbiota. Recently, the beneficial effect of butyrate on gut health and performance of production animals, including the mode of action, is well described, indicating the interest of the industry in developing butyrate based feed additives.
The objective of this trial was to evaluate the effect of a synergistic butyrate based product on performance and diarrhea incidence in weaned piglets.

Materials and methods

A trial was carried out at the Swine Research Unit of PUCPR, located in Fazenda Rio Grande, Brazil. 48 weaned piglets (24 males and 24 females), with an average body weight of 7 kg, were housed in 1,92m2 nursery pens during 35 days and divided into 2 groups with 8 replicates each: control (C) and butyrate (B). 2 corn and soybean meal based diets were formulated according to animals' age: prestarter (1 to 14 days of evaluation) and starter feed (15 to 35 days of evaluation). Treatments consisted of a negative control (standard feed without inclusion of additives) and a supplementation with a synergistic coated calcium butyrate based product (Butifour® NF) at 0,15% in prestarter and 0,075% in starter phase. Body weight (BW) and feed consumption were controlled weekly to provide data on feed conversion ratio (FCR). Diarrhea incidence was verified on a daily basis, and a score was attributed according to VASSALO and coauthors (1997) (0 – normal feces; 1 – soft feces, 2 – slurry feces and 3 – aqueous feces). Data were analyzed using the software JMP® (SAS Institute Inc.). All statements of difference were made considering $p \leq 0,05$.

Results

A significant decrease in FCR was observed in B (1,651) comparing to C (1,687). Body weight was numerically increased in B (24,592kg) comparing to C (24,088kg). Diarrhea incidence (weighted mean) was significantly lower in B (1,75) comparing to C (1,96).

Conclusion

Findings from this trial revealed that the inclusion of a synergistic butyrate based product (Butifour® NF) could improve performance and diarrhea incidence in piglets.

P011

EFFECT OF DIETARY PLANT ALKALOIDS ON STRESS AND PRODUCTION PERFORMANCE OF SOWS

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Introduction:
The time around farrowing and the subsequent lactation period are particularly critical in sows because of changes in housing conditions, piglet birth and initiation of milk production. Hence, suboptimal feed intake, poor litter performance along with elevated levels of stress and the incidence of mastitis, metritis and agalactia (MMA) negatively influence overall performance in piglet production. Aim of this study was to determine the effect of dietary supplementation with a standardized blend of plant alkaloids with active ingredients from the group of quaternary benzophenanthridine and protopine alkaloids (QBA+PA) on stress level and production performance of sows under hot climatic conditions.

Materials and Methods:
90 Yorkshire x Landrace sows with an initial body weight of 211 kg were assigned to three dietary treatments: (1) Control (basal corn-rice-soy diet); (2) QBA+PA 1 (Sangrovit® 50 g/t); (3) QBA+PA 2 (Sangrovit® 100 g/t). Sows were in the second and third parity; the average parity in treatments 1, 2 and 3 was 3.5, 3.3 and 3.2, respectively. Sows were kept in a confinement facility starting on day 100 of gestation and fed individually. Daily feed allowance was 2.5 kg during gestation. At farrowing sows were placed in farrowing crates and fed individually ad libitum. On day 3 after farrowing, litters were standardized to ten piglets per sow. Diets in mash form were formulated to meet NRC (1998) requirement recommendation. Blood samples were collected at the day of farrowing and analyzed for cortisol, C-reactive protein (CRP) and complete blood count profile. Sow body weight, lactation feed intake and piglet birth and weaning weights at 21 days were determined.

Results:
Sow body condition score (BCS) at d 100 of gestation and farrowing did not differ ($p > 0.10$). At d 21 of lactation BCS of the Control group was lower than both of treatment groups ($p < 0.05$). QBA+PA supplementation reduced ($p < 0.05$) levels of white blood cells, CRP and cortisol. Sows in the Control group had more MMA scores than the sows fed QBA+PA during day 1-3 of lactation ($p < 0.05$). Sow lactation feed intake tended to increase by QBA+PA supplementation. There were no differences in litter size at birth or litter birth weight ($p > 0.05$). QBA+PA supplementation in sow diets increased ($p < 0.05$) litter weaning weight and litter weight gain and reduced ($p < 0.05$) pre-weaning mortality of piglets.

Conclusion:
Supplementation of sow gestation and lactation diets with a standardized blend of QBA+PA is a suitable strategy to reduce stress levels and enhance piglet growth performance.

P012

THE EFFECT OF A NATURAL FEED ADDITIVE (MACLEAYA CORDATA), CONTAINING SANGUINARINE, ON THE PERFORMANCE AND HEALTH STATUS OF WEANING PIGS

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Introduction
The aim of this study was to investigate the efficacy of Sangrovit® , a plant-derived feed additive, given throughout the nursery stage via feed at 15 ppm and 50 ppm, on the health status and performance of weaners against negative controls.
Materials and Methods
Sangrovit® (Phytobiotics Futterzusatzstoffe GmbH) is a natural, plant (Macleaya cordata) derived supplement of swine feed containing benzo[c]phenanthridine alkaloids compounds of which sanguinarine is the most abundant (minimum of 1.5% sanguinarine).
In a 900-sow farrow-to-finish farm, a total of 864 piglets were divided into three groups: (i) negative controls (NC); (ii) Sang 15: same feed as NCs, plus 15 g Sangrovit® /t of feed; (iii) Sang 50: same feed as NCs plus 50 g Sangrovit® /t of feed. The piglets of the NC group were fed standard feeds as follows: for the first 2 weeks after weaning, creep feed was offered; while for the period from week 6 until week 10 the piglets received weaners' feed. The pigs of the Sang 15 group were fed the same feeds as of the NC group, plus 15 ppm Sangrovit® / tn feed and the Sang 50 group were fed also NC group's feed with the addition of 50 ppm Sangrovit® / tn feed.
Trial design included the weighing of piglets at weaning and at a weekly basis until the end of the trial period, as well as blood sampling of all groups at days 1, 7 and 14 for the assessment of biochemical parameters. A number of 72 blood samples from each group were analyzed for their concentration in acute phase proteins, haptoglobin (Hp) and serum amuloid A (SAA). All samples including standards were determined in duplicate.
Furthermore, clinical examination of the piglets was carried out throughout the whole trial period at regular intervals. Morbidity and mortality rate, as well as diarrhea score (DS) were also calculated. The feed consumption on pen basis was recorded, as well as Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) was calculated per production stage and in total.
Results
The results indicated that administration of 50 ppm- Sangrovit® had the most beneficial effects on growth performance in weaning pigs. Specifically there was increase of body weight and average daily gain, as well as reduction of feed conversion ratio. Blood analysis from the Sang groups and especially the Sang 50 group revealed low values of haptoglobin and serum amyloid A.
Conclusion
Based on the results of this study, evidence is provided that administration of a phytogenic product containing high amounts of the alkaloid sanguinarine in weaners feed, has beneficial effects on their growth performance and stimulates anti-inflammatory activity.

P013

EVALUATION OF SIX DIFFERENT AMINO ACID CONCENTRATIONS IN RELATION TO COBALAMIN–DEPENDENT METABOLITES IN SERUM OF PIGS BETWEEN 6– AND 26–WEEKS OF AGE.

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The B vitamins such as cobalamin play an important role in amino acid metabolism and nucleic acid synthesis. In humans, low serum cobalamin concentrations have been associated with increased serum methylmalonic acid (MMA) and homocysteine (HCY) concentrations, which are negatively correlated with this vitamin on a cellular level. Increased serum MMA and HCY concentrations have been described in pigs with acute, chronic or subclinical Lawsonia intracellularis infection. Also, pigs may show age-dependent changes of serum MMA and HCY concentrations post-weaning, which may reflect a decreased availability of intracellular cobalamin for RNA and DNA synthesis and amino acid metabolism. Therefore, the aim of this study was to evaluate serum concentrations of six amino acids and their relationship with serum MMA and HCY concentrations in pigs post-weaning.
For this retrospective study, serum samples were collected from six pigs over time at week 6, 10, 15, 22 and 26 and handled in the same manner. The serum concentrations of MMA, HCY and the six amino acids (i.e., glycine [GLY], glutamic acid [GLU], tyrosine [TYR], methionine [MET], lysine [LYS], and tryptophan [TRP]) were measured using gas-chromatography/mass spectrometry. Amino acid concentrations were compared among the different age-groups of pigs by using an ANOVA or a non-parametric Kruskal-Wallis test, as applicable. Also, a Spearman rank sum correlation (rho: ρ) was performed to test for correlations between MMA and HCY, respectively, and each of the six amino acids.
In post-weaning pigs, concentrations of serum GLY ($p=0.0215$), GLU ($p=0.0173$), MET ($p=0.0015$) and TYR ($p=0.0005$) differed significantly among time-points, except for TRP and LYS (both $p>0.05$). Also, a correlation was observed between concentrations of serum MMA and GLU ($\rho = 0.60$; $p=0.0004$) or TYR ($\rho = 0.36$; $p=0.0482$) but not for serum MMA concentrations and the remaining amino acids. Furthermore, a correlation was found between serum HCY and TYR ($\rho = -0.36$; $p=0.0491$) concentrations, but not for the remaining comparisons.
In conclusion, a comparison of six amino acids between groups of post-weaning pigs revealed differences for four amino acids (i.e., GLY, GLU, MET and TYR). Interestingly, MMA and HCY, respectively, two main cobalamin-dependent metabolites showed significant correlations with GLU and TYR, and TYR, respectively. This study suggests that concentrations of serum MMA and HCY might have an influence on certain serum amino acid concentrations in post-weaning pigs. Whether supplementation of cobalamin might be valuable in post-weaning pigs with regard to optimal amino acid maintenance requires further prospective studies.

P014

EPISODE OF SALT POISONING IN FATTENING PIGS

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Introduction
Pigs are one of the most susceptible species to salt poisoning which can result from inadequate water intake or too much salt in the feed. This clinical case confirms that the likelihood of sodium poisoning in pigs is exacerbated when they are given a diet rich in salt and when access to water is limited.

Farm description and context
This farm in Brittany houses 3,390 fattening pigs which are fed with liquid feed consisting of a mixture of lactoserum B (37%), a supplement (8%) and a presoup (55%). The presoup is composed of 39% bakery by-products, 20% water and 41% lactoserum A.

Situation analysis and preliminary investigations
November 24, 2014, the farmer registered the death of 36 animals, between 60 and 100 kg. Almost 2% of the animals were supine or apathetic. No other symptom was noticed. No macroscopic lesions were detected at necropsy. Given this picture, salt poisoning was suspected straight away.
A number of tests were carried out on Lactosera A* and B*, the presoup (pH, dry matter content, sodium concentration) and the supplement (sodium concentration).

Results
Testing of the various components of the diet showed high levels of dry matter and sodium in the Lactoserum B used to make up the pre-soup. In the 48 hours before the deaths, the pigs had been given feed containing 8.4 grams of salt per kilogram of complete feed while commercially available “finishing” products contain between 2 and 5 grams of salt per kilogram.

Conclusions and Discussion
In this case, a concatenation of circumstances led to sodium poisoning: salt intake two times higher than in a standard diet coupled with inadequate water intake (between 7% and 10% live weight).
Two measures were recommended: first, replacing lactoserum B with water. Second, increasing water intake, in order to continue dispensing the high-salt presoup safely.

P015

INFLUENCE OF ALGAE–BASED COMPLEMENTARY FEED ON THE DEVELOPMENT OF THE SMALL INTESTINE OF PIGLETS DURING LACTATION

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At birth, the piglet's small intestine is not mature and is still challenged by pathogenic and environmental factors with consequences on health and growth performances. Preserving the intestinal integrity of piglets during lactation is a challenge in the aim of combining well-being and productivity. The objective of the present study was to evaluate the effects of a seaweed-based complementary feed on health parameters and growth performance of piglets during lactation. Seventy-two litters (833 piglets) were included in the study that lasted from birth (day 0) to weaning (day 21). Animals were randomly allocated to one of two groups, balanced in litter weight, sow parity and farrowing date. Thirty-six litters (T1) did not receive the complementary feed and 36 litters (T2) received 100 g of complementary feed per day from day 5 to weaning. The results showed no significant influence of the treatment on the average daily gain of the piglets. A significant decrease was observed in diarrhoea incidence ($P < 0.05$) and medication use ($P < 0.001$). No significant differences were observed on histology parameters of the small intestine, survival rate, runts and weaning weight homogeneity. Under the conditions of this study, the seaweed-based complementary feed decreased diarrhoea incidence in piglets as well as concomitant treatments. Therefore, its use in lactation could be helpful to improve health of piglets.

P016

GLOBAL MYCOTOXIN SURVEY IN 2014

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Mycotoxin occurrence in all kinds of commodities is a worldwide phenomenon. BIOMIN conducts an annual Mycotoxin Survey to raise awareness of the incidences of mycotoxins found in agricultural commodities intended for animal feed. The focus of this study is to evaluate the extent of mycotoxin contamination in various samples from different regions worldwide.
From January 2014 to September 2014, more than 3,900 samples sourced from around the world (1,677 from Europe) were analysed for aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Samples were analyzed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS, Spectrum 380®), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). The latter method was only chosen for single commodities.
In total, 80 % of all samples contained at least one of the five main mycotoxins. In 51 % of all animal feed and ingredient samples, more than one type of mycotoxin was found. Afla was present in 23 % of all samples at an average concentration of 82 ppb. ZEN was present in 50 % of samples at an average level of 108 ppb. OTA was detected in 24 % of all samples at an average of 4 ppb. DON was found in 59 % and FUM in 60 % of all samples. These results show once again that DON and FUM are the most prevalent groups of mycotoxins worldwide with an average contamination of 543 and 1366 ppb, respectively.
The mycotoxin survey results presented above indicate that these toxic substances are a serious concern in agricultural production. Multi-mycotoxin occurrences continue to be a global threat, with Fusarium mycotoxins being the most common. An effective mycotoxin risk management program should be applied to protect animals from the negative effects of mycotoxins.

P017

FUSARIUM MYCOTOXINS – NEW INSIGHTS INTO METABOLISM AND BIOMARKER AP–PROACHES

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Introduction
Deoxynivalenol (DON) and fumonisin B1 (FB1), produced by various Fusarium spp., are among the economically most relevant mycotoxins in pig production. Whilst research has primarily focused on the effects of high levels of mycotoxins in the last decades, significant knowledge gaps still exist concerning the metabolism of mycotoxins and the appropriate diagnosis of mycotoxicosis. For the latter aspect, the biomarker approach – measuring either the mycotoxin (metabolites) or specific biological responses in body fluids – holds promising potential. Recent studies of our research group provide new insights into the metabolism of DON and their implications for exposure assessment in pigs. Furthermore, we show the current state of mechanism-based biomarkers using the example of FB1.

Materials and Methods
In a first experiment, 4 crossbred piglets (ca. 7 kg) received water and 75 µg/kg b.w. DON per gavage on days 1 and 9, respectively. After each of the treatments, urine and feces were collected for 24 h and analyzed for DON, deepoxy-DON and DON-glucuronide (DON-GlcA) by liquid chromatography-tandem mass spectrometry (LC-MS/MS).
In a second trial, 24 crossbred piglets (ca. 10 kg) were randomly assigned to four different groups, receiving either blank feed, blank feed + fumonisin degrading enzyme preparation FUMzyme (100 units/kg), FB1 contaminated feed (6 ppm) or FB1 contaminated feed + FUMzyme for 35 days. Plasma samples were taken weekly and analyzed for levels of sphinganine (Sa) and sphingosine (So) by LC-MS/MS. Thereof, the Sa/So ratio, a mechanism based biomarker of FB1 exposure, was calculated.
Results
After oral DON administration, two different DON-GlcA isomers were identified for the first time in pig urine. DON-3-GlcA and DON-15-GlcA accounted for 19 ± 7% and 15 ± 2% of the given dose. Notably, ratios of DON-15-GlcA to DON-3-GlcA varied between individual pigs (0.5–2.2).
In plasma samples of piglets exposed to FB1, a significant increase of the Sa/So ratio (1.04 ± 0.09, d35) was observed. In contrast, no elevation of the Sa/So ratio was determined after supplementation of FUMzyme (0.18 ± 0.01) or in piglets receiving the blank diet (0.20 ± 0.01).

Conclusions
The broad range of mycotoxin metabolites formed in vivo requires sophisticated analytical techniques, which allow the accurate determination of biomarkers. As demonstrated for FB1, mechanism-based biomarkers are very valuable tools in scientific studies e.g. to assess the effect of mycotoxin deactivating agents. However, more knowledge is needed on factors influencing the bioavailability of mycotoxins and kinetics of biomarkers before they can be used in practice on farms.

P018

EVALUATION OF WELFARE ASPECTS IN SUCKLING PIGLETS AFTER INTRADERMAL VACCINE APPLICATION WITH THE IDAL INJECTOR

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INTRODUCTION:
The purpose of this study was the scientific evaluation of welfare aspects of an intradermal vaccination method for suckling piglets under field conditions. Local reactions within three days after vaccination, behavioural responses of piglets and performance data of the piglets vaccinated intradermally (ID) were compared to piglets conventionally immunized by intramuscular (IM) injection.
MATERIALS AND METHODS:
The study was carried out on a commercial German pig farm. On the 7th day of life, a total of 672 suckling piglets in three batches were vaccinated with Porcilis® M Hyo ID ONCE (off label use); 338 of those with the IDAL injector, and 334 with a conventional, commercially available IM M Hyo vaccine administered with a needle/syringe. On the following three days, the site of injection was evaluated, scoring the size of swelling from 0 to 5, and rubor from 0 up to 3. Piglets were weighed individually one day before vaccination and eight days later. Video-recordings were performed for ten days, starting two days before vaccination in order to assess piglets' resting and activity behaviour. Per batch, two litters vaccinated intradermally and two control litters were observed. All data was statistically analysed using the software IBM SPSS Statistics, version 22.
RESULTS:
Vaccination using IDAL injector took 11 seconds (s) on average compared with 17 s for conventional vaccination. Daily weight gain was not significantly different between piglets following IM injection (258g/d) or ID vaccination (257g/d). On the first day after vaccination, 72.3% of IM vaccinated piglets had no swelling versus 2.7% of the IDAL group. A very small swelling was observed in 27.9% of IM vaccinated piglets versus 49.7% of the IDAL group. In contrast, 0.9% of IM vaccinated piglets had a larger swelling versus 47.7% of the IDAL group.
On the third day after vaccination 97.6% of IM vaccinated piglets had no swelling and 2.4% had a very small swelling, whilst 10.4% in IDAL group had no swelling and 60.4% had a very small swelling. On the 7th day no swelling was observed.
Initial video-analysis suggest no differences between activity behaviour between experimental and control groups, however more data has to be gained out of these observations.
CONCLUSION:
The IDAL injector was easy to handle and vaccination was done fast.
In piglets vaccinated intradermally, more transient soft tissue reactions lasting less than 7 days were found, suggestive of a desired local immune reaction. Vaccination method did not impact daily weight gain. Further video analyses have to be done to assess additional welfare aspects of the two vaccination methods.

P019

DO SUCKLING PIGLETS USE ENVIRONMENTAL ENRICHMENT MATERIALS IN FARROWING PENS?

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Introduction
Modern pig production systems are often characterized by a barren environment with scant opportunities for playing or exploring, and thus often not fulfilling natural behavioural needs of pigs. It is known that environmental enrichment can increase animal welfare in fattening pigs and weaned piglets by reducing boredom and abnormal behaviours. However, the offer of enrichment materials for suckling piglets in the farrowing pen is not common practice and it is still unknown whether young piglets already feel the need for exploration and manipulation of objects. Therefore, the aim of our study was to investigate the use of different enrichment materials by piglets from the first to the 29th day of life. Particularly, the time of initial use and the impact of enrichment on the behaviour of piglets, especially on the occurrence of mutual oral manipulation were analysed.

Animals, Materials and Methods
The study was carried out on the research farm of University of Veterinary Medicine Hannover Foundation, Germany. In each of two rounds, two conventional farrowing pens were equipped with various enrichment materials for piglets (straw tower, paper dispenser, chain with plastic bones, dog toy ropes). Two other litters reared in pens without enrichment material in the same compartment were used as control group. Behaviour of piglets was video-recorded once per week for 24 h and analysed using scan sampling method. One day before weaning, skin lesions were detected for each piglet using a lesion sore (0-3) for different body parts. ANOVA analysis followed by posthoc test (SNK) was conducted.

Results
There were no significant differences concerning activity, resting and suckling behaviour between experimental and control groups. Piglets in control groups showed more mutual oral manipulation than piglets in enriched environment (0.5 % vs. 0.3 %, $p<0.05$). Piglets started to use the materials on the 2nd day of life. On day 20, 4.7 % of piglets of a litter were using the materials at the same time. Preferred materials were straw tower and paper dispenser. Piglets in control groups had a higher total lesion score than piglets in enriched groups (8.0 vs. 6.5; $p<0.05$).

Conclusions
We conclude that young suckling piglets are already interested in enrichment materials and it seems that early enrichment can reduce mutual oral manipulation and skin lesions. Thus, the offer of enrichment materials in the farrowing pen can be recommended on commercial farms.

P020

THE INFLUENCE OF BIO-ACTIVE PEPTIDES FROM FPP ON THE FATTENING PIG PERFORMANCE AND CARCASS QUALITY

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Introduction:
Previous research demonstrated a positive effect of Lianol®, a complementary feed based on fermented potato protein, on plasma insulin-like growth factor-1 (IGF-1) levels. There is considerable circumstantial evidence that the actions of growth hormone on protein accretion in skeletal muscle and other lean tissues are mediated by IGF-1.
This trial investigates the effect of this new feedstuff on lean meat content and performance in fattening pigs.

Material and methods:
This research is a summary of a well-controlled field trial performed on a Dutch closed cycle farm. In total 5 equal compartments containing 320 pigs were started up between June 2012 and July 2012. The animals originate from Topigs 20 with PIC Piétrain boar. The pigs were slaughtered in Germany.
In a cross mixed set-up 3 compartments were allocated to the Lianol® treatment and 2 were allocated to the control. The diets were equally formulated. The diet of the Lianol® group was supplemented with 300 grams Lianol® Solapro/mT for pigs from 40kg until slaughter.
The daily gain, feed conversion (FCR) and lean meat percentage on the carcass classification (SEUROP classification and Autofoam) was evaluated.

Results:
The trial showed an improved SEUROP carcass classification from 81.3 % to 85.9 % in the control and Lianol® group respectively. In the German Autofoam classification, 10 % more pigs had a score over 100 points. As a result of this improved carcass quality, the FCR improved 4.6% in the Lianol® group.

Conclusions:
The supplementation of fattening feed with Lianol® Solapro from of 40kg body weight improved the SEUROP classification and FCR in fattening pigs. Under German conditions this improved carcass classification gave an extra benefit of 1.99€ per carcass. The improved feed conversion resulted in another extra benefit of 3.29€ per slaughtered pig. This brings the total benefit of the Lianol® treatment to € 5.28 per slaughtered pig with a ROI of 4.

P021

POSTPRANDIAL NUTRIENT METABOLISM IN LIPOPOLYSACCHARIDE–CHALLENGED GROWING PIGS REARED AT THERMONEUTRALITY OR ACCLIMATED TO HIGH AMBIENT TEMPERATURE

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The effect of exposure to high temperatures on the health status of pigs has not been investigated thoroughly. It is not clear how the ambient temperature (Ta) could influence the responses to an inflammatory challenge in pigs, inflammation being known to induce a high metabolic and physiologic cost. We recently showed that acclimation to high Ta might limit the physiological disturbances, and the associated impact on growth rate and feed intake, caused by repeated administration of Escherichia coli lipopolysaccharide (LPS). The aim of the present study was to evaluate the effects of high Ta on postprandial metabolism of growing pigs subjected to repeated administration of LPS.
Thirty-seven pigs fitted with a jugular catheter were assigned to thermo-neutral (TN, 24 °C) or high (HT, 30 °C) Ta conditions. After a 21-day adaptation period, pigs were administered 5 injections of LPS, each at 2-day intervals. Pigs were submitted to a meal test procedure before the first LPS injection (T1), then 24h after the second (T2) and the fourth injections (T3): after being fasted overnight, pigs were fed a meal standardized for size (300 g) and composition, and serial blood samples were taken before the meal then over 4 hours to measure plasma insulin and metabolite concentrations.
Before the LPS injection, HT has negligible direct effects on the postprandial profiles of insulin, energy and nitrogen related metabolites. Each LPS injection induced a hyperthermia and increased plasma concentrations of haptoglobin, an acute phase protein. Compared to T1, post-prandial plasma concentrations of glucose and insulin were greater and those of lactate, NEFA, α-amino nitrogen and urea were lower after the LPS injections. The lactate profiles were more affected by LPS in TN than in HT pigs whereas those of nitrogen related metabolites were more affected by LPS in HT than in TN pigs. Conversely, Ta did not modify the effect of LPS on NEFA profiles. Postprandial plasma glucose and insulin were increased by LPS at T2 and T3 in HT pigs whereas those changes were noticed only at T2 for TN pigs. This revealed that LPS induced a state of insulin resistance that persisted longer in pigs acclimated to HT. This metabolic status may contribute to limit glucose utilisation by muscle and adipose tissue and could be a potential mechanism modulating pig capacity to overcome an inflammatory challenge.

P022

INVESTIGATION OF A NOVEL GROUP HOUSING SYSTEM FOR SOWS DURING LACTATION: HEALTH STATUS OF THE SOWS

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Introduction
In conventional pig production, it is common practice to keep sows in single housing systems with farrowing crates from parturition until the end of lactation. The restriction of free body movement can cause negative consequences for health and wellbeing of the sows. The aim of this study was to investigate a novel group housing system from an ethological point of view as well as concerning the health of the sows and piglets and their biological performance.

Materials and methods
The study was carried out at the research farm of the University of Veterinary Medicine Hannover, Germany. The novel group housing system has five single pens for farrowing and a shared area between the pens including a feeding system. Instead of farrowing crates, the pens are equipped with special flexible iron bars and rubber bollards to prevent crushing of piglets by the sow. The sows can move freely all the time, they can turn around inside the pens, leave them and meet other sows in the shared area. A flexible step at the entrance of the pens prevents the piglets from leaving the pens during the first days of life. The group housing system was compared to conventional single housing with farrowing crates in the same piggery. In both systems, the lactation period was 35 days. In five batches, data of 23 sows and their litters in group housing system and 16 sows in conventional system were collected. Concerning the animal health status of the sows, skin lesions were assessed when the sows entered the farrowing system, three days later and when the sows left the system. Several parts of the body were scored from 0 to 3 and a cumulated scoring index (min.:0/ max.:51) was calculated for each sow. Furthermore the occurrence of shoulder lesions was recorded. For each sow reproductive parameters including the number of piglets born alive, stillborn piglets and piglet losses were documented.

Results
When the sows entered the group housing system, their scoring index was 19, on average. Three days later, the scoring index was about 16 and until the end of the lactation the index declined to 11. The increase of shoulder lesions during the lactation period was 5% in group housing versus 41% in conventional pens. The average mortality rate of piglets was 20.4% in group housing system and 20.9% in conventional systems with farrowing crates.

Conclusions
Despite the possibility to interact, the sows in group housing system did not show an increase in skin injuries caused by aggressive interactions. Shoulder lesions were found less in sows in the group housing system compared to conventional farrowing crates. The housing system did not influence the piglet mortality.

P023

PIG JEJUNAL EXPLANTS: AN EX–VIVO MODEL REDUCING ANIMAL EXPERIMEN–TATION (3RS) FOR STUDYING THE EFFECTS OF MYCOTOXINS ON THE INTESTINAL MUCOSA, ALONE AND IN COMBINATION

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Introduction In the context of implementing the 3Rs by reducing the numbers of animals used, we developed pig jejunal explants for studying the effects of mycotoxins. Deoxynivalenol (DON) and nivalenol (NIV) are type B trichothecenes fusariotoxins, targeting intestinal mucosa (Pinton et al, 2009, 2012). Pig jejunal explants were used to characterize the effects of DON and NIV, alone or in combination, on the intestinal tissue of pig, the most sensitive animal species.
Materials and methods Crossbreed weanling piglets of 4–5 week-old (n = 6) were used for explanting jejunal tissue (Kolf-Clauw et al., 2009). Explants were exposed to DON, NIV, and the mixture DON+NIV (1:1) for 4 hours, at 0.1 to 30µM for each mycotoxin or for the mixture 1:1. Mucosal lesions were assessed by using histopathological scores. Realistic in vitro concentrations compared to pig in vivo digestive exposure to contaminated feed were used.
Results The individual treatment with the mycotoxins DON and NIV resulted in a significant decrease of the histopathological score from doses of 3µM and 1µM, respectively. The main morphological and lesional changes were flattening of epithelial cells, villi fusion, apical denudation of villi with the highest dose of NIV, villi with absence of epithelia were observed. The interaction effects were evaluated by the isobologram method. The DON+NIV combination demonstrated synergism for IC50 whereas antagonism was observed at the lower doses. Taken together, the present data provide strong evidence that NIV and DON mycotoxins alone or in combinations at low exposure alter the intestinal health. Our results are in accordance with previous investigations on intestinal and non-intestinal cells lines, showing less severe toxicity of DON compared to NIV.
Conclusion Pig explants represent a sensitive model to assess the digestive barrier alterations following toxins exposure.

P024

RISK FACTORS AT THE INDIVIDUAL PIG LEVEL FOR BEING A TAIL–BITTEN PIG

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Introduction
The risk of being a victim of tail biting depends not only on the biters but also on characteristics of the victim itself. Entire and castrated males are more likely to be tail-bitten than females. One study suggests that the higher-ranking and heavier pigs are more likely to become victims than their pen mates. An association between poor health and subsequent tail lesions at the individual pig level has not been established. The objective of this study was to identify factors relating to the individual pig that are associated with an increased risk of being tail-bitten.

Materials and Methods
An existing data base comprising data from nine herds was used for the analysis. In each herd, a cohort of 672-924 pigs was followed from weaning to slaughter or death/euthanasia. The birth date of each pig was known. All pigs were tail-docked, and males castrated. Pigs were weighed individually at weaning and at transfer to the finishing unit. For each pig, the farm staff recorded the cause and date of any individual medical treatments. Pigs were not grouped by gender. According to the treatment data, only three of the nine herds had more than sporadic cases of tail lesions and were included for further analyses. In herd A, tail biting was recorded only in the weaner unit – in herds B and C, only in the finishing units. The association between the risk of being tail-bitten (according to the treatment data) and potential risk factors related to gender, growth and health was analysed by logistic regression analysis.

Results
Herd A (weaners): thirteen (1.9%) out of 671 pigs were recorded as being tail-bitten at least once, mainly during days 35-42 after weaning. Castrates had a higher risk of being tail-bitten than females (OR=5, P=0.03), whereas weight and age at weaning were not associated with the risk of being tail-bitten. Herds B and C (finishers): a total of 35 pigs (2.3%) out of 1526 were recorded as being tail-bitten. Most tail lesions were first detected within 32 days of transfer of the pigs to the finishing units. Castrates had a higher risk of being tail-bitten than females (OR=5, P=0.02). Weight and age at transfer to the finishing unit were not associated with the risk of being tail-bitten. Pigs that had received individual medical treatment in the weaner unit did not have a higher risk of being tail-bitten in the finishing unit. However, only 2.9% of the pigs had received medical treatment in the weaner unit.

Conclusion
In accordance with previous studies, castrates were more often tail-bitten than females. The data did not support the assumption that the risk of being tail-bitten depended on weight, previous growth rate or previous disease.

P025

NUTRIENT METABOLISM ALTERATIONS CAUSED BY CO-INFECTION BY SWINE INFLUENZA VIRUS AND MYCOPLASMA HYOPNEUMONIAE IN PIGS – MITIGATING EFFECTS OF FEED RESTRICTION

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Nutritional strategies can be effective to improve the ability of pigs to cope with disease. Indeed, inflammation is responsible for changes in nutrient partitioning between growth and functions devoted to body defense. Reciprocally, many nutrients are involved in metabolic pathways modulating inflammatory and immune responses. European avian-like swine H1N1 is a major pathogen of the porcine respiratory disease complex together with Mycoplasma hyopneumoniae (Mhp). We studied the impact of a moderate feed restriction on the ability of pigs pre-infected with Mhp to cope with an H1N1 influenza challenge and its consequences on nutrient metabolism and performance. Post-prandial nutrient utilization was used to identify nutrients whose metabolism was modified.
Two groups of 8 SPF pigs were intra-tracheally inoculated with Mhp and H1N1 21 days apart. One group was fed ad libitum (AL) whereas the other one was applied a 40% feed restriction (FR) 1 week before H1N1 infection. One mock-inoculated AL and 1 mock-inoculated FR group of 4 pigs each were included. All pigs were fitted with a jugular catheter. Three days post-H1N1 infection and after an overnight fast, 200g of feed was given to all animals and serial blood samples were performed during 4h for measuring post-prandial plasma nutrient concentrations. Pigs were slaughtered 7d post-H1N1 infection. Clinical signs were observed throughout the study.
Both FR and infection modified postprandial kinetics of glucose and amino acid (AA) concentrations showing dramatic changes in nutrient metabolism. Glucose, arginine and threonine plasma concentrations were lower in infected pigs. The lower threonine concentrations might be related to the greater concentrations in plasma immunoglobulins that are rich in this AA. Feed restriction hardly modified the plasma nutrient response to infection. However, FR pigs presented a shorter hyperthermia as well as a positive mean weight gain over the 3 first days following H1N1 infection while animals fed AL lost weight during that period. This trial confirmed that feeding practices could be a strategy to help animals to overcome an influenza infection.

P026

IMPACT OF ZINC OXIDE ON THE IMMEDIATE POST WEANING COLONIZATION OF ENTEROBACTERIA IN PIGS

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Introduction: Dietary zinc oxide shows beneficial effects on E. coli induced diarrhea in pigs after weaning, but no data is available on the development of enterobacteria directly after weaning.
Material and Methods: Twenty weaned piglets were fed diets containing 150 or 3000mg/kg zinc from standard zinc oxide sources and 150 or 300mg/kg zinc from a commercial zinc oxide preparation. Fecal samples were taken daily from day 0 to day 4 and additionally on day 6, 8, 10 and 14. A range of relevant enterobacterial genes were detected via PCR assays of fecal DNA extracts. Statistical analysis was carried out by ANOVA for comparison of single days as well as by repeated measures GLM.
Results: The development of the Escherichia group (measured by 16S rDNA) showed an increase until four days after weaning in pigs fed the 150mg/kg zinc diets, followed by a drastic decline until day 10 after weaning. However, the 300mg/kg as well as the 3000mg/kg diets showed an earlier decline already two days after weaning, which led to less colonization of the Escherichia group than in animals fed the lower zinc diets. A renewed increase of colonization was visible 14 days after weaning. The most prevalent enterobacterial toxin gene was the E. coli estIIb. Contrary to the colonization of enterobacteria, a severe decrease of the amount of estIIb genes was visible in animals fed the 3000mg/kg diet already after the first day after weaning. A steady decline was observed in all other trial groups; the 300mg/kg commercial zinc preparation led to a sharper decrease than both 150mg/kg zinc diets. Generally, the impact of dietary zinc was lessened in all experimental groups 14 days after weaning.
Conclusion : This study showed that there is an immediate effect of zinc oxide ranging from three to four days after weaning until eight to ten days after weaning.

P027

DOSE–RESPONSE EFFECT OF β–ALANINE SUPPLEMENTATION FOR WEANED PI–GLETS

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Previous trials in growing pigs have indicated that addition of β-alanine to the diet improved average daily gain (ADG) and average daily feed intake (ADFI). To determine the optimal dose of supplementation for weaned piglets, a dose-response trial was done. A total of 84 gilts and 84 barrows, all weaned at the age of 24 days, were divided over 48 pens. In order to have equal stocking density, half of the pens housed 3 piglets of the same sex and the other half 4 piglets of the same sex. All piglets were fed a commercial weaning and starter diet supplemented with 0, 250, 500 or 750 mg/kg β-alanine. Each dose was assigned to six pens of gilts and six pens of barrows. Piglets were weighed biweekly and total feed intake of each pen was recorded simultaneously with each weighing. Based on the total feed intake and the growth during the 42 days trial period ADG, ADFI and feed conversion ratio (FCR) on pen level was calculated. Statistical analyses were performed in RStudio, using a linear mixed effect model including dose and gender as fixed effects and number of piglets per pen as random effect (lme in nlme package). On day 28 and 42, pigs supplemented with 250 ppm β-alanine were significantly heavier (1.03kg and 1.94kg, respectively) compared to the control group. At the dose of 250 mg/kg, ADG was 66 g higher (P=0.018) and ADFI was 94 g higher (P=0.027) compared to the pigs not supplemented with β-alanine. The corresponding FCR was only numerically improved. For the two other doses tested, no significant effects were recorded. It can be concluded that for this type of weaned piglets, 250 mg/kg β-alanine was the optimal dose improving both growth and feed intake, without negative impact on the efficiency of the piglet.

P028

EFFECT OF ACIDIFIERS AND THEIR COMBINATION ON GROWTH PERFORMANCE OF WEANING PIGS

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Introduction
Organic acids as alternatives to antibiotic growth promoters have increasingly and successfully been supplemented in feed. However, the efficacy of some organic acids can be improved by combining them with other acids. An experiment was conducted to study the effects of dietary supplementation with an enhanced acidifier (EA) consisting of a blend of organic acids, cinnamaldehyde and a permeabilizing substance PS (Biotronic® Top3, BIOMIN, Austria) in a diet based on corn-soybean meal on growth performance. Additionally the efficacy of benzoic acid and the combination with EA was tested in the same trial.
Materials and Methods
The trial was conducted in a swine trial facility in Austria using 140 mixed sex weaning piglets [(Landrace x Large White) x Pietrain]. Pigs were weaned at 28 days of age and assigned to 4 treatments with 3 replicates per treatment and 10 piglets in each replicate.
The control group diet contained no feed additives, whereas the diet of the first trial group was supplemented with NGP at an inclusion rate of 2.0 kg/t feed, second trial group with benzoic acid at the inclusion rate of 5 kg/t and third trial group with the combination of NGP and benzoic acid at the inclusion level of 1 and 3 kg/t feed respectively. The duration of the trial was 42 days.
Results
The inclusion of EA showed positive effects on growth performance of piglets. Final weight of pigs fed EA was higher in comparison to the control group although the difference was not significant. Feed conversion ratio (FCR) was significantly lower in the group fed EA in comparison to the group fed benzoic acid. the Although the inclusion of benzoic acid alone did not show numerical difference of performance data to the control group, combination of EA and benzoic acid showed highest final weight numbers in comparison to other groups. FCR of the group fed the combination was significantly lower than the group fed benzoic acid.
Conclusion
The inclusion of the combination of EA and benzoic acid to pig's diet showed improved final weight in comparison to the inclusion of EA or benzoic acid alone.

P029

IMPACT OF A WHEAT AND BARLEY GRAIN REPLACEMENT BY THEIR DRIED SILAGES ON PERFORMANCE AND FAECES CONSISTENCY OF WEANED PIGLETS

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Introduction: Ensiling of wheat and barley grain is a reasonable storage alternative, even if the content of lysine decreases during the preservation process. However, in ensiled grain both beneficial and adverse effects on prececal digestibility of amino acids were found. Nevertheless such effects may influence growth performance. Additionally beneficial effects on the intestinal microbiome and, as a result, a decreased occurrence of diarrhoea may be assumed. The aim of this study was to investigate the impact of both ensiled and untreated wheat and barley grain directly included in a pelleted diet on performance and occurrence of diarrhoea in weaned piglets. We hypothesized that days of diarrhea (DD) are minimized and growth performance improved.
Material and Method: In total, 60 weaned, 28 d old, castrated male piglets (initial body weight 6.1 ± 0.56 kg) were divided into 3 groups. Piglets were individually caged and randomly distributed on a flat deck. They received a pelleted diet ad libitum for 5 weeks. The control diet based on wheat grain, barley grain, soybean meal, and whey powder (13.1 MJ ME, 1.31% lysine). According to the dry matter content wheat and barley were gradually replaced by cracked and dried wheat silage and barley silage, both ensiled with Lac. plantarum (DMS 8862, DMS 8866). Body weight gain (BWG), feed intake, and feed conversion ratio were determined weekly. Within the first 10 trial days consistency of faeces was scored (1-5). Days with score 3-5 are considered as DD. One way ANOVA (SPSS 20.0) and Chi-square test (2 groups per process) were performed.
Results: Crude protein content was highest (19.9) and pH values (5.3) lowest in treatment group (TG) 2, but without developing beneficial effects on BWG or feed intake. Feed conversion ratio was significantly improved in TG 2 ($P<0.05$). DD were significantly reduced ($P<0.05$) in TG 2 (15 DD) compared to control (31 DD), but not to TG 1 (28 DD).
Conclusions: Results indicate that a complete replacement of untreated by ensiled wheat and barley in piglet diets had beneficial effects on feed conversion ratio, but not on BWG and feed intake. However, occurrence of diarrhoea seems to be beneficially reduced when cereal grains are completely replaced by their silages. Further investigations have to examine whether ensiling products like lactate as well as the reduced pH beneficially influence the intestinal microbiome of piglets.

P030

HEALTH DISORDERS ON PIG FARMS DUE TO FAULTS IN MINERAL SUPPLY

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To ensure health and performance of pigs a mineral supply that meets the requirement is essential. Risks occurr in cases of an under- or oversupply as well as due to imbalances of minerals.
Reasons for those problems could be mixing errors, that might happen on the farm (mixing by the owner himself) or by manufacturers. It can be explained by faults in the calculation (wrong data concerning individual feeds are used in the calculation program), technical problems during mixing process (inaccuracy of the scale), missing cleaning of the pipelines (unwanted carry-over of critical additives, cross contamination) or a wrong selection of ingredients (interaction between minerals). Furthermore, there is a risk using faulty mixed supplementary feeds (e.g. mineral feeds).

But even in cases of a well mixing process during transport or blowing into the silo or offering to the pigs demixing processes are possible, that lead to a faulty mineral supply of pigs.

On the basis of analyses of feedstuffs and diets that were sent in to the consultation service of the institute in the years 2010 until 2014 possible deviations of the mineralization of diets and the following consequences for pig health` will be presented by several case reports.

P031

REDUCED FEED INTAKE IN PIGS – DUE TO DISORDERS OR DIETS`QUALITY?

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A low feed intake belongs to one of the common problems on pig farms and results in a reduced performance combined with high economic losses. Causes of a reduced feed intake are possibly an illness of the pigs but also aberrations within the feed. Besides variations of the botanical composition (high levels of components with a low taste due to antinutritive factors like rape seed meal, peas a.s.o.) specifics of the chemical composition (e.g. an excess of nutrients like calcium, sodium or copper) can also lead to a low palatability in pigs. Moreover, a microbial contamination (e.g. a reduced hygienic status due to higher levels of colony forming units of yeasts), mycotoxines (especially vomitoxine) or a physical contamination (e.g. foreign bodies like plastic particles from building of a new fooder silo) have to be mentioned. Furthermore, diets` physical form (indirect by gastric ulcers) and an insufficient dry matter content in liquid feeding systems (dry matter intake capacity of pigs as limiting factor) may lead to a lower amount of ingested feed. Last but not least the water supply and its quality might influence the feed intake. Beside an evaluation of 215 feed samples sent in to the consulting service within the last three years with the nutritional history of a reduced feed intake the diversity of reasons that come into consideration in this situation will be spotlighted by several case reports in which a reduced feed intake was observed and reported by the owner or veterinarian.

P032

UNCONVENTIONAL FEEDSTUFFS/BYPRODUCTS IN SWINE FEEDING

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Introduction: Omnivorous species like pigs are predisposed to utilize byproducts with high carbohydrates, fat or low fiber contents. In the last years a complete ban on feeding leftovers in pigs was discussed frequently due to their role in spreading ESP virus, the possible occurrence of packaging material, disposal of undesirable substances via unconventional products (e.g. MCPA in molasses) a.s.o.. But the judiciary allows products derived from production, transport, processing or consumption of food and their usage for further years. Therefore, the chemical composition of such unconventional feedstuffs (high variability between the diverse products and within different batches of the same product) as well as possible contaminations should be proofed.

Materials/Methods: Various unconventional feedstuffs were analysed. Chemical composition (nutrients, amino acids, minerals) was determined by standard methods of feed analyses, the particle size was proofed by sieve analysis. Furthermore, detailed sensory and microbiological examinations (cultural standard methods) were carried out. Results of microbiological analyses was interpreted corresponding to recommended data of the group Feed microbiology of the VDLUFA. Moreover, a possible contamination with packaging material or other undesirable substances was considered.

Results and discussion: Some unconventional products showed typical nutrient levels depending of their origin (for example high level of fat in potatoe chips or in the skin of hazelnuts). However, not only crude protein contents but also macro minerals showed a high variation of their levels. Whereas some products could be characterized by low calcium levels (e.g. chocolates), others showed high sodium levels (e.g. potatoe chips). Furthermore, great differences could be observed concerning trace elements (original? contamination?). Besides variations between different by-products also feed specific differences of a product occurred. A common problem of by-products are their prone to spoilage processes (liquid products: microbial spoilage; dried products like old bread: moulds; products rich in carbohydrates, especially sugar: mainly yeasts).
Some products showed contaminations like packaging material (aluminium foil or plastics) in amounts of 0.5 – 2.5%. Up to now packaging materials belong to the prohibited substances, but it is discussed to classify these products like undesirable substances.

Summary: To asses by-products typical nutrients of these products (e.g. fat content in food leftovers or husks of hazelnuts β soft fat of the sward?) or levels of sodium chloride (risks of intoxication when water availability is restricted?) are recommended. Besides these well known risks further aspects like the particle size of these products (size/structure of wine gum or broken biscuits in GIT β gastric ulcers?) have to be considered.

P033

EVALUATION OF A STRATEGIC DEWORMING PROGRAM WITH FLUBENOL (ELANCO AH) FOR CONTROLLING MILK SPOT PREVALENCE AND ASCARIS SUUM INFESTATION IN FATTENING PIGS

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Introduction
Infestation by Ascaris suum is the most important parasitism of pigs worldwide, causing a negative effect on performance and a high economic cost [Greve JH, 2012. Diseases of Swine, p. 910-912]. As a part of A. suum hepatotracheal migration route, L3-larvae travel through the liver damaging it. Consequently, whitish healing foci occur in the livers known as milk spot lesions.
The aim of this study was to assess the efficacy of Flubenol® (Elanco AH) for controlling milk spot prevalence and A. suum infestation when used according to a strategic deworming program based on the prepatent period of the parasite.
Materials and Methods
This study was conducted in a 150-farrow to finish farm, with a 3-week batch farrowing management. The farm had a recent history of A. suum infestation, with a sustained milk spot liver prevalence >60%.
Piglets from 4 consecutive batches of production were randomly allocated into two experimental groups: Flubenol® Group (n=386), pigs were treated with 30 mg/kg of flubendazole (Flubenol®, Elanco AH) for five consecutive days at 9, 15 and 21 weeks of age. Control Group (n=383), pigs remained untreated. Groups of pigs were weighed at 9, 13 and 23 weeks of age and average daily gain (ADG) calculated. Pooled faecal samples from 5 individual pigs were taken from both experimental groups at 14, 20 and 26 weeks of age in batch 1 (n=16); at 13, 17 and 23 weeks in batch 2 (n=12), at 14 and 20 weeks in batch 3 (n=8) and at 11 and 17 weeks of age in batch 4 (n=8) and faecal egg counting (FEC) was performed by McMaster flotation method (Taylor et al., 2007). Presence of liver milk spot were evaluated at the slaughterhouse.
Results
The parasitological examination revealed significant differences in the number of A. suum eggs between experimental groups. An average of 2,627 eggs/g faeces (SD 1081) in the non-treated pigs compared to 72 eggs/g faeces (SD 193) in the Flubenol® treated pigs ($p<0.05$) was observed. In addition, a significant reduction ($p<0.05$) in the number of milk spot livers at slaughter was recorded in the group treated with Flubenol® (33%) when compared to the control group (56%). When ADG was examined, there was not a significant difference between experimental groups. However, pigs in the Flubenol® group were on average 1.4 kg heavier at the end of the finishing period (86.2 and 87.6 kg, respectively; $p>0.05$).
Conclusion
The use of Flubenol® in a strategic deworming program was efficacious in controlling the infestation by A. suum as shown by the significant reduction in FEC and milk spot lesions at the abattoir. A strategic deworming program with Flubenol® should be considered together with other management and disinfection practices in order to decrease the parasite burden, control A. suum and reduce milk spot lesions.

P034

CLINICAL PRESENTATION OF TRICHURIS SUIS IN A CONVENTIONAL HERD

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INTRODUCTION
Internal parasites are common in pigs worldwide and infection may reduce productivity and in severe cases also cause overt clinical disease. Ascaris suum is most common and Trichuris suis occurs more sporadically but eggs of both nematodes are long-lived and highly resistant why infection may build up over time. The aim of this report is to describe the clinical presentation of T. suis and the prevalence of internal parasites in a conventional farrow-to-finish herd.

MATERIALS & METHODS
A farrow-to finish, 180 sow herd, free from Brachyspira hyodysenteriae, experienced wasting and diarrhea, sometimes hemorrhagic, of fattening pigs in one pen. Treatments with tylosin or trimethoprim sulphonamides were not effective.
Sows were group housed on deep-straw during lactation and farrowed in individual pens with peat as bedding material which was reused in pens where weaned sows were kept. Fatteners were kept in pens with partially slatted floors. Peat and straw was used as rooting material. Sows were routinely dewormed with 5 mg/kg febantel one week before farrowing and weaners at 9 weeks of age before they were transferred to a fattening unit.
Two fatteners with diarrhea were euthanized and submitted for post mortem. Individual fecal samples were collected from two groups of either pregnant or lactating sows. Pooled samples were collected from one batch of growers, two fattening batches and two groups of gilts. Individual and pooled samples, respectively, were examined for nematode eggs with a modified sugar-salt flotation method based on 30 g of faeces.

RESULTS
The post mortem revealed a severe, mucohemorrhagic, necrotizing inflammation of the cecum, colon spiral and the large intestine of both pigs. Numerous T. suis were found in the intestinal mucosa and lumen in both pigs who were emaciated.
Fecal sampling demonstrated T. suis in 3 out of 16 sow samples and in 5 out of 8 samples from pregnant gilts. T. suis was only demonstrated in one pooled sample from fattening pigs. Oesophagostomum sp. were detected in all sampled categories and A. suum was detected in all categories except for weaners aged eight weeks and pregnant sows.

CONCLUSION
Routine dewormings were effective in reducing A. suum in sows and their offspring. The use of deep-straw bedding for sows and reusing of peat could have contributed to an increased pathogen load of T. suis that with time, caused severe clinical disease in fatteners even though the fattening units were emptied, cleaned and allowed to dry between batches. Therefore, regular strategic fecal samplings are important in designing targeted deworming programs to control internal parasites in pig herds.

P035

NATIONAL GUIDELINE FOR TREATMENT OF RESPIRATORY DISEASE IN PIGS

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Motive: the Dutch government urges veterinarians in the livestock industry to reduce antimicrobial use, as well as to be more transparent about the legitimacy of this use. The Dutch Royal Veterinary Association (KNMvD) responded with the development of national guidelines for veterinary conduct for various indications in various species. The present guideline has been developed to set out a framework for the justification of antimicrobial use in the treatment of respiratory disease in pigs.
Method: research questions for urgent matters were posed by a panel of practicing veterinarians. A working group of 5 veterinarians and scientists supported by a secretary of the KNMvD is set to work in confidentiality, after having si gned a declaration of conflict of interest. Discussion sessions are held to address specific issues in order to start a review of the scientific literature. The draft guideline is subsequently send out to at least 25 randomly chosen swine veterinarians and multiple stakeholders and scientists for an external review. The comments of the reviewers are judged on its merits, commented by the working group. Thereafter, the revised guideline is assessed by an authorization committee (3 scientists), according to the AGREE II instrument (a). Finally, the board of the KNMvD will adopt the guidelines for use.
Results: The final guideline will likely be adopted by the KNMvD board in Q1 2015. For the guideline on respiratory disease these major conclusions to the research activities were drawn:

- Literature is extremely biased by industry sponsored studies on drug efficacy.
- There is no scientific evidence available on levels of prevalence or incidence of disease that may help to justify group treatment with antimicrobials.
- Necropsy investigations are an essential tool to differentiate between pneumonia of viral and bacterial origin in the field.
- ELISA or PCR techniques have very little added value for making a diagnosis of acute respiratory disease that may justify the use of antimicrobial use.
- Scientific literature on the use of NSAIDs as complementary to antimicrobials for enhanced treatment effect in respiratory disease is sparse and inconclusive.

a) AGREE Next Steps Consortium (2009). Appraisal of Guidelines for Research & Evaluation (AGREE) II Instrument. www.agreetrust.org

P036

IMPACT OF ASCARIS SUUM ON TECHNICAL PERFORMANCES AND ON LUNG LESIONS IN 104 PIG FARMS IN FRANCE, USING SERASCA TEST AS A DIAGNOSTIC TOOL

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Introduction
Infestations of pigs by Ascaris suum are known to have an impact on swine performances and on swine health. Serasca, a new serological test for the diagnosis of this parasite is available. The goal of this study was to evaluate the impact of Ascaris suum on swine performances and on lung lesions, using this new serological test to detect the presence of the parasite.

Materials and Methods
The study was conducted in 104 pig farms in France between February and September 2014.
For each farm, the following data were collected:
- Age at 115 kg.
- Type of flooring in gestation, post-weaning and finishing.
- Parasite treatments: for sows, during post-weaning and finishing.
- Lung lesion score (24 point scale) were evaluated at slaughter. A minimum of 50 lungs per farm were controlled.
- Blood sampling: 10 pigs per farm were samples at the slaughterhouse. Samples were sent to Labocea, Ploufragan, France for sera extraction and subsequently submitted to Gent University, Belgium for Serasca analysis.

Results
38% of the farms were positives for Ascaris suum at the time of slaughter.
The average lung lesion score was 2.0. Farms negative for Ascaris had an average lung lesions score of 1.5 whereas farms positives for Ascaris had an average lung lesions score of 2.9. Student test showed a significant differences between those 2 groups (p=0.0016).
The average age at 115 kg was 174.3 days. Farms negative for Ascaris had an average age at 115 kg of 173.4 days whereas farms positives for Ascaris had an average age at 115 kg of 175.5 days (no significant difference).
In our study, 18 farms had gone over a total depopulation – repopulation program and only one farm was positive for Ascaris. Over the other 86 farms of this study, 39 were positives for Ascaris.
23% of the farms raised sows on straw. 92% of the post-weaning barns and 82% of the finishing barns were on full slated floor. The number of farms raising the pigs on straw was too low in our study to do a statistical analysis.
10%, 32% and 60% of the farms did not perform any treatment against parasites respectively for the sows, during the post-weaning and during finishing. There was no difference between the number of treatments against parasites and sera results.

Conclusions
This study shows a high prevalence of Ascaris suum at slaughter, 38% of the farms being positives. When the farm was positive for Ascaris, lung lesion score was increased. When the farm had gone under a depopulation – repopulation program, sera were negatives. This new serological test seems to be interesting to evaluate the infection pressure of Ascaris suum and the deworming protocol.

P037

SWEDISH PIG FARMERS KNOWLEDGE OF ANTIMICROBIALS AND ANTIMICROBIAL RESISTANCE

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Introduction
Sales of antimicrobials (AM) in Europe differ, and Sweden is one of the countries with the lowest sales figures. It is assumed that knowledge of AM and AM resistance among farmers has an impact on use. The aim of this survey conducted within the MINAPIG project was to characterise the knowledge of AM and AM resistance among Swedish farmers. Farmers' relations to their veterinarians were also investigated.
Materials and Methods
A questionnaire survey was constructed and sent to pig farmers in Sweden. The survey was constructed based on 8 mental constructs related to AM and AM resistance, of which one construct with 18 statements explored farmers' knowledge. Farmers were asked to decide whether each of the statements was true or false or whether they did not know. The proportions of correct answers were calculated ("do not know" was treated as wrong answer), and differences between gender and between farmers with great vs. little trust in their veterinarian were analysed.
Results
A total of 390 farmers (45%) completed the survey. Of those, 75% were male and 25% female. More than 50% of farmers answered correctly on 17 of the 18 statements. Almost all (90%) farmers knew that AM are not effective against viruses, that the use of broad-spectrum AM to treat all diseases in pigs does not prevent AM resistance and that systematic cleaning and disinfection, biosecurity and rodent control belong to the most effective alternative measures to antimicrobials. However, 53% of the farmers incorrectly believed that AM resistance means that bacteria can resist the effect of all AM. Most farmers (90%) stated that their vet was the most important source of information about pig diseases and that they fully trust their advice and 77% indicated that their vet supported them in implementing or using alternative measures to antibiotics. The proportion of female farmers that provided correct answers was higher ($p<0.05$) than male farmers on 9 of the statements. The proportion of right answers were higher ($p<0.05$) for 4 of the statements in the group of farmers with a supporting vet. However, farmers with a higher trust in their vet had a significantly lower proportion of right answers for 3 statements.
Conclusion
Swedish pig farmers are well informed about AM and AM resistance, especially female farmers. Veterinarians' impact on farmers' knowledge was partially surprising. The results could imply that a small, well informed group of farmers expect more from their vets. The reason for the gender difference could perhaps be attributed to educational differences but that needs further studies.

P038

ELISA SEROLOGY TO ASSESS ASCARIS SUUM INFECTION IN GILTS: FIELD TRIALS IN SIX FRENCH FARROW TO FINISH PIG FARMS.

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Health status of gilts is critical in pig farms because it has a strong effect on whole herd health status. Regardless of the pathogen, infected gilts can easily be a source of infection for the sow population and their own litters, as well as decrease their own performance in terms of fertility and prolificacy. Ascaris suum, a large roundworm, is an ascarid nematode and is a highly prevalent gastrointestinal parasite in pigs. Infected breeders can eliminate thousands of eggs and contaminate pens, barns and their own litters. A new Elisa serology test, Serasca, was developed by the veterinary parasitology laboratory of Genth University (Belgium). The test assesses antibodies against Ascaris suum haemoglobin in blood of pigs aged more than 25 weeks and is mainly used in fatteners. The aim of this study was to evaluate this test in gilts naturally infected with Ascaris suum in commercial farms.
In six different French farrow-to-finish pig farms (A1, A2, B1, B2, C1, C2), six batches of young sows were sampled. Three batches of gilts were sampled at the beginning and end of quarantine. Three other batches of gilts were sampled at the entrance of gestating facilities, and later, at the farrowing unit entry. Each sampling group of gilts was 6 to 10 animals. Serasca test was performed on every blood sample and results was expressed as Optical Density ratio (ODr). Final results of the test for each sampling group was the average of the individual ODr. Cut-off of the test is 0,5. Results under 0,5; between 0,5 and 0,8; and above 0,8 indicate respectively low, medium and high Ascaris suum infectious pressure.
Seventeen sampling groups at quarantine entry had low (14/17) or medium (3/17) infectious pressure. Sixteen sampling groups at the end of quarantine had low (5/16), medium (7/16) or high (4/16) infectious pressure. Farms B1 and C1 had the highest seroconversion during quarantine. Sampling groups at gestating unit entry had low (3/16), medium (7/16) or high (6/16) infectious pressure. Sampling groups at farrowing unit entry had low (7/17), medium (4/17) or high (3/17) infectious pressure. Farms A1, A2 and C1 had the higher serological levels during gilts gestation.
Risk factors for Ascaris suum contamination during quarantine or gestation of gilts will be discussed.
As a conclusion, this study suggest that Serasca tests on gilts sampled at the entrance of Gestating unit could be of interest to evaluate Ascaris suum infectious pressure, biosecurity practices and gilts deworming program applied at farm level.

P039

ETIOLOGY AND DIAGNOSTICS OF PORCINE RESPIRATORY SYNDROME ON A PIG FARM IN THE REPUBLIC OF SERBIA

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Introduction. Respiratory diseases of swine are among the most important problems causing huge economic losses to the swine industry worldwide and in the Republic of Serbia as well. Intensifying of swine industry increases the frequency and economic importance of this disease regardless of the applied measures for their suppression and eradication. Respiratory diseases cause great economic losses expressed through increased rate of deaths and forced slaughter on one hand, and reduced daily gain, increased feed consumption per average daily gain (kg), prolonged time of fattening, high percentage of light-weight pigs as well as increased costs of medical treatments on the other hand. Occurrence of swine respiratory diseases in Serbia is strongly dependent on the whole range of factors, including farming system, nutrition, genetic factors, zoohygienic conditions, overall health status of the herd, etc. However, infectious agents (viruses, bacterial agents) are often introduced into the herd by purchasing latently infected animals or infected semen for artificial insemination from another farm.
In everyday farming practice, assessment of economical impact of respiratory diseases at herd level relies on the following information: data records on the diseases within the herd (occurrence of clinical form of respiratory disease and/or other diseases, mortality rate, autopsy results, material costs of applied therapy and prophylactic strategies), productivity data (current weight gain, number of days until slaughter, conversion) and slaughterhouse records (the percentage of low-weight pigs, number of diseased animals, severity of lesions observed at slaughter and amount of discarded organs and carcasses). The inspection at slaughter might be a useful tool for monitoring the health status of animals and data source for further epidemiological studies. In many countries, health recording protocols for collecting relevant data at the slaughterhouses were developed.
This study was aimed at establishing etiological diagnosis of respiratory syndrome on a pig farm characterized by apparent increase in the fluctuation in body weight and high mortality rate of fatteners, as well as implementing of appropriate control measures.

Material and Methods. The examined material included respiratory organs of fatteners obtained at slaughter line (90 samples) and 30 blood samples. The examination was performed applying standard bacteriological diagnostic methods (aerobic and microaerophillic cultivation) and molecular diagnostic methods (PCR and real time PCR) aimed at confirming presence of particular viral species (PRRSV, PCV2). Blood serum samples were examined using immunoenzyme (ELISA) tests for specific agents: PRRSV (Ingenasa, Madrid), PCV2 (Ingenasa, Madrid), Mycoplasma hyopneumoniae (IDEXX M. hyo. Ab test), Influenza virus H1N1 and H3N2 (IDEXX Swine Influenca Virus Ab test).

Results. The examination of respiratory organs of fatteners at slaughter line, no changes in lung tissue were observed in 19.21% animals. In other animals, examination of respiratory organs revealed the following pathological changes: Pneumonia catarrhalis (32.39%), Pneumonia catarrhalis disseminata (14.08%), Pneumonia fibrinosa partum necrotica (8.45%), Pneumonia apostematosa disseminata (2.81%). Besides, the changes in the pleura were established, manifested as local pleuritis (28.17%) and chronic diffuse pleuritis (8.45%), as well as the changes in the heart muscle (Pericarditis villosa, 16.9%). Serological examination of blood samples revealed presence of specific antibodies against H1N1 in two samples, Mycoplasma hyopneumoniae in 17 samples, ant PRRS in 20 blood samples. Specific antibodies against PCV2 were detected in 20 (IgG) and 5 (IgM) examined samples. The presence of H3N2-specific antibodies has not been established. Molecular methods were applied for the examination of 30 samples of changed tissues (lungs and mediastinal lymph nodes) collected directly at slaughter line. The presence of viral genome PRRS-EU was established in 16 samples, whereas PCV2 and Mycoplasma spp. were detected in 11 and 13 examined samples, respectively. Bacteriological examination revealed Pasteurella multocida in 26.83%, Haemophilus parasuis in 24.39%, Escherichia coli in 19.51%, Trueperella pyogenes in 14.63%, Arcanobacterium haemolyticum in 9.76%, Streptococcus suis type 1 and Streptococcus dysgalactiae in 2.44% of examined samples of pathologically change lung tissue. The testing of antimicrobial susceptibility of isolated bacteria demonstrated that none of the isolated agents was resistant to ceftriaxone and amoxicillin, whereas resistance towards colistin, gentamicin and neomycin was established in the majority of agents.

Conclusion. Changes in the respiratory organs are evident at high rate in clinically healthy fatteners, resulting in pronounced inconsistency in body mass, leanness and carcass quality. Apparent high-grade changes in thoracic cavity organs of examined pigs raise the issue of economical feasibility of their raising. Moreover, such fatlings are potential carriers of infectious agents. In spite of applied pharmaco-prophylactic measures on the investigated farm, the presence of numerous agents responsible for swine respiratory syndrome was established. The obtained results, at the slaughter line strongly suggested the necessity of designing and implementation of a new, updated vaccination program, taking into consideration the isolated causative viral and bacterial agents.

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P040

MYCOPLASMA HYOPNEUMONIAE, PRRS VIRUS AND ASCARIS SUUM INVOLVED IN AN OUTBREAK OF PORCINE RESPIRATORY DISEASE COMPLEX: A CASE REPORT

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Porcine Respiratory Disease Complex (PRDC) is one of the most important contributors to disease associated losses in swine production worldwide. It is now described as an infection with multiple pathogens, incl. Mycoplasma hyopneumoniae (MHyo), Pasteurella multocida, Porcine Reproductive and Respiratory Syndrome (PRRS) virus, Porcine Circovirus type 2 (PCV2) , and other infectious agents.
Ascaris suum (As), a large roundworm, is an ascarid nematode and is a highly prevalent gastrointestinal parasite in pigs. Its biological life cycle includes larveal migrations through blood and lymphatic vessels, liver, heart and lungs of infected pigs via “the hepato-tracheal route”. Ascarid infections are known to modulate protective immunity following natural infection with various pathogens or vaccination against several diseases.
This paper reports a field case of PRDC in a French farrow to finish swine farm, and the contribution of MHyo, PRRS virus and As. It provides accurate data and information about clinical signs, gross and microscopic lung and lymph node lesions, PRRS, Mhyo and As serology and qPCR, production parameters, PRDC control and return on investment. Relevance for practitioners of a new ELISA As serologic test, strategic use of a fenbendazole, a larvicidal benzimidazole drug, and economic consequences of this particular case of PRDC are discussed.
This paper provides also the outcome of a recent serological study, in 467 different French farms where serum from fattener pigs was tested in the As ELISA. The results confirm the high prevalence of As in fatteners, and we can assume that other farms could be confronted with the same PRDC problem as described in this case report.

P041

PHARMACOKINETICS AND ORAL BIOAVAILABILITY OF A NEW DEVELOPED 200 MG/G FLORFENICOL DRINKING WATER FORMULATION IN PIGS

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.Introduction
Florfenicol is a synthetic, broad-spectrum antibiotic in the phenicol group active against most Gram-positive and Gram-negative organisms isolated from domestic animals and can be used for the treatment of bacterial respiratory infections in pigs. In this study, the pharmacokinetic parameters in pigs of a new-developed florfenicol drinking water formulation (Amphen® 200 mg/g, Huvepharma) are determined.

Material and methods
Six healthy animals were treated via a cross-over study design. A dose of 10 mg florfenicol /kg body weight (BW) was administered intravenously and orally (bolus administration) by gavage of Amphen® 200 mg/g oral solution) to fasted and non-fasted pigs. In a second multiple oral dosing study, Amphen® 200 mg/g was applied continuously via drinking water for 3 days. The florfenicol concentration in each plasma sample was determined by using a validated HPLC-UV method.

Results
A high oral bioavailibility of 88 ±20% and 64 ±6 % respectively was obtained after bolus administration to fasted and non-fasted pigs, indicating an influence of feed status on the oral bioavailability. The mean terminal half-life was 5.6 hours and mean AUC0-24 of 44.7 µg•h/mL.
The multiple administration via drinking water resulted in continuous plasma concentrations varying between 0.5 and 5µg/ml. This means that a very favourable T > MIC90 = 100% could be obtained for bacterial infections such as Actinobacillus pleuropneumoniae (MIC90 = 0,5 µg/ml) and Pasteurella multocida (MIC90 = 0,5 µg/ml) in the pig.

Conclusions
It can be concluded that favourable pharmacokinetic parameters are obtained for the new developed florfenicol drinking water formulation (Amphen® 200mg/g) for the treatment of bacterial infections.

P042

COMPARISON BETWEEN WHITE SPOTS IN THE LIVER AND SEROLOGICAL RESPONSE IN THE SERASCA® TEST AS A CONSEQUENCE OF ASCARIS SUUM INFECTIONS IN DANISH FINISHING HERDS

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Introduction
The SERASCA® test is a serological test that can be used to determine the lifelong exposure to larval migration of Ascaris suum in slaughter pigs. Detection of white spots at slaughter is the traditional way of determining the level of infection with migrating A. suum, but has the limitation to only reflect infections that are a few weeks old.
The prevalence of A. suum positive conventional farms in Denmark is 39% when using the SERASCA® test.
The aim of this study was to compare the SERASCA® test's ability to detect the presence of migrating A. suum in pigs during the growing phase with the ability of detection of white spots registered at the slaughterhouse as an indicator of larval migration.

Materials and Methods
Blood was sampled shortly after killing of pigs from 172 conventional farms at the slaughter line. From each farm 10 blood samples were collected and screened with the SERASCA® test. The result of blood sampling was compared to the frequency of white spots registered on the farm from time of blood sampling and the following half year.
Results
A relationship was found between infection of A. suum by prevalence of white spots at slaughter over ½ year and the serological result by the SERASCA® test from blood sampled at slaughter house. At one sampling, 30 % of serologically positive farms had no white spots at slaughter over half a year. On the other hand, 45 % of farms that were serologically negative were white spot positive on one or more occasions.

Conclusions
The results suggest that a single serological test will give a false negative result at the farm level in 45 % of cases. This finding can be caused by differences in different batches, by very late infections or because of a very low reaction due to only low infection or few pigs positive in a farm. As a consequence, it is advised to combine white spots reported at slaughter and the SERASCA® test to make the right farm diagnosis of A. suum infection level during the growing phase.

P043

APLICATION OF COMPUTED TOMOGRAPHY IN THE STUDY OF INTERNAL LESIONS OF LIMBS FROM PIGS

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INTRODUCTION
Computed tomography (CT) scanning is a nondestructive technique based on the fact that different body tissues attenuate X-rays at different rates. Thus, it can be used for detailed examination of body components in live animals at different stages of their growth with a great degree of accuracy.

MATERIAL AND METHOD
In the present study CT scans of 42 forelimbs and 44 hind limbs from pigs condemned due to generalized lesions associated to lymphadenitis were performed with a helical CT scanner (CT Hi Speed CT/e Dual; General Electric, Japan). Technical factors were 55 mA and 120 kVp. Contiguous 3-mm transverse slices (pitch 0.75/1) were made approximately from the level of the proximal border of the scapula to the radiocarpal joint (forelimbs) and from the proximal border of the iliac crest to the tarsocrural joint (hind limbs). To enhance the detection of soft tissue structures a narrow window was used (window width: 350 Hounsfiled units; window level: 40 Hounsfield units). The original CT data were transferred as DICOM images to an image analysis with DICOM viewer (OsiriX v.3.3.1. 32-bit Open Source) to perform image analysis. The original transverse slices were reformatted into the sagittal and dorsal planes.

RESULTS
None of the 86 examined pieces presented lesions within the musculature. However, CT scanning allowed detecting small size granulomatous lesions in 4.65% of the pieces. These lesions were located in superficial (i.e. superficial inguinal lymph node) and deep (i.e. popliteal lymph node) lymph nodes as well as in subcutaneous lymphatic vessels. CT scan evidenced septic arthritis and peri-arthritis in 3 hind limbs (3.49% of the examined pieces) and one abscess in the musculature, which were not detected in the examination ante-mortem together with the extension of the lesion. Traumatic lesions were observed in 8.14% of the examined pieces, with a marked proliferation of bone and soft tissue in some of them. In addition, osteochondritis dissecans (OCD) was detected in the femur of 2 pigs. OCD is a genetic disease with an alimentary origin, which induces lameness and a low performance of the animals; thus, CT is nowadays being used in animal selection programs.

CONCLUSION
Our results support previous findings and highlight the suitability of CT to evaluate internal lesions of limbs from pigs.

P044

FATTY LIVERS IN SOWS AND ROLE OF TRACE ELEMENTS

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Introduction
Around farrowing a sow is facing some major metabolic changes. Metabolic disturbances can result in slow farrowing, weak piglets and post partum dysgalactia syndrome. A relation with fatty livers has been suggested but so far not investigated. Poor feed intake could result in fat mobilisation from adipose tissue giving rise to non-esterified fatty acids (NEFA's) and subsequently accumulation of triglycerids in the liver. In addition, it is unclear to which extent build-up of trace elements in the liver is associated with fatty livers in sows. In order to get a first impression on both the prevalence of fatty livers and the possible role of trace elements, a pilot study was conducted by GD Animal Health (Deventer, Netherlands).

Material & Method
Extensive post mortem examination and histopathological investigation were done in 25 sows submitted to GD Animal Health between August 2012 and October 2013. The sows were euthanized because of impaired health or died around farrowing. Liver tissue was tested for trace elements (As, Cd, Cr, Co, Fe, Cu, Pb, Mo. Ni, V, Zn) and triacylglycerol (triglycerids). Histological liver preparations were coloured with Sudan fat staining. Additional information from sows and farms was collected using a questionnaire.

Results
The levels of As, Co, Pb, Cd, V were almost always below the detection limit. Detected trace elements were Cr (0.3 mg/kg), Fe (1,366 mg/kg), Cu (133 mg/kg), Mo (4.3 mg/kg) and Zn (214 mg/kg). The average concentration of triglycerids was 21.4 g/kg. In one sow the concentration was 60 g/kg which was marked as fatty liver. The Sudan score for this sow was also elevated. Fat concentrations in the liver were slightly negatively correlated with number of live born piglets (P=0.2). Molybdene concentration was related to parity of the sows. Zinc levels were lower in fattier livers. Other correlations between investigated parameters were not established.

Discussion & Conclusion
In this study only one out of 25 examined sows showed signs of a fatty liver. It has to be considered that not all sows were submitted for PME because of typical clinical signs associated with fatty livers. Even so, from these results it can not be concluded that fatty livers in sows are of practical relevance in the Netherlands. So called 'yellow livers' as sometimes reported by practitioners or at slaughter are not necessarily related to fatty livers.
Trace element concentrations in sow livers were in the same range as found earlier in the biochemistry laboratory of GD Animal Health. Molybdene is regarded as a contamination that can accumulate during life time. The relation of Zinc with fatty livers is unclear.

P045

DEVELOPMENT OF THE FIRST REGIONAL CONTROL EXPERIENCE ON PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) IN SPAIN

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Introduction
Pig production in Spain comprises a high diversity of typologies of farms including from big pig-producing companies to small family-owned farms. Within this frame, cooperatives are a particular system of organization representing small-to-medium farms deeply rooted in the territory and highly bound to the population of the places where they are located. One of the challenges for the future of cooperatives is PRRS and the costs associated to the disease. Current trends for controlling PRRS aim to regional programs that are starting to develop in Spain. A first step in control programs is to have a precise picture of the situation in the region. With the aim to prepare the basis for future regional control plans of PRRS, the Catalan Federation of Agricultural Cooperatives (FCAC), started a project directed to have the situation picture making special emphasis on biosecurity and categorization of farms based on PRRS status financed partially by the Spanish Ministry of Agriculture (MAGRAMA). This communication presents the preliminary results of the categorization and biosecurity characteristics of the examined farms.

Material and Methods
The study focused on farms belonging to cooperatives of Catalonia and Aragon (Spain). Of the 12 pig-producing cooperatives in those regions, 6 were willing to participate. Seventy-six pig farms belonging to 6 cooperatives were included based on a voluntary enrolment. A 139-question survey, including questions related to the apparent health status and internal and external biosecurity measures was filled for each farm. Also, 60 farms were selected for a categorization scheme based on the determination of the PRRS-status of sows and piglets. Briefly, serum samples from 20 sows and 10 9-week-old piglets were analyzed by ELISA and up to 20 weak-born piglets in farrowing units were analyzed by RT-PCR.

Results
Thirty-five percent of the farms reported a PRRS outbreak in the last 12-month period. Of the known biosecurity risk factors associated with PRRS, 65% of the farms did not analyze replacement gilts before entering them to the stock, 65% did not have a policy for eliminating little viable suckling piglets and 21% use semen from positive or unknown sources. Up to now, 18/29 categorized farms had evidences of PRRS virus circulation in newborns or at weaning.

Conclusion
Preliminary results suggest that there is a considerable way for improvement in biosecurity and monitoring. The steps taken in the project are seen as very positive by the farms and are already contributing to improve health conditions of the farms. Also, the methodology used may serve to the immediate development of regional control plans.

P046

"PROFESSIONAL PIG PRACTICE" – AN INNOVATIVE APPROACH OF PRACTISING CLINICAL SKILLS

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Introduction
Herd visits and animal contact are essential in the training of veterinary competences during education. However, veterinary students have little possibility to reach a proper level of confidentiality in their skills/abilities, as they have no "training-facilities" and many students have only visited pig herds very few times. One possible solution is to provide a safe, virtual environment (game-based) where students can practice interdisciplinary clinical skills in an easy-accessible, interactive setting. A playable demo using Classical Swine Fewer in a pig herd as example has been produced.
To tailor the game concept to the veterinary learning environment and in the effort to assure compliance with both learning objectives and the actual learning processes of the veterinary students, the project contains a developmental aspect (iterative user centred game development) and an exploration of the different (academic and profession) learning contexts.
Materials/Methods
In the different learning contexts of a veterinary course in Herd Health Management (Pig module), ethnographic studies have been conducted, using multiple data collection methods: participant observation, spontaneous dialogues, videos and interviews. All course related activities in the different learning spaces (commercial pig herds, auditoriums, post-mortem examinations, independent group work) have been followed in five consecutive courses. In a recent course, the playable demo was introduced and groups of students followed in workshops/game-sessions (observation and video).
Results
Initial studies have provided important knowledge on students´ competences and confidence as well as which elements of the learning module (game content and game features) to incorporate in the final game. In total 75.4% (135/179) answered the questionnaire, hereof 17 % never visited a pig herd, 45 % visited a herd 1-3 times and 28 % of students have had more than 5 visits.
Students value the use of videos and engage readily in improving the game module. The presentation will include close-up analysis providing a better understanding of clinical veterinary courses, the use of commercial pig herds for teaching, game based learning in veterinary courses related to pig diseases/herd health management and the veterinary students of today.

P047

DIAGNOSIS AND TREATMENT OF DIFFERENT DISORDERS IN THE ABDOMEN OF SLAUGHTERPIGS IN DENMARK.

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Diagnosis and treatment of different disorders in the abdomen of slaughterpigs in Denmark.
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Introduction: As a cause of death in slaughterpigs in the abdomen you find about 13 different kind of diagnosis with more or less blood.
Material and Methods: It is of outmost importance that the veterinarian has developed a quick, safe and easy technique to do the autopsy. Further it is essential that the whole pig is examined, so that it is cut from the anterior sternum to the pubis.
After cutting the sternum on both sides and lifted it, then cut down the sides along the rib curvature and take then a pause and look carefull before you go on. Make an overwiev and check the positions of the organs. Then with your right hand push the intestines to the right to make sure that it is NOT a torsion ! Wrong diagnosis due to a bad or improper technique are endless.
The 13 diagnosis:
1: Acute stomach ulcer is easy seen. Correct feed .
2: More chronic stomach ulcer with blood in the intestines is more difficult and you have to look careful.
3: Chronic stomach ulcer. Open the stomach and look for the typical lesions in the white part.
4: Lawsonia intracellularis infections:
- PHE. Can be very difficult as a differential to HBS. See photos! Treat with Tylosine. The KLM-rule.
- Hosepipe syndrome. Treat with an effective antibiotic. Vaccination ?
5: Hemorrhagic bowel syndrome (HBS) has a lot of courses. Find the course and correct it.
- Saltpoison from whey or seawater ! The term "Whey bloat" is wrong !
- Too much crude protein in the ration. Connected with diaree in the pens.
- Cold liquid feed, lower than 12 degrees of centigrade.
- Mycotoxins.
- Corrosion caused between metals and minerals in the vitamin/mineralmixture.
- Fine grinded feed.
- etc. etc
6: Clostridial infections. Drumskin pigs. You need small gasbladders on the liver surface.
Correct feed and feeding hours and add acid to the diet.
7: Glasser with elongated bloodclots in the abdomen. Treat with an effective antibiotic.
8: Vit K-block or - defiency. Serohaemorrhagic fluid in the abdomen. Add vitamin K.
9: All kind of torsions, invaginations in the intestines. Find the "torsion-screw" .
10: Torsion of the stomach or liver or spleen.
11: The abdominal catastrophe.
12: All kind of ruptures from external forces .
13: No diagnosis !
Results and conclusion: With this technique and these diagnosis in mind, the veterinarian can obtain good and consistent diagnostic work in this field.

P048

APPLICATION OF IMAGING TECHNIQUES FOR VISUALIZATION OF MAMMARY GLAND IN LACTAGING AND PREGNANT SOW

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Introduction. The increased litter size and the fact that the most significant limiting factor in piglets' growth is the milk yield of sows provoked functional anatomy study of mammary glands. Based on many advantages of computer tomography (CT) and magnetic resonance (MR) imaging techniques, we conducted a pilot study with a lactating and a pregnant sow with a special regard to gross volume and blood supply of mammary glands.

Materials and methods. The imaging was performed under general anaesthesia. The scans covered the region of the mammary glands.
CT imaging was performed on a lactating sow by a dual-source CT scanner (Siemens Definition Flash; Siemens AG, Germany). The sow was positioned head-first and supine. After the native examination, the sow received i.v. iodinated contrast material (Iomeron 350, Bracco Imaging S.p.A.). Bolus triggered acquisition timing was used to initiate the CTA (CT Angiography). Data were acquired in a cranio-caudal direction using a dual-energy body angiographic protocol.
MR imaging scan was conducted on a pregnant sow on the 45th day of pregnancy by a 1.5T MR system (MAGNETOM Avanto; Siemens AG, Germany). The sow was positioned head-first and prone. After the sow received i.v. gadobutrol contrast agent (Gadovist ®, Bayer Healthcare) the images were performed using a dynamic T1 weighted sequence in the coronal plane.
The volumetric evaluation was performed on the native CT scans. Both CTA and MRA (MR Angiography) data were reconstructed as 3D maximum intensity projections for evaluation.

Results and discussion. Both CT and MR imaging are suitable non-invasive, in vivo method to study the parenchymal volume and structure as well as geometry and network of blood vessels of the mammary gland. The diameter of sow's body is a limiting factor of feasibility of imaging. Because of the radiation load, the CT imaging is contraindicated on pregnant sows. Three-dimensional images can be generated to get more functional anatomy information.
If this abstract will be accepted for oral presentation 3D CT and MR video imaging will be presented.

P049

ACUTE PHASE RESPONSE IN PIGS AFTER INGUINAL HERNIA AND CRYPTORCHISM SURGERY

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Inguinal hernia and cryptorchism together occur in about 1% of all male piglets in Norway. Cryptorchid testicles and hernias are traditionally removed surgically. By many veterinarians this method is still in use, despite today's access to medical alternatives. Trauma to body tissue triggers physiological responses that aim to protect the organism against potential damage. This includes changes in the composition of serum proteins and immune cells in blood. A clinical intervention study was performed to map these changes after cryptorchism and inguinal hernia surgery. The study involved a population of 32 pigs. Veterinary students at the Faculty of Veterinary Medicine and Biosciences, NMBU, performed surgery on the pigs in the period of January-April 2014. Blood samples were collected from each pig on the day before surgery and on the first, third, sixth and fourteenth day post-surgery. The initial samples were used as individual controls. Changes in the blood parameters were calculated based on these control samples. Results from one point in time to another were compared by the means of a two-sided paired t-test.
The study showed an average increase of 47.41 mg/L in C - reactive protein (CRP) from the basal levels from the day before surgery until the day after surgery ($p<<0.001$). From the day before surgery until the day after surgery there was no significant change in total protein, α-globulin, β-globulin, γ-globulin or albumin. On day three after surgery, α-($p<0.001$), β- ($p<0.0001$) and γ-globulin ($p<0.05$) were significantly higher than before surgery. Albumin was significantly decreased on day three ($p=0.0002$) and six ($p<<0.001$) after surgery. On day fourteen after surgery, total protein ($p<0.01$), α-($p<0.01$), β-($p<0.001$) and γ-globulin ($p<<0,0001$) were still significantly elevated from the levels pre surgery.
This study showed that cryptorchism and inguinal hernia surgery lead to an acute phase reaction in the pigs. α-, β- and γ-globulins are groups of proteins that will show an increased occurrence post trauma. α- and β-globulins include acute phase proteins, immediately produced in the liver as a response to cytokine release from peripheral immunologic cells i.e. after trauma. CRP is one of the most important acute phase protein in pigs. γ-globulins are mostly made up of the antibodies produced by lymphocytes after reacting to "foreign" antigens. This process is relatively slow, compared to the acute phase proteins, and the increase of γ-globulin concentration will hence occur later in the inflammation process.

P050

WHITE BLOOD CELLS IN PIGS AFTER INGUINAL HERNIA AND CRYPTORCHISM SURGERY

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Inguinal hernia and cryptorchism together occur in about 1% of all male piglets in Norway. Cryptorchid testicles and hernias are traditionally removed surgically. By many veterinarians this method is still in use, despite today's access to medical alternatives.
Trauma to body tissue triggers physiological responses that aim to protect the organism against potentially threatening agents. The body increases the excretion of a number of immunologic cell to the bloodstream. The bone marrow then increases its production of these cells, and they will occur in a higher number. A clinical intervention study was performed to analyse the changes in haematologic parameters after trauma. The study involved 32 pigs during and after surgery of cryptorchism and inguinal hernia. Veterinary students at the Faculty of Veterinary medicine and Biosciences, NMBU, performed the operations in the period January-April 2014.
The pigs underwent clinical examination and blood collection the day before surgery. On the first, third, sixth and fourteenth day post-surgery, blood was collected again, and the surgical wounds were evaluated. The initial blood samples were used as individual controls. The average changes in white blood cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils were calculated based on these controls. Results from one point of time to another were compared by means of a two-sided paired t-test.
The study showed a significant increase in the numbers of white blood cells ($p<0.001$) from the day before surgery until the day after surgery, and it stayed significantly elevated until the sixth day post-surgery (day three ($p<0.01$), day six ($p<0.001$), day fourteen ($p>0.05$)). There was also a significant increase in neutrophil granulocytes ($p<<0.001$) from the day before surgery until the day after surgery. This increase could also be seen in the percentage amount of neutrophils of the total white blood cell count. The average percentage significantly increased from 39.64 to 44.82 from the day before surgery until the day after surgery ($p<0.01$). The neutrophil number stayed significantly increased on the third ($p<0.001$), sixth ($p<<0.0001$) and fourteenth ($p<0.05$) day after surgery. There was no significant changes in the average number of lymphocytes throughout the whole test period. However, the percentage amount of lymphocytes showed a significant decrease from 51.44 to 46.68 ($p<0.01$) from the day before surgery until the day after surgery due to the elevated number of neutrophils.
This study showed that cryptorchism and inguinal hernia surgery lead to a response in the white blood cells, and a shift in the cellular picture in the blood of pigs. Before surgery, the white blood cell composition was dominated by lymphocytes. By day three after surgery, a neutrophilic shift had occurred, and by day fourteen after surgery the cell composition was again dominated by lymphocytes.

P050a

FIELD EVALUATION OF THE EFFICACY AND SAFETY OF FORCYL® SWINE IN THE TREATMENT OF SRD IN NATURALLY INFECTED PIGS

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Introduction
Actinobacillus pleuropneumoniae (APP) is a causative agent of porcine pleuropneumonia, causing significant economic losses worldwide. Antibiotherapy is the best approach with respect to animal welfare and consumer protection, especially when the treatment is efficacious and at the same time does not support the development of antimicrobial resistance in the targeted bacteria and commensal flora. This study evaluated the efficacy and safety of Forcyl® Swine, a single-injection short acting marbofloxacin, for the treatment of swine respiratory disease (SRD) associated with APP.
Materials and Methods
A total of 242 fattening pigs presenting with clinical signs of SRD was enrolled in a controlled, block-randomized blinded multicentre clinical field study. Pigs were enrolled during SRD outbreaks in 4 German and Hungarian farms after an APP infection was diagnosed based on gross pathology in sentinels. Pasteurella multocida and Haemophilus parasuis strains were also isolated in enrolled animals.
According to the randomization, the animals received either Forcyl® Swine (8 mg of marbofloxacin /kg bodyweight, 1 ml/20 kg IM, once on D0, n=122) or Baytril® 1nject (7.5 mg of enrofloxacin /kg bodyweight, 0.75 ml/kg IM on D0 and on D2 if needed, n=120). In vitro susceptibility of isolated pathogens towards both antibiotics was high. A 3-week follow up of included animals was performed with a record of clinical signs (respiratory score, depression score, rectal temperature) on D0, D1, D2, D3, D7 and D21, and in case of premature follow up until stop.
Results
Cure rates were 81.8% in Forcyl® treated animals versus 81.4% in Baytril® 1nject group on D7 and 84.2% versus 82.2% on D21, respectively. 17.8% of Baytril® 1nject treated animals underwent a second injection. One treated animal (Baytril® 1nject group) relapsed between D8 and D21. Development of clinical parameters did not differ significantly between groups. Statistical analysis allowed the conclusion that Forcyl® Swine was at least as efficient as the control product for the treatment of SRD (rejection of the hypothesis of inferiority, $p<0.05$).
No suspected adverse drug reactions were observed. General and local safety were good and were not different between groups ($p>0.21$).
Conclusion
In this field study, Forcyl® Swine was highly efficient and safe for the treatment of naturally-occurring SRD associated with APP and complicated by Pasteurella multocida and/or Haemophilus parasuis. A single high dose short-acting marbofloxacin treatment is therefore a treatment of choice for animals with acute clinical signs of SRD.

P050b

COMPARISON OF TWO PCV VACCINES IN A CONTROLLED FIELD TRIAL: EVALUATION OF PIG PRODUCTION PARAMETERS

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Comparison of two PCV vaccines in a controlled field trial: Evaluation of pig production parameters
Introduction
A comparative, controlled and contemporary trial was conducted in a 180-sow farm in Britany (France) in order to compare the growth performances obtained using two porcine circovirus vaccines: Suvaxyn PCV and Ingelvac Circoflex. The piglets were vaccinated against M. hyopneumoniae at 4 weeks of age and against Circovirus at 6 weeks of age.
Material and method
Prior to the trial, Ingelvac Circoflex was the usual vaccine applied on the farm. The trial was performed on 4 consecutive batches of 248 animals, each of them divided in two equal groups, either vaccinated with Suvaxyn® PCV (SuvPCV) or Ingelvac® Circoflex (IngCir). Each vaccine group was weighted the day of vaccination and properly identified. Mortality and weight at slaughterhouse were recorded, each piglet being considered as the statistical unit. Average Daily Weight Gain (ADWG) from vaccination to slaughter was analysed by ANOVA and age at slaughter was analysed by a non parametric test of Mann-Withney.
Results
The two vaccine groups were comparable with regard to the sex ratio. The mean weight at inclusion was higher (+ 1.6 Kg) for the IngCir group. The ADWG was significantly higher in the SuvPCV group with a difference of + 25 g/d ($p<0.001$). A significant difference was found in each batch in favor of SuvPCV that showed a quantitative interaction ($p=0.003$). The comparison of the variances also showed an homogeneity of ADWG significantly better for the SuvPCV group ($p<0.001$).The time to slaughter was significantly decreased (- 3d) in favor of SuvPCV group ($p<0.001$). Mortality rates were not seen to be significantly different between the 2 groups (around 4.5%).

Group	Number	Weight at vaccination (Kg)		ADWG (g/d)		Age at slaughter (d)	% mortality
SuvaxynPCV	474	14.1	867a	158a	4.24		
Ingelvac Circoflex	473	15.7	842b	161b	4.64		

Different letters (a and b) indicate statistically significant differences ($P < 0.001$) between groups.

Conclusion
Since there was no evidence of PCV clinical disease over the course of the study, the significantly higher ADWG and its extent observed in the Suvaxyn PCV group tends to demonstrate that Suvaxyn PCV allowed a better control of the sub-clinical consequences of PCV2. This finding is also supported by a better homogeneity of ADWG and age to slaughter with Suvaxyn PCV.

P051

PCV2 VIRAEMIA IN BATCHES OF PCV2-VACCINATED AND NON-VACCINATED WEANER PIGS

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Introduction
Previous studies suggest that PCV2 vaccination can reduce the risk of PCV2 infection in pigs produced under all-in all-out (AIAO) conditions and that entire batches of pigs could be free from PCV2 infection. However, the documentation on this is sparse. Studies also suggest that, within a production system, both PCV2-infected and non-infected batches can be produced. The objective of this study was to investigate whether PCV2 vaccination of pigs combined with AIAO management by room in the weaner unit could prevent PCV2 viraemia at the end of the weaner period. An additional objective was to explore the variation of PCV2 viraemia among batches of weaner pigs.

Materials and Methods
Three herds (A, B and C) were included in the study. Each herd consisted of a sow unit with 1300-2100 sows and a weaner unit managed AIAO by room. In total, 37 batches of weaner pigs were included in the study (herd A: 16 batches, B: 15, C: 6). Pigs in herd A were not vaccinated against PCV2, while pigs from herds B and C were vaccinated before weaning (herd C) or at weaning (herd B). At the end of the weaner period (11-12 weeks of age), 20-40 pigs per batch were bled. All of the serum samples were pooled, with each pool consisting of ten samples. Thus, two-four pools per batch were available for analysis. Serum was analysed for PCV2 by a real-time qPCR. A batch was defined as PCV2-positive if at least one of the pooled samples was positive by PCR (≥3 log10 copies/mL).

Results
In herd A (non-vaccinated pigs), eight out of 16 batches were PCV2-positive at the end of the weaner period. Over time, PCV2-positive and -negative batches occurred in a non-systematic way. In herds B and C (PCV2-vaccinated pigs), a total of ten out of 21 batches were PCV2-positive. In herd B, all PCV2-positive batches except for one occurred during the latter half of the study period. In herd C, only the first batch of six was PCV2-positive.

Conclusion
The results show that PCV2 vaccination before or at weaning combined with AIAO management by room in the weaner unit does not necessarily prevent viraemia at the end of the weaner period. However, this does not exclude that vaccination could have had a beneficial effect on the viral load and on the productivity of the pigs. The positive effect of vaccination on productivity is well established. Within each of the three herds, both PCV2-positive and -negative batches were found. This variation implies that it will often be necessary to include a significant number of batches when performing diagnostics to establish the PCV2 infection patterns in a herd.

P052

COMPARATIVE EFFICACY OF TWO PCV2 VACCINES IN 4-WEEK-OLD PIGLETS UNDER SPANISH FIELD CONDITIONS

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Introduction
Several commercial PCV2 vaccines are available for piglet in Europe. The objective of the present study was to compare the efficacy of two PCV2 vaccines: CIRCOVAC® (Merial, France) and a subunit vaccine.
Material and methods
The trial was conducted in a 900-sow farrow-to-finish farm, positive to PRRSv, M. hyo and SIV. A total of 600 piglets were weaned at 4 weeks of age and randomly allocated to vaccination with 0.5 ml of CIRCOVAC (Group N, n=291) or with 2 ml of a PCV2 subunit vaccine (Group R, n=309). Pigs from the two vaccine groups were commingled both in the post-weaning and in the fattening units. They were individually weighed at weaning, placement on the fattening unit and immediately before the first delivery of pigs to the slaughterhouse. A subsample of 15 animals per vaccination group was identified and placed in pairs of both vaccination groups in 15 different pens distributed throughout the post-weaning and fattening facilities and blood sampled at 4, 8, 11, 15, 19 and 23 weeks of age. Sera were tested for PCV2 antibodies using two commercial ELISA kits (IgG/IgM blocking ELISA and indirect ELISA, INGENASA). Data was analysed through a 2-way ANOVA model for group and sex factors or using Wilcoxon test within each gender stratum if not applicable.
Results
A total of 25 piglets (4.2%) died from several causes (wasting, diarrhea, meningitis, etc.) during the post-weaning period; 20 additional pigs (3.5%) were found dead during the fattening period. Mortality rate was 7.5% from weaning to slaughter with no statistical difference between the vaccine groups. Causes of death were not recorded in this trial. No statistical difference was observed in live weight between groups at weaning, thus validating the randomization process. The pigs administered CIRCOVAC had higher average live weights at the end of the post-weaning period (Group N: 20.53kg vs Group R: 19.73kg, p=0.03), attributable to better growth in the nursery (Group N: +264g/day vs Group R: +252g/day, p=0.04). There was also a numerical improvement during finishing: Group N: +58.95kg vs Group R: +58.33kg). At slaughter, the pigs in group N were 1.52 kg found heavier on average : Group N: 79.58kg vs Group R: 78.06kg, p=0.17. The serological results confirmed both vaccine take and a limitation of the circulation of the field virus in each group.
Conclusion
In conclusion: in the conditions of this trial, CIRCOVAC 0.5 ml IM in piglets at 4 week of age was shown to be at least as efficient as the other PCV2 vaccine in comparison with a slightly faster growth in the post weaning.

P053

INCLUSION BODY RHINITIS DISEASE IN PIGS

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Introduction
Persistent sneezing, nasal discharge, abdominal panting and loss of body condition were seen in 3 to 5 week old piglets. These clinical signs were observed during the last three years. Morbidity was 20 – 30%. Mortality was 95%.
Affected piglets were from both gilts and sows.
The farm is a closed herd with no live pig introductions. It breeds its own replacement gilts.

Materials and Methods
Clinical investigations consisted of several farm visits over the last 3 years. Nasal swabs, blood samples and euthanised pigs were submitted in 2011 to AFBI (Northern Ireland) for bacteriology and histology. Serum was analysed for Porcine Reproductive and Respiratory Syndrome virus (PRRSv) (ELISAS, IDEXX) and Swine Influenza Virus (SIV) (ELISA IDvet).
A typically affected 5 week old weaner was euthanised in 2014. Lymph nodes (mesenteric, inguinal, bronchomediastinic), tonsil, spleen, liver, different sections of the snout, and lung tissues (apical, cardiac and diaphragmatic lobes from the right and left lung) were collected and fixed in 10% formaldehyde. These samples were submitted to the Diagnostic Service of Veterinary Pathology in the UAB (Barcelona). Histology was performed in the tissue samples submitted. PRRSv and Porcine Circovirus type 2 (PCV-2) immunoperoxidase tests were carried out on these samples.

Results
Results came back negative to significant bacteria, PRRSv, SIV and PCV-2.
Histology from the nasal sections showed extensive erosion of the mucosa with neutrophilic exocytosis and micropustules. Thickening of the lamina propria with lymphoplasmatic inflammatory infiltration was identified. Kariomegalia of the epithelial cells of the mucosal glands caused by intranuclear inclusion bodies was detected. Finally, subacute interstitial pneumonia was detected in the lung.

Conclusion
The clinical signs and the lesions identified in the nasal mucosa indicated infection by Porcine Cytomegalovirus (PCMV). This herpesvirus is responsible for Inclusion Body Rhinitis (IBR) disease in pigs.
This virus is ubiquitous in the pig population. Clinical disease is rare. Why this unit developed clinical signs remains unknown. However, PCMV is usually underestimated and/or misdiagnosed. This was part of the reason it took longer than expected to make a final diagnosis. In addition to this, not all laboratories can identify this virus.
The inclusion of 200ppm of tilmicosin in the sow's diet 5 days before and 3 days after farrowing reduced the prevalence of the disease in this unit. Tilmicosin has been used with some success for the treatment of PRRSv & PCV-2 infections, and has also been in this case.

P054

PRRS PREVALENCE IN EUROPE: PERCEPTION OF THE PIG VETERINARY PRACTITIONERS

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Introduction
Since its appearance in the late 1980's and its identification in 1991, the majority of the pig producing countries have reported porcine reproductive and respiratory syndrome virus (PRRSV) infections and nowadays it is considered endemic in most of them. The disease in sows and gilts causes mild to severe reproductive problems, while in nursery and fattening pigs the typical signs of the disease are respiratory problems and increased mortality, resulting in reduction of average daily weight gain (ADG) and feed efficiency. However, in some cases a lack of clinical signs and/or poor recognition of them can cause that PRRSV infections remain undetected on positive herds.
The goal of this study was to to obtain the perception of PRRSv prevalence by swine practitioners in different European countries

Material and Methods
A Europe-wide survey was undertaken to assess PRRS prevalence in European pig production. 515 swine veterinary practitioners were interviewed in 11 countries across Europe between January and March of 2014 in relation to the presence of PRRS in the farms under their care. The distribution of pig producers interviewed was as followed: Germany: 50; France: 50; Netherlands: 48; Denmark: 20; Austria: 36; UK: 26; Italy: 44; Belgium: 50; Poland 51; Russia 50
Each practitioner was asked to estimate the percentage of the animals under his or her supervision that were PRRS-positive. In fact, the veterinarians could respond by saying if pigs were infected and showed clinical signs, or were infected without clinical signs, or were not infected with the virus.

Results/Conclusion
According to the interviewed practitioners, on average PRRS is present in 71% of sows and in 68% of weaned or growing pigs. While on average, clinical cases of the disease were estimated to occur in 17% of sows and in 23% of weaned or growing pigs. However, the incidence in sows in particular varied widely by country, from a high of 47% reported from Italy to just 4% for Russia.

Conclusion
The survey demonstrates a wide variation between countries, but also a clear general indication that most pigs in Europe are PRRS-positive: the virus remains highly prevalent in sows as well as in pigs from the nursery to the finishing pen.
What is more, the replies of the veterinarians suggest that a considerable proportion of pigs in Europe are not only infected, but also clinically affected by PRRS.
This information underlines the need for European pig herds to stay vigilant against PRRS virus.

P055

SWINE INFLUENZA VIRUS SURVEILLANCE IN NORTHERN IRELAND

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Introduction
Routine surveillance is an essential component in protecting animal health, production efficiency and public health. The aim of this study was to find the changes in prevalence of Swine Influenza Virus (SIV) in Northern Ireland (NI) from 2011 to 2013. SIV causes respiratory and reproductive failure in pigs. Furthermore, it is significantly cited as one of the contributing agents of pleurisy in the lungs of pigs in NI.

Materials and Methods
Eight blood samples per batch were collected at the bleeding area of the slaughterhouse in May/June 2011 and from October to December 2013. A total of 141 batches of pigs which represented 130 producers were checked in 2011. In 2013, 174 batches of pigs which represented 141 producers were checked. This sampling represented 95% of the pig producers in NI in 2011 and 2013, respectively. The blood samples were analysed for SIV (ELISA, IDvet). Any SIV positive sample in 2013 was differentiated for H1N1 Eng92 Avian Like, H1N1 Belg79 Classical, H3N2 NI Isolate & H1N1 Pan Flu by Haemagglutination Inhibition. All laboratory tests were performed in AFBI, Stormont (Belfast).
Positive and negative herds were geographically mapped.

Results
Batches of pigs infected with SIV increased by 6.3% (41.13% vs 47.43%) from 2011 to 2013. This represented a 6.86% increase of producers (46.09% vs 39.23%).
H1N1 Pan Flu was the main subtype detected in 2013 (86.15% producers; 40.23% batches), followed by H1N1 Eng92 Avian Like (32.3% producers; 40.23% batches), H1N1 Belg79 Classical (15.38% producers; 6.32% batches) and H3N2 NI Isolate (6.15% producers; 2.29% batches).
Some producers had more than one subtype in the herd. The combination H1N1 Eng92 Avian Like + H1N1 Pan Flu being the most frequent (13.92% producers; 6.89% batches) followed by H1N1 Eng92 Avian Like + H1N1 Belg79 Classical + H1N1 Pan Flu (13.84% producers; 6.32% batches), H3N2 NI Isolate + H1N1 Pan Flu (6.15% producers; 2.29% batches) and H1N1 Belg79 Classical + H1N1 Pan Flu (1.53% producers; 0.57% batches).

Conclusion
There was an increase of producers and batches affected with SIV. A number of factors could have contributed to this spread such as purchase of infected livestock; poor biosecurity measures in place; and, infected neighbours.
When infected farms are geographically mapped, the majority of infections occur in pig dense areas.
H1N1 Pan Flu is the most commonly isolated subtype. In 2009, NI was the first European country where this subtype was recorded after its initial appearance in Mexico. This subtype is always involved in other SIV subtype combinations in NI.
H1N1 Pan Flu should be included in modern SIV vaccines in order to control infection in pigs.

P056

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS SURVEILLANCE IN NORTHERN IRELAND

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Introduction. Routine surveillance is an essential component in protecting animal health, production efficiency and public health. The aim of this study was to find the changes in prevalence of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) in Northern Ireland (NI) from 2011 to 2013. PRRSv causes respiratory and reproductive failure in pigs. Furthermore, it is significantly cited as one of the contributing agents of pleurisy in the lungs of pigs in NI.

Materials and Methods. Eight blood samples per batch were collected at the bleeding area of the slaughterhouse in May/June 2011 and from October to December 2013. A total of 141 batches of pigs which represented 130 producers were checked in 2011. In 2013, 174 batches of pigs which represented 141 producers were checked. This sampling represented 95% of the pig producers in NI in 2011 and 2013, respectively. The blood samples were analysed for PRRSv (ELISA, IDEXX) in AFBI, Stormont (Belfast). Positive and negative herds were geographically mapped.

Results. Batches of pigs infected with PRRSv increased by 7.12% (36.88% vs 44%) from 2011 to 2013. This translated to an 8.59% increase of producers (35.38% vs 43.49%). When infected farms are geographically mapped, the majority of infections occur in pig dense areas.

Conclusion. There was an increase of producers and batches affected with PRRSv between 2011 and 2013. A number of factors could have contributed to this spread such as purchase of infected livestock; poor biosecurity measures in place; and, infected neighbours. A major contributing factor for PRRSv spread in NI was the purchase of infected semen from a major boar stud supplier in April 2013. Better biosecurity controls are required to be implemented in stud farms in order to avoid the spread of PRRSv to naïve herds. PRRSv is enlisted as a Notifiable Disease in NI.

P057

INACTIVATION OF PRRSV BY A NOVEL METHOD OF ON BOARD HEATING OF TRUCKS IS WITHIN RANGE FOR THE DAILY ROUTINE IN PIG TRANSPORT

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Introduction. Trucks for transportation of pigs are an important risk factor in the spread of PRRSv. Research in the USA has led to the conclusion that heating and drying of transport trucks (by using the Thermo Assisted Drying and Decontamination or TADD system) will block the spread of PRRSv effectively. Experiments in the USA included the use of detergents and disinfectants and a time period of 2 hours. However, under Dutch and European circumstances questions arising from the daily practice are different from the situation in the US: trucks are used on more than one swine farm each day. As a consequence, the time available for cleaning and disinfecting trucks between different transports is limited to 30 minutes, and proper use of detergents and disinfectants is often not possible.Therefore, a research project funded by the Dutch Product Board for Meat and Livestock was set up to investigate which combination of time, temperature and ventilation will lead to inactivation of PRRSv, on a truck that was not cleaned and disinfected, but only rinsed with water.

Materials and Methods. The used truck during the experiments was equipped with an on board heater, which is intended to heat the air in the truck while traveling between farms. Experiments included the application of vaccine virus in a dose of 5x 10^5 TCID50 in a full size truck (4 sites on the bottom floor, duplicate testing). Experiments included a time period of 30 and 60 minutes and an air temperature of 70 oC. Inactivation of the virus was tested by using virus infectivity assay on MA 104 cell cultures. Air temperature and relative humidity in the truck were recorded, the drying of wet floor parts was checked visually. Samples were taken by using swabs on the sites where the virus was applied.

Results and Conclusions. An average air temperature of 74 oC on lower floor level appeared to be feasible, and resulted in inactivation of PRRSv on nearly all parts of the floor, in a time period of 60 minutes. Air temperatures on floor level varied from 71 to 78 oC in the front of the truck, and from 49 to 70 oC in the back of the truck. Relative humidity in the air on floor level was 4% (3-5%) in the front of the truck, and 13% (8-18%) in the back. At the end of the time period, the floor was dry, except in the back. Inactivation of PRRSv was only achieved on dry floor parts. Dutch truck constructors are currently adjusting the air circulation in order to improve the heating and drying process in the back of the truck. Further testing is needed to confirm the inactivation of PRRSv in a desired time period of 30 minutes.

P058

USING REALTIME PCR TO QUANTIFY ULTRAVIOLET INDUCED RNA DAMAGE OF PRRS VIRUS

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Introduction. Porcine respiratory and reproductive syndrome virus (PRRSV) as RNA virus causes devastating swine diseases with massive economic losses to the swine industry worldwide. In order to prevent economic losses from PRRS, many swine producers use UV light as a sterilizer for the workers, equipment, surface of farm units etc. However, it is very little known about its actual degree of UV-induced RNA damage and effectiveness. The aim of the experiment was to measure levels of UV-induced RNA damage by utilizing the property that damaged RNA of PRRSV can inhibit PCR.

Materials and Methods. PRRS virus strain, ATCC VR2332, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV lamp (Enputech Co., Ltd., Korea) with wavelength output at 254nm, 150uW•sec/Cm2, for 0.5, 1, 2, 4, 8, 16, and 32 min, respectively. RT-PCR amplified a 100bp (1154-1253) region (Table. 1) and the amplicons were diluted 10-5-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. Statistical evaluation was performed by Excel (Microsoft, USA), Using regression normalization (a linear trendline correction).

Table 1. Oligonucleotide primer

Primer	Primer Seq.	Product Size
PRRSV-f	5'ACGGACCTATCGTCGTACAG3'	100bp
PRRSV-r	5'AGGAGGTCCTCAAACCCAGA3'	

Results. The inactivation ratio of PRRSV was relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control sample showed the linear correlation (R2=0.999). The inactivation of PRRSV by UV light was dose dependent (Table 1). Table 2 showed inactivation ratio of PRRSV using a linear trendline correction.

Table 1. The result of Ct value and inactivation ratio by exposure time using ReTi-PCR

Exposure Time (min)	Ct value	Inactivation ratio (%)
0.5	23.92	61.68
1	25,5	86.9
2	26.54	93.53
4	28.14	97.82
8	29.31	99.01
16	29.86	99.32
32	31.43	99.77

Table 2. Calculated inactivation ratio of PRRSV

Virus	1D	2D	3D
PRRSV	10.72	72	638.2

*D=1log10, unit is mW/cm2 (Erwin et al., 2004)

Conclusions. In Table 2&3, PRRSV was sensitive to UV light irradiation. The UV light is very effective and environment friendly for disinfection in pig farm units. It is thought that these results can be a useful data for sterilizing of PRRSV.

P059

APPLICATION OF REALTIME PCR TO ASSESSMENT OF UV C EFFECT ON PED VIRUS

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Introduction. Recently, porcine epidemic diarrhea virus (PEDV) was detected in the United States of America. PEDV have continued to cause ongoing disease challenges for pork producers. Economic losses from the disease are very serious. In order to prevent economic losses from PED, many pork producers use UV light as a sterilizer. However, it is very little known about its actual degree of UV-induced RNA damage and effectiveness. The purpose of this experiment was to measure levels of UV-induced RNA damage by using the property that damaged RNA of PEDV can inhibit PCR.

Materials and Methods. The PED virus strain, P-5v, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by ultraviolet C lamp (Enputech Co., Ltd., Korea) with wavelength output at 254nm, 150μW•sec/cm2, for 0.5, 1, 2, 4, 8 and 16 min, respectively. RT-PCR amplified a 90bp of PEDV membrane protein (m) gene (337-426) region (Table. 1) and the amplicons were diluted 10-5-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. To evaluate effects of UV induced RNA damage, Regression normalization (a linear-trendline correction) was used.

Table 1. Oligonucleotide primer

Primer	Primer Seq.	Product Size
PEDV-f	5`AATCCTGAAACTGACGCGCT3`	90bp
PEDV-r	5`TAGCGTTACACCAGTTGGGTC3`	

Results. The inactivation ratio of PEDV was relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control sample showed the linear correlation (R2=0.9999)
The inactivation of PEDV by UVC light was increased with exposed time (Table. 2).

Table 2. The results of Ct value and inactivation ratio by exposed time using ReTi-PCR

Exposure Time (min)	Ct value	Inactivation ratio (%)
0.5	18.24	89.89
1	18.57	92.13
2	19.22	95.19
4	21.45	99.11
8	22.04	99.43
16	22.90	99.71

In the setup of the experiment, 1D, 2D, and 3D (D=1log10, unit is mW/cm2) inactivation were found 5.97, 46.33, and 359.47mJ/cm2, respectively. (Erwin et al., 2004)

Conclusions. The UVC light was highly effective to inactivation of PEDV. But substantial viral inactivation occurred after exposure of 4minutes. Increasing output of the UV lamp is an effective way to reduce the time of exposure. The data reported in this experiment suggest that PEDV is killed by UVC light when it has absorbed the required amount of radiant energy in the lethal range. It is thought that these results can be a useful data for sterilizing PEDV.

P060

CHOICE OF PCV2–VACCINE AFFFECTS THE MORTALITY IN WEANERS

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Introduction. Several vaccines against PCV2 are available and sometimes, huge price differences are found. For the piglet producer, the price is often the main driver, when he has to choose between products. This paper describes a case, where a piglet producer chose a PCV2 vaccine due to a lower price, but observed severe local reactions after weaning. A side-by-side study examined whether the side effects affected the productivity compared to piglets vaccinated with another PCV2 vaccine.
Material and methods. Case herd: 1350 sows with PCV2 vaccination of piglets at 3 weeks of age and weaning to an off-site unit 1 week later. All piglets are exported at 30 kg live weight.
Design: Side-by-side study with 2 vaccinated groups and allocation balanced according to piglet weight within litters. The groups were identified by ear tags. The study included all piglets in 3 consecutive week batches (1650 piglets), and the grouping was blinded for the people managing piglets after weaning.
Treatments:
Group 1: Vaccine 1, 2 ml i.m.
Group 2: Vaccine 2, 1 ml i.m.
Vaccines were applied according to manufacturer's instructions, including warming of vaccine 1 in hot water before injection.
Data collection: Mortality was recorded from weaning until sale. Piglets were weighed at vaccination, at weaning, 3 weeks after weaning and before sale of the 1st load from the current batch. Oral fluid samples were taken at each weighing in the weaning unit and examined for PCV2 by PCR.
Data treatment: Statistical comparison of weights was made with Students T-test, and comparison of mortality was done with Fishers Exact Test. An economical calculation was done using key figures from the Danish Pig Producers organization.
Results. Piglets vaccinated with vaccine 1 had a higher mortality than piglets vaccinated with vaccine 2 in all 3 batches. In total, the mortality was 1.5% higher when vaccine 1 was used (p=0.06). The mean weight of the piglets at weaning was 6.2 kg, and the mean weight before the first piglets were sold was 24.2 kg. The weight was equal in the 2 groups throughout the study. PCV2 virus was not found in any oral fluid samples. A comparison of the economic value of the 2 different vaccines showed, that keeping vaccine 1 would create a deficit of more than 4000 € in 6 months due to the higher piglet mortality.
Conclusion. This blinded side-by-side shows an increased mortality in weaners after vaccination with vaccine 1 compared to vaccine 2. The increased mortality will decrease the gross margin for the piglet producer, even if vaccine 1 is sold at a lower price. The losses for the piglet producer will increase with increasing piglet prices.

P061

IMPACT OF DIFFERENT PCV2 VACCINES ON NURSERY WEIGHT GAIN

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INTRODUCTION. The present study was set up because considered changing to an alternative PCV2 vaccine due to a lower price. A blinded side-by-side study was set up to compare the nursery productivity after vaccination with the 2 different vaccines.

MATERIALS AND METHODS. Case herd 650 sow herd with weekly batch farrowing. Piglets are vaccinated against PCV2 at 3 weeks of age (woa) and weaned to an off-site unit one week later and exported at 30 kg. Side-by-side study with 2 vaccinated groups. Piglets were allocated at vaccination, where litters were alternatingly allocated to treatment group 1 or 2. The study included all piglets of 7 batches (2165 piglet total), and the treatment groups were blinded for the people managing pigs after weaning.

Group 1: Original vaccine, 1 ml i.m. (Ingelvac CircoFLEX®, Boehringer Ingelheim Vetmedica)
Group 2: Alternative vaccine, 2 ml i.m. (Suvaxyn PCV, Scanvet Animal Health)

Piglets were weighed at weaning and before sale of the first load from each batch. Average daily gain (ADG) was calculated for individual pigs. Mortality was recorded from weaning until sale.

Statistical comparison of weights and weight gain was made with Students T-test, and comparison of mortality and underweight pigs was done with Fishers Exact Test, with p=0.05 as level of significance. An economical calculation was done using key figures from the Danish Pig Producers organization.

RESULTS. The mean weight at weaning was 7.4 kg in both groups. ADG in pigs vaccinated with the alternative vaccine was significantly lower (p=0.010). The difference in ADG was 15 g/day, meaning that when pigs vaccinated with the original vaccine reached a weight of 30 kg, pigs vaccinated with the alternative vaccine would weigh 792 g less. A comparison of the weight distribution in the 2 groups showed significantly (p=0.019) more underweight pigs in the group vaccinated with the alternative vaccine. The mortality in the nursery was 0.96% higher, when the alternative vaccine was used, however, this difference was not significant (p=0.232). The economic evaluation of the alternative vaccine showed that the producer would lose more money on the decreased ADG than could be saved on the lower vaccine price.

CONCLUSION. This side-by-side study shows that vaccination with the alternative vaccine significantly decreased weight gain in the nursery. Furthermore, a significantly higher number of underweight pigs and a numerically higher mortality was found among pigs vaccinated with the alternative vaccine. The decreased weight gain will decrease the gross margin, if a piglet producer chooses the alternative vaccine, and the economic losses will increase with increasing piglet prices.

P062

MONITORING OF PRRSV IN A COHERENT AREA IN SOUTHERN GERMANY

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Introduction
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is considered to be one of the major pathogens in pigs and causes significant economic impact on the swine industry worldwide. While globally a variety of voluntary regional monitoring and control programs for PRRSV already exist, the current efforts in Germany concentrate on the elimination of PRRSV from individual farms. Due to the risk of re-infection by surrounding PRRSV positive farms only a coordinated regional control approach can be regarded successful on a long-term basis.
Basic requirement for a regional monitoring program is an initial evaluation of the infection status of farms within the particular region.

Materials and Methods
The aim of the present study was to define the PRRSV status of pig farms in a coherent area in southern Germany. The status was investigated using a combination of direct and indirect virus detection methods. For the detection of PRRSV specific antibodies by ELISA, 10 blood samples from sows and fattening pigs were examined in sow farms and fattening farms, respectively.
Oral fluids were used for the detection of PRRSV by PCR. In farrow to feeder farms oral fluids were collected from pigs at the age of 8 to 10 weeks, while in fattening farms pigs at the age of 16 to 18 weeks were sampled. In addition to the investigation of the PRRSV status of farms, a risk analysis was performed to determine potential risk factors influencing PRRSV positivity.

Results
The study was conducted in an area of 269.57 km2. A total of 49 (87.5%) out of 56 pig farms in the assigned region agreed to participate in the program. Samples were collected between January and July 2014. PRRSV specific genome fragments were detected in 12 (5.3%) out of 228 oral fluid samples. PRRSV-Genotyp I could be identified in 8 samples, whereas PRRSV genome detected in 4 samples were classified as Genotyp II. 269 (55.5%) out of 485 blood samples were positive for PRRSV specific antibodies by ELISA.
Due to these laboratory results 30.6% of the farms were categorized as PRRSV positive, while 38.8% of the farms were identified as PRRSV vaccinated. In 30.6% of the farms neither PRRSV specific genome fragments nor PRRSV specific antibodies could be detected. The data, including geographic coordinates, production type and PRRSV status were entered into ESRI's ArcGIS.
For all farm types, the access of other animal species into the farm building and the proximity to the next PRRSV positive farm were associated with a higher probability of PRRSV detection. Other factors increasing the risk of PRRSV-positivity of fattening farms were multiple sources as well as variable sources of feeder pigs.

P063

PRRS PREVALENCE IN EUROPE: PERCEPTION OF THE PIG PRODUCERS

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Introduction
Since its appearance in the late 1980's and its identification in 1991, the majority of the pig producing countries have reported porcine reproductive and respiratory syndrome virus (PRRSV) infections and nowadays it is considered endemic in most of them. The disease in sows and gilts causes mild to severe reproductive problems, while in nursery and fattening pigs the typical signs of the disease are respiratory problems and increased mortality leading to reduction of average daily weight gain (ADG) and feed efficiency. However, in some cases a lack of clinical signs and/or poor recognition of them can lead to PRRSV infections remaining undetected on positive herds.
The goal of this study was to obtain an estimate of PRRSv prevalence by surveying pig producers in different European countries.

Material and Methods
A telephone survey including 390 pig producers was carried out to assess the perceived PRRS status of their herds. 390 pig producers were interviewed in 8 European countries between January and March of 2014 in relation to the presence of PRRS in their farms. The distribution of pig producers interviewed was as follows: Germany: 50; France: 51; Netherlands: 50; Denmark: 50; Austria: 50; UK: 51; Italy: 38; Belgium: 50. Each producer was asked to estimate the percentage of his or her animals that were PRRS-positive. They could respond by saying if pigs were infected and showing clinical signs, or were infected without showing clinical signs, or not infected with the virus.

Results
According to the interviewed producers, on average PRRSv is present in 75% of sows and in 67% of nursery or growing pigs. While on average, clinical cases of the disease were estimated to occur in 10% of sows and in 14% of weaned or growing pigs. However, the incidence in sows in particular varied widely by country, from as high as 21% reported from Italy to only 2% for Russia.

Conclusion
Based on a similar survey conducted with swine practitioners in Europe, disease awareness amongst pig producers is lower than swine practitioners. This survey demonstrates a wide variation between countries in the perceived prevalence of PRRS amongst European farmers, and underlines the need for improved epidemiological understanding of PRRS in European pig herds.

P064

AGE AND STRAIN DEPENDENT DIFFERENCES IN THE OUTCOME OF EXPERIMENTAL INFECTIONS OF DOMESTIC PIGS WITH BELGIAN WILD BOAR PSEUDORABIES VIRUS ISOLATES

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Introduction
Aujeszky's disease is an economically important disease in domestic swine caused by the porcine alphaherpesvirus, pseudorabies virus (PRV). Although the virus is eradicated in domestic pigs in a large part of Europe, serological studies show that the virus is still circulating in wild boars. Infection studies suggest an attenuated nature of these wild boar strains, but care should be taken in generalizing these observations.

Material and Method
To get more insight into the virulence of Belgian wild boar PRV strains, an infection study of 28 days using two genetically distinct wild boar isolates and the NIA3 strain was performed. Six female domestic pigs of 15 weeks old were intranasally inoculated with 105 TCID50 of the NIA3 strain or 106 TCID50 of the wild boar isolates BEL24043 or BEL20075. Furthermore, six contact animals were added to the boxes at 24hpi to assess virus transmission. Since clinical disease induced by PRV is age dependent, also six animals of two weeks old were inoculated with the NIA3 strain or the isolate BEL24043.

Results
A clear difference between clinical symptoms induced by the NIA3 strain and the wild boar isolates BEL24043 and BEL20075 was observed. In 15 weeks old pigs, infection with the NIA3 strain led to severe respiratory and neurological symptoms whereas no clinical symptoms were observed after inoculation with the isolates BEL24043 or BEL20075. In two weeks old piglets, the symptoms induced by the NIA3 strain were even more pronounced, but also the isolate BEL24043 induced respiratory and neurological disease, although less severe compared to NIA3 infection. Inoculation of 15 weeks old pigs with both the NIA3 strain and both wild boar isolates and inoculation of two weeks old piglets with the isolate BEL24043 resulted in seroconversion. PCR analysis of swabs showed a prolonged nasal and vaginal virus excretion after inoculation of 15 week old pigs with the isolate BEL24043. Importantly, efficient transmission of the virus to contact animals was evidenced by nasal and vaginal excretion, seroconversion and the presence of virus in the tonsils of these contact animals. On the other hand, transmission of isolate BEL24043 was only evidenced to one contact animal.

Conclusion
Our study identified differences between Belgian wild boar isolates in their capacity to infect and spread between domestic pigs. The difference in clinical symptoms in two and 15 weeks old piglets showed that the known age dependency of pigs towards the outcome of infection with PRV isolates from domestic pigs is also valid for wild boar strains. These results indicate that a reintroduction could have serious economic consequences.

P065

COMPARISON OF FOUR PCV2–VACCINES IN WEANERS

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INTRODUCTION. This study was a side-by-side study, where pigs vaccinated with 4 different PCV2 vaccines were compared to non-vaccinated pigs in the first 5½ weeks after weaning to evaluate, whether vaccination had a negative influence on performance.

MATERIALS AND METHODS. Blinded side-by-side study with 5 groups, including piglets from 3 weekly batches (165 pigs/group). Pigs were allocated within litters, balanced by weight, and ear tagged. Pigs were vaccinated i.m. at 3 weeks of age, weaned 1 week later and exported for finishing at 30 kg live weight.

Group 1) Non-vaccinates: 2 ml Saline
Group 2) Vaccine 1: 0,5 ml
Group 3) Vaccine 2: 2 ml
Group 4) Vaccine 3: 2 ml
Group 5) Vaccine 4: 1 ml

Vaccines were applied according to the manufacturer instructions, including warming of vaccine 3 before administration.
Pigs were weighed at vaccination, at weaning, 2 weeks after weaning and before sale of the first load from the current batch (5½ weeks after weaning). Mortality was recorded from weaning until sale. Pigs sorted out due to low performance (runts) were counted.
The parameters compared were weight, % lightweight pigs at 5½ weeks of age and number of lost pigs, defined as the sum of dead pigs and runts. Statistical comparison of weights was made with Kruskal-Wallis test, and comparison of lightweight pigs and lost pigs was made with Fishers Exact Test. The level of significance was p=0.05.

RESULTS. The mean weight of the pigs in the 5 groups was not significantly different at any time point, but at 5½ weeks of age, there was a numerically lower mean weight in the non-vaccinated group (-0,5 kg) and in the group vaccinated with vaccine 2 (-0,6 kg). Vaccine 4 significantly reduced the percentage of lightweight pigs compared to the non-vaccinated group (p=0.008).
The number of lost pigs was lowest in the group vaccinated with vaccine 4. In the groups vaccinated with the other 3 vaccines, the number of lost pigs was higher than in the non-vaccinated group, and vaccine 1 had a significantly higher number of lost pigs (7.9%) than vaccine 4 (1.8%) (p=0.022).

CONCLUSION. This blinded side-by-side shows significant differences between the four vaccines against PCV2 with regard to pig performance in the nursery. Pigs vaccinated with vaccine 4 had the best performance and was the only group performing better than the non-vaccinates on all parameters.
Vaccination with vaccine 4 resulted in a significantly lower number of lightweight pigs than in the non-vaccinated group and a significantly lower number of pigs lost than vaccine 1.

P066

A PRACTICAL ENVIRONMENTAL SAMPLING PROTOCOL TO DETECT PRRS GENOTYPE 1 VIRUS IN LIVESTOCK TRANSPORTATION VEHICLES

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Introduction. It has been proved that pigs can become infected with PRRSv through contact with contaminated transport vehicles1. Some studies have shown the efficacy of disinfection and thermal assisted drying to eliminate PRRSv from transport vehicles2,3. However, there is only one study looking at the detection of the virus in livestock trailers before and after disinfection and drying processes in a commercial setting4, but this study was performed under US conditions and focused on Type II virus.Considering that in Europe, PRRS Genotype 1 is more prevalent, the aim of this study was the development of a practical technique to detect genotype I virus in transportat vehciles. Furthermore, the detection level at the diagnostic laboratory was determined in order to validate this methodology.

Materials and Methods. The trailer used had 4 floors with 56 pens of 1,2 x 1,75 m each. This vehicle had only transported PRRS negative piglets. It was disinfected twice and was allowed to dry for a day before the trial.
We did serial dilutions of a quantified Type 1 commercially available modified live vaccine (MLV) (106.2 TCID50) using MilliQ water to generate 40 ml of each dilution from undiluted to 10-7.
Every dilution was sprayed into a one meter per one meter square. Three replicates of each dilution were sprayed in different corridors of the trucks.
Dilutions were removed from the floor of the truck by using SodiBox Kits (wipes) and squeezed into a 50 ml tube. A total of 27 samples were collected, identified and refrigerated.
The refrigerated tubes were sent by a one day delivery service to BI-Veterinary Research Center (BIVRC) in Hannover Germany. Hannover Germany) were PRRS PCR was performed.

Results. PRRSv RNA was detected in 3 out of 3 samples in the followings dilutions: Undiluted, 10-1
10-2, 10-3 whereas only 1 out of 3 samples were resulted to be positive in the 10-4 dilution.

Discussion and Conclusions. According to the results obtained by PCRs, we were able to detect in 100% of the cases the dilution 10-3. This means taking into account the title of the MLV, that we were able to detect 0.1- 103 TCID50. In 1 out of the 3 replicates we were able to detect the 10-4 dilution that means that we were able to detect 0,01- 102 TCID50.
According with the minimum infective dose via oral established in 10-5,3 TCID50, we are able to detect even lower level of PRRSV necessary to infect an animal in field conditions. These results confirm that the technique is ready to put in place in a Type 1 predominant scenario representing a great tool for trucks decontamination protocol evaluations as part of biosecurity programs in farms, systems or even regions.

P067

REDUCING PCV2 VIREMIA IN NEONATAL PIGS THROUGH SOW MASS VACCINATION

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Introduction. In utero infection of piglets with PCV2 may serve as a potential source of PCV2 vertical transmission to the offspring. This infection might make newborn pigs more susceptible to co-infections with other pathogens and therefore may be associated with PCVAD in the growing pig.
Piglet vaccination for PCV2 is routinely used in the pig production globally. It has been shown before that Ingelvac CircoFLEX® is safe when used in sows .
The objective of this study was to determine the prevalence of PCV2 viremia in pre-suckle piglets before and after sow mass vaccination.

Materials and Methods. This study was conducted in a 2200-sow, three sites commercial herd in Cuenca, Spain. The herd is positive for PRRS, M. hyo. Pigs are weaned and vaccinated with FLEXcombo® (PCV2 and Mycoplasma fresh mix combination) weekly at 3 weeks of age. The herd was selected for this trial because PCV2 was detected in 4 week old piglet. Nevertheless the sow herd did not have obvious problems in terms of reproductive performance nor clinical signs or performance problems during nursery phase. To evaluate the prevalence of PCV2 in presuckle piglets 39 animals were bled with the following protocol:
- 3 piglets per litter from 5 parity 1-2 sows
- 3 piglets per litter from 4 parity 3-4 sows
- 3 piglets per litter from 4 parity $\geq$ 5 sows.

This sample collection was followed by a mass vaccination of the whole sow herd with 1 ml of Ingelvac CircoFLEX®.
The sampling was repeated 2 months after mass vaccination, following the same protocol.
All statistical analyses were performed using SPSS v.15 (SPSS Inc. Chicago, IL, USA). Differences were considered statistically significant at $p<0.05$

Results.In the first sampling corresponding to piglets from non-vaccinated sows, PCV2 virus was detected in 43% of the samples whereas after sow vaccination only 7,7% of the piglets were tested positive (P<0,001).When looking at the different parity groups, the results show a statistically significant reduction of PCV2 positive piglets in the P1-2 group (64,3% vs 6,6%) P<0,001 and the P3-4 group (28,6 vs 0%) P<0,05, and a numerical difference in the P>5 group (33% vs 16%) when comparing pigs from non-vaccinated and vaccinated sows.

Discussion and Conclusions. According to the results obtained by PCRs, we can conclude that is possible to reduce prevalence of PCV2 in presuckle piglets by using Ingelvac CircoFLEX® in a mass vaccination protocol in sows. Further studies are necessary to determine whether there is a correlation between prevalence of PCV2 in presuckles pigs and performance parameters in nursery and finishing. Acknowledgements: Boehringer-ingelheim Spain technical service.

P068

PRRSV AND PCV–2 DETECTION IN BLOOD AND ORAL FLUID COLLECTED WITH GE–NOTUBE SWABS

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV-2) are among the most significant viral pathogens of swine. Direct detection by PCR of these viruses in live pigs is usually performed in serum. In the presented work, we compared PRRSV and PCV-2 detection in blood and oral fluid collected with GenoTube (GenoTube Livestock, Prionics) swabs (GT swabs) with matched serum samples, to determine whether GT swabs represent efficient sampling tools for the diagnosis of PRRSV and PCV-2 infection.
Material and Methods
Twenty piglets from a PRRSV negative but PCV-2 positive farm were divided into two groups: Group 1 (10 piglets) received an inactivated PRRS vaccine (Progressis, Merial) at the age of 2 and 4 weeks. Group 2 consisted of 10 unvaccinated piglets. At the age of 7 weeks, both groups were intradermally injected with a HP PRRSV type 2 isolate. Blood was collected from all pigs just before virus challenge (day 0) and at 3, 7, 10 and 14 days post infection (dpi). A total of 31 GT blood and 45 GT oral fluid swabs were collected from selected pigs at 3, 7, 10 and 14 dpi. Real-time quantitative PCR (qPCR) for both PRRSV and PCV-2 was performed on all sera, GT blood and GT oral fluid swabs.
Results
All pigs that tested positive for PRRSV in serum also tested positive in matched GT blood swabs; however, median PRRSV loads in GT blood swabs were about 25 times lower. In GT oral fluid swabs, the sensitivity of PRRSV detection was reduced as compared to serum (69%), with median quantitative differences between serum and GT swabs of approximately 500-fold. Correlation between quantitative results obtained from GT blood swabs and serum was substantial, while there was no correlation between results from GT oral fluid swabs and serum. Interestingly, more animals tested PCV-2 positive in GT blood swabs (29/31) than in serum (24/31). PCV-2 quantitative GT blood swab results were almost identical to serum over a wide range of concentrations. All GT oral fluid swabs tested positive for PCV-2, while only 32/45 sera tested PCV-2 positive. Nevertheless, agreement between quantitative test outcomes obtained from GT oral fluid swabs and serum was substantial.
Conclusion
To our knowledge, this is the first study determining the usability of GT swabs for PCR detection of PRRSV and PCV-2. The presented results suggest that GT blood swabs can be used for both PRRSV and PCV-2 PCR detection, with no impact on test sensitivity as compared to serum. GT oral fluid swabs were found to be less sensitive for detection of PRRSV, but not for PCV-2 infection, most likely due to comparatively low PRRSV loads in oral fluid.

P069

EFFICACY OF PCV2 VACCINATION WITH CIRCOVAC® UNDER SWISS FIELD CONDITIONS

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Introduction
The objective of this randomized controlled trial was to compare the production parameters during the castration-to-slaughter period in piglets vaccinated against PCV2 with CIRCOVAC or not-vaccinated, under Swiss field conditions.
Material and methods
The study was conducted in the Nord-Eastern part of Switzerland in an organic 90-sow farrow-to-finish farm. At the time of castration A total of 413 piglets was tagged, weighted and allocated to two homogenous treatment groups according to weight at castration, sex, sow parity and litter. At weaning, 208 piglets were vaccinated with CIRCOVAC, 0.5mL, IM in the neck. The other piglets remained unvaccinated against PCV2. All piglets were individually weighed again at slaughter. Mortality was recorded was well. To compare weights and Average Daily Weight Gain (ADWG), between the two groups, a Student's t-test or a Kruskall-Wallis test was used depending on the conditions of use of the tests. Since some pigs were sold before the end of the study to comply with organic farm certification, comparability of group performance was checked by excluding these animals from the data analysis.
Results and conclusion
The piglets were castrated at the same age (4.6 days of age, p=0.66) and no difference in average bodyweight was evidenced at castration between groups (p=0.87). The pigs were also weaned at the same age on average (p= 0.78) i.e. at 26.2 days of age (range between 21 and 34 days of age). ADWG between castration and slaughter was higher in the vaccinated group by 15g/day (V: 571g/day vs NV:556 g/day, p<0.01). Slaughter weight was reached 4 days earlier (p=0.01) for the group vaccinated with CIRCOVAC and mean bodyweights at slaughter were not different (100.6kg, p=0.95) between the groups. Six unvaccinated pigs were found dead as well as 3 vaccinated pigs. Mortality rate was consequently very low in each group with no difference among groups.
In conclusion and under the conditions of the study, CIRCOVAC vaccination proved better growth performance and led to reach the targeted weight for slaughterhouse earlier than in non-vaccinated piglets.

P070

IMPROVEMENT OF THE RELIABILITY OF A PORCINE PARVOVIRUS (PPV) ELISA TEST BY LOGISTIC REGRESSION FOR VACCINATION COMPLIANCE PURPOSE

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Introduction
Porcine Parvovirus (PPV) vaccines have been widely used in the field to prevent PPV induced reproductive problems since the early 80's with a high level of satisfaction. However, the pattern of seroconversion after vaccination is often lower and more irregular than after field virus infection. Indeed, it may eventually cause some concern about the level of protection induced by vaccination in spite of the lack of correlation between the test results and the degree of protection (Edwards et al. 1986. Efficacy of porcine parvovirus vaccines, Vet. Rec., 119, 203-205). Moreover, farmers and vets usually check sera from vaccinated animals for antibodies in order to test vaccination compliance or immunological response. Therefore, the aim of this trial was to assess the serological response to PARVORUVAX® with a commercial ELISA test and to evaluate its reliability according to the interpretation criteria.
Material and methods
Twenty-nine gilts and sows of a farrow-to-finish farm were selected and classified according to their age and their vaccination status in three groups: 10 randomly selected unvaccinated gilts from the gilt pool, 10 pregnant gilts that had been vaccinated twice with PARVORUVAX before mating (BV), and 9 pregnant multiparous (4th to 6th parity) sows that have received several vaccinations with the same vaccine (twice as gilt and every lactation). Blood samples were taken and tested for PPV antibodies by an indirect ELISA (Ingezim PPV, Ingenasa). The optical density readings were statistically analysed by logistic regression to evaluate the sensitivity (Se) and specificity (Sp) of the test according to two criteria: known vaccination status or classification according to the test interpretation guidelines. Receiver Operating Characteristic curves were designed for each model.
Results and discussion
The Se and Sp following the guidelines of the test were 47% and 100% respectively. whereas they were 94.7% and 100% when the threshold limit was corrected to 0.100 instead of 0.300 according to our logistic regression model. As a consequence the area under the curve changed from 0.763 to 0.989. Furthermore, our data indicate heteroscedasticity because the variance increased according to the number of vaccinations received: nil, 2 or >2, indicating the need to take parity into consideration for a correct interpretation of test results.
Conclusion
PPV serological tests have been developped to evaluate infection. According to our results, when using serological tests to evaluate PPV vaccination compliance, it is advisable to reconsider the threshold of positivity recommended for this test in order to maximize its sensitivity and specificity.

P071

REDUCTION OF ANTIBIOTIC USE AFTER IMPLEMENTATION OF INGELVAC® PRRS MLV PIGLET VACCINATION IN A BELGIAN WEAN TO FINISH FARM

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INTRODUCTION
Infections with Porcine Reproductive and Respiratory Syndrome (PRRS) virus play an important role in the Porcine Respiratory Disease Complex (PRDC). The aim of this study was to evaluate the effect of implementing a PRRS modified live virus (MLV) vaccine as an aid to control PRDC, on the antibiotic use in piglets and fatteners.

MATERIALS AND METHODS
The study was performed in a Belgian wean to finish farm with 800 nursery and 1200 fattening places. Piglets were vaccinated upon arrival in the farm. In 2012, piglets were only vaccinated against Mycoplasma hyopneumoniae (M. hyo) with Ingelvac MycoFLEX® (1 ml, I.M.). As from January 1st 2013, a vaccination with Ingelvac® PRRS MLV (2 ml, I.M.) was added to the vaccination scheme. This vaccine was applied at the same time as the M. hyo vaccine, at the other side of the neck. The reason for implementing the PRRS MLV vaccine were PRDC problems occurring at the end of 2012, including cough and poor performance in the nursery and fattening period. The presence of the PRRS virus was confirmed by means of serology.
A retrospective analysis of the antibiotic use and costs was performed over a period of 1 year before implementation of the PRRS MLV vaccine (January 1st - December 31st 2012), an intermediate period of 6 months in which both PRRS vaccinated and non-vaccinated pigs were present (January 1st - June 30th 2013) and a period of 1 year in which only PRRS vaccinated pigs were present on the farm (July 1st 2013 - June 30th 2014).

RESULTS
In the one-year period before PRRS MLV vaccination, 18.56 g active substance per pig place per year was used, equal to an average of 29.3 daily doses per animal year. In the transition period of 6 months, the antibiotic consumption equaled 20.82 g active substance per pig place per year or 33.6 daily doses per animal year. In the period 6 to 18 months after the PRRS vaccine implementation, 12.04 g active substance per pig place per year or 13.5 daily doses per animal year was used. The antibiotic costs equaled respectively 3.40€, 4.35€, 1.85€ per pig place per year in the 3 subsequent periods.
This means a reduction of the antibiotic use expressed in average daily doses per animal year with 53.9% and a reduction of the antibiotic costs of 45.6% after implementation of the PRRS MLV vaccine compared to the period before PRRS vaccination. Besides the reduction in antibiotic use, an improvement of the technical performance of the pigs was also observed.

CONCLUSION
On PRRS virus infected farms suffering from PRDC, the implementation of a PRRS MLV piglet vaccination can be used as an aid to control respiratory problems and to reduce the antimicrobial consumption.

P072

DETECTION AND GENETIC CHARACTERIZATION OF PORCINE EPIDEMIC DIARRHEA VIRUS CIRCULATING IN NORTHERN ITALY

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Introduction: Porcine epidemic diarrhea (PED) causes watery diarrhea, dehydration and a high death rate among suckling pigs. Nowadays, PED is considered an emergent infectious disease in United States and Asia, causing important economic losses. In Europe, the last reported outbreak occurred in 2005-2006 in Italy. Since then, PED detection is included in the diagnostic procedure of swine enteric diseases and only sporadic outbreaks with mild clinical signs were observed in Northern Italy. In this study, we report the presence PEDV infection in pig farms and the genetic characterization of PEDV variants circulating in Italy from 2007 to 2014.
Materials and Methods: A total of 1563 samples collected from clinical cases of pig enteritis were investigated through electron microscopy, ELISA and PCR. Fifty-four positive samples were molecular characterized by sequencing of one to four regions of the genome, including 349 nt of the RNA-dependent RNA Polymerase (RdRp), 553 or 2581 nt of the spike S1 gene, 439 nt of the matrix (M) and 360-599 nt of the ORF3 gene. The nucleotide and amino acid sequences were aligned and compared to selected PEDV sequences available from the GenBank database. Phylogenetic trees were generated by the neighbor-joining method by MEGA 5.
Results: PEDV was diagnosed in 61 outbreaks and a total of 54 were then confirmed by RT-PCR, using PEDV specific primers. Twenty-eight, 21, 22 and 5 sequences were obtained for the S1, RdRp, M and ORF3 regions, respectively. The phylogenetic analysis evidenced that genetically distant coronaviruses entered in Italian farms during the period 2007-2014. In particular 3 independent entries occurred in 2007, 2009 and 2014. Moreover, the virus detected in 2014 showed more than 99% nt identity with the U.S. “Ohio” variant considered less pathogenic.
Conclusion: These results indicate an endemic presence of PEDV in Northern Italy. The PEDV strains circulating since 2007 showed a high genetic variability. The characterization of the circulating strains is important to keep updated the diagnostic tools that are in use for PED diagnosis and to understand the cross-protection ability of the different strains.

P073

PRRSV MONITORING USING ORAL FLUID SAMPLING: A CASE REPORT IN TWO STABLE FARROW–TO–FINISH FRENCH FARMS

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Introduction
Nowadays, oral fluid sampling is of great interest for Porcine Reproductive and Respiratory Syndrome virus (PRRSv) monitoring. This method is easier to use, faster and causes less stress to pigs when compared to the standard blood sampling. The objective of this study was to compare PRRSv antibody profiles obtained with these two techniques in two positive but stable herds.
Material and methods
Two farrow-to-finish farms located in Brittany (France) known to be PRRSv-stable were selected. Mixed vaccination programs using modified live and killed vaccines were in force on the farms in addition to basic hygiene and all-in all-out management. The absence of clinical signs and recent seroprofiles indicated no to low virus circulation. A transversal study was performed at 4 different ages, farm A: from 6 to 23 and farm B from 5 to 26 weeks of age (WoA). The pigs in each pen were individually blood sampled and given a rope to chew. A total of 146 sera and 8 rope samples were collected. The samples were assayed for PRRSv antibodies using two commercially available kits (IDEXX PRRS 3XR, IDEXX PRRSV Antibody for Oral Fluid). Samples with S/P ratio ≥0.4 were considered as positive according to the manufacturer instructions.
Results and Discussion
In farm A, at 6, 9, 12, and 23 WoA, the percentages of positive pigs in the ELISA were 78%, 58%, 8% and 0% and OF E/P ratios were 7.0, 3.3, 0.9 and 0.2, respectively. In farm B at 5, 8, 11 and 26 WoA, the percentages of positives in the ELISA were 43%, 73%, 12% and 0% whereas the OF E/P results were 1.4, 2.2, 0.1, 0, respectively. No linear correlation between serum prevalence and OF E/P ratio was observed. However, the higher the percentage of positive pigs, the higher the OF E/P and in both farms, profiles of percentage of positive pigs and OF E/P ratio were qualitatively similar. Data demonstrated a steady decline of maternal antibody level in farm A and possibly a weak and controlled PRRSv circulation during post-weaning in farm B. Fatteners were antibody negative to PRRSv at slaughter age according to the two techniques. These observations confirmed the stable status of the two farms towards PRRSv as well as the effective strategy implemented to control the virus circulation.
Conclusion
Under the conditions of the study, oral fluid samples tested with this PRRSv oral fluid antibody ELISA kit provided extensive information at low cost and confirmed its reliability for PRRSv monitoring in stable herds.

P074

STABILIZATION OF PRRSV CIRCULATION IN A NURSERY AND FATTENING UNIT FOLLOWING THE IMPLEMENTATION OF MIXED PRRSV VACCINE PROGRAM IN BREEDERS

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Introduction
PRRS is one of the most significant pig diseases. Current vaccination strategies are based on the use of MLV (Modified Live Vaccines) or KV (Killed Vaccines). This case report describes the stabilization of PRRSv circulation in a nursery and fattening unit, after the introduction of a PRRSv vaccination at day 90 of gestation with a KV vaccine in breeders vaccinated at day 6 of lactation with a MLV-EU type vaccine.
Case description
The field study was carried out in a 400-sow PRRSv-positive farrow-to-finish farm operating in 5 batches of 80 sows in Brittany, France. During lactation, vaccination against PRRSv (MLV) is performed. No clinical signs are observed in the maternity unit.
Piglets are vaccinated against M.hyopneumoniae. During the nursery and fattening period, respiratory symptoms are common and antibacterial treatments were frequently performed against PRDC. In December 2013, PRRSv infection was confirmed in two batches in the nursery, by positive results of both PCR and ELISA (IDEXX) on sera and oral fluids.
In January 2014, one batch of breeders received a second PRRSv vaccination at day 90 of gestation with a KV (PROGRESSIS®, Merial). A cohort of 10 piglets born from these sows were monitored for the detection of PRRSv infection (PCR on oral fluids) and serologic profiling (ELISA) at 8, 12 and 16 weeks of age.
The offspring of the vaccinated sows did not show any respiratory symptoms until slaughter. All except one (S/P ratio 0,66 at 8 weeks of age) ELISA results at 8 and 12 weeks of age were negative. Delayed PRRSv circulation was shown by positive results on both sera and oral fluid samples at 16 weeks of age.
In February 2014, while sows were vaccinated according to the historical vaccination protocol (only at day 6 in lactation with the MLV vaccine), respiratory symptoms reoccured in the nursery, suggesting an early PRRSv infection.
In June 2014, the KV vaccination at 90 days of gestation was introduced as a routine practice. In October 2014, a transversal oral fluid sampling showed the persistence of maternal antibodies until 10 weeks of age, and the absence of PRRSv circulation until 12 weeks of age.
Conclusion
Under the field conditions of the study, the implementation of mixed PRRSv vaccination program in sows combining MLV vaccination at day 6 of lactation and PROGRESSIS vaccination at 90 days of gestation resulted in a delayed PRRSv circulation, as well as a clear reduction of associated clinical signs. These results indicates that the use of PROGRESSIS 3 weeks before farrowing can be an efficient tool for the control of early virus circulation in piglets and total herd stabilization.

P075

USE OF PHYLOGENETIC ANALYSIS OF PRRSV TO MONITOR OF THE EFFICACY OF THE ACCLIMATIZATION OF REPLACEMENT GILTS IN A PYRAMID PRODUCTION SYS–TEM

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Introduction
In area of high pig density the eradication of PRRSV is challenging because of a high probability of reinfection . The control of the disease pass through a proper management of replacement gilts and a good acclimation help in the achievement of herd stability.

Material and method
This study was carried out in a pyramid system of 1,500 females of three productive units: Grand-parent sows are reared in unit B and gilts are moved to farrow-to-finish units identified as M and L, a portion of growers of units M and L are moved in separate finishing units. Before spring 2013 the replacement females were introduced at 20kg to 40kg b.w. and quarantine were not applied. The gilts were vaccinated with a PRRSV MLV but acclimation to the resident strain of PRRSv before the introduction in productive units was not applied. M, B and L herds, were not stable and reproductive disorders were constantly observed. Starting from April 2013 an acclimatization protocol was implemented and gilts from unit B were moved to units M and L at 6kg of b.w. After that, the replacement breeding females received a longer period of time to get exposed to the resident virus, to develop an appropriate immunity and to eliminate viremia and the shedding of the virus. At 130kg the replacement gilts were tested both by ELISA and PCR to evaluate the success of the natural exposure to PRRSV and the "freezing" of the infection (no shedders). Since 2010, ORF7 sequences were constantly obtained. The alignment and phylogenetic analysis was performed using MEGA6. Time scaled phylogenetic analysis and estimation of nucleotide substitution rates were generate using BEAST v1.8.1. A model of relaxed molecular clock was used (HKY with gamma distribution). A chain length of 8M was used to generate a clade credibility trees from 80k trees.

Results
From farm M,B and L between 2010 and 2014 a total of 14 ORF7 nucleotide sequence data was obtained. The degree of nucleotide identity ranged from 100% to 84.6%. The phylogenetic analysis revealed 4 well separated clades. In clade A, B and C were spanned only sequences obtained from herd M, B and L, respectively. In clade D were grouped sequence from farms M, B and L obtained before the decision to change the system designed to acclimatize the replacement gilts. The substitution rate was estimated as 1.99E-3 (95% HPD [3.69E-4, 4.02E-3]) and the maximum rate of diversity observed within a clade was 2.1% acquired in 14 months.

Conclusion
The implemented acclimatization strategy induced PRRS stabilization among farms so that only one resident PRRSV was present. Phylogenetic analysis contributes to evaluate the efficiency of acclimation system.

P076

MULTIPLE GENES SEQUENCING OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS FROM FIELD SAMPLES BY NEXT GENERATION SEQUENCING

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Introduction: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes a highly contagious viral disease in swine population worldwide, resulting in huge economic losses and sanitary problems. PRRSV leads to complications during pregnancy, neonatal loss, or affliction of the respiratory system in young piglets. PRRSV, which belongs to the Arteriviridae family, has a 15-kb positive-sense single-stranded RNA genome harbouring eight open reading frames (ORFs). The ORF1a and ORF1b located at the 5'end of the genome represent nearly 75% of the viral genome and encode for proteins involved in replicase and polymerase activities. ORFs 2-7 encode for structural proteins associated with the virion: GP 2,3,4,5, membrane protein (M) and nucleocapsid protein (N), respectively. Two genetically distinct PRRSV genotypes are described: Type 1 (European genotype) and Type 2 (North American genotype), both sharing only 50-60% genome sequence homology due to a high degree of genetic variability. Type 1 PRRSV includes, four genetic subtypes: 1.1, 1.2, 1.3 and 1.4. PRRSV genetic diversity have a direct impact on immunobiology, epidemiology, diagnosis, and vaccine efficacy. The majority of PRRSV genotyping is limited to two viral genes: ORF5 or ORF7. As Mateu's Team recently showed, the existence of mosaic viruses caused by recombination events, leads to reconsider the use of a single-gene sequence strategy to genotype PRRSV.
Materials and Methods: In order to answer this issue, we developed a next generation sequencing (NGS) method to sequence multiple PRRSV genes, on field samples. Our strategy is to analyze PRRSV genes that display both high genetic diversity and encode for potential lymphocyte B cells activating epitopes resulting in virus neutralizing antibody production. Thus, we selected Nsp2, ORF2, ORF3 and ORF4 in addition to ORF5 and ORF7. The main problems of field samples rests on poor RNA quality, presence of large amount of PCR inhibitors and low viral load. Thus, we set up a fast and robust method based on small size fragments RT-PCR amplification and subsequent NGS using the Ion Torrent PGMTM Sequencer.
Results: We are able to sequence PPRSV target genes covering nearly 50% of the PRRSV's genome from low viral load field samples with a low failure rate.
Conclusion: This approach enables to analyze several samples in parallel, directly from the field sample avoiding cell culture viral isolation.

P077

FIELD EFFICACY OF A PCV2 VACCINE IN A HIGH-HEALTH DANISH FARM UNDER SUBCLINICAL PCVD CONDITIONS

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Introduction
PCV2 is a widespread virus, present in the swine population all over the world. Even in subclinical conditions, it has been proven to decrease pig performance in the whole production cycle, hence decreasing profitability. The objective of this trial was to demonstrate that PCV2 piglet vaccination can improve weaning-to-slaughter performance in a high-health status and high-performing farm under subclinical PCVD field conditions in Denmark.
Material and Methods
The trial was conducted on a well-managed Danish wean-to-slaughter pig farm producing 22,000 pigs per year with no PCVD clinical signs and good production data. The farm was classified SPF Myco. hyo.-positive. Prior to the trial, a serological survey was performed by collecting three times five blood samples at 12, 16 & 20 weeks of age. The sera were pooled and assayed for PCV2 DNA using a qPCR technique. Five weekly batches of piglets received over a two-month period were included in the study. The first group was left as an unvaccinated control whereas the four other groups were vaccinated against PCV2 at arrival at the wean-to-finish farm in their fourth week of life with 0.5 mL, IM in the neck. Group-based weights at arrival and individual carcass weights were recorded. Live weights at slaughter were calculated based on a 76.3% live weight-carcass weight ratio. Statistical analyses were performed non-parametric tests.
Results
The three pools of blood samples all demonstrated positive with log10 PCV2 DNA copy values:3.84, 5.92 & 6.76 confirming subclinical PCV2 circulation on the trial farm. In this trial, including 2.063 pigs at slaughter, an improvement of growth was observed in each batch of PCV2-vaccinated pigs as compared to unvaccinated pigs: ADWG and days to market were highly-significantly ($p<0.01$) improved by resp. 34 g/day [24 to 42 g/day] and 5.3 days [4.2 to 6.3 days]. The carcasses were numerically heavier in all vaccinated groups as compared to the unvaccinated group except in one batch displaying a non-significant difference ($p=0.24$) of 0.21kg.
From the farm production data recordings, mortality dropped from 1.7 % to 0.8 % from weaning to 30 kg and from 2.5-2.0 % to 1.5-1.0 % from 30 kg to slaughter following the implementation of the PCV2 vaccination. The number of treated pigs on the farm was reduced. The health remarks recorded at the slaughterhouse was also reduced.
Discussion and conclusion
These results demonstrated the presence of subclinical PCVD, even in a well-managed farm. In this context, the initiation of a PCV2 piglet vaccination program successfully improved production performance of this high-health and high-performing Danish herd.

P078

COLLECTION OF ORAL FLUID SAMPLES TO SURVEY FOR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN 20 BELGIAN FARMS

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Introduction
The use of oral fluid (OF) samples for the detection and monitoring of PRRSV has gained in popularity over the last years. It is a less labor-intensive and more cost-effective method than the traditional serum sampling. Yet, interpretation of the results from PRRSV endemically infected and/or vaccinated farms can be difficult. The objective of the present study was to gain more insights into the relationship between sample-to-positive (S/P) ratios in OF and sera on one hand and into infection profiles on farms on the other hand.
Material and Methods
In 20 Belgian farrow-to-finish operations, 3 OF samples were collected in each farm in pens of animals of three age categories as follows: (1) 4 to 7 weeks, (2) 8 to 12 weeks, (3) 13 to 21 weeks. Each pen housed 5 to 13 pigs. Corresponding serum samples were collected from all pigs. All serum and OF samples were assayed for PRRSV antibodies using commercial indirect ELISA kits (PRRS X3 AB and PRRS OF respectively, IDEXX). A positive result was defined as a S/P ratio ≥ 0.4. In addition, presence of viral RNA was detected on the OF samples by RT-PCR (Virotype PRRS).
Results
The correlation coefficient between the average serum S/P ratio and the OF S/P ratio was 0.68; 0.87 and 0.78 respectively in the 3 different age groups. In the two youngest groups, PRRS S/P ratios of both OF and serum showed large variation within farms. In the oldest group however, PRRS serum and OF S/P values were both either very low or very high. The former were farms in which PRRSV circulation, indicated by positive RT-PCR results in the OF samples, was low/absent. The number of PRRS RT-PCR positive OF samples out of 20 was 6, 14 and 8 in the 3 different age groups respectively. All isolates belonged to the European type.
Discussion
The lower correlation coefficient in the pigs from the early nursery likely resulted from the high variation in S/P results between piglets from different farms, as this diverse group included animals with high and low levels of maternal antibodies as well as recently infected animals. Furthermore, the results indicated that most PRRSV infections was occurring between the end of the nursery and the beginning of the fattening period. Therefore, understanding the degree of farm infection using RT-PCR may be the most successful at the late nursery or early fattening phase, and should include antibody monitoring at multiple times during the fattening phase as well.
Conclusion
In conclusion, the results indicated that OF can be a useful tool for PRRSV monitoring, even under endemic conditions. Sampling in multiple age groups on a regular basis provides timely information for improved PRRSV management.

P079

REDUCTION OF MORTALITY AND ANTIBIOTIC USE BY PIGLET VACCINATION AGAINST PCV2

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Introduction
PCV2 infections are widespread and therefore vaccination is regularly implemented to control infection. However, under field conditions, the infection status at the moment of vaccination may be variable and is generally unknown. The current report describes the effects of CIRCOVAC® (Merial, Lyon, France) vaccination during the nursery period in a farm where piglets were already exposed/infected at the moment of vaccination.
Material and methods
The farm consisted of a farrow-to-finishing farm system with 450 Hypor sows kept in a four-week batch system. Most of the pigs are transferred to external fattening farms after the nursery period (10 weeks of age). The pigs of the current case report were transferred to a fattening farm with a history of severe respiratory problems. In a group of 959 piglets, 480 were IM vaccinated (V) with 0.5 ml of CIRCOVAC at the age of 7 weeks. The remaining 479 piglets served as non-vaccinated (NV) control pigs. During the fattening period the two groups were housed in two different compartments. Two
oral fluid samples per vaccination group were collected at the age of 7, 10, 15 and 21 weeks for serological monitoring by an in-house ELISA (LABOCEA). In addition, a PCR assay (CODA-CERVA) was used to evaluate for viral DNA detection. Group treatments with antibiotics, mortality and weight at the beginning and the end of the fattening period of the 2 groups were recorded.
Results
The PCV2 PCR on the oral fluids, indicated that the pigs were in contact with the virus at vaccination. This was confirmed with high antibody titers in oral fluid samples of both V and NV animals from 10 weeks onwards. By the end of the fattening period, the V group displayed a statistical tendency to a reduced mortality (V: 0.62% vs NV: 2.3%, Fisher's exact test p=0.055). Also a difference in treatment frequency between the two groups was observed: the NV pigs received three group treatments with doxycycline (during 5 days each due to respiratory problems), leading to a higher treatment cost of € 340.2. The ADWG was 671 g/day in NV group and 680 g/day in the V group.
Discussion
In this study, vaccination of piglets, despite the contact with PCV2 at the moment of vaccination, decreased finishing mortality rates and antibiotic use. Taken in account the differences in antibiotic treatment and the improved production figures (ADWG), vaccination was economically justified. In this case with € 1.15 per slaughterpig added value.

P080

IMPACT OF PCV2 VACCINATION ON LYMPHOID LESIONS AND PERFORMANCE IN A NON– VACCINATED HERD WITH SUBCLINICAL PCV2 INFECTION

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Introduction
Porcine Circovirus Type 2 (PCV2) is endemic in most commercial pig producing countries. A farrow to finish unit with 300 sows, experiencing subclinical PCV2 infection, no PCV2 associated clinical disease and low wean to slaughter mortality (below 3%), (PCV2 circulation was diagnosed by serology and virology) was followed to assess the impact of PCV2 piglet vaccination on health and performance. An assessment was made on the return of investment (ROI) of this intervention. Years prior to this and during the study the breeding herd was vaccinated with a licensed PCV2 vaccine.

Methods
For this study, 124 pigs were individually ear tagged and weighed at weaning, grower stage and pre slaughter. Pigs were randomly allocated to a PCV2 vaccinated (vac) group, n=61 (Porcilis PCV) or a non-vaccinated control group (n = 63). Pigs were blood sampled at the three time points. The inguinal lymph nodes (ILN) were collected at slaughter and assessed for lymphoid depletion and PCV2 immunohistochemical antigen (a.g.) staining. Data was analyzed with proprietary statistical software.

Results
No significant difference was found between the live weight (LW) of the pigs in the two groups at the time of inclusion in the study and grower stage 7.6, 7.8 kg and 51.9 and 54.2 kg for control and vac group respectively. Before slaughter, pigs in the control and vac group weighed 75.6 and 81.4 kg of LW ($p<0.05$). The PCV2 antibody (a.b.) titer of the vac group at the grower stage were significantly higher than the a.b. titer for the control group, 5419 and 3214 respectively ($p = 0.003$). The ILN analysis showed a higher proportion of pigs with higher scores for PCV2 a.g. staining in the control group than in the vac group (Mann Whitney test, $p <0.0024$).

Conclusions
The ROI of interventions should always be assessed. In this case, the difference in LW pre slaughter between the two groups was 5.8 kg representing an extra £5 / pig or 7 days less to slaughter. The proportion of animals with a high score of PCV2 a.g. in the ILN was higher in the control pigs. This may indicate that PCV2 a.g. in ILN could be used as a measure for PCV2 vaccine efficacy but further studies are needed. In addition, the results suggest that vaccinated pigs are healthier and less prone to PCV2 circulation at a later stage in life.
Overall this study demonstrated that PCV2 vaccination of the feeding herd is beneficial for farms affected by subclinical PCV2 infection. Vaccination of the feeding herd improved pig health and performance, resulting in a ROI to the use of the vaccine of 4.4.

P081

DETECTION OF PRRSV ANTIBODIES IN SERUM, ORAL FLUID AND DRY SWABS IN PIGS AFTER INTRADERMAL APPLICATION OF HIGHLY PATHOGENIC PRRSV TYPE 2

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Introduction
Highly pathogenic (HP) PRRSV type 2 causes high economic losses in Southeast Asia. Therefore, efficient immunization strategies are necessary to minimize the problems in affected farms. Aim of the study was to measure the antibody reaction in pig serum and oral fluid after intradermal application of a HP PRRSV type 2 isolate.
Material and methods:
Twenty piglets from a PRRSV negative farm were divided into two groups: Group 1 included 10 piglets vaccinated twice with an inactivated PRRS vaccine (Progressis, Merial) at the age of 2 and 4 weeks. Group 2 contained 10 not pre-vaccinated piglets. At the age of 7 weeks, all piglets were injected intradermal with virus culture supernatant of a HP PRRSV type 2 isolate diluted 1:1 with an adjuvant. Blood samples and individual oral fluid via dry swabs (GenoTube Livestock, Prionics, Switzerland) were taken from each piglet at days 0 (before inoculation), 3, 7, 10 and 14 after inoculation. Pen-wise oral fluid was collected daily from each group with a cotton rope. All serum samples were tested by PRRSV RT-qPCR. PRRSV antibodies were measured in serum by IDEXX PRRS X3 ELISA and in oral fluid by IDEXX PRRS OF ELISA.
Results:
At day 0, all serum samples, individual oral fluid samples re-constituted from dry swabs and oral fluid from cotton ropes tested negative for PRRSV antibodies and PCR. From day 3 onwards, all serum samples were positive in PRRSV RT qPCR. In serum samples, eight piglets of group 1 and seven piglets of group 2 had seroconverted at day 10, the remaining piglets at day 14. One piglet, however, did not seroconvert during the study. In oral fluid from dry swabs, nine piglets from each group tested positive for PRRSV antibodies at day 10, the two remaining piglets were positive at day 14. At day 14, however, four piglets that were PRRSV antibody positive at day 10, tested negative in oral fluid dry swabs. In oral fluid from cotton ropes, from day 9 on until end of the study at day 14, PRRSV antibodies were found in both groups.
Conclusions:
Intradermal application of highly pathogenic PRRSV type 2 caused viremia in all pigs and seroconversion between day 7 and 14 after application, independent of pre-vaccination with an inactivated vaccine. PRRSV antibodies could be detected in oral fluid from dry swabs and in group-wise collected oral fluid via cotton rope as well. Therefore, individual oral fluid samples collected with dry swabs and oral fluids collected with cotton rope are useful alternatives for detection of PRRSV antibodies. Through daily oral fluid collection, PRRSV antibodies could be detected earlier than in serum.

P082

INFLUENCE OF SPRAY–DRIED PLASMA IN SWINE FEED ON PRRSV ANTIBODY DIAGNOSTIC IN ORAL FLUID

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Introduction
Spray-dried plasma is sometimes used as source of high-quality proteins in feed for pre-weaned and weaning piglets. In the EU, the plasma is produced from animals slaughtered at abattoirs under veterinary inspection. These animals are intended for human consumption. Because the plasma is collected from many different pigs from different farms it is to be expected that it contains antibodies against various infectious diseases. Aim of the study was to test the occurrence of PRRSV antibodies in feed enriched with spray-dried porcine plasma and to test the influence on oral fluid diagnostic in a farm using such feed.
Material and methods:
A whole of 39 feed samples was included in the study. 20 pig feed samples contained spray-dried plasma. For comparison, 15 pig feed samples contained no plasma and 4 were feed samples for poultry or horses.
Furthermore, in a continuously monitored PRRSV negative farm using feed with spray-dried plasma as milk replacer, piglet starter and weaning diet, serum and oral fluid samples were collected in piglet groups of different ages: Individual oral fluid samples of four suckling piglets one week old, group-wise oral fluid samples (two samples of each age group) of suckling piglets 4 weeks old and nursery piglets 7 and 10 weeks old. PRRSV antibodies were measured in all serum and feed samples with the IDEX PRRS X3 ELISA (IDEXX, Ludwigsburg, Germany). Oral fluid samples were tested with the IDEXX PRRS OF ELISA.
Results:
All feed samples not containing plasma were PRRSV antibody negative. Out of the 20 feed samples with plasma, 18 were PRRSV antibody positive. This includes the milk replacer and both piglet feeds containing spray-dried plasma from the farm where the serum and oral fluid samples were taken. All serum samples of the piglets were PRRSV antibody negative. Three out of four oral fluid samples from suckling piglets, one week old, fed with milk replacer containing spray-dried plasma and all group-wise oral fluid samples from piglets 4 and 7 weeks old, fed with feed containing plasma, were PRRSV antibody positive. Oral fluid samples from 10-week old piglets with no plasma in feed were PRRSV antibody negative.
Conclusions:
Especially in weaning piglets, oral fluid diagnostics is more and more advertised. The collected oral fluid often contains feed remains from the pig mouth. In feed enriched with porcine spray-dried plasma, usually PRRSV antibodies can be found. These antibodies are able to influence the results of PRRSV ELISA determined in oral fluid collected from pigs fed with this feed.

P083

EPIDEMIOLOGICAL SITUATION OF ASF IN CENTRAL–EASTERN EUROPEAN COUNTRIES

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Objectives
From 2007 ASFV is circulating close to eastern border of the EU. In 2014 it get territory of EU. In Poland, from February 14th till the middle of November, 21 cases of ASF in wild boar (WB) involved 46 animals (44 died and 2 hunting) and 2 outbreaks involved 6 backyard pigs were detected in Podlaskie voivodeship, from 0 up to 17 km from the Polish border with Belarus.
NRL for ASF located in NVRI in Pulawy is monitoring the current epidemiological status of Poland by providing diagnostic investigations.
Materials and methods
Biological samples were collected from both, WB - dead, from traffic accidents or shot in hunting season, as well as from domestic pigs (DP) died with symptoms of infectious diseases or transported.
To the middle of November in total 33481 animals - 20297 DP and 13184 WB were tested
In serological screening 6420 sera - 1653 from DP and 4767 from WB were tested using commercial ELISA (PPA Ingezim Compact 11.PPA.K3). Positive or questionable samples were retested using immunobloting (IB) or immunoperoxidase test (IPT), as confirmatory techniques.
In molecular screening in total 40092 biological samples from 33473 animals – 20646 from DP (18313 blood, 80 serum, 2 clots, 155 kidneys, 159 spleens, 42 tonsils, 60 lymph nodes, 106 lungs, 37 livers, 50 hearts, 47 bones, 20 pieces of skin, 1547 pieces of muscles, 26 feed samples, 2 gelatines) and 19446 from WB (8571 blood, 180 sera, 48 clots, 2577 kidneys, 2920 spleens, 713 tonsils, 1004 lymph nodes, 2111 lungs, 28 livers, 15 hearts, 1 urinary bladder, 37 salivary glands, 2 intestines, 1 peritoneal fluid, 1238 bones) were examined using the Real-Time PCR.
Results
In Poland, till middle of November 2014, using commercial ELISA all samples from DP except 2 blood samples which gave the questionable and the 7 others which gave weak positive results were negative. Using confirmatory test – IPT, all those samples were negative.
From WB, 112 questionable and 238 positive samples were detected using commercial ELISA. Only 4 of them gave positive result using confirmatory techniques.
In the Real-Time PCR only 46 from 13184 wild boar and 6 from 20297 pigs were positive.
Polish ASFV isolated belong to genotype II and are identical as isolated from Belarus from 2013 and Eastern European countries from 2014.
Conclusions
Monitoring studies were very useful to prove epidemiological status of Poland. In Poland the most intensive laboratory survey is conducted in the world. From 2011 nearly 50000 of animals were tested therefore the declared status is well defined.
Currently ASF was detected only in restricted and protection areas in 4 municipalities of 2 districts in 1 voivodeship - Podlaskie, but still 99.5% of our territory is free from ASF.
In Baltic countries (Estonia, Lithuania and Latvia) and in Russia more cases and outbreaks were detected. The epidemiological situation in Eastern Europe is currently unfavorable and the risk of further spreading of ASFV is very high.
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P084

IMPROVED REPRODUCTIVE PERFORMANCE AFTER PROGRESSIS® VACCINATION AT THE END OF GESTATION IN A PRRSV INFECTED FARM

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) is endemic in swine producing countries worldwide and causes important losses due to reproductive and respiratory disorders. Additionally, the virus variability and the immune evading properties of the virus are leading to increased difficulties in the control of the disease. As a result, both attenuated and inactivated commercial vaccines are applied under field conditions according to different schedules. Recently, increased transfer of colostrum derived antibodies by vaccinating sows at the end of the gestation period (D90) with an inactivated vaccine, has gained interest as an additional control strategy.
Material and methods
In a conventional 2000-Topigs-sows farm, sows, divided into 20 groups of about 105 animals kept in a one week batch system, used to be initially vaccinated with an attenuated EU based PRRSV vaccine at day 60 of gestation and at day 6 of lactation. Since one and a half year, the vaccination schedule has changed by including an additional vaccination with PROGRESSIS® at day 90 of gestation. Reproductive parameters such as the number of pigs born alive, piglet mortality in the farrowing unit and number of weaned piglets were compared in one year before the implementation of the D90 vaccination with 79 groups after the change in vaccination strategy. Statistical comparison between the weekly reproductive parameters recorded during the two periods were performed using a Wilcoxon test.
Results
The average number of pigs born alive increased from 13.80 (s.d. 0.61) to 14.35 (s.d. 0.48) after the implementation of the D90 vaccination ($p<0.01$). The percentage of piglet mortality in the farrowing unit was significantly reduced from 15.39% (s.d. 0.79) to 12.19% (s.d. 1.83) on average after implementation of day 90 PROGRESSIS vaccination ($p<0.01$). This resulted in an significant increased number of weaned piglets per litter ($p<0.01$) from 11.61 (s.d. 0.79) to 12.58 (s.d. 0.45) after the change in vaccination schedule.
Discussion and conclusion
In this farm, a clear and long lasting positive effect of the additional PROGRESSIS vaccination at D90 on the piglet mortality and health in the farrowing unit was observed. Since no other management factors were changed during the observation period, it was clear that the improved sow immunity resulted in an increased survival rate of piglets before weaning. This effect could be explained by a lower infection rate of piglets and by a reduced infection pressure of PRRSV.

P085

PRRSV TYPE 1 DETECTION IN AEROSOLS FROM THREE SWINE HERDS IN DENMARK

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PRRS is one of the most important diseases in Danish swine and has a significant impact on the production economics, antibiotic consumption and animal welfare. Approximately 40 % of Danish swineherds are positive for PRRSV type 1 or/and type 2. Air borne transmission via aerosols has previously been described for PRRS type 2 viruses, but there have been no reports of aerosol transmission of PRRSV type 1 under field conditions. Information on aerosol transmission is important for the control of the disease and methods to detect PRRSV in the air could be an effective tool in control and eradication programs. The aim of this study was to investigate if PRRSV could be detected in the air outside four (4) PRRSV-positive swine herds in Denmark.

Case description
The study was performed during the winter months in four (4) PRRSV-positive swineherds located in the Southern part of Jutland. Pigs in all herds had high levels of IMPA antibody titers to PRRSV indicating acute infections and thus shedding of PRRSV.
Descriptions of the herds: Herd 1: Weaning-to-finisher (positive for type 1 PRRSV); Herd 2: Finishers (type 1+2 positive); Herd 3, site 1: Finishers (type 2 positive); Herd 3, site 2: Weaning-to-finisher (type 2 positive).

Materials and methods
A liquid cyclonic collector (Midwest MicroTek, Brookings, SD, USA) was placed approximately 30 meters downwind from the herds. Air was collected in the cyclone for 30 minutes. During the collection period the air was centrifuged into a collection bowl, where 10 mL of Phosphate Buffer Saline (PBS) was added. If present, PRRSV was captured in the buffer. A total of 20 air samples were collected. The samples were subsequently tested for PRRSV by a specific real time RT-PCR assay. During the air sampling period meteorological data (temperature, humidity, pressure, wind- and gust speed) were also collected by a mobile weather station.

Results and discussion
In total 4 out of 20 samples were found PRRSV-positive and all were type 1. In herd 1, 2 and 3 (site 2), 2/4, 1/4 and 1/6 of the samples were positive, respectively. All air samples collected at Herd 3, site 1 were PRRSV negative. The four positive air samples had a Ct-value of 35.01-38.8 indicating, that the level of virus were relatively low in all samples.
Ranges of the weather variables on the four days when PRRSV-positive samples were collected were as follows: Temperature (°C): 6.3-8.3; humidity (%): 83-93; pressure (hPa): 983.1-1017; wind speed (m/s): 0.6-2.5 and gust speed (m/s): 1.0-3.0.

The results showed that PRRSV type 1 could be detected in air samples collected 30 meters from three PRRSV-positive swine herds.

P086

PRRSV TYPE 1 DETECTION IN AEROSOLS INSIDE A PRRSV–POSITIVE SWINE HERD IN DENMARK, A COMPARISON ANALYSIS OF AIR SAMPLING VS BLOOD SAMPLING

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PRRS is one of the most important diseases in Danish swine and has a significant impact on the production economics, antibiotic consumption and animal welfare. Approximately 40 % of Danish swineherds are positive for PRRSV type 1 or/and type 2. Air borne transmission via aerosols has previously been described for PRRS type 2 viruses, but there have been no reports of aerosol transmission of genotype 1 PRRSV under field conditions. This aim of the study was to test if PRRSV could be detected in the air collected inside a PRRSV-positive swine herd in Denmark. The results of test of air samples were compared with test of blood samples from pigs in the same section/pen.

Case description
The case herd was a PRRSV-positive wean-to-finisher herd. High IMPA antibody titres to PRRSV type 2 were found in the pigs prior to the study, indicating possible shedding of PRRSV. A mass vaccination (sows, gilts, boars and piglets from 1 week and up to 30 kg) using Ingelvac®PRRSV MLV was conducted prior the study, because of an acute outbreak of PRRSV in the sow herd. All pigs entering the herd were thereafter vaccinated with Ingelvac®MLV.

Materials and methods
A liquid cyclonic collector (Midwest MicroTek, Brookings, SD, USA) was placed inside the herd in the middle of each room, 50 cm from the ground. Air was collected in the cyclone for 30 minutes. During this period the air was centrifuged into a collection bowl, where 10 mL of PBS was added. If present, PRRSV was captured in the buffer. Air and blood samples were collected weekly for a period of 10 weeks from 3 groups of pigs: pigs 14 weeks, non-vaccinated (Gr. 1), pigs 13 weeks (Gr. 2) and pigs at end nursery, 10 weeks (Gr.3). The samples were subsequently tested for PRRSV by a specific real time RT-PCR assay. ORF5 of selected samples was sequenced.

Results and discussion
Twice as many air samples compared with blood samples were PRRSV positive in Gr. 1 and Gr. 2. All positive results from air- (6/9) and blood (8/8) at Gr. 3 were PRRSV type 2. Ct-values of the air samples were significantly higher (p=0.0003) compared to the Ct-values in the blood samples indicating, that only a low amount of virus was shed in the air.
No significantly difference (p=0.683) was found between air and blood samples in detecting PRRSV, indicating that the cyclone was an effective tool for detection of PRRSV within a section. PRRSV in all 8 air and blood samples tested showed high nucleotide sequence homology to vaccine strain in ORF5 (98.51-99.83%).
In conclusion, collection and test of air samples were as sensitive as blood samples for detection of PRRSV inside a PRRSV-positive herd.

P087

USE OF A STANDARD TISSUE DIAGNOSTIC PROTOCOL TO PROPERLY DIAGNOSE PRDC IN HERDS FOR VACCINE PROTOCOL DECISION–MAKING

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Introduction
Proper diagnosis of disease is necessary to develop a comprehensive prevention protocol. Improper diagnosis could delay treatment, lead to unsuitable medications or development of inadequate vaccine protocols. Identifying porcine respiratory disease complex (PRDC) antigens or antibodies via serology or oral fluid testing is useful to identify exposure to pathogens. However, most often this does not indicate whether the interventions put in place properly address antigen exposure. Proper tissue diagnosis allows practitioners to identify the pathogen and histologic damage in the target tissue indicative of disease from the etiologic agent.
Materials and Methods
Eight finisher farms from six production flows in NC, USA had finishing pigs exhibiting clinical signs of coughing plus/minus sneezing, nasal discharge, and labored breathing. M. hyopneumoniae (M.hyo) was listed as a top differential. A minimum of three acutely clinical, non-treated pigs were selected from each farm and humanely euthanized. Fresh and 10% formalin-fixed samples included: four lung sections (transition areas of normal/pathology), lymph nodes and tonsil. Cooled samples were shipped overnight and screened on histopathology, immunohistochemistry, PCR, and culture. Farms were considered diseased when one or more pigs tested positive for both pathology and antigen.
Results
One of the eight finisher farms was not diagnosed with disease. Two farms were diagnosed with a single disease, one with PRRS and one with Influenza Type A virus (IAV). Four farms were diagnosed with dual diseases: three with PRRS and IAV and one with IAV and M.hyo, concurrently. The eighth farm was diagnosed with PRRS, M.hyo and IAV. All PRRSv sequenced were type II field strains. IAV typing indicated either H1N1 or H1N2 infection. All six flows were PRRS infected and five were IAV infected. No bacteria were identified as primary pathogens.
Conclusions
All pigs were vaccinated for PCV2 and M.hyo, but not for PRRS. Co-infections with PRRS, by flow, then IAV, by site, were most common. M.hyo was the least common finding even though all flows were considered M.hyo positive and presented with clinical signs suggestive of M.hyo. In both cases, there were viral co-infections. In response to this investigation, all six flows have added modified live PRRS vaccine vaccination of wean-age pigs. Three flows have elected to use a commercial trivalent PCV2, M.hyo and PRRS vaccine.

P088

SEROCONVERSION IN PIGLETS EXPERIMENTALLY INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS

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Introduction
The virus Bovine Viral Diarrhea (BVDV), has enveloped virus with a single-stranded RNA genome and positive polarity, belonging being of the family Flaviviridae, genus Pestivirus member. Congenitally infected piglets seem to excrete large amounts of virus, young piglets kept in contact with the virus possessed a fast seroconversion with high antibodies levels. It's evident that in swine's BVDV infection there is the neutralizing antibodies development. The BVDV infection may manifest many clinical forms, from subclinical conditions until the animal's death.
Materials and methods
Six seronegative pigs of 21 days had been selected. The zero day (D0) was the day of animal's inoculation and then, placed in the isolators. Randomly divided into three groups in pairs in the insulators. The Bovine Viral Diarrhea Virus Type-1 (BVDV-1), was inoculated by the oronasal route with a dose of 1 x 107 TCID50 in 2 ml of BDV EMEM (Eagles Minimal Essential Medium). The inoculum was administered through a nasal catheter (0.5 ml per nostril) and 1 mL administered orally, simulating a natural infection. The animals were kept in stainless steel insulators (0,80m x 0,80m x 1,30m), fully enclosed and specially designed for epidemiological studies. The model was developed from prototype by TORREMORELL et al. (1997) and used by Oliveira et. al. (2010). Blood samples were taken to obtain the antibody response on days D-7, D0, D4, D8, D12, D16 and D18. The laboratory test used for analysis of seroconversion of piglets was the virus neutralization (VN). All serum samples were tested in duplicate, with successive dilutions of 1:10 to 1: 5120 as recommended by the "Manual of Diagnostic Tests and Vaccines of Terrestrial Animals Code" (OIE, 2009).
Results
In this period, the piglets had normal temperature in daily reviews. The group infected with BVDV-1 showed diarrheal frames up to the D7 and showed low clotting in blood collection, and DHF are clinical signs of BVDV in pigs. Appetite and water consumption were normal. The control group of piglets not seroconverted during the experimental period, which was expected. The pigs of infected groups seroconverted on the sixteenth day (D16) of the experimental period, and one of the piglets showed viral titration 1x10-5 and another got 1x10-7 of viral titration.
Conclusion
The pigs inoculated with the virus Bovine Viral Diarrhea Type 1 seroconverted on the 16th day of the experimental period and persisted until the day of slaughter.
Financial support by FAPESP (2014/13590-3)

P089

RISK FACTORS ASSOCIATED WITH BVDV-2 INFECTION IN FINISHING PIGS

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Introduction
Epidemiological studies consider cattle as the main BVDv host and the most important infection source to swines and other ruminants (Vannier; Albina, 1999; Ridpath, 2010). Deng et al. (2012) claims that the BVDv prevalence in swine herds is closely related to the disease occurrence in cattles of the property. The transmission can also occur due the use of or other milk derivates from infected cattle in the feeding of swines (Terpstra and Wensvoort, 1988) or by the use of contaminated fomites (Carbrey et al.,1976). This study focused on evaluating the anti-BVDv-2 antibodies occurrence and the associated risk factors.
Material and methods
1705 samples were tested using the virusneutralization test (VN) to detect antibodies anti-BVDv, the methodology used was the one according to "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals" (OIE, 2014). The strain used in th VN was VD-253 that belongs to genotype 2 of the virus.
A structured epidemiological questionnaire was applied in 33 farms where the animals were originated. All farms that had one positive animal were considered as "cases" and the univariate analysis was done. The logistic multivariate regression was done for all variables with p value < 0.2 in the univariate statistics using Epi Info 7 Software.
Results
63.6% (21/33) of the herds were positive in the detection of antibodies antiBVDv-2. Significant association with0 BVDv-2 positive cases were observed on the logistic regression to the risk factors: trucks were not washed and disinfected (p=0.0077) and the presence of visitors that did not respect 72 hours fallowing (p=0.0491).
Conclusion
The application of biosecurity measure is of utter importance to the non-occurrence or reduction of the prevalence of infections on animal rearing. The risk factors observed in this study should be taken into account since preventing them helps to reduce or eliminate the infectious agent introduction when correctly applied.
Financial support by FAPESP (2014/13590-3)

P090

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTION: FINDINGS OF A BASELINE SURVEY OF SLAUGHTER PIGS IN THE UK

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Introduction:
Porcine reproductive and respiratory syndrome virus (PRRSv) was first confirmed in the UK in 1991 and is now considered endemic. A study in 2008-09 estimated that there was active PRRSv circulation on 35.1% of farms in Britain. The economic impact of PRRS on pig farming is significant with costs associated with production losses, increased mortality, treatment, and disruption to breeding programmes. Better understanding of the current prevalence and the epidemiology of PRRSv in the UK, with confirmation that only geotype 1 has been detected so far, is needed in order to target control measures appropriately and assess the effectiveness of interventions.

Materials and Methods:
Between January and May 2013, samples were collected from pigs in 14 abattoirs in England and Northern Ireland to study the prevalence of a variety of pathogens.
Plasma samples were tested for antibodies to PRRSv by ELISA using the IDEXX PRRS X3 assay.
Tonsil samples from pigs which tested positive or inconclusive in the ELISA were then tested by a real-time RT-PCR detecting both genotype 1 and genotype 2 PRRSv.
Sequencing of the PRRSv ORF5 gene was then performed on several PCR-positive tonsil samples. The resulting sequences were analysed and compared to a library of British PRRSv strains.
Pigs were classified as PRRSv-positive if they tested positive in either the antibody ELISA and/or the diagnostic PCR.

Results:
A total of 621 plasma samples were tested for antibodies to PRRSv, of which 362 were seropositive. After accounting for clustering of pigs within farms the seroprevalence was 58.3% (95% CI 53.1-63.4).
Of the 372 tonsil samples subsequently tested by PCR, 31 were positive for PRRS genotype 1viral RNA. After accounting for within-farm clustering, the prevalence of PRRSv RNA-positive tonsils in seropositive/ inconclusive pigs was thus 8.3% (95% CI 5.5-11.2).
The sequence of the ORF5 PRRSv gene was determined from 23 PCR-positive tonsil samples. The diversity of these sequences was similar to that of other PRRSv samples from Britain, and no particular clustering was observed.

Conclusion:
This was the first structured UK-wide prevalence study for PRRSv in pigs at slaughter and the seroprevalence indicates significant exposure of UK pigs to PRRSv. The detection of PRRSv RNA in 8.3% of seropositive pigs suggests that a proportion of healthy pigs are infectious at slaughter. No genotype 2 PRRSv was detected, supporting the absence of this type from UK pigs. The sequences of the viruses reflect the continuing increase in diversity of PRRSv also detected in recent years in other British submissions, with no distinct novel clades being found.

P091

MANAGEMENT CHARACTERISTICS OF PCV2 PUCS STABLE SOW HERDS

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Introduction
Porcine circovirus type 2 (PCV2) vaccines administered at weaning age have been shown to protect pigs effectively in downstream production. Understanding why some sow herds have elevated PCV2 vertical transmission rates may allow us to further improve the health of the entire herd. Previously, Baumert described six sow herds with varying degrees of PCV2 sow herd stability; four of which had 10 of their 12 sampling periods with >80% of the litters positive for PCV2 at birth. To compare and contrast stable and unstable herds, the authors repeated PCV2 testing on six herds that were expected to be stable/low shedding and two herds with high levels of vertical transmission based on previous colostrum sampling.
Materials and Methods
A demographic, production and health survey, along with placental umbilical cord serum samples (PUCS, n=692, ~30/month for 3 month period per farm) for 8 sow herds (herd size R:1,258-4,424 sows, avg parity R:2.7-3.2) were completed. To collect a PUCS sample, the expelled placenta was inverted and 3-4 placental umbilical cords not visibly contaminated by the environment were milked into a single serum tube. All PUCS samples were tested as individuals, in triplicate, using TaqMan real-time PCR reagents (Life Technologies Corp., NY, USA, HMC, Ames, IA, USA) with a detection limit of below 3.5 genomic equivalents/reaction.
Results
Only four of six expected low positive herds had positive samples (R:1.0-4.4%). The two expected unstable herds were 50.6-63.5% positive. Two noticeable differences identified in the survey between former participants (high percent positive) plus the two high positive herds in this study and the low percent positive/stable herds were 1) PCV2 vaccination of select age gilts and 2) recent herd closures in the current study's low positive/stable herds.
Conclusions
Both gilt PCV2 vaccination and whole herd closure could have an impact on the stability of PCV2 due to alteration of the immune system through repeat vaccination of gilts and decreased pathogen propagation. This has been proven in the cases of PRRS as well. These observations support Baumert's work showing herd PCV2 stability coincided with either dam/gilt PCV2 vaccination or sow herd closure. Although piglet PCV2 vaccination must continue, we may be able to alter the vertical transmission of PCV2. Future studies specifically addressing the questions of gilt and sow herd vaccination and stability following herd closures are drafted.

P092

ASSESSING SOW HERD PCV2 STABILITY AND CROSS-SECTIONAL ANTIBODY STATUS OF DAMS

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Introduction
Although piglet vaccination is necessary to prevent PCVD downstream, more studies are targeted to understand the vertical transmission of PCV2. With a large variation in placental umbilical cord serum (PUCS) percent positive between herds, veterinarians inquire how the measureable antibodies in sows could be used to assess PCV2 stability/protection. A comparison between percent positive PUCS and cross-sectional sow antibody status was evaluated.
Methods and Materials
Nine herds (A-I, R:1,200-3,800 sows) were evaluated in fall 2014. PUCS samples (n=692, 52-111/herd within a 3 month period) and sow serum (n=274, 20-44/herd across multiple parities, ≥75d of gestation) were collected. To collect PUCS, expelled placenta was inverted and 3-4 placental umbilical cords not visibly contaminated were milked into a single serum tube. All PUCS samples were tested as individuals using TaqMan real-time PCR reagents (Life Technologies Corp., NY, USA) with a detection limit of below 3.5 genomic equivalents/reaction. Sow antibodies were measured by PCV2 ORF2 ELISA (Iowa State VDL, IA, USA) with a 0.3 S/P ratio cut-off.
Results
Two of nine herds (B,D) were PUCS negative. PUCS positive herds were 1%(E), 2%(F), 4%(C,G), 28%(A), 51%(I) and 64%(H) positive. The percent of sows ELISA positive for the entire study group was 93% (R:80-100%). There were significant differences between PCR negative herds (mean S/P ratios B=0.50, D=0.73, $p<0.0001$), between low percent PCR positive herds C,E,F and G (mean S/P ratios 0.48, 0.95, 0.81, 0.81, respectively, $p<0.0001$), between high percent PCR positive herds H and I (mean S/P ratios 0.57, 0.74, respectively, $p<0.001$), between herds H and B or D mean S/P ratios ($p<0.0001$) or between herds I and D ($p<0.0001$). Parity 2 ELISAs were significantly different than parity 1 ($p<0.0001$) but neither were significantly different than the other parities.
Conclusions
S/P ratios did not provide value in making stability or protection conclusions. Unstable herds had S/P ratios similar to the no detectable transmission herds. None of the herds vaccinate sows for PCV2, but herds B-G routinely vaccinate select gilts for PCV2. Sows maintain a PCV2 positive ELISA in all of these conditions regardless of gilt PCV2 vaccination, vertical transmission status and no PCV2 sow vaccination. From this study, there is a recommendation to use PUCS sampling but not PCV2 ELISA to monitor sow herds.

P093

HISTOLOGICAL LESIONS AND DISTRIBUTION OF VIRAL NUCLEIC ACID IN INTESTINAL SAMPLES FROM PIGLETS WITH ACUTE PORCINE EPIDEMIC DIARRHEA

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Introduction
Porcine epidemic diarrhea (PED) has not been diagnosed in Europe for many years. Following recent outbreaks in Asia and America, infections with a PEDV strain originally described in the USA emerged in southwestern Germany.
Material and Methods
Small intestinal samples (n=25) from an acute outbreak of PED on a farrow-nursery farmwere fixed in 10% formalin and subjected to histological investigation. In order to study the quantity and distribution of enterocytes with viral replication, an oligonucleotide probe-based in situ hybridization (ISH) was established.
Results
The entire procedure was set up within five days which proved the suitability of this assay for rapid tissue localization of novel infectious agents. Only few samples showed severe histological lesions, like shortening and fusion of villi with focal necrosis of epithelial cells. The majority of the samples showed only moderate to mild lesions and in some samples there were no objective differences to control samples.
By ISH, viral signals were found in the cytoplasm of enterocytes. The vast majority of infected cells was present in the villi but occasionally there were positive crypt epithelia too. Approximately one third each of the cases was graded as severe, moderate or mild. The amount of positive cells did not exactly correspond with the histological scores.
Conclusion
This investigation shows that not all clinically diseased piglets exhibit pronounced histological lesions and that procedures enabling tissue localization of the infectious agent, like ISH, facilitate etiological diagnosis.

P097

PCV2 SEROLOGICAL STATUS OF SOW HERDS AND A POSSIBLE RELATIONSHIP WITH REPRODUCTIVE DISORDERS: AN ORIENTATION STUDY

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Introduction:
One of the outcomes of PCV2 infections is reproductive disorders. Neutralizing antibodies together with cell-mediated immunity play an important role in protection and amount of neutralizing antibodies is correlated with amount of PCV2-antibodies (IPMA).
The aim of this study was to determine the seroprevalence of PCV2 in sows on different farms, and to investigate a possible relation between the sows' PCV2 profiles and reproductive disorders. The influence of PCV2 sow vaccination and serological status of gilts on sow profiles were also studied. Data originated from ResPig 2013, a program that includes regular cross-serological investigations of 5 samples per production group for 6 diseases (incl. PCV2) as well as clinical/vaccination history.

Materials and methods:
Prevalence: The PCV2 profiles of the sampled sows were categorized in 3 groups: all negative (Cat. 1), mixed negative and positive samples (Cat. 2) and all positive samples (Cat. 3).
The PCV2 sow profiles were compared with notes in the clinical records about sow reproductive disorders to establish a possible relationship. The effect of PCV2 sow vaccination and effect of the PCV2 profiles of incoming gilts on the sow profile were also investigated.

Results:
-PCV2 seroprevalence sows: 88% (35: all sows neg. / 285: tot. farms)
-PCV2 sow profile and % anamneses with reproductive disorders: Cat.1→26%(9/35) Cat.2→24%(51/216) Cat.3→15%(5/34)
- Eleven (11) % of farms vaccinated sows (53%Porcilis PCV,37%CF,10%CV). The relationship between sow vaccination and sow herd seropositivity was significant: 44% of sow vaccinating farms in cat.3 vs 7% in sow non-vaccinating farms (P<0.0001).
- The relationship between gilt and sow PCV2 profiles was also significant (P<0.0001).

Conclusion:
The PCV2 seroprevalence in Dutch sow farms is high (88%). There are more reports of reproductive disorders in farms with a higher % of seronegative Sows, suggesting a protective effect of PCV2 antibodies. The statistical analyses regarding a possible relationship revealed a tendency rather than statistical significance. This may be explained because observations such as abortions and repeat breeders are most likely caused by infections other than PCV2. In addition, the level of antibodies in the sow may have an influence, but this info is not in the ResPig database. Therefore, further analysis of the raw data is needed (titers, specific reproductive problems and presence of other infections). Such analysis is important to manage expectations regarding the protective effect of PCV2 sow and gilt vaccination on reproductive disorders as there is a significant relationship between sow vaccination, incoming gilt status, and sow herd seropositivity.

P098

INFLUENCE OF DIFFERENT BATCH–REARING SYSTEMS ON SWINE INFLUENZA VIRUS SPREAD AND PERSISTENCE IN FARROW–TO–FINISH FARMS USING A STOCHASTIC MODELING APPROACH

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Introduction
Swine Influenza, formerly described as an episodic disease, has been shown to persist in an enzootic form in farrow-to-finish farms. Previous studies on commercial farms showed that Swine Influenza A Virus (SIV) infection generally affects weaned piglets in nursery at a particular age and occurs on each successive batch. However, the transmission process between batches is still not well understood. The aim of this study was to analyze the conditions for persistence and recurrence of SIV in farrow-to-finish pig herds reared with different batch-rearing systems (4-, 5-, 7-, 10- and 20-batches).

Materials and Methods
The within-farm dynamics of SIV was modeled using a stochastic metapopulation compartmental model. A population dynamics model, accounting for different batch-rearing systems, was coupled with a SIV epidemiological model. Two subpopulations were considered, corresponding to breeding sows and growing pigs respectively, and interacting during lactating stage. The infectious process was represented by batch-specific SIRS models, to which were added different compartments accounting for the impact of Maternally Derived Antibodies (MDAs) on piglets infection (partial protection and impairment of the post-infectious immune response). Moreover, an indirect between-batch transmission rate was considered. For each batch system, one infected gilt was introduced in batch 1 in insemination room. Gestating sows were housed in a large dynamic group. The model has been used to evaluate the minimal conditions for virus transmission between batches and persistence at the population level. The impact of batch-rearing systems was also evaluated.

Results
Whatever the batch system of the farms, the introduction of one infected gilt in insemination room resulted in SIV persistence for several months in pig population. Relatively low transmission rate between animals from different batches was found sufficient to produce recurrent infections at fixed age in growing pigs population, a close to field observations. The batch system was shown as a pivotal factor favoring the spread and persistence when short time-intervals were implemented between successive batches.

Conclusion
The introduction of an infected gilt in the breeding sows population reproduced the same dynamic in this modeling approach as observed in commercial farms (i.e. infection of consecutive batches of piglets in nursery). The results of the model showed that a lack of internal biosecurity between batches in the nursery part could partially explain the propagation and maintenance of the virus from batch to batch. Moreover, several batch-rearing systems were found more at risk for SIV persistence at the metapopulation level.

P099

EFFECT OF PORCILIS® PCV VACCINATION ON GROWTH PERFORMANCE IN LATE SUBCLINICAL PCV2 INFECTION

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Introduction
Pigs around the world are infected with PCV2 and it is well known that even low levels of PCV2 virus can have significant negative effects on production, and therefore negative economic impact. The aim of this study was to prove that, even in case of late subclinical infection, vaccination with Porcilis® PCV improves growth performance
Materials and Methods
The trial was carried out in a 4500 sow farm. Before the start of the study a cross-sectional sero profile was completed, and PCV2 infection was confirmed at 18 weeks of age. A total of 999 piglets, all from the same batch, were included in the trial. Piglets were weaned at 24 days of age, transferred to a wean-to-finish unit, and 4 days later, randomly allocated into 2 groups of treatment, according to gender, dam and weight, and were individually identified by ear-tag. The treatment groups were as follows: G1: 500 piglets vaccinated with Porcilis® PCV (2ml/dose) and G2: 499 piglets treated with aqueous adjuvant (2ml/dose) All piglets were individually weighted at 4, 10, 18 and 24 weeks of age, and the Average Daily Weight Gain (ADWG) of each group was determined. Mortality data was also recorded.
The individual pig served as the statistical unit, and the statistical tools used were ANOVA and Tukey's HSD Test.
All management and other factors remained the same for all treatment groups.
Results
The vaccinated animals had a significantly higher weight at 18 weeks (G1 73,33 kg vs G2 71,74kg; $p<0,001$) and 24 weeks of age (G1 108,53kg vs G2 105,65 kg; $p<0,001$). This significantly higher growth also resulted in statistically higher ADWG during the fattening period. From 10 to 24 weeks, the ADWG of the vaccinated group was 32g/day higher compared to the control group (G1 820g/d vs G2 788 g/d; $p>0,001$).
The mortality rate was not significantly different between groups throughout the observation period from 3 to 24 weeks (G1 4,8% vs G2 5%; $p=0,2$).
Discussion
Porcilis® PCV is a highly efficacious and immunogenic vaccine based on the capsular protein of PCV2 virus, with X-Solve as adjuvant. This study confirms the data obtained in previous trials and supports that Porcilis® PCV has a positive impact on performance even in farms with late and subclinical PCV2 infection. Considering only the increase in market weight, the ROI of vaccination was 3.5, demonstrating that Porcilis® PCV is a profitable investment.

P100

VALIDATION OF A PRRS SEROLOGICAL DIAGNOSIS USING SOW COLOSTRUM

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Introduction
Although several PRRS serological methods exist, collecting blood or oral fluid samples from sows for such testing is difficult. The aim of this study was to investigate the possibility of using individual or pooled sow colostrum samples for PRRS antibody detection, which is an easy sample in the field and pooling samples lowers the cost of individual analysis.
Materials and Methods
The study included 18 PRRS free herds with various vaccination and feeding protocols to control the specificity of the diagnosis. In each herd, colostrum of 10 young sows (First or second parity) and 10 older ones (i.e third parity or more) were sampled. In addition, 6 known PRRS infected herds were selected, in which a total of 72 paired sera/colostrum were collected to test the sensitivity of this method. All samples were tested with the Idexx 3XR PRRS Antibody Elisa kit at Labocea Diagnostic Laboratory.
Results
The specificity of the diagnosis on individual colostrum was calculated as 98.8 % (confidence interval 96,7 – 99,6), and the specificity on pooled colostrum was 100 % (CI pools: 71,5 – 99,8). At the herd level, the specificity, was 82,4% on individual samples (taking into account 3 false positive results among 260 individual colostrum samples, originating from 3 out of 17 herds), while the herd's specificity on pooled colostrum was 100 %. The sensitivity of the diagnosis on individual and pooled samples was optimal (100%) (CI individuals: 95-100; CI pools: 71,5-100).

Conclusion

Based on the results from this study, PRRS serological diagnosis on sow colostrum appears to be a new and simple way to monitor the status of PRRS negative herds, and the rate of positive animals in infected sow herds.

P101

DEVELOPMENT OF ACUTE PHASE PROTEINS FOLLOWING IMMUNIZATION WITH TWO COMMERCIAL PCV2 VACCINES IN PRE–WEANING PIGLETS.

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Introduction
Vaccination rates against PCV2 are close to 100% in many swine markets. Acute phase proteins (APPs) have been proposed as suitable veterinary biomarkers to monitor stress, for detection of inflammation and infections and for disease monitoring. In addition, C-reactive protein (CRP) has recently been proposed as a potential biomarker in vaccine safety studies. The aim of this study was to evaluate the development of haptoglobin (Hp) and CRP after vaccination with two commercially available PCV2 vaccines in piglets around weaning.
Materials and Methods
Two groups of 16 piglets(1 male and 1 female in each of the 8 liters per group) were vaccinated, 3 days before weaning (22 days of age) with a single injection (1 mL) of Ingelvac CircoFLEX® (Boehringer Ingelheim, Spain, SA) or with a single injection (2 mL) of Suvaxyn PCV® (Zoetis Spain, SLU). Blood samples and weight of each animal were taken before vaccination, 24 h after vaccination (24 h Post-V) and 48 h after vaccination (48 h Post-V). The rectal temperature was recorded before and 8 h after immunization. The Hp and CRP concentrations in serum were determined using an automatic biochemical analyzer.
Results
For both treatment groups the CRP and HP levels were significantly higher at 24 and 48 hours after vaccination when compared to the baseline values before vaccination. This increase was significantly greater in the group of piglets vaccinated with Suvaxyn PCV®. After 24 h Post-V, Hp concentrations were higher in animals vaccinated with Suvaxyn PCV® that in animals vaccinated with CircoFLEX®. Similarly, the CRP concentrations were also significantly higher in animals vaccinated with Suvaxyn PCV®. 8 h post immunization, the rectal temperature was significantly higher in animals vaccinated with Suvaxyn® (40.2 ºC) compared with the animals vaccinated with Ingelvac CircoFLEX® (39.8 ºC) ($P < 0,05$). The average weight gain for the 24 hours period post vaccination was significantly higher in animals vaccinated with Ingelvac CircoFLEX® (100 gr) compared to the animals vaccinated with Suvaxyn PCV® (0.06 gr) ($P < 0,05$).
Discussion and Conclusions
The immunization with both vaccines increased significantly the APPs concentrations compared with basal levels. However, the production of APPs, and mainly the CRP, has been higher and more persistent in animals vaccinated with Suvaxyn PCV® compared to CircoFLEX®. This more pronounced response in APP(stress and inflammation biomarkers) levels after vaccination can explain the differences in rectal temperature, weight gain and local reactions between animals vaccinated with different products which were observed in this and other studies .

P102

SAFETY OF PORCILIS® PCV: EFFECT OF VACCINATION ON GROWTH PERFORMANCE DURING NURSERY PERIOD IN A FARM WITH A LATE PCV2 INFECTION

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Introduction
PCV2 vaccines are an essential tool when designing disease control strategies. Usually they are applied at 3 or 4 weeks of age, during a period when piglets are subjected to quite a lot of stress. Therefore, some concerns have been raised about the safety of these vaccines and their effect on feed intake and growth performance in the post-vaccination period. The objective of this trial was to study the safety of Porcilis® PCV, evaluating its effect on growth performance during the nursery period compared with a control group.
Materials and Methods
A blinded, randomised and controlled trial was conducted in a 4500 sow farm located in the north-east of Spain. Before the start of the study PCV2 age of infection was confirmed at 18 weeks. Piglets were weaned at 24 days of age and moved to a wean-to-finish unit. Four (4) days later, 999 piglets were individually ear tagged and randomly allocated into 2 treatment groups according to gender, dam and weight. (G1: 500 piglets vaccinated with Porcilis® PCV; G2: 499 piglets treated with aqueous adjuvant).
Vaccine was pre-heated (25-28ºC) and shaken before injection. Twenty (20) control animals were bled at 4, 7 and 10 weeks of age to ensure that no contact with PCV2 virus had occurred during the study period (Ingezim PCV ELISA).
Local and systemic reactions were recorded at the time of vaccination and for the next 24 hours.
All piglets were individually weighed at 4 and 10 weeks of age, and the ADWG for each group was determined. Mortality data was also recorded. The individual pig served as the statistical unit (test used ANOVA and Pearson's Chi-square test).
Results
No local reactions were recorded in either group. In G1, 5 piglets had minor systemic signs that disappeared spontaneously a few minutes after injection.
Serological results of G2 confirmed no contact with PCV2 field virus. Initial weight was comparable at the beginning of the trial (G1 6,33kg vs G2 6,34kg). At 10 weeks of age, no statistical differences were found in regards to weight (G1 23,49kg vs G2 23,89kg; $p>0,05$) nor ADWG (G1 390gr vs G2 398 gr; $p>0,05$). Mortality was also the same between treatment groups (1,6%).
Discussion
Vaccination with Porcilis® PCV has no negative effect on nursery mortality and growth performance. In addition, vaccinated pigs had increased growth rate compared to the controls during the fattening period, demonstrating therefore the efficacy of the vaccine in the face of a PCV2 infection.

P103

THYMOCYTE DEPLETION IN EXPERIMENTAL HIGHLY VIRULENT PRRSV-1 INFECTION

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Introduction:Porcine reproductive and respiratory syndrome virus (PRRSV) shows high differences among and within genotypes. The aim of this study was to evaluated changes in the thymus of piglets infected with PRRSV-1 strains of different pathogenicity. Material and Method: Fifty-four, 5-week-old, male piglets were inoculated by intranasal route with sterile medium (control group) or with one of three different PRRSV-1 strains (105.0 TCID50): Lelystad virus strain (LV), the British field strain 215-06 and the highly pathogenic Eastern European strain SU1-bel. Animals were clinically monitored and euthanised at 3, 7 and 35 days post infection (dpi). Samples from thymus were routinely processed for histopathological and immunohistochemical studies by using specific antibodies against PRRSV, CD3 and TUNEL. Results:PRRSV antigen was detected in thymus from 3dpi until the end of the study in all virus-infected animals except control group. The cortex/medulla ratio and the number of total thymocytes and CD3-positive cells per mm2 were lower in all inoculated animals than in controls. A higher number of tingible-body macrophages was detected in animals infected with SU1-bel strain from 3dpi onwards, and in a lesser extent in LV and 215/06 infected animals. The detection of cell death by TUNEL was increased in the cortex of the thymus in all infected groups with respect to control group at 3 and 7dpi, and in turn in SU1-bel infected animals with respect to the other infected groups. SU1-bel group showed more severe changes than the other PRRSV-1 infected animals at 7dpi. Conclusion: Our results point out that different PRRSV-1 strains induce a different degree of depletion of the thymic cortex , with an increased number of tingible-body macrophages and cell death. Moreover, the highly pathogenic SU1-bel strain induced a higher depletion of thymocytes in the cortex than the other PRRSV-1 strains included in this study, producing a moderate atrophy of the thymus.

P104

SWINE INFLUENZA AND BACTERIAL RESPIRATORY DISEASE OUTBREAKS IN FINNISH FATTENING PIGS

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Viral agents like swine influenza have been suspected to predispose the pig to bacterial respiratory infections. Our aim was to find out presence of swine influenza virus (SIV) antibodies with bacterial infections in acute respiratory outbreaks in fattening pigs.

The study was carried out in Finland from May 2011 to October 2013. The study population consisted of herds with acute respiratory symptoms in fattening pigs in 20 farms. The herds had a median of 765 fatteners (100-2184). Samples were taken within two days after receiving the information about the acute respiratory disease outbreak. In each farm, 3 pigs (1-4) were euthanized and their lungs were sent to laboratory examination including bacterial culture. A blood sample and nasal swab was taken from 20 pigs. All nasal swabs were subjected to SIV determination with PCR and 15 serum samples were tested for SIV antibodies with ELISA and hemagglutination inhibition test, the latter only for ELISA positive samples. A herd was considered SIV positive with at least two positive SIV antibody result. Accordingly, a herd was considered to have a bacterial infection, if at least one bacterial sample from the lung yielded a positive result. After one month each farm was visited again and same pigs were bled again. A herd was considered having increased SIV antibodies if the number of positive samples was bigger than during the first farm visit. Fifteen samples per herd were analyzed for PRRS and swine enzootic pneumonia (SEP) with ELISA.

18 out of 20 farms had a bacterial infection. Out of these, 16 herds had APP, and 2 had other bacterial infections; S. aureus, Haemophilus spp. or Streptococcus spp. Seven bacterially positive farms were serologically SIV-positive (H1N1) at the first visit, which is 39% of all farms with bacterial infection. Three farms of those seven had increasing SIV antibodies in the second sample. The rest of the herds (n=13) had no SIV antibodies in any of the samples. All nasal swabs were negative to SIV. All herds were free from PRRS and SEP.

Swine influenza H1N1 introduced in Finland for the first time in 2008, and since then sporadic influenza outbreaks have occurred. According to our results, active swine influenza infection diagnosed as rising SIV antibodies occurred together with a bacterial infection in about 17% of the cases. SIV might be one predisposing factor for acute bacterial respiratory disease in some outbreak cases, but certainly not the only one. Increasing the number of SIV antibodies during this one month period implies that there is a co-infection with SIV and bacterial agent.

P105

RING TRIALS ON THE DETECTION OF PATHOGENS IN ORAL FLUID BY SEROLOGY OR PCR IN A EUROPEAN INFORMAL NETWORK

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Members of 18 diagnostic laboratories from 13 EU countries, regrouped in an informal 'European network of diagnostic laboratories on swine oral fluid', performed two ring trials on the detection of PRRSV, PCV2 and SIV, coordinated by Labocea (France) and funded by Merial.
First ring trial: field samples of oral fluid (OF)
Phase 1 compared the capability of each network member to perform common and separate analytical methods for the detection of PRRSV, PCV2 or SIV in shared samples of native (i.e. unspiked, field-collected OF. This ring trial was organised by Merial and Labocea, which prepared and froze multiple batches of four sample panels (one per test), with 6 samples per panel. Each batch was simultaneously sent to all participating laboratories (n=11). They performed four tests: a semi-quantitative ELISA specific for anti-PCV2 antibodies (Ab), a qualitative ELISA specific for anti-PRRSV Ab, a qualitative RT-PCR method specific for the genome of SIV and a qualitative RT-PCR method specific for PRRSV genome. Results, collected and processed by Labocea were found to be satisfactory for serology (concordant results in 7/9 laboratories for PCV2 and 7/10 for PRRS), pointing at a good potential for serology standardisation on native OF in the network. Results were highly heterogeneous for the RT-PCR methods.
Second ring trial: PRRSV-spiked samples
Phase 2 focused on the detection of PRRSV by the RT-PCR methods and using sera and OF collected in a Specific Pathogen Free farm, spiked with two different EU-PRRSV strains. For this second ring trial, OF samples were pooled and centrifuged at Labocea, and were sent frozen (along with the sera panels) to the participating laboratories (n=14). They performed the spiking, and ran two different RT-PCR methods: the method they routinely used for diagnostic purposes, and a common 'troubleshooting' method, kindly provided by Thermofischer. Results were collected and processed by Labocea. At the 10-2 spike dilution, all but one of the participating laboratories detected the sera and OF spiked with either PRRSV strain, by both methods. One laboratory failed to detect one strain (but detected the other). For further dilution values, high differences were observed between the individual laboratories (up to two orders of magnitude). These results may explain the discrepancies observed between laboratories in ring trial #1. They also demonstrate that a coordinated and multidisciplinary work is feasible with a large number of laboratories, to improve the reliability of analytical results in Europe.

P106

THYMOCYTE DEPLETION IN EXPERIMENTAL HIGHLY VIRULENT PRRSV–1 INFECTION

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INTRODUCTION
Porcine reproductive and respiratory syndrome virus (PRRSV) shows high differences among and within genotypes. The aim of this study was to evaluated changes in the thymus of piglets infected with PRRSV-1 strains of different pathogenicity.

MATERIAL AND METHOD
Fifty-four, 5-week-old, male piglets were inoculated by intranasal route with sterile medium (control group) or with one of three different PRRSV-1 strains (105.0 TCID50): Lelystad virus strain (LV), the British field strain 215-06 and the highly pathogenic Eastern European strain SU1-bel. Animals were clinically monitored and euthanised at 3, 7 and 35 days post infection (dpi). Samples from thymus were routinely processed for histopathological and immunohistochemical studies by using specific antibodies against PRRSV, CD3 and TUNEL.

RESULTS
PRRSV antigen was detected in thymus from 3dpi until the end of the study in all virus-infected animals except control group. The cortex/medulla ratio and the number of total thymocytes and CD3-positive cells per mm2 were lower in all inoculated animals than in controls. A higher number of tingible-body macrophages was detected in the thymic cortex of SU1-bel-infected animals from 3dpi onwards, and in a lesser extent in LV and 215/06 infected animals. The detection of cell death by TUNEL was increased in the cortex of the thymus in all infected groups with respect to control group at 3 and 7dpi, and in turn in SU1-bel infected animals with respect to the other infected groups. SU1-bel group showed more severe changes than the other PRRSV-1 infected animals at 7dpi.

CONLUSION
Our results point out that different PRRSV-1 strains induce a different degree of depletion the thymic cortex, with an increased number of tingible-body macrophages and cell death. Moreover, the highly pathogenic SU1-bel strain induced a higher depletion of thymocytes in the cortex than the other PRRSV-1 strains included in this study, producing a moderate thymic atrophy.

P107

USE OF AIR FILTRATION SEGREGATE FLOWS (NEGATIVE AND POSITIVE) IN THE FACE OF A PRRS BREAK

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Air filtration has gained popularity in the USA swine industry as the final part of a complete herd biosecurity program. This is particularly important for farrow to wean farms located in pig dense areas. Air filtration in conjunction with a complete biosecurity program has been successful in reducing the incidence of PRRS and Mycoplasma outbreaks. . Using historical records, herds that were breaking approximately 50% of the herds every year before air filtration are now only breaking 12% of the herds after air filtration. This is a dramatic reduction and technology in filtration practices has continued to improve, reducing the rate even further. The herd in this case study had used air filtration to separate Mycoplasma hyopneumoniae positive and negative populations in a successful elimination program.
Materials and Methods:
This site is 8000 sow farrow to wean site weaning 6-7 kg pigs to offsite nurseries. The sow farm had a PRRS break within the main population of the farm. When the outbreak started the gilts that were all naive to natural infection but that had been vaccinated with PPRS MLV were showing more severe clinical signs in farrowing, experiencing high morbidity and mortality. The pregnant gilts (due to farrow in 2 days) were housed in a separate facility located across the road from the sow site in the gilt developer that grows the gilts up from 6-7 kg gilts to entry into the herd at the time of farrowing. The gilts are from a PRRS negative source.
Because the facility had been developed from a number of expansions, there were a number of different farrowing buildings. It was decided that one of the newer buildings located on the perimeter of the complex was the right size to farrow all the gilt litters and keep them separate until weaning. Also offspring could go to a different flow in the nursery. The hallway was closed off/sealed. Gilts were delivered through a temporary load in area. Workers showered in the company office and came through the same temporary entrance.
Results
The farm was able to wean approximately 11000 pigs (approximately 10 weeks' worth of gilts) that were PRRS negative in the face of the outbreak that otherwise would have had high preweaning mortality There was one room that had a PRRS-positive litter, but they were weaned out early to avoid contamination.
Conclusions
Air filtration allowed for different PRRS status management within the same complex, allowing for management of the PRRS outbreak in a new way. More pigs were able to be weaned in this time frame resulting in a better cash flow through the outbreak. This case study demonstrates that with the tools of air filtration and biosecurity veterinarians and producers can be more creative in managing herds during disease outbreaks.

P108

NEW NEONATAL PORCINE DIARRHOEA SYNDROME IN DANISH PIGS: CHARACTERISATION OF VIRAL FINDINGS IN DISEASED AND HEALTHY CONTROL ANIMALS

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Introduction
Since 2008, field experiences on a new diarrhoeic syndrome in neonatal piglets referred to as New Neonatal Porcine Diarrhoea Syndrome (NNPDS) have been reported in Denmark and elsewhere. The prevalence of well-known enteric pathogens, gross- and histological findings in age-matched diarrhoeic- and non-diarrhoeic piglets from four Danish herds has previously been reported. Briefly, no association between the presence of diarrhoea and the detection of enterotoxigenic Eherichica coli, Clostridium perfringens type A or C, Rotavirus A (tested by antigen ELISA), Coronavirus, Clostridium difficile, Cryptosporidium spp, Giardia spp, Cystoisospora suis or Strongyloides ransomi was revealed.
The aim of the present study was to complete a more detailed investigation on possible viral factors involvement in NNPDS.
Material and methods
Four well-managed herds experiencing neonatal diarrhoea suspected to be NNPDS, were selected and 989 piglets within these herds were clinically examined on a daily basis. Samples from diarrhoeic and non-diarrhoeic piglets at the age of three to seven days were selected for extensive virological examination using specific PCRs and general virus detection methods.
Results
Of the tested animals, 9% were rotavirus A positive when tested by PCR. A total of 47 case and 49 control animals were tested for porcine astrovirus type 1-5, kobuvirus and teschovirus. 93.6% of the case animals and 89.9% of the control animals were positive for kobuvirus. All animals tested negative for astrovirus type 1, 2, 4 and 5, while 63.8% of the case and 75.5% of the control animals were positive for type 3 astrovirus. For teschovirus only three out of 96 animals were positive. No animals tested positive for rotavirus C, coronavirus (TGEV/ PEDV), Sapovirus, Enterovirus, Parechovirus, Saffoldvirus, Cosavirus, Aichivirus or Klassevirus. Microarray-analyses performed on a total of 18 animals were all negative, as were eight animals examined by Electron Microscopy. Using Next Generation de novo sequencing on pools of samples from case animals within all herds, kobuvirus, rotavirus A, rotavirus C and teschovirus were detected in 4, 1, 1 and 1 of the four herds, respectively.
Conclusion
The analyses performed on enteric samples in the study did not suggest a significant contribution of viruses to NNPDS. Still, a systemic virus may play a role in the pathogenesis of NNPDS and further investigations are needed to scrutinize that possibility.

P109

COURSE OF PCV2 VIREMIA IN PIGS FROM GERMAN FARROW–FINISH FARMS WITH DIFFERENT VACCINATION STRATEGIES AGAINST PCV2

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Introduction
Immunization of piglets against PCV2 can be carried out through passive immunization via antibody-containing colostrum from PCV2 vaccinated dams or as an active immunization using a piglet vaccine. Both strategies are known to be effective in preventing pigs from PCVD. In the present study the course of PCV2 viremia in pigs from farms with different vaccination strategies against PCV2 was followed from birth until slaughter.
Material Methods
A total of 9 German farrow to finish farms (piglet vaccination: group A, n=3; 43 sows, 127 piglets; sow vaccination: group B, n=3, 43 sows, 129 piglets; no vaccination against PCV2: group C, n=3, 43 sows, 127 piglets) were included into this investigation. Blood was collected from sows and piglets from one farrowing batch / farm. Blood samples were collected within 3 days after birth (sows and piglets) and at 3, 8, 12, 16, 20, 24 weeks of age (piglets). Antibodies against PCV2 (in ELISA units, EU) in the serum of the sows as well as PCV2 viral load (genome copies / ml serum) and in the serum of the sows and piglets were quantitatively analyzed.
Results
At time of farrowing sows of group B (6.28*103 EU) had significantly higher antibody titers than sows of groups A (1.34*103 EU) and C (9.09*102 EU). Non of the sows were found to be viremic at time of sampling. Within the investigational period viremia was detected in 7.8%, 65.4% and 94.5% of piglets from group A, B and C, respectively. The chance to become viremic for unvaccinated animals was 204 times higher than for actively immunized piglets and 9 times higher than for passively immnized piglets. For the passively immunized piglets the chance to become viremic was 21 times higher than for actively imunized piglets. None of the active immunized piglets reached levels of PCV2 viremia > 105 genome copies / ml, whereas 10.1% of the unvaccinated and 7% of the passively immunized animals had levels of viremia > 105 genome copies / ml serum. Onset of viremia was similar for pigs from groups B and C (12th week of life) whereas pigs from group A showed a later onset of viremia (16th week of life).
Conclusion
Although both, active and passive immunization against PCV2 are known to be effective in preventing pigs from PCVD active immunization seems to be superior in reducing the amount of viremic pigs and levels of viremia in finishing pigs compared to passively immunized piglets. Additionally onset of viremia is later for active immunized pigs compared to passively immunized pigs or pigs from farms without vaccination against PCV2.

P110

DISTRIBUTION OF PCV2 GENOTYPES A AND B IN FARMS WITH DIFFERENT VACCINATION STRATEGIES IN GERMAN FARROW TO FINISH FARMS

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Introduction
Since 2004/2005 the predominant PCV2 genotype in the domestic pig population has become PCV2b. All commercially available vaccines against PCV2 registered in Germany are based on PCV2a. In this field examination a look inside farms with different vaccination strategies against PCV2 should give information about the distribution of PCV2a and PCV2b under different vaccination strategies against PCV2.
Material and methods
A total of 383 pigs from 9 German farrow to finish farms (piglet vaccination: group A, n=3; 127 pigs; sow vaccination: group B, n=3,129 pigs; no vaccination against PCV2: group C, 127 pigs) were included into this investigation. Blood samples were collected within 3 days after birth and at 3, 8, 12, 16, 20, 24 weeks of age and quantitatively examined. PCV2 positive samples were further analyzed to differentiate between PCV2a and PCV2b.
Results
Within the entire study period 52.7% out of all 383 pigs were positive for PCV2b whereas only 3.9% where positive for PCV2a. The mean viral load over the time for PCV2b (4.53x10β) was significantly higher than for PCV2a (8.49x10β) (p = 0.002). On farms with piglet vaccination no PCV2a positive pigs were detected whereas 7.8% of the pigs were positive for PCV2b. For pigs from farms with sow vaccination against PCV2 the distribution was 6.3% and 59.1% for PCV2a and PCV2b respectively. On farms with no vaccination against PCV2 the distribution was 5.5% PCV2a and 92.1% PCV2b. Within the pigs from non-vaccinating farms 4, 2 and 1 pig/s were positive for both, PCV2a and PCV2b in the 16th, 20th, and 24th week of life respectively. 16 PCV2 strains were sequenced exemplarily. 11 strains could be assigned to PCV2b clade 1A and 2 to PCV2b clade 1B, 3 PCV2a strains were assignable to clade 2D.
Conclusions
PCV2a can still be detected in low amounts within the German domestic pig population. Interestingly no PCV2a was detectable within the group of actively vaccinated pigs but in pigs passively vaccinated or non-vaccinated against PCV2. Additionally, the overall mean viral load of PCV2 was significantly higher for PCV2b than for PCV2a. Further investigations are needed to determine whether these findings can be reproduced based on a higher amount of farms with different vaccination strategies under field conditions.

P111

MEANING OF THE PARITY OF SOWS FOR PCV2 VIREMIA IN THEIR OFFSPRING

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Materials and Methods
129 sows and 383 piglets from farms with different vaccination schemes against PCV2 (3 farms: group A sow vaccination; 3 farms: group B piglet vaccination; 3 farms: group C no vaccination) were included into this investigation. Piglets were assigned to groups accordingly to the parity of their mothers (Table 1). The amount of viremic pigs and their viral load were evaluated from birth until slaughter (blood sampling at 3 days after birth and at 3, 8, 12, 16, 20, 24 weeks of age).
Results
The total amount of viremic pigs within the investigational period for gilts, 2nd-4th litter sows and >4th litter sows was 55.6%, 52.0%, 51.4%, respectively (group A: 63.2%, 54.3%, 63.0%; group B: 15.6%, 4.8%, 2.4% and for group C: 92.7, 97.7, 92.9%). A significant difference concerning the amount of viremic pigs within the entire study period was observed for pigs from group B. In this group 15.6% of the offspring from gilts and only 2.4% of the offspring from > 4 litter sows became viremic (p= 0.034, OR: 5.28).
Regarding the vaccination schemes of the farms a significant difference concerning the amount of viremic pigs within the entire study period pigs was detected for pigs from group B. Within group C pigs from sows > 4 litters from had significantly higher viral loads in the serum at 12 weeks of age (5.54*106) than piglets from sows with 2-4 litters (2.59*102) (p = 0.01).
Conclusion
In this investigation the parity of the sows had a significant influence on the amount of viremic pigs within the observational period in farms with piglet vaccination. In those farms pigs that originate from gilts had a higher risk to become viremic than pigs from sows with a higher parity (OR: 5.28). In non-vaccinating farms pigs that originate from sows >4 litters had significantly higher mean viral loads in the serum than pigs from sows with 2-4 litters.

P112

EFFICACY OF PORCILIS PCV M HYO IN REDUCING DISEASE IN A SPANISH HERD AFFECTED BY POST–WEANING MULTI–SYSTEMIC WASTING SYNDROME

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Introduction
Vaccination against PCV2 and M hyo is standard practice in the pig industry. The investigational product, Porcilis PCV M Hyo, is a new combined vaccine that induces immunity against both porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo). The aim of the present study was to assess the efficacy of this new vaccine after single vaccination of 3 weeks old piglets under field conditions in a Spanish farm.
Material and Methods
The study was designed as a controlled, randomized and blinded field trial and conducted in a farrow-to-finish herd with confirmed PCV2 and M. hyo infection. Healthy 3-week age piglets were allocated randomly, within litters, to one of two groups of 303 piglets each: The test (2 ml Porcilis® PCV M Hyo) and the control (2ml Unisol) group. The pigs were weighed individually at admission, at transfer to the finishing unit, and just before slaughter, and average daily weight gain (ADWG) calculated. Medication and mortality were recorded daily. The lungs of the pigs were examined individually at slaughter and scored for typical M. hyo lesions (LLS) according to the method of Goodwin and Whittlestone, and for pleurisy. Fifty-four pigs total were selected for blood and swab (nasal and rectal) sampling at regular intervals, to analyse antibody titres, PCV2 viremia and PCV2 shedding. Safety was also assessed based on systemic and local reactions after vaccination.
Results
Vaccination with Porcilis® PCV M Hyo promoted a reduction in PCV2 viral load and an improvement in ADWG in the finishing period (+35 g/day). Vaccination also reduced PCV2 viremia and shedding. Mean pleurisy and LLS were not significantly reduced by the vaccination. However, the low mean LLS and the low percentage of seropositive pigs at the end of the study are indicative for a late M. hyo infection, and therefore lesions might not have fully developed at time of slaughter. Mortality and morbidity were not different between groups. Safety was also supported by the absence of vaccine related local or systemic reactions and lack of differences in general health scores between both treatment groups.
Conclusion
Porcilis® PCV M Hyo was found to be safe and efficacious in reducing the level of PCV2 viremia and shedding and in improving the ADWG during the finishing period in pigs infected with PCV2 and M. hyo. Although M hyo lesions were not different between treatment groups due to the low challenge in this farm, Porcilis® PCV M Hyo was shown effective against M. hyo in several other field studies.

P113

DEVELOPMENT AND VALIDATION OF A NEW ASFV REAL TIME PCR

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Introduction:
African Swine Fever Virus (ASFV) is a notifiable, highly contagious disease that can cause enormous economic losses. The disease is widely endemic in many part of Africa, in parts of South Europe and increasingly becoming a threat in Eastern Europe. As there is still no vaccine or treatment available monitoring and controlling of the disease is of outmost importance. In order to improve diagnostics we developed and validated a new duplex real time PCR kit.

Material and Methods:
For the development of a reliable, sensitive and specific rtPCR system more than 450 different ASFV sequences representing the p72 protein encoding region were aligned. The new assay composes of a duplex rtPCR including an internal control to ensure reliable DNA extraction. For extraction of viral DNA from field samples the MagMax Pathogen RNA/DNA Kit and the MagVet Universal Isolation Kit were validated.
In order to demonstrate the sensitivity and specificity of the new LSI VetMAX™ ASFV kit different internal and field studies including animal infection experiments (INIA, Valdeolmos, Spain; CVI, Netherlands; Germany) were carried out. In total about 1600 negative samples from ASFV free regions (Germany/ Spain) and additionally 33 different pathogens were tested to demonstrate specificity of the assay.
For validation of the sensitivity about 100 ASFV positive samples from Africa and Europe were tested. The Limit of detection (LDPCR) was determined by serial dilution of a plasmid carrying a specific ASF sequence (pASF). The efficiency of the PCR reaction was identified by using dilutions of 10-4 to 10-11 of the pASF plasmid.

Results:
Test results of the ASFV positive samples showed 100% sensitivity in all tested sample materials (blood, serum and tissue samples). A serial dilution of the ASF target sequence led to a limit of detection (LOD) of 16 genome copies per PCR reaction (95 % confidence interval). The experimental LOD was 5x103 copies per ml in serum and 1x104 copies per ml in blood.
By testing 1600 negative samples a specificity of 100% was demonstrated. Additionally, all tested samples positive for different pathogens scored negative in the ASFV specific assay.

Summary:
In conclusion, the LSI VetMAX™ ASFV kit fulfils all the validation criteria of PCR characteristics and complete method required by the U 47-600-2 standard.

P114

NEW INSIGHTS INTO ORAL FLUIDS AS A DIAGNOSIS PROCEDURE TO DETECT AND DETERMINE THE PREVALENCE OF PORCINE RESPIRATORY AND REPRODUCTIVE VIRUS SYNDROME (PRRSV) UNDER FIELD CONDITIONS.

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INTRODUCTION : Diagnostic tests are often used to assess the PRRSV infection status of pig herds. For routine settings, ELISA test methods and reversed transcriptase qPCR (RT-qPCR) are used to determine antibody titers and detect antigen. Until now, RT-qPCR on serum/blood and tissue samples is the most used technique to detect PRRSV antigen. Recently, the detection of this virus in oral fluids is worldwide being used as an alternative technique. The main goal of this study was to establish clear recommendations in herd management for swine practitioners when oral fluids are used as a diagnosis procedure depending on the goal: detection of a disease versus prevalence determination with a focus on PRRSV.
MATERIALS AND METHODS : Raw Data coming from 2 different experiments were included. In both cases, before starting the studies, oral fluids and blood/serum samples were collected and analyzed by RT-qPCR and ELISA to confirm PRRSV negative status. Experiment 1: A challenge with a PRRSV European strain. After carrying out the challenge with the virus, a weekly sampling of blood and Oral fluid of 80 pigs was carried out during 3 months. Experiment 2: A PRRSV experimental infection with a North American strain was carried out in naïve pigs. Animals were sampled repeatedly over the 40 days post-infection, individual blood samples and oral fluids samples of each pen. In both cases, sample extraction was carried out with magnetic beads sample extraction (MagMAX™ Pathogen RNA/DNA kit and MagVet Universal Isolation Kit 4x 96 tests)and RT-qPCR with LSI VetMAX™ PRRS EU/NA and TaqMan® NA and EU PRRSV Reagents. A logistic regression analysis was carried out in SPSS 15.0 (SPSS Inc., 1989-2006) to calculate the probability of virus detection in a pen by oral fluid (PCR positive results) taking into account the prevalence of PRRSV in serum as independent variable. With this probability, basic epidemiologic information (Thrusfield, 1997)was used to correctly estimate the number of pens necessary to be sampled in order to detect a disease.
RESULTS : Diagnostic sensitivity is 87.5% (78.8%, 96.2%) and diagnostic specificity is 100.0% (100.0%, 100.0%) in experiment 1. Based on the data of experiment 1 and 2, the probability to detect PRRSV virus in oral fluids is significantly associated to the PRRSV prevalence in serum ($p<0.0001$) and the determination coefficient of the logistic regression analysis is 82%. The probability (0-1) to detect PRRSV in oral fluid samples was 0.15, 0.40, 0.72, 0.91 and 0.97 for a serum prevalence (%) of 10, 20, 30, 40 and 50, respectively. If the serum prevalence is higher than 50%, the probability to detect this virus in oral fluid samples is close to 1. The number of oral fluids samples on herd level to be taken in order to find, at least, 1 positive PRRSV oral fluid sample was 1, 1, 2, 2, 2, 3, 4, 8, 19 and 58 for a serum prevalence (%) of 100, 90, 80, 70, 60, 50, 40, 30, 20 and 10, respectively.
DISCUSSION AND CONCLUSIONS: Oral fluid sample is a good tool to detect PRRSV at herd level but it is not suitable to determine the prevalence of this disease. Testing the sampling recommendation under field conditions is requested and these studies are being carried out.

P115

AGE OF PRRSV INFECTION IN COMMERCIAL BELGIAN FARROW–TO–FINISH HERDS

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Introduction:
Porcine Reproductive and Respiratory Syndrome virus (PRRSv) can cause severe economic losses through growth retardation, susceptibility to other infections and respiratory problems during the nursery and finishing periods. The object of the present study was to determine at what age pigs mainly become infected in commercial farrow-to-finish herds in Flanders, with a history of PRRSv related problems.
Material and Methods:
In 20 Belgian farrow-to-finish herds, blood and oral fluid samples were collected in 3 age categories: (1) 4 to 7 weeks, (2) 8 to 12 weeks and (3) 13 to 21 weeks. Per age category one pen was selected in which 10 to 13 pigs were housed. This pen was first sampled by means of one cotton rope/pen and afterwards individual blood samples of all pigs in the pen were taken by jugular venipuncture. Oral fluid was collected after 20 minutes and immediately chilled for transportation to the laboratory. All serum samples were analysed for PRRSv antibodies using a commercial indirect ELISA (PRRS X3 AB, Idexx). A positive result was defined as an s/p ratio $\geq$ 0.4. Additionally, presence of viral RNA was detected on the oral fluid samples by RT-PCR (Virotype PRRS). Vaccination of the piglets against PRRSv was not allowed during the trial, whereas sow vaccination was optional and comprised several vaccination schemes.
Results:
The mean s/p ratio (± standard deviation) in the sera in the different age categories was 0.9 (± 0.7), 0.9 (± 0.9) and 2.0 (± 0.7) respectively and median values were 0.6, 0.4 and 2.1 respectively, with large herd differences. The analyses of the RT-PCR revealed 68%, 25% and 56% negative results for the three subsequent age categories. In the majority of the PCR positive samples, the European type was identified apart for one herd where both the European and North American strain was detected in the youngest age category.
Conclusion:
In conclusion, most variation was seen in the antibody titres of the pigs between 8 and 12 weeks old. Most positive PCRs were also found in that age category, indicating that PRRSv infection mostly occurs near the end of the nursery and the beginning of the fattening unit. A subsequent trial investigates the effect of piglet vaccination in these herds on (1) the level of antibody titres during the fattening period and on (2) the stabilization of PRRSv infection pressure.

P116

CONTROL OF CLINICAL CASES OF DIARRHOEA IN LACTATING PIGLETS CAUSED BY PORCINE EPIDEMIC DIARRHOEA VIRUS (PEDV) AND ROTAVIRUS.

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Introduction
Since early this year several pig farms reported an increase in the number of cases of diarrhoea in lactating piglets that do not respond to antibiotic therapy. The severity of the symptoms, morbidity and mortality are not as high as the ones reported by authors from North America.
First signs of diarrhoea begin at 3 to 5 days of age. Piglets from all parity sows become affected with yellow to brownish watery diarrhoea, with clots and a distinct smell. Vomiting of some piglets is also found. Antibiotic treatments with quinolones and cephalosporins have little or no results. Piglets become dehydrated, reluctant to suckle, loose weight and die if no supportive treatment is done. Average mortality is 10-12%.
The purpose of this paper is to report the strategy used to control the symptoms of the disease in the absence of commercial vaccines.

Materials and methods
Rectal swabs of affected piglets and portions of the small intestines from dead ones were collected and sent to the laboratory. Histopathology, bacteriology, parasitology and PCR for enteric viruses were requested.
Results were negative to: haemolytic E. coli, Clostridium perfringens, Clostridium difficile type A&B, Salmonella sp., Isospora sp..
Results were positive to: PCR for PEDv , Coronavirus and non-haemolytic E. coli. Histopathology revealed lesions of atrophic enteritis.

Symptomatic treatment included hydration and supply of an energy source plus antibiotic treatment for secondary bacteria. Coca-cola was supplied 3 times a day to affected piglets for 3-4 days with fast recovery of body condition and appetite. A solution of electrolytes was also provided. Lincomicin, sulfa-trimetoprim, colistin and amoxicilin+clavulanic acid were the antibiotics with better results. Strict biosecurity rules were applied to avoid spreading of the disease between litters. All in/ all out per farrowing room with washing and disinfection with Sodium hidroxyde 10%.

To prevent new cases we tried to establish immunity in the sows in order to protect the piglets via passive immunity. Maternal derived antibodies seem to protect piglets until the second week of age.
A protocol of controlled exposure of gestating sows to viral material was implemented.
A solution of non-chlorinated water with 5% of milk was prepared. Diarrhoea from affected litters was collected and diluted in this solution. 100 ml of the mixture (inoculum) was then supplied to each gestating sow with 11, 12 and 13 weeks of gestation, at least 2 times in each week, meaning that each sow has received at least 6 doses of the mixture during 3 consecutive weeks.

Results and Conclusions
The first litters from sows exposed to the inoculum during late gestation had almost no cases of diarrhoea. The few cases recorded were easier to treat and probably were due to other causes than viral diarrhoea.
As a conclusion, in this group of farms, the controlled natural exposure of gestating sows to PEDv/ Rotavirus and the biosecurity procedures helped to control the clinical symptoms in piglets.

P117

MONITORING OF PRRSV CIRCULATION IN GROWING PIGS FROM THREE POLISH FARMS OVER TIME USING ORAL FLUID AND SERUM SAMPLES.

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The objective of our study was to monitor the circulation of PRRSV in growing pigs from three Polish farms over time, using PCR, ELISA and DNA sequencing on oral fluid and serum.
The study was performed in three Polish farms, reportedly PRRSV positive. Farm A was a multisite system with 800 sows. Replacement gilts were vaccinated with Porcilis® PRRS at the beginning of quarantine. Farm B was a multisite system with 800 sows. Immunization was implemented with Porcilis® PRRS vaccination in piglets, at 3 weeks of age (WOA). Farm C was a farrow to finish farm with 1000 sows and no vaccination against PRRSV. Three or four pens of pigs were randomly selected, and oral fluid (OF) samples were collected at 5, 7, 9, 11, 13, 15 and 17 or 18 WOA. Serum samples were collected at 5, 9, 13 and 17 or 18 WOA. OF was collected using the Oral Fluid Collection Kit (IDEXX). The RNA extraction was performed using a QIAmp Viral RNA Mini Kit (Qiagen). Real-time PCR for the detection of PRRSV was performed with EZ-PRRSV™ MPX 4.0 (Tetracore) using the Rotor Gene-Q 6000 (Qiagen). Detection of PRRSV antibodies in OF and serum was performed using IDEXX PRRS X3 and IDEXX PRRS Oral Fluids Ab test, respectively. From positive farms, serum and OF samples with the lowest Ct values were selected for ORF5 amplification and sequencing.
PRRSV was not detected by PCR in any sample from farm A. In farm B, Type 1 PRRSV was detected in OF in pigs from nearly all pens from 5 to 15 WOA, but it was negative in pigs at 17 WOA. In farm C, Type 1 PRRSV was detected in samples from pigs at 5 to 17 WOA. Seroconversion in farm A was detected in serum of 5 week old pigs in all 3 pens sampled, in farms B and C was detected in all pens from 5 to 17 WOA.
The sequencing of OF samples was largely unsuccessful and only one ORF5 sequence was obtained in farm C. In Farm C, in pigs of 5, 9, 11, 13 and 17 WOA, >98% sequences were detected, about 85% identical to Porcilis® PRRS and to Amervac (Hipra). In Farm B two strains were detected. In one pig, 5 WOA, Porcilis PRRS was detected (>99% identity). In one pig, 9 WOA, a wild type PRRSV was identified, that was about 84% identical to Porcilis and 93% identical to Amervac.
In farm B the result might indicate lack of efficacy of the vaccination protocol. The gilt vaccination in farm A performed with Porcilis® PRRS was successful in eliminating PRRSV from nursery and growing pigs.
The study was supported by NCN grant 2013/11/B/NZ7/04950. The Rotor Gene-Q 6000, EZ-PRRSV™ MPX 4.0 kit and QIAmp Viral RNA Mini Kit were provided free of charge by Tetracore, and IDEXX PRRS X3 and IDEXX PRRS Oral Fluid and oral fluid collection kit were provided free of charge by IDEXX.

P118

DISEASE DUE TO RUMINANT PESTIVIRUS INFECTION IN A PIG HERD

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Introduction
Persistent infection of pigs with Border disease virus (BDV) was diagnosed as the cause of low morbidity ill-thrift and anaemia in grower pigs on a mixed livestock holding including a 170-sow, indoor, farrow-to-finish pig unit and a 250-ewe sheep flock.
Materials and methods
Six live pale and wasted pigs aged 12-16-weeks-old were submitted from the herd for post-mortem examination between September 2012 and March 2013. Nucleic acid extracted from kidney, spleen and blood was tested in a virus microarray and, subsequently, in a BDV-specific RT-PCR. Histopathology and BDV immunohistochemistry were undertaken on lymphoid and other tissues. The epidemiology of the disease was investigated by a veterinary visit to the farm.
Results
Gross pathology in all submitted cases included pallor which was confirmed haematologically as anaemia. There were multifocal variably-sized red areas of discolouration throughout the kidneys, thymus, liver and lymph nodes which were confirmed histologically as extramedullary haematopoiesis. This resulted in a red-cream marbling of affected lymph nodes which were also enlarged. BDV immunohistochemistry on tissues, including brain from some pigs revealed patterns of labelling closely similar to those observed in ruminants with congenital persistent pestivirus infections. A virus microarray detected the presence of BDV, which was confirmed by BDV-specific RT-PCR. The farm visit revealed that about 30 typical cases were suspected over a two-year period. Sheep handling facilities were immediately adjacent to the dry sow yard with no separation of air space and shared staff. In-pig gilts were housed within the fattener shed with a shared scrape-through dung channel and empty boar pens in the service house housed growing pigs with illthrift.
Conclusion
The confirmation of BDV in submitted pigs indicated that infection was present in the herd over a period of at least nine months and the farm reported earlier typical cases suggesting active infection over a period of about 24 months. Immunohistochemistry supported the suspicion that the pigs were likely to have been infected in utero following early gestational pestivirus infection in the sows. Features of the pig and sheep accommodation, and farm management practices, were identified which were considered likely to have provide the opportunity for transmission of BDV between sheep and pregnant sows and favoured continued transmission of BDV within the pig herd. It is worth noting that the clinical presentation of BDV in pigs in this incident was similar to that which might initially be seen if breeding sows were infected with Classical Swine Fever (CSF) virus of low virulence. CSF was not formally suspected primarily due to the absence of pyrexia, low morbidity and mortality and the fact that in-contact pigs were healthy. The case also illustrates the usefulness of new virus discovery methods. Ruminant pestivirus infection should be considered a differential diagnosis for low morbidity anaemia and ill thrift in pigs, especially if there is a history of ruminant contact.

P118a

A REAL–TIME PCR TEST FOR THE DETECTION AND DIFFERENTIATION OF PORCINE EPIDEMIC DIARRHEA VIRUS AND PORCINE DELTACORONAVIRUS

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Introduction
Porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) represent new threats to the swine industry. To aid in early detection of virus, monitor shedding, or differentiate viral species, PCR has been a useful diagnostic tool. To this end, IDEXX has developed a multiplex real-time PCR test to detect and differentiate the presence of viral RNA from PEDV and PDCoV. Additionally, tests for PEDV, PDCoV and TGEV have been developed. All tests use an internal control approach based on detection of endogenous swine RNA, referred to as the Internal Sample Control (ISC) reaction.

Materials and Methods
Reaction mixes contained equal parts RealPCR™ RNA Master Mix and target-specific IDEXX detection mix for a total volume of 20 µL x number of samples tested. Samples (5ul per reaction) consisted of either synthetic oligonucleotides or nucleic acid purified from clinical samples. Clinical samples (fecal swabs and oral fluids) were purified using a commercial total nucleic acid extraction kit. The cycling program consisted of one cycle at 50°C for 15 minutes and 95°C for 1 minute, followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds.

Results
The efficiencies and correlation coefficients for each test design were determined using serial dilutions of synthetic DNA. All test designs maintained efficiencies of 95%–105% with R2 values of ≥0.994 and detected at least 10 copies per reaction. To ensure no interference and/or competition between target and ISC reactions, multiplexed sensitivity testing was performed for all test designs. Copies of the target sequence (PEDV or PDCoV) were amplified in the presence or absence of artificially high concentrations of ISC. High levels of ISC had no impact on the detection of 10 copies of either PEDV or PDCoV. To confirm the ISC design detects swine RNA and not genomic DNA, the reverse transcriptase (RT) contained in the RealPCR RNA Master Mix was inactivated before addition of sample. Inactivation of RT resulted in complete loss of ISC signal. Test sensitivity and specificity for PEDV and PDCoV were evaluated using purified total nucleic acid from samples of known status. The PEDV/PDCoV multiplex test had PEDV sensitivity of 99.5% (n=191) and PDCoV sensitivity of 100% (n=44). Both designs had 100% specificity.

Conclusions
These results demonstrate the high sensitivity and specificity of the IDEXX RealPCR swine coronavirus tests. The tests are configured as either single target, PEDV, PDCoV, and TGEV tests, or as a PEDV/PDCoV multiplex test. All configurations include an ISC for the detection of swine RNA as an internal control.

P119

EVALUATION OF A COMBINATION OF CEFTIOFUR FREE ACID AND KETOPROFEN IN NEWBORN PIGLETS FOR THE CONTROL OF STREPTOCOCCUS SUIS.

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Introduction: The aim of this study was to evaluate the efficacy of ceftiofur free acid plus a non-steroidal anti-inflammatory such as ketoprofen administered to newborn piglets against Streptococcus suis.

Materials and Methods: The experiment was conducted on a complete cycle farm with 830 farrowing sows located in the province of Buenos Aires. Birthing and weaning areas work with an all-in-all-out system; while growing and finishing is a continuous flow system. Deliveries (births) are weekly and each gestation area has 24 cages. For this experiment, we utilized 2 gestation rooms per week (one for the Control group and one for the Treated group) for 3 weeks (72 births per group). The piglets in the control group were inoculated with 0.1 ml of saline IM at 24 hours after birth, while piglets in the treated group were inoculated with 0.1 ml of Ceftiofur 12% + Ketoprofen 8% (corresponding to a dose of 0.5 mg / Kg) IM at 24 hours after birth. Animals that died during the first 20 days of the trial, from birth to weaning, were necropsied and samples of central nervous system, mesenteric lymph and liver were sent for bacterial isolation of Streptococcus spp and / or Escherichia coli and subsequently analyzed for antibiotic susceptibility. Analysis parameters for the experiment included: mortality rate, etiologic isolation and anti-biograms of isolated bacteria.

Results: Info Stata V2014 program was used to compare differences in mortality between treatments. There were 9 deaths out of 238 piglets, (3.785%) in the treated group, whereas in control group the mortality was 25 about 234 pigs (10.68%), giving a p-value of 0.041. 14 of the death piglets had demostrated neurological symptoms; Streptococcus suis was isolated and identified in these animals. Diarrhea was observed in the remaining 11 death piglets; Escherichia coli was isolated and identified in these 11 animals. From these 9 piglets,Streptococcus suis was isolated from 6 piglets and no bacteria was recovered from the remaining 3 piglets. Susceptibility results from the control group showed that the 100% of the isolates were Ceftiofur and Amoxicilin sensitive, 56% were sensitive to enrofloxacin, 48% were sensitive to Florfenicol and 100% of the samples showed resistantance to tetracycline. In the treated group, 100% of the isolations were susceptible to Ceftiofur and Amoxicillin, 50% were sensitive to enrofloxacin, 66%were sensitive to Florfenicol, and 100% of the samples were resistant to tetracycline.

Conclusion: The strategic use of a combination of ceftiofur free acid with ketoprofen, in newborn piglets significantly decreases mortality associated with Streptococcus suis.

P120

EFFICACY OF A VACCINATION AGAINST MYCOPLASMA HYOPNEUMONIAE AT 3 DAYS OF AGE IN THE PRESENCE OF MATERNALLY-DERIVED ANTIBODIES

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Introduction
The efficacy of a vaccination against Mycoplasma hyopneumoniae (M.hyo) in 7-days-old piglets has been largely documented (Meyns et al., 2006; Reynolds et al., 2009, Villarreal et al., 2011; Vranckx et al., 2012). A vaccination against M.hyo at 3 days of age is more consistent with routine management practices than a vaccination at 7 days of age, since 3-days-old piglets undergo several manipulations, such as castration, tail docking and/or teeth clipping. The objective of this study was to assess the efficacy of a vaccination against M.hyo at 3 days of age in piglets having high levels of maternally-derived antibodies.

Materials and Methods
A total of 110 M.hyo-seropositive piglets, originating from dams who had been vaccinated twice against M.hyo at 6 and 4 weeks pre-farrowing respectively, were divided into 2 groups of each 55 piglets: one group was vaccinated with Stellamune® One (Elanco) at 3 days of age following manufacturer's recommendations, the other group was vaccinated at the same age with a placebo vaccine, containing the same components as Stellamune® One, except for the M.hyo antigens. At 39 days post-vaccination (42 days of age), all piglets were challenged by both intranasal and intratracheal route with a virulent M.hyo strain. Piglets were necropsied 28 days later and the extent of their lung lesions was determined, using the lung lesion scoring method described in the European Pharmacopoeia monograph 2448. Broncho-alveolar lavage fluids (BALF) were collected to confirm the presence of M.hyo by polymerase chain reaction (PCR).

Results
The piglets vaccinated with Stellamune® One had a mean lung lesion score of 4.69%, which was significantly (P=0.005) lower than the one of the piglets vaccinated with the placebo (8.50%). BALFs taken from excised lungs were positive for M.hyo confirming the observed lung lesions were caused by M.hyo. None of the pigs displayed adverse reactions following the vaccination with either Stellamune® One or the placebo.

Conclusion
The present study showed that a single vaccination of 3-days-old seropositive piglets with Stellamune® One significantly reduced lung lesions due to M.hyo. Vaccination against M.hyo at 3 days of age with Stellamune® One allows the development of active immunity against M.hyo before weaning. Moreover, less pathogens that can interfere with a proper development of the immune response following vaccination, such as porcine reproductive and respiratory syndrome virus (PRRSV), are present at that time (Maes et al., 2008). Since M.hyo infection is widespread in 3-weeks-old piglets (Villarreal et al., 2010) and is readily transmitted to in-contact pigs during the nursery period (Meyns et al., 2006), early vaccination with Stellamune® One should be considered in programs for controlling enzootic pneumonia.

P121

COMPARISON OF 1-SHOT VERSUS 2-SHOT VACCINATION SCHEDULES FOR THE CONTROL OF M HYO INFECTIONS IN THE FIELD

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Introduction
Mycoplasma hyopneumoniae (M hyo) is the causative agent of Enzootic Pneumonia (EP), a chronic respiratory disease that affects pigs worldwide and it is a major contributor to the porcine respiratory disease complex with high economic losses in the swine industry. Many vaccines are available for the control of M hyo but vaccination schedules to best protect pigs are controversially discussed since many years. This study evaluates the efficacy of a one shot vaccination program vs. a 2 shot program against M hyo in a side by side trial in a farm in France.
Materials and Methods
The study was conducted during the winter 2012/2013 on a 350 sows farrow to finish farm, positive for M. hyo and PRRSv. In total 1146 piglets from 3 batches were included at the age of 8 days. Piglets within litters were randomly assigned to one of 2 treatment groups. Pigs of Group A (n=565) were vaccinated with 2ml of a 2-dose vaccine (Stellamune©Mycoplasma, Elanco) at 8 days of age (doa) and at weaning (28 doa). Group B (n=551) received 2 ml of a one-shot vaccine (Ingelvac© M.hyo, Boehringer Ingelheim) at weaning. 10 non-vaccinated sentinels were added to demonstrate pathogens and time of seroconversion per batch. Individual weights were assessed at the age of 8 and 28 days and at slaughter. Blood samples for the investigation of M hyo (DAKO ELISA) and PRRSv antibodies (IDEXX ELISA) were taken from 30 pigs/study group at 8 doa, end of nursery, mid finishing and end finishing. Lung lesions were assessed using a modified method of Madec and Kobisch from main slaughter batches of 334 and 312 pig from groups A and B, respectively. ADG was calculated based on the number of days to slaughter. Differences in losses were statistically analysed by ChiSquare. Weights, age, and ADG assessed with t-test. As lung scores were not normally distributed the Mann-Whitney-U test was used for this parameter.
Results
Serological results of the sentinels group confirmed the presence of M hyo and PRRSv during the study with M hyo seroconversion in mid/end finishing and PRRSv sero¬conversion in mid finishing. No statistical significant differences were observed between the two treatment groups with regard to weight at inclusion, sow parity and losses, total ADG or lung scores. Statistical significant differences were observed with regard to weights at 28 doa, growth from 8 to 28 doa and the age at slaughter.
Conclusions and Discussion
Under the conditions of this study pigs with the one shot M hyo program developed significantly better in the suckling phase and reached slaughter about 1.5 day earlier. The tolerance of a vaccine has already been demonstrated to have a significant influence on feed intake and the well-being of pigs. It can be assumed that this influence is even more relevant in the suckling phase than it has been demonstrated in weaned pigs. The study demonstrated the negative effect of 1st week vaccination in regards to performance of suckling piglets and overall efficacy of the 1 shot vaccine compared to the 2 shot vaccine Stellamune© Mycoplasma.

P122

COMPARISON OF VACCINATION SCHEDULES FOR THE CONTROL OF PCV2 AND M HYO INFECTIONS IN THE FIELD

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Introduction
Mycoplasma hyopneumoniae (M hyo) and Porcine Circovirus type 2 (PCV2) are globally widespread pathogens in the swine industry. Vaccines are available for the control of both pathogens and veterinarians as well as farmers are looking for the best strategy and schedule to protect their pigs with these vaccines. This study evaluates two vaccination schedules against PCV2 and M hyo in a side by side trial in a farm in Brittany, France.
Materials and Methods
The study was conducted on a farrow to finish, 350 sows farm, positive for M. hyo and PCV2. In total 1217 piglets from 3 consecutive batches were included at the age of 10 days. Piglets were randomly assigned to one of 2 treatment groups. Pigs of Group A (n=594) were vaccinated with Suvaxyn© M hyo (2 x 2mL - Zoetis) at 10 and 31 days of age (doa) and with Circovac© (0,5 mL - Merial) at 24 doa. Group B (n=593) received Ingelvac CircoFLEX© (1 mL) at 24 doa and Ingelvac© M hyo (2 mL, both Boehringer Ingelheim) at 31 doa. 10 non-vaccinated sentinels were added to demonstrate pathogens and time of seroconversion per batch. Due to management issues, about 20% of pigs had to be fattened off-site and were therefore excluded from the study. Results were calculated from data of on-site pigs only. Individual weight was assessed at the age of 10, 31, 84 and 166 doa. Blood samples for the investigation of M hyo antibodies (IDEXX ELISA) and PCV2 viremia (qPCR, pools of 5 sera) were taken from 30 pigs/study group at 10, 31, 110 and 150 doa. ADG was calculated using the different weighting timings. Losses were evaluated for the period between 10 doa and slaughter. Differences in losses were statistically analysed with ChiSquare and ADG with the t-test.
Results
Pigs at inclusion as well as the sub-group "on-site pigs" were similar regarding to their age, weights, parity or sex distribution in both study groups. Serological results of the sentinel group confirmed the presence of M hyo and PCV2 during the study. M hyo seroconversion was observed in late finishing and PCV2 antigen was detected in samples of the control group already at 30 doa and up to slaughter with highest genome equivalents (GE) in mid finishing (10 exp 6.7 GE). PCV2 was detected in 1 of 6 pools of group A with 10 exp 6.95 GE at 150 doa. In group B two sample pools were PCV2 positive with low GE: 1 of 6 pools at the age of 30 days (10 exp 3.3 GE) which was only 7 days after vaccination so that protection could not yet be expected and in 1 of 6 pools at 110 doa (10 exp 4.8 GE).
The Boehringer group performed better than the Zoetis-Merial group with differences already in the ADG during the suckling period and statistical significant differences at the end of fattening.
Conclusions and Discussion
In this study, the Boehringer vaccination program provided a numerically better growth of piglets in the suckling phase. As safety of a vaccine has already been demonstrated to have a significant influence on feed intake and the well-being of pigs1, the well tolerated one shot M hyo vaccine was beneficial for these young piglets. Finally, the Boehringer vaccination program provided clinical protection till the end of fattening and an overall superior performance with 2.21 kg more slaughter weight.

P123

IMPROVING FARM BUILDING DISINFECTION BY APPLYING A SECOND DISINFECTION WITH ULTRADIFFUSION®

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Introduction
The implementation of terminal disinfection to improve the overall quality of the cleaning and disinfection protocol and the risk reduction of persistence of pathogens such as salmonella is common in poultry. In pig, Corrégé et al. (2003) showed recontamination of the room during the down time phase and a reduction of bacterial contamination after applying a second disinfection (liquid or nebulisation). The humidity is one of the factors affecting the persistence of pathogens, the objective is to evaluate the application of dry disinfection, the Ultradiffusion®, on the final result of disinfection and destruction of specific pathogens.
Materials and Methods
In two farrowing rooms, a recommended cleaning and disinfecting protocol was implemented. After complete drying of the rooms (controlled by monitoring the relative humidity), a second disinfection by Ultradiffusion® was carried out. The effectiveness of surface disinfection was evaluated by counting in ten sample sites the total number of bacteria in Petri dishes before and after Ultradiffusion®. At the same time, the assessment of the efficiency of the process on specific pathogens (Salmonella sp, Staphylococcus sp and Streptococcus sp) was performed by the use of germ carrier steel discs under a test protocol compliant with the French Standard (AFNOR NFT72281) recommended for measuring the effectiveness of disinfecting by the air way disinfection methods.
Results
Results for total bacteria counts in Petri dishes suggest an improvement in the final disinfection (60% of the samples show fewer bacterial colonies after the second disinfection) but some uncertainty remains on the level of this gain, due to the very low initial level of contamination (before any disinfection). Thus, the results with the method of microbiological carrier test shows a reduction of contamination exceeding 2 log after disinfection by Ultradiffusion® for the 3 types of pathogenic microorganisms considered, with values of up to 2.6 log. These results demonstrate a significant efficacy from 99% to 99.8%.
Conclusion
The method of microbiological carrier test confirms, in field conditions, the efficacy of the here-applied global disinfection process to decrease the number of the 3 types of pathogenic microorganisms considered, with a reduction of contamination exceeding 2 log. The Ultradiffusion® after the recommended cleaning and disinfecting protocol could be a preventive measure in programs to control salmonella and other pathogens.

P124

EVALUATION OF IDEXX PRRS 3X ELISA AND PCR FOR MONITORING AREA ELIMINATION OF PRRSV TYPE 2 USING INGELVAC® PRRS MLV IN A LOAD, CLOSE AND HOMOGENIZE MODEL (L-C-H).

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Introduction
The objective of this study is to evaluate IDEXX3X and PCR as a tool to monitor PRRSV elimination project in Horne peninsula, Denmark. The aim of the elimination project was to achieve SPF status for all herds in the area, and avoid lateral spread of PRRS virus between herds. Thirteen farms are located in this small pig dense area (10 x 7 km); 4 sow farms (400 sows in avg.), 2 wean to finish (W-F) farms (producing about 14000 finishers a year) and 6 finisher farms (together producing 36000 finishers a year).
Materials and methods
L-C-H was performed in 2 of the sow sites as follows; the acclimatization site was loaded with gilts down to 10 woa. The sites with sows and gilts were closed for 29 weeks. The first batch of gilts was introduced after herd closure were vaccinated and placed in quarantine for 3 month, before introduction to the sow site. The first PRRS negative gilts were introduced in May 2014. All sows, gilts, boars, and piglets in the farrowing room and the nursery, were homogenized by vaccination with Ingelvac® PRRS MLV during the first week of July 2013. Each of the following 3 weeks, 7 day old piglets were vaccinated until the mass vaccination of sows, gilts and boars was repeated four weeks after the initial mass vaccination. Following the second mass vaccination, piglet vaccination was moved to immediately post-weaning, in the nursery for the next 10 weeks. Monthly monitoring by PRRS PCR and IDEXX 3X Elisa was implemented in sow nursery sites and W-F sites. Cross sectional profiles in nursery and finisher sites (avg. 29 pigs, 5 pigs per age group) were implemented during the project.
Results & Discussion
3 week old prewean piglets remained ELISA positive (IDEXX PRRS 3X) until 51 weeks after L-C-H. The ELISA antibodies are presumed to be maternal antibodies (MA), as no samples were PCR positive in that age group at any time point. Some of the tested piglets came from the second batch of gilts that were introduced after LCH. MA could be detected in 0 – 20 % of pigs until 6 – 7 woa, still being PCR negative. After 8 woa no MA's were detected.
Results show ELISA (IDEXX3X) as a useful tool for monitoring in PRRS elimination programs in pigs older than 8 woa. For younger pigs pre and post wean, PCR is recommended, as some pigs remained ELISA positive until 6 – 7 woa most probably due to Maternal Antibodies.

P125

ANTIBIOTIC REDUCTION IN A SWINE FARM USING A HEALTH PLAN

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Introduction
Antibiotic reduction using a Health Plan (HP) is needed considering both wellness and health demands aimed to reduce the bacterial resistance and genetic transmission to the human population. The HPs should not disregard the following principles: Efficiency - meet the objective; Efficacy - using the fewest resources possible; Sustainability - perpetuation throughout time.
The study is focused on the control of the respiratory complex main diseases through a HP based on population immunity. The overall intent is to reduce the therapeutic control in reference to antibiotic uses (AU), and to enhance main health indicators.
Materials and Methods
A historical (2 years) result from PRRS-free, farrow-to-finish, 500 sow farm was taken as starting point. At that time, the vaccination plan for sows was M. Hyo, 60/80 gestation days, for fattening period, PCV2 21 days, M. Hyo 40 days. AU expressed as antibiotic mg per pork kg weight was 1.18 mg/kg.
The following HP was taken later (during 2 years) in order to reduce antibiotic use in farm: for sows two PCV2 and Mh doses, 60-80 gestation days, and for fattening period 40-60 days PCV2 and Mh. Sensitivity efficiency variables were as follows: mortality and runts, monthly control; lesion score (Iowa St. University), slaughtering house prevalence, quarterly control. At the same time, the AU amount in mg/kg swine live body weight sold and use profile as efficacy sensitive variables and HP challenge. (MSD)
Results
Mortality and runts were reduced from 12.27%±0.04, to 7.08%±0.02*, lesion score dropped from 10.22±2.57 to 6.32±3.63 and mg of antibiotic use reduced from 1.18 mg/kg to0.65* mg/kg. * (P<0,05)
Conclusion and Discussion
Main strategy is focused on the PCV2 control as an immunosuppressor agent which, in addition to Mh, promotes disease by secondary pathogens that, as a whole, may produce therapeutic failure and subsequent resistance. Immunosuppression might increase mg AU/kg sold with progressive switch to more complex, state-of-the-art AUs. The HP proved efficient, efficacious and sustainable because it is still in force. Immunosuppression control might enhance the scenario for more complex HPs which, together with state-of-the-art vaccines against other agents, might reduce even more the AU.

P126

USE OF BRIX REFRACTOMETER TO EVALUATE COLOSTRUM IGG CONTENT AT FARM LEVEL

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Introduction
Colostrum plays an essential role in piglet survival and growth providing immunoglobulin (IgG and IgA) and being a source of energy. The neonatal piglets lack globulins, relaying on colostrum as the sole source of antibody. Colostrum IgG composition might be highly variable among sows, due to age, feeding, and immunological background (vaccinations, environmental pathogen pressure). At the moment, it is impossible to obtain information about colostrum IgG concentration at herd level. However, a variety of methods are available to evaluate colostrum quality. Brix refractometer, measuring the total solid % in fluids, has been proposed as a reliable, cheap and fast means to estimate IgG concentration of maternal colostrum in cow, sheep, and horse. This report concerns a study in which sow's colostrum quality was evaluated on-farm by a refractometer and the performance was compared with that of an ELISA in laboratory.

Materials and Methods
Colostrum samples (5-6 ml) (N = 80) were obtained between 0-4 h and between 20-24 h after the birth of first piglet, in order to have maximum and minimum levels of IgG, respectively.
A drop of freshly drawn colostrum samples was used for the on-farm measurement of Brix percentages, by a digital refractometer shortly after collection. The remaining part of the samples was frozen before laboratory analysis of total IgG, using a commercially available pig IgG ELISA kit.

Results
The Brix percentage of the 0-4 h and the 20-24 h samples was 25.1 ± 3.01 (mean ± SD) and 19.85 ± 4.73, respectively. IgG concentration, measured by ELISA, was 66.74 ± 22.94 mg/ml and 33.13 ± 28.71 respectively. The Brix percentage was highly correlated (r = 0.71) with colostrum IgG concentration.

Conclusion
In conclusion, the Brix refractometer has an acceptable sensitivity as a test for practical use at farm level. Present results indicate that the Brix measurement of total solids in fresh sow's colostrum is an inexpensive, rapid, and satisfactorily accurate method of estimating IgG concentration, giving indication to differentiate between good and poor quality colostrum.

P127

ANTIMICROBIAL USE EVOLUTION BETWEEN 2010 AND 2013 IN A GROUP OF PIG FARMS – TRENDS AND DRIVERS

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Introduction
To reduce and prevent antimicrobial resistance - one of the major emerging threats to human health – a reduction of antimicrobial usage in veterinary medicine has been scheduled in France through a national plan. The objectives of this study were to determine trends and drivers of antibiotic use between 2010 and 2013 in a sample of farms, to identify the factors that account for variations in use and to assess farmers' perception on these variations.

Material and Method
The study included 46 farrow-to-finish farms in the West of France. These farms were enrolled in 2011 in a study in which their use of antibiotics for the year 2010 were quantified using a standardized method. During the renewal of the survey, the same farmers were asked about the technical and sanitary changes that occurred between 2010 and 2013. The quantities of antibiotics used in 2010 and 2013 were expressed as the number of Course Dose (nCD) per produced pig per year. Finally, a comparison was made between the calculated variation of antibiotic use over three years and farmers' perception.

Results
In our sample, antibiotic use decreased markedly over three years (- 31%), in a large majority of farms (74%). For all animal categories, increases were uncommon and concerned less than 15 % of farms. In 57% of farms, difference between 2010 and 2013 reached at least one nCD / produced pig in post-weaning units. In a majority of farms, much work has been carried on disease prevention through better control of herd management, hygiene, food and/or buildings. Treatments were also optimized, especially vaccines, de-wormers and alternative products, whereas antimicrobial treatments were reassessed. Finally, the perception of farmers on their variations in antibiotic use over three years was incorrect in 60% of cases. Particularly, 49 % of farmers thought erroneously that they administered the same or a higher quantity of antibiotics in 2013 compared to 2010.

Conclusion
The marked and frequent decrease in antibiotic use illustrates the collective efforts made over the last years and demonstrates margins of decrease, sometimes substantial, that are achievable in farms. This work also emphasizes the need for farmers of a self-assessment tool of antibiotic usages.

P128

POSITIVE RETURN ON INVESTMENT AFTER VACCINATION AGAINST PCV2 AND MYCOPLASMA HYOPNEUMONIAE IN HIGH PERFORMING FINISHING HERD

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INTRODUCTION
PCV2 and M hyo can have a huge impact on pig performance, but herds can also be infected without clinical signs. The present study examined the benefit from vaccination in a herd with excellent performance and no obvious signs of infection.

MATERIALS AND METHODS
The study was carried out in 3 consecutive week batches in a finishing herd with an average daily gain (ADG) of 1013 g/day and a feed conversion rate (FCR) of 2.51 FE/kg gain.
The study included 2 groups side-by-side: A non-vaccinated group and a group vaccinated against PCV2 and M hyo (FLEXcombo®, Boehringer Ingelheim). Half of the pigs were vaccinated on the day of weaning by the piglet supplier and vaccinates and non-vaccinates were differentiated by eartags. Groups were blinded for the finishing herd.
When the study pigs arrived to the finishing herd, they were sorted by size for each wetfeed-valve according to the herds normal procedures. Vaccinated and non-vaccinated pigs were placed at separate valves. Totally, 238 pigs at 7 valves were included for each group.
Every 2nd week, the number of coughs were counted, and oral fluid (OF) was collected and examined for PCV2 by PCR. During finishing, the total bodyweight and feed consumption per valve was recorded weekly.
Statistical comparison of coughing and level of PCV2 in OF in the 2 groups was made with Fishers Exact Test, with p=0.05 as level of significance. Because bodyweight, FCR and ADG was calculated by valve, statistical comparison was not possible. An economical evaluation was done with key figures from the Danish Pig Producers Organization

RESULTS
The frequency of coughs was significantly lower in vaccinated pigs (p=0.008). Vaccination significantly (p<0.001) reduced the level of PCV2 in OF; with 58% of samples from non-vaccinates having a level of PCV2 copies > 5 log compared to only 4% of samples from vaccinates.
Vaccinated and non-vaccinated pigs had the same weight at the start of finishing, but vaccinates grew heavier compared to non-vaccinates during finishing, with an increased ADG of 67 g/day. The kg feed consumed in the 2 groups was equal until the pigs were close to slaughter. Hence, FCR per kg gain was lower for vaccinates and in total, vaccination reduced FCR by 0.21 FE/kg gain.
An economic evaluation of the results shows that the combination of increased end weight and reduced FCR resulted in an economic benefit of 6.8 €/ produced pig after vaccination.

CONCLUSION
Vaccination against PCV2 and M hyo reduced the negative effect of the infections and increased the good productivity to a higher level, with a higher ADG and end-weight and a lower FCR. The result is a large economic surplus.

P129

MONITORING OF PRRSV AND IAV INFECTION IN A CONVENTIONAL FATTENING FARM BY DETECTION OF VIRUS ANTIBODIES IN PEN-BASED ORAL FLUID SPECIMENS

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Introduction
Analysis of pen-based oral fluid samples by PCR testing has proven to be an easy and efficient method for monitoring and surveillance of various infectious diseases in swine populations based on the detection of the infectious agents, e.g. porcine reproductive and respiratory syndrome virus (PRRSV). Another established method to prove an infection by detection of antibodies against some agents is ELISA, and commercial ELISA for serum has been adapted to analyze oral fluids, as well. It was shown that monitoring of an influenza A virus (IAV) infection is feasible by detecting virus antibodies in pen-based oral fluid specimens.
Material and Methods
Oral fluids were collected weekly on a conventional fattening farm in Germany from two consecutive batches of approximately 25 pigs in four pens, from 12 to 24 or 22 weeks of age. For comparison, serum samples were also collected monthly from two randomly selected pigs of each pen starting at the age of 12 weeks. Oral fluid and serum samples were tested for antibodies against PRRSV and IAV by commercial ELISA test kits. Oral fluids were also tested for viruses by PCR. The herd had a history of circulating PRRSV and IAV.
Results
One week after initial detection of IAV in oral fluid samples by RT-PCR, antibodies against the virus were detected both in oral fluids and in serum samples. Oral fluids continued to test positive for antibodies four to seven weeks after initial detection of virus, but with a decreasing trend, and all samples in batch one tested negative after nine weeks, whereas all serum samples continuously tested positive. Because PRRSV was circulating in the herd, as was detected by PCR, almost all samples, both oral fluids and serum, tested positive for PRRSV antibodies; however, antibody profiles based on oral fluids reflected the infection dynamics in a much more differentiated way than profiles based on serum samples. Waves of PRRSV infection occurrences within the herd were mirrored by the corresponding changes in the profiles of antibodies detected in pen-based oral fluids but not in serum.
Conclusion
Profiles of antibody content in oral fluids reflected the dynamics of PRRSV and IAV infection in a more differentiated way than antibody profiling using serum samples. Furthermore, longitudinal studies confirmed the usefulness of monitoring PRRS and IAV infection by detection of virus antibodies in pen-based oral fluid specimens.

P130

EVALUATION OF THE PERFORMANCES OF TWO SAMPLING METHODS FOR QPCR DETECTION OF MYCOPLASMAS IN LIVE PIGS USING A BAYESIAN APPROACH

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Mycoplasma hyopneumoniae and Mycoplasma hyorhinis are involved in the pathogenesis of Porcine Respiratory Complex, which is common in modern pork production and considered one of the main challenges for the swine industry. In order to monitor the contamination by both of those pathogens in live pigs, quantitative PCR tests have been developed. Various sampling sites can be used to detect Mycoplasma on live pigs. In order to design suitably timed management strategies, the performances of PCR techniques need to be quantitatively evaluated under field conditions and for various sampling sites. In this study, two hundred and sixty seven finishing pigs were randomly selected from one batch of pigs in 19 herds purposively selected. Each pig was submitted to tracheo-bronchial swabbing (TBS) and tracheo-bronchial washing (TBW). All samples were analyzed using quantitative PCR assays. A Bayesian analysis framework was applied to estimate the sensitivity and specificity of two tests in two populations (pigs with or without cough considered as cases or controls respectively) without a gold standard. The cross classified results of the two sampling methods for M. hyopneumoniae in the case population were as followed: 57 (65%) of the 87 pigs sampled were found positive by TBW only, 72 (83%) tested positive by TBS and 56 (64%) were positive by the two sampling methods. In the control population, 89 pigs (49%) of the 180 pigs sampled were TBW positive and 106 (59%) by TBS. For M. hyorhinis, in the case population, 12 pigs (14%) of the 87 pigs sampled were positive by TBW and 25 (29%) by TBS. Seven pigs (8%) were found positive by the two sampling techniques. In the control population, seven pigs (4%) of the 180 pigs sampled were positive by TBW and 24 (13%) by TBS. Three pigs (2%) were positive by both TBW and TWS. For both mycoplasmas, models converged solely when sampling techniques were considered independent. For M. hyopneumoniae, the best latent class model was obtained when the prior for specificity was a beta distribution with a mean Sp of over 0.9 and a mode of 0.98. It rendered a mean Se of 79% and a mean Sp of 96% for TBW with a 95% credibility interval of posterior distribution (95% CrI) ranging from 72% to 86%, and 91% to 99% respectively. The TBS was found more sensitive with a mean of 97% (95% CrI: 92%-99%) and equally specific with a mean of 96% (95% CrI: 89%-99%). When sensitivity was introduced as an informative prior drawn from literature data, results were similar to that of the previous study considered. In the case of M. hyorhinis, the best model was obtained with a fixed specificity of 1. Both sampling methods appeared less sensitive for TBW with a mean Se of 20% (95% CrI: 11%-32%) and for TBS a mean Se of 50% (95% CrI: 31%-71%). Again TBS was found more sensitive. Results for M. hyopneumoniae were similar to those found in previous work although slightly higher. Our study also indicates that TBS associated with PCR appears to be an accurate diagnostic tool for assessing M.hyopneumoniae infection dynamics in pig herds but more research is needed to draw similar conclusions for M.hyorhinis.

P131

NOVEL INSIGHTS IN THE PREVALENCE OF ASCARIS SUUM IN COMMERICAL PIG FARMS IN EUROPE

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Introduction
Although several studies have already shown that infections with the roundworm Ascaris suum are still highly prevalent in intensive pig production systems, farmers are most often unaware of worm infections on their farm and the economic losses caused by this parasite.

Material and Method/ Results
The first goal of this project was therefore to assess the prevalence of A. suum infections in fatteners throughout Europe using the SERASCA-test, a novel serodiagnostic test developed to measure exposure to A. suum and to investigate potential associations between infection levels and growth, feed conversion and applied deworming programs. Blood samples were collected and analyzed from 1838 different fattening farms throughout Europe. Of these farms 48,4% tested serological as 'highly infected'. From 132 Belgian farms, technical performance parameters and management factors were obtained to analyze for potential associations with serology. This was done by a Spearman's rank correlation test and a two-tailed Mann-Whitney test (nonparametric) (P-values <0,05 were statistically significant). Analysis on the associations between serology and the data provided in the questionnaires revealed a significant negative correlation between serology and the average daily growth.
Based on the high prevalences observed in fattening pigs, the question arises whether exposure to A. suum mainly occurs in the fattening units or earlier on in farrowing and nursery units. To address this question, the second aim of this project was to investigate seroprevalence for A. suum in piglets at both weaning and at the end of the nursery period on a representative farm. Surprisingly, the outcome of this analysis indicated that at the time of weaning, the piglets were already highly positive for anti-Ascaris antibodies and that these antibody levels correlated significantly with anti-Ascaris antibody levels in the respective sows, suggesting maternal transfer. This was further supported by the fact that anti-Ascaris antibody levels in the piglets further dropped till 5 weeks post weaning after which the animals remained seronegative. Interestingly, further research showed that seropositive piglets were protected against an artificial infection with A. suum, suggesting the protective nature of this maternal immunity. The mechanism and duration of the maternal immunity and the potential implications it has on the deworming strategies of both sows and piglets are currently being further investigated. In addition, a large scale seroprevalence study is currently being performed to investigate the status of sows and piglets on commercial farms.

P132

PADRAP – PRODUCTION ANIMAL DISEASE RISK ASSESSMENT PROGRAM – A TOOL TO EVALUATE AND SHARE BEST BIOSECURITY PRACTICES IN EUROPE

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Introduction
Production Animal Disease Risk Assessment Program (PADRAP) is an epidemiologically-based initiative to help producers and veterinarians manage disease risks. It is a set of web-based risk assessment surveys used to evaluate and benchmark internal and external risk factors. It was originally created in the USA in 2003 by Boehringer Ingelheim Vetmedica and offered to the American Association of Swine Veterinarians (AASV) as a gift in 2005. AASV took this tool and it is managed by Iowa State University College of Veterinary Medicine since 2006. This tool can be applied to evaluate current biosecurity protocols and/or develop new ones to avoid risks, to demonstrate improvement in biosecurity over time, as an aid in the decision to initiate a project to eliminate PRRSv from a site, and as a tool for regional elimination or control projects. In this paper we focus on the use of the tool aligning a group of sow farms in order to share best biosecurity practices.

Material and Methods
During the summer 2014 we used the breeding herd survey to assess biosecurity in 8 sow farms. The reporting structure of the PADRAP tool develops internal and external risk scores within all farms at all different levels. Based on the scoring of each criteria for each farm, we identify best practices already in place as well as gaps. The methodology was at first to compile all individual reports (Pareto chart) of the considered farms. Secondly using a pivot table structure, the sow farms could be compared on strengths (scores ≤ 10) and weaknesses (score ≥ 21.5). Once the pivot table is created all criteria can be identified.

Results/Conclusion
In our group of 8 farms criteria such as the existence of a written biosecurity protocol, a high level of compliance of the employee with the protocol, sanitation procedure for employees and visitors entering site or disposal of dead animal done by dedicated equipment can be considered as best practices or gaps. In conclusion beyond the assessments of the biosecurity measures in place on an individual farm, PADRAP can be used for sharing practices between farms and help to create development plans by comparing farms sharing information.

P133

EFFECTS OF PCV2 VACCINES ON POST WEANING PIG PERFORMANCE

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Introduction
Vaccination of pigs for PCV2 and Mycoplasma hyopneumonia is a routine measure in pig production globally. In many cases vaccination for these two pathogens is done around weaning. Pigs have to cope with many stressors at weaning. At the same time the pig's performance around weaning is critical for the later performance and the degree of variability after weaning has a substantial impact on the variability at the end of finishing. The objective of the trial presented here was to compare two PCV2 vaccines on their impact on piglet performance shortly after vaccination.
Material and Methods
The trial was conducted in a PRRSv negative, 1200 sow farrow-to-finish farm in France. The farm operates in a weekly batch system and weans piglets at about 3 weeks of age. After weaning pigs are kept in a nursery unit on dry feed for about 3 weeks before they are moved to a rearing unit with liquid feed. From weaning to slaughter the mortality rate in this farm is about 4% and the feed conversion rate from weaning to slaughter is at about 2.55. For this trial one day before weaning pigs were included into the study, weighed individually, and piglets of each litter were alternatingly allocated randomly by first pick to either treatment group 1 or 2 and marked individually with an ear tag. In total 1158 pigs of 2 following farrowing batches were included. On the day of weaning the pigs were vaccinated either with Ingelvac MycoFLEX (1 ml) and Porcilis PCV (2 ml, both products according to label instructions, treatment group 1) or with Ingelvac MycoFLEX and Ingelvac CircoFLEX (according to the label, 1 ml of each product, treatment group 2). In the nursery unit pigs of the two treatment groups were kept comingled in pens of 65 to 70 animals and were weighed again individually 14 days after inclusion.
Results
At inclusion mean body weights, weight distribution as well as sex ratio and age were comparable for the two treatment groups. For treatment group 1, 4 out of 578 (0,8%) animals died during the trial period whereas for treatment group 2 it was only 1 out of 580 animals (0,2%) . The average daily weight gain was significantly different between the treatment groups (200g/d in treatment group 1 vs. 216 g/d in treatment group 2, $p < 0{,}0001$). This difference was consistent over the 2 repetitions of the trial.
Conclusion
The findings of this study are in line with other studies that found a difference in local and systemic reactivity between commercial PCV2 vaccines. The results shown here indicate that the choice of PCV2 vaccine has an impact on performance after weaning, which might also impact performance through to finishing.

P134

MONITORING PIG HEALTH IN THE NETHERLANDS: LOCOMOTION PROBLEMS IN FINISHERS

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Introduction
Since 2002 GD Animal Health in Deventer, the Netherlands, runs a monitoring system on pig health in the Netherlands. This system is funded by the commodity board for the pig industry, the ministry of agriculture and by individual farmers. The sources used for the monitor are GD databases containing information from laboratory tests, post mortem examinations and veterinary consultations (telephone, farm visits). This reactive system is very well suitable to detect notifiable diseases and new and unusual findings. Shortly the system will be extended with an online network of veterinary practitioners to add a more pro-active tool in order to monitor trends in pig health more accurately. Quarterly reports are produced for the stakeholders. If there is a need for it, additional pilot studies are conducted to gain more insight in specific health issues. Examples of the latter are locomotion problems. In the past ten years a gradual increase of the percentage (from 4 to 10%) of veterinary questions about locomotion was observed. In the first half year of 2014 even 16% of all pig health questions to GD veterinarians were related to locomotion.

Material & Method
A detailed questionnaire was designed to investigate systematically the nature of the discussed locomotion problems. In the first quarter of 2014 the questionnaire was used in 20 telephone calls.

Results
Most questions about locomotion (14) concerned young finishers. Most pigs received water from an own well (10) which was often acidified (7). Locomotion problems started roughly at the age of 3 to 4 months, often (8) in pigs with heavy muscles and relatively delicate legs. Symptoms mostly concerned the hind quarters: stiff gait, bursitis, swollen joints, and difficulties in standing up. Additional symptoms like poor appetite or diarrhea were seldom mentioned. De estimated prevalence ranges from 10% to more than 25%. In general, mortality is low. In some cases extra diagnostic tests were conducted and diagnoses were made like osteochondrosis (2), cartilage defects (1) and impaired bone development (2).

Conclusion
This example shows how the pig health monitoring system in the Netherland works in practice. In relation to locomotion problems in finishers, it has been suggested that long term acidification of drinking water and feed negatively effects bone mineralisation and development. The described symptoms are also suggestive for infection with Mycoplasma hyosynoviae. Unfortunately at present testing for M. hyosynoviae (culture, PCR) is not common practice in Dutch laboratories. The findings in this study have prompted further research proposals.

P135

CASE REPORT: PRRS–CONTROL IN A HIGH DENSE AREA OF

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CASE REPORT: PRRS-CONTROL IN A HIGH DENSE AREA OF GERMANY

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PRRSV was first detected on German pig farms in early the nineties. It is still an unsolved problem with huge economic impact on pork industry worldwide. Costs in Europe can be estimated at an average of 128 € per sow per outbreak. Using modified live vaccines for PRRSV has been successful to stabilize the infection, but once farms are affected by PRRS problems are often flaring up again when mass vaccination of the whole herd is stopped. The case report describes control and stabilization of an acute PRRSV-outbreak in an endemically infected breeding herd, using PRRS-vaccines for long term (2006 till present).

Located in an area of high pig density in North Rhine Westphalia, close to Germany`s biggest abattoir and main transport routes the so far as PRRS-negative described herd started with a decrease in reproductive performance at the beginning of 2006. Pathogens causing this performance subsidence were ruled out except PRRSV, later confirmed as EU-type (14 % homology to Lelystad; ORF 5).Vaccination started September 2006 with Ingelvac® PRRS KV then changing to a modified live virus vaccine (Ingelvac® PRRS MLV; US strain VR 2332) in 2008 with mass vaccination of the 450 productive sows every 4 months, suckling piglets vaccination at an age of two weeks, gilts revaccination at an age of 120 days.

First term of 2006, parameters like return to service- and liveborn piglets (13,9% respectively 10/litter; average of half term) were non-satisfying when starting mass vaccination of sows with Ingelvac® PRRS KV quarterly. While there was a slight improvement in first term of 2007, return to service increased to 15, 9% in the second term of 2007. Changing to Ingelvac® PRRS MLV in 2008 and adding vaccination of suckling piglets and gilts stabilized the performance. This was maintained throughout time with improvement of return to service and liveborn piglets (5% respectively 13/per litter average of half term 2010-2013).

In this case it was not possible to set the improved performance in proportion to other herds in this dense area, but it is clear that undulating recurrence of PRRS-clinic is absent. One difference compared to other PRRSV-positive farms may be the consequent implementation of the whole herd vaccination, what is also reflected in unremarkable results for pneumonia at slaughtercheck over the years.

While PRRS-infection requires more than tools like vaccination and monitoring the production performance for control, this report shows that it is possible to control PRRS in the long term throughout production systems even in high dense areas of Germany by combining these methods.
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Table 1: Reproductive performance parameters of sows (average of half term/year)

term of year		return to service (%)	liveborn piglets/litter		piglet mortality (%)	Vaccine-Type
2cd -06	14,0	10,0	6,6	KV		
1st - 07	8,9	10,6	5,1	KV		
2cd - 07	15,9	10,2	9,4	KV		
1st - 10	5,3	12,3	9,1	MLV		
1st - 11	5,9	13,3	13,1	MLV		
1st - 13	5,2	14,0	10,0	MLV		

Table 2: Results of slaughterchecks at abattoir (%; according to QS company guidelines, Germany)

year	animals	negative	pneumonia		enzootic pneumonia	pleurisy
2010	24	89,3	0,10	0,00	0,00	
2011	68	96,3	0,10	0,00	0,00	
2013	26	64,0	0,00	0,00	0,00	
2014	30	96,7	0,00	0,10	0,10	

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SUDDEN MORTALITY OF AI BOARS DUE TO MYCOTOXINS IN THE NETHERLANDS

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Varkens KI Nederland is one of the largest swine AI organizations in the world, producing more than 3 million insemination doses per year with 1500 boars divided over 8 AI stations.

Introduction: In 3 weeks time, from 3 until 25 September 2014, in an AI center housing 260 boars, 7 boars died and 11 animals showed elevated body-temperature, varying from 39,2°C to 40,9°C.

Materials and methods; All boars that died were sent for autopsy. Unfortunately, the results did not help to find a cause, as it gave multiple pathological findings (pericarditis, pleuritis, stress, spleen torsion, renal cysts, arthritis of both knees).
All boars with elevated body temperature recovered after treatment with anti–inflammatory drugs (Meloxicam). Paired blood sampling was done with three weeks interval on four of these animals in the acute phase of disease and checked for antibodies for PRRS, PCV2 (IgG and IgM) , Haemophilus parasuis, and porcine influenza type H1N2, H3N2 and H1N1. None of these converted between the first and second sampling, so blood testing was inconclusive on the cause of mortality and fever.

Results: The AI station exists of 6 barns, with bigger and smaller compartments, all affected animals came from the same compartment, this was the biggest with 56 boars in one room. It is the first room after entering the barn from the shower, and everybody has to pass through this room, to reach the rest of the barns. In case of an infection, it would be spread from this room to all other rooms easily. This made an infectious cause unlikely and the conclusion was drawn, that environmental causes could play an important role. This was the only room in which bedding, consisting of cut straw, was used. The batch of straw used the past month, was almost finished and a new batch was already delivered. Samples of 1 kg of straw were taken from the stock of both batches and were tested on the presence of mycotoxins (Biomin). In the "old" batch of straw the level of DON was high (592 ppb), while in the new batch, it was low (71 ppb). After changing the straw, no new cases of mortality and fever appeared, the feed intake improved to normal and the boars were more vital. Also, a mycotoxin binder was added to the feed in a double concentration, to solve the problems.

Conclusion: Mycotoxins (DON) in straw are the most probable cause for the sudden death of seven adult boars. DON is known for its immunosuppressive effect, which may explain the various pathological findings.

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EVALUATION OF TWO DIFFERENT VACCINATION PROGRAMS FOR CONTROL OF RESPIRATORY DISEASE ON A FARROW TO FINISH UNIT IN THE UK

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Introduction
Porcine Circovirus Type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhyo) are two pathogens that affect pigs worldwide, their immunesuppressive effect reduces the ability of the respiratory tract (RT) to eliminate disease. A farrow to finish unit with 380 sows, batch farrowing every 3 weeks, affected by high respiratory disease levels in late finishing and high enzootic pneumonia like lesions (EPL) decided to assess the effect of two different Mhyo and PCV2 control programs on the performance and respiratory health.

Methods
For this study, 164 pigs were individually identified and weighed at weaning (28 days of age), grower stage and before slaughter. Pigs were randomly allocated to one of two treatment groups, group A was vaccinated with Porcilis PCV and M+PAC concurrently (n=83) and group B was vaccinated with PCV2 and Mhyo vaccines licensed to be mixed (n=81). Blood samples were collected for serological and virological testing. The respiratory tracts of 87 pigs were assessed at slaughter following the Goodwin et al (1967) system to evaluate the EPL.

Results
No significant difference was found between the live weights at weaning, grower and finishing stage, for Group A and Group B, 7.1, 7.24 kg, 49.1, 50 kg, 91.5 (±9.4) and 91.8 (±12.2) kg respectively. The weight gain over the finishing stage for Group A and Group B was 41.9 and 41.7 kg, respectively, the coefficient of variation for Group A and B finishing weights was 10% and 13%, respectively.
The average EPL was significantly (Fisher test) lower for Group A (n= 55; 3.1±6) than Group B (n=32; 5.9±9). Serological results were not significantly different due to the small number of samples taken.

Conclusions
Efficacy of control programs should always be assessed to ensure that the interventions are satisfactory. In this study, no differences were observed between the two groups regarding the finisher weights, however Group A had a higher weight gain in the finishing period than Group B, this may be due to the significantly higher level of EPL in Group B. This may indicate that Group A, (Porcilis PCV and M+PAC) was better protected against Mhyo, with a significantly lower EPL, a marginally better finishing performance and less variation in finishing weights.
Under the conditions of this study, we observed that different control programs offer varying levels of protection against Mhyo. Therefore, a close assessment of EPL should be performed, even in vaccinated pigs, when respiratory disease increases.

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EFFICACY OF TWO DOSING REGIMENS OF PIGFEN® AGAINST THE ADULT STAGES OF ASCARIS SUUM

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Introduction
Ascaris suum, also known as the large round worm, is still the most important helminth in pigs under intensive rearing conditions. A sound anthelmintic control program is of major economic importance, because in addition to unthriftiness and weight loss, the migrating larval stages of Ascaris suum also causes respiratory disease. Pigfen® is a 40 mg fenbendazole per gram containing anthelmintic for pigs. The standard dose rate of Pigfen® is 5mg fenbendazole /kg bodyweight (BW) which can be administered as a single dose treatment (one day) or which can be divided over several days. In this study the efficacy of two different dosing regimens of Pigfen® against adult stages of Ascaris suum were compared in an experimental challenge model
Materials and methods
Three groups (A,B,C) of 19 piglets with a bodyweight of 13 to 20 kilo were individually challenged with 1000 embryonated eggs of Ascaris suum as induced infection at day 0. Group A were the infected non-medicated control group, Group B received one treatment of 5mg/kg BW as Pigfen® at day 56 post infection (PI) and Group C received a 7 day consecutive day treatment of 5 mg/ kg BW as Pigfen® (=0.71 mg/kg BW per day) from day 50 to 56 PI. All animals were necropsied for worm counts at day 64 PI (8 days after treatment). At days 56 and 64 PI faecal samples were collected for egg counts.
Results
Adult Ascaris suum worms were found in 7 out of 19 pigs from the control group (Group A). No adult worms were found in the treated groups. This resulted in a total reduction in number of worms of 100% for both Group B and C. At the time of treatment pigs from all groups excreted eggs which indicated that the experimental infection was successful in the 3 infected groups. At D64 the arithmetic mean (AM) EPG excretion in Group B and C decreased significantly compared to the control group, respectively with 95.07 % and 100%.
Conclusions
Pigfen® showed to be very effective against adult stages of Ascaris suum. No differences were observed between Pigfen® administered for 7 consecutive days and a single dose treatment with Pigfen®

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EFFICACY OF TWO DOSING REGIMENS OF PIGFEN® AGAINST THE LARVAL STAGES OF ASCARIS SUUM

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Introduction
Ascaris suum, also known as the large round worm, is still the most important helminth in pigs under intensive rearing conditions. A sound anthelmintic control program is of major economic importance, because in addition to unthriftiness and weight loss, the migrating larval stage of Ascaris suum also causes respiratory disease. Pigfen® is a 40 mg fenbendazole per gram containing anthelmintic for pigs. The standard dose rate of Pigfen® is 5mg fenbendazole /kg bodyweight (BW) and can be administered as a single dose treatment (one day) or the dose can be divided over several days. The aim of the study was to evaluate the efficacy of two dosage regimens of Pigfen® against the larval stages of the swine helminth Ascaris suum.
Material and methods
Three groups (D,E,F) of 8 piglets with a bodyweight of 13 to 20 kilo were individually challenged with 1000 embryonated eggs of Ascaris suum as induced infection at day 0. Group D was the non-medicated control group, Group E received one treatment of 5mg/kg bodyweight (BW) at day 8 post infection (PI) and Group F received a 7 day consecutive day treatment of 5 mg/ kg BW (=0.71 mg/kg BW per day) from day 2 to 8 PI. All animals were necropsied for worm counts (larval stages) at day 14 PI.
Results
Larval stages of A. suum worms were found in 7 out of 8 pigs form the control group (Group D). The arithmetic mean (AM) number of larvae found in Group D (=control) was 45. In contrast, only 2 pigs harboured only 1 larva in the single dose treated group (Group E) i.e. a reduction of 99.4%. In the 7 day treated group (Group F) no larvae were found i.e. a reduction of 100%.
Conclusions
To conclude Pigfen®, administered for 7 days or as a single dose treatment, was very effective against larval stages of Ascaris suum.

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THE EFFECT OF SUPPLYING LIANOL® COLOSTRO TO NEWLY BORN PIGLETS ON PRE WEANING MORTALITY AND ANTIBIOTIC USE.

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Introduction:
Previous research demonstrated a positive effect of Lianol® Colostro, a complementary feed based on fermented potato protein, on plasma insulin-like growth factor-1 (IGF-1) levels. After birth, IGF-1 has a predominant role in regulating growth. If a beneficial effect on IGF-1 could be demonstrated in just born piglets, this new feedstuff could have a beneficial effect on neonatal piglet mortality.

The objective of this research was to evaluate the effect of this new complementary feed stuff added to the lactation and flushing feed on fertility parameters and litter size in sows.

Material and methods:
This research was a well-controlled field trial in Italy. In total 806 piglets belonging to 71 sows were allotted to the control and treatment group based on similar sow parity.
In the Lianol® Colostro group, piglets received 2 doses of 1 ml of Lianol® Colostro into the mouth: the first immediately after birth, the second 7 to 12 hours later.
For each litter, the daily mortality of piglets from farrowing to weaning was recorded distinguishing between death due to crushing, starvation, diarrhea or other causes. Furthermore, medication and antibiotic use were monitored throughout the whole testing period.

Results:
Administrating Lianol® Colostro to newly born piglets significantly reduced the piglet mortality due to starvation by 1.83 piglets or 11 %. The overall mortality was reduced by 2.41 piglets or 23 % versus the control. The antibiotics use was significantly reduced by 83 % in the Lianol® Colostro group versus the control.

Conclusions:
The supplementation of Lianol® Colostro improved the number of weaned piglets by 2.41 piglet / litter. This trial also demonstrates that Lianol® Colostro can help to reduce the antibiotic use in the farrowing compartment.

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EFFECTS OF ENERGY SUPPLEMENTATION TO NEONATAL LOW BIRTH WEIGHT PIGLETS ON MORTALITY, DAILY WEIGHT GAIN, WEANING WEIGHT AND COLOSTRUM INTAKE

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Pre-weaning piglet mortality is an important economic and welfare problem in pig industry and is mainly due to an energy deficit. Energy supplements may provide directly energy to neonatal piglets as well as improve their colostrum intake. As colostrum is the main source of energy to neonatal piglets, the effect of energy supplementation on mortality and performance as well as on colostrum intake need to be investigated. In practice, energy supplementation can easily be implemented in the farrowing management. Field trials are needed to investigate the efficacy of energy supplements.

The study was performed at a commercial farm with 1700 PIC sows in a 2-week batch system. In total, 25 sows in both groups were enrolled. Low (LBW) and normal (NBW) birth weight piglets were defined as live born piglets with a birth weight of less or more than 1.2 kg, respectively. In the treatment group, LBW were supplemented (Vigorol®, Ecuphar) at birth and 8 to 12 hours after birth. From birth till weaning, dead piglets were daily registered. At weaning, all piglets were individually weighted. Colostrum intake was estimated by a regression equation.

The overall mortality was lower in the treatment than in the control group at day 3 (16% vs. 23%; $P < 0.05$), day 7 (21% vs. 28%; $P < 0.05$) and day 21 (23% vs. 31 %; $P < 0.05$). The mortality of LBW piglets was lower in the treatment than in the control group at day 7 (29% vs. 41%; $P < 0.05$) and day 21 (33% vs. 46%; $P < 0.05$). There was no difference in mortality between the groups for the normal birth weight (NBW) piglets (>1.2 kg). The overall daily weight gain (DWG) and weaning weight (WW) were lower in the treatment (187 g; 5.18 kg) than in the control group 209 g ($P < 0.001$) and 5.69 kg ($P < 0.001$), respectively. The overall lower weaning weight and daily growth in the treatment group might be attributed to the lower LBW piglet mortality in the treatment group. Regarding the LBW and NBW piglets, no difference for DWG nor WW between the treatment and control group was observed. The colostrum intake per piglet, per LBW or NBW piglet did not differ between the treatment (229; 193; 265 g) and control group (238; 191; 274 g). Colostrum intake by the litter was numerically lower (3 054 g vs. 3 308 g) but more uniform (coefficient of variation 57% vs. 74%) in the treatment than in the control group.

Energy supplementation to neonatal LBW piglets reduced pre-weaning mortality of all and LBW piglets but there was no measurable effect on colostrum intake. Pig producers can easily implement this measure in their farrowing management to increase pre-weaning survival.

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CORRELATION BETWEEN NUMBER OF EGGS AND SEROLOGICAL RESPONSES IN THE SERASCA® TEST DUE TO ASCARIS SUUM INFECTIONS IN 4 DANISH ORGANIC FARMS

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Introduction
The SERASCA® test is a serological test that can be used to determine the exposure to both larval and adult Ascaris suum in pigs.
Traditionally the diagnosis is based on detection of endoparasites in swine by counting eggs in feces, using the McMaster technic, but the presence and concentration of eggs do not always reflect the actual infection pressure from Ascaris suum in a farm. Due to the intermittent shedding of eggs, there is a risk of false negative diagnosis based on the McMaster test, and this diagnosis will then not warn against a possible negative impact on productivity.
The aim of this study was to compare the use of the SERASCA® test on blood to determine the prevalence of positivity with the counting of eggs by using the McMaster technic on fecal samples. Additionally the aim was to understand the infection dynamics of Ascaris suum in organic farms.

Materials and Methods
Blood and feces were collected from 4 commercially organic farms in Denmark. From each farm, 3 x 5 feces samples were taken from rectum of pigs at the age of 7, 12 and 17 weeks. Furthermore 3 x 10 blood samples were collected from pigs in the ages of 10, 15 and 20 weeks. The samples were taken as a cross-sectional investigation. By sampling blood from pigs 3 weeks older than the once exposed to fecal sampling, it was assumed that blood sampling reflected the level of infection present three weeks earlier due to the delay in development of antibodies. A positive SERASCA® test was registered as "positive in SERASCA®" and finding of shedding in one or more samples at a given time in the McMaster was recorded as "shedding of eggs".

Results
In 100% (9 of 9) cases where a positive McMaster was found, the concurrent SERASCA® was positive. On the other hand a positive SERASCA® was related to a positive McMaster in 82 % of cases (9 of 11).

Conclusions
The strong correlation between demonstrating the eggs in McMaster and the serological response in SERASCA® underlines the value of the SERASCA® test to determine whether or not a farm is infected with A. suum.
Although there were too few farms to make a statistical evaluation, it was interesting to find that pigs at all 4 organic farms were infected.
Pigs at 2 of the 4 organic farms were already infected at 7 weeks of age, indicating an early infection during farrowing that is maintained and further developed during the growing phase.
The study results indicate the importance of investigating the presence of parasites in organic farms, as any level of infection is suspected to impact productivity and may negatively interfere with vaccine efficacy if piglets are infected already around 7 weeks of age.

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EFFICACY OF INTRADERMAL MYCOPLASMA VACCINATION COMPARED TO CONVENTIONAL CONTROL STRATEGIES

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Introduction
Although intramuscular (IM) vaccination is most commonly used to administer swine vaccines, intradermal (ID) vaccination has demonstrated a lot of advantages. MSD Animal Health has an ID needle-free device (IDAL) that reduces iatrogenic transmission of systemic pathogens and reduces pain and stress. Intradermal vaccination is highly immunogenic, and due to the high presence of dendritic cells in the dermis, the immune response is in some cases even higher than via IM. The aim of this study was to prove safety and efficacy of an ID vaccination against M.hyopneumoniae, and compare results with conventional IM control strategies against enzootic pneumonia.
Materials and Methods
The trial was done in 2 farms located in Murcia (Spain). Farm A (1250 sows) was PRRSv positive and unstable. Until July 2012, an M.hyo commercial vaccine was applied (IM) at 1 week of age. From July 2012 to March 2013, M.hyo vaccination was substituted by a macrolide treatment administered in fattening feed. The results were not as expected, and protocol was changed to vaccination of piglets with Porcilis MHyo ID ONCE. Farm B (1175 sows) was also positive to PRRS virus. In this farm, piglets were vaccinated at 1 week of age with a commercial Mhyo vaccine. Due to a PRRS outbreak in sows in March 2013, Porcilis Mhyo ID ONCE at 14 days of age was introduced to reduce the potential iatrogenic PRRS transmission and stress in viremic piglets. To measure efficacy of the control strategies, Mhyo lung lesions were evaluated at slaughter (1 to 5 score; 2000 lungs-Farm A and 1800-Farm B). A historical comparison was done of performance data from 75 batches of fattening pigs.
Results
Lung Lesion Score at slaughterhouse of Farm A was as follows: Prevalence of lesions 32.47% Macrolide group vs 16.85% IDAL group; Disease Index 0.6 in Macrolide Group vs 0.32 in IDAL; and severe lesions 3,07% in Macrolide group vs 1,9% in IDAL group (all parameters $p>0.05$). In Farm B, the results of IM vs IDAL vaccination were 19.4% vs 20.9% in prevalence of lesions, 0.41 vs 0.36 in Disease Index and 3.55 vs 1.6% of severe lesions ($p>0.05$). In regards to productive data, in Farm A, macrolide group had ADWG of 656g/d and FCR of 2.53 vs 667 g/d and 2.46 with IDAL ($p>0.05$). In Farm B, ADWG and FCR improved from 651.2 g/d and 2.58 with IM vaccination to 690.2 g/d and 2.49 with IDAL ($p<0,01$).
Conclusion
Intradermal needle-free IDAL Mhyo vaccination demonstrated to be safe and efficacious when compared to previously used conventional IM vaccination or antibiotic treatment strategies in the study farms, with comparable results in lung lesion score and improved productivity parameters such as ADWG and FCR

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EXPLAINING PIG FARMERS' INTENTION TO REDUCE THEIR ANTIMICROBIAL USAGE: FINDINGS FROM A SURVEY IN SIX EUROPEAN COUNTRIES

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Introduction
Antimicrobial (AM) resistance is an increasing problem in human and animal populations and has been related, among other factors, to the use of AM in pig farming. To be able to tackle this problem, it is important to know how farmers perceive AM usage and AM resistance, and which personal and psychosocial factors are related to farmers' intention to reduce the AM usage at their farm ("intention to reduce"). Intention is a prerequisite for actually taking measures to reduce the AM usage.
Method and Materials
We conducted a survey among pig farmers (N = 1,309) in Belgium, Denmark, France, Germany, Sweden and Switzerland, which was part of the European MINAPIG project (www.minapig.eu). The questionnaire included items about, among others, the risks and benefits of AM usage, AM treatment practices, intention to reduce, knowledge about AM, personal experiences with AM resistance, and control (i.e., ability and effectiveness) over reducing their AM usage. Most items were measured on 6-point Likert scales.
Results
We found several significant differences between the six countries regarding farmers' perceived benefits and risks of AM, relation to their veterinarian and perceived control of reducing their AM usage. For example, Danish farmers perceived fewer risks of AM usage than their colleagues from the other countries. Moreover, a regression analysis showed that farmers reported more intention to reduce when they reported to have had personal experience with AM resistance, to use a higher amount of AM compared to other farmers, to perceive more risks of AM usage and less need to use them, to have a better relation with their veterinarian and, most importantly, to perceive more control over a reduction of the AM usage. Knowledge about AM did not influence farmers' intention to reduce.
Conclusion
In sum, to increase farmers' intention to reduce AM usage, just providing them with more information about AM resistance will be insufficient. It seems more important to show farmers how they can control AM reduction at their farm. This can for example be done by showing them alternative methods to keep their pigs healthy, ways to improve the biosecurity at their farm and that small reductions at their farm may have large effects on a country level.

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SEX IS A RISK FACTOR FOR IRON DEFICIENCY ANAEMIA IN PIGLETS AT WEANING

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Introduction
Piglets are born with very limited iron reserves. Therefore, injection of iron (200mg) at early days of life has become routine management practice in commercial herds for the prevention of anaemia and iron deficiency. Whether all the piglets receive adequate iron is still a matter of discussion. Therefore, we aimed at investigating important sow and piglet characteristics that may be potential risk factors for the development of iron deficiency and anaemia in piglets.
Materials and Methods
Nine conventional farrow-finish sow herds were selected for the study. Within each herd, 20 litters belonging to a weekly farrowing batch were selected randomly at weaning. From each litter, the largest piglet based on visual judgement and a random piglet were selected. Blood samples of each piglet were analysed for complete haematology including serum iron. The effects of the explanatory variables type of piglet, sex, parity of the sow, litter size at weaning and weight of piglet at weaning on haematological parameters was tested using general linear mixed model with herd as a random effect. Statistical significance was set at $P < 0.05$.
Results
A total of 360 piglets belonging to 180 litters from nine herds were included in the study. Anaemia defined as haemoglobin < 90 g/L was observed in 20 castrated male piglets (11.7%) and 7 female piglets (4.7%) (P=0.02). The mean haemoglobin concentration in males was lower compared to female piglets (111.2 ± 20.0 vs. 114.9 ± 15.2 g/l) (P=0.02). Similarly, compared to females, males had lower haematocrit, mean corpuscular volume (MCV), mean cell haemoglobin concentration (MCHC) and serum iron. Males had higher percentage of reticulocytes compared to females. All these differences were either significant or tended to be significant based on the final model. Only serum iron differed according to piglet type. Parity and litter effects were non-significant for all the parameters. However, weight at weaning had significant effects on MCV and MCHC.
Conclusion and discussion
Several haematological parameters differed significantly between castrated males and female piglets. Lower haemoglobin in males may be related to bleeding during castration or other physiological processes that is still unknown. Previously, we have reported that large piglets in litters are at a higher risk of developing iron deficiency anaemia compared to smaller ones. Hence, it seems that large male piglets may be regarded as risk animals when diagnosing anaemia and iron deficiency at the herds.

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A CASE REPORT OF AN ERYTHEMA MULTIFORME, ASSOCIATED WITH A RESPIRATORY DISEASE, IN A COMMERCIAL BREEDING HERD

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Introduction
Symmetrical red lesions all over the skin, more prominent around the eyes and ears compatible with erythema mutliforme were observed in the majority of sows/gilts of a large-scale farrow-to-finish commercial pig farm at the 30-35 days of gestation (moving to the group housing stable). The affected animals present depression, decreased appetite, high fever, posture difficulties and respiratory signs without increased mortality. In addition, the return to oestrus rate of was increased in inseminated sows/gilts.
Materials and methods
Blood samples from 7 diseased sows were tested by qPCR for PRRSV (PRRSV EU, US) and PCV2, and serology for A. pleuropneumoniae, E. rhusiopathiae, PRRSV, Aujeszky, ASFV, CSFV and Leptospira spp. Nasal swabs were also examined by PCR for apx-IV gen of App, B. bronchiseptica, H. parasuis, P. multocida, S.suis, PCMV and SIV.
Complete blood count was also performed in blood samples. At slaughterhouse, samples from 7 diseased sows (skin, liver, kidney, lung) for histopathology, were also collected. Finally, feed from gestation diet was examined for mycotoxines.
Results
All sows were seropositive for App and PRRSV, but seronegative for ASFV, CSFV, ADV and Leptospira spp. The seroconversion to PRRSV of sows was due to the booster vaccination at 6th day of lactation (MLV vaccine). No viraemia for PRRSV and PCV2 was detected. S.suis was detected in all nasal swabs samples, but one sample was positive for App, H. parasuis and S.suis and one nasal swabs sample was positive for SIV.
The CBC count with differential revealed moderate leukocytosis with atypical lymphocytes and lymphopenia, possibly secondary to the depletion of CD4 lymphocytes. An eosinophil count greater than 3000/μL was also seen in two cases and neutropenia occurred in the most severe case. A severely elevated total WBC counts in two cases was consistent with infection. Mild anemia was also present, and thrombocytopenia was found in one case. Mycotoxins were not found at detectable levels in the diet. The histopathological examination of the skin revealed increased vascularization of the superficial and middle dermis mainly. No remarkable lesions were found in the other examined organs. No mycotoxins were detected in the feed.
Conclusion
Erythema mutliforme is possibly mediated by deposition of immune complex in the superficial microvasculature of the skin that usually follows an infection or other various triggers. In present study, under stressful group-housing conditions a subclinical infection or an interaction of different respiratory pathogens seems to have been activated, affecting negative the health status and performance of the breeding stock.

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PRRSV EXPOSURE DYNAMIC IN GROWING PIGS IN EUROPEAN FARMS PART I

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Introduction
The objectives of this large study were to determine PRRSv exposure pattern and virus genetic diversity in growing pigs in European farms; to capture key information about common farm practices on biosecurity, diagnostic monitoring, and control tools and to capture mortality impact at the moment of sampling.

Material and Methods
Thirty four farms were identified across Europe in thirteen countries: Germany n:2; Ireland n:1; UK n:2; Italy n:3; France n:4; Spain n:3; Belgium n:3; Poland n:3; Hungary n:2; Romania n:2; Austria n:3; Denmark n:3; The Netherlands n:3. These farms were typical production systems for each country and with PRRSv clinical problem history. All farms represented 41,329 sows. At each farm, a cross sectional sampling was implemented taking three sampling points: at weaning; end of nursery and mid finisher. At each sampling point, 20 blood samples were taken running PRRS IDEXX ELISA 3x and PRRS rt-PCR in all samples, getting serum individual results for ELISA (60 samples per farm) and pooled (1:5) for PCR (12 pools per farm). In total, 1,980 individual ELISAs, 396 pooled PCRs and 50 ORF5 sequences from selected positive results were added in the analysis. In addition to diagnostic work; an on-farm questionnaire was implemented at each farm at the moment of sampling, capturing information about: production system; mortality; PRRS status; semen source; previous information about PRRS sequencing; biosecurity and PRRS monitoring programs in place and PRRS control strategies at different levels of production system: gilts, breeding herd and growing pigs. This part I shows results on farms stats description and cross sectional serum profiles ELISA and PCR from farms. F-test for SP ratio variability and Mann-Whitney tests were used for diagnostic results analysis.

Results
According to the questionnaire, the description of the sample was: 63% of farms as Farrow to finish; 17% farrow to nursery and the rest multi-sites or two sites. Median size: 800 sows, having a max of 9,500 and a min of 60, distributed with positive/right Skew (3.88). Prevalence by pig flow was 85.2 %, where PRRS genotype I was identified in 28 pig-flows out of 29, and genotype II only in one. Cross sectional profile on ELISA (N: 1620 samples; 540 per sampling point) and PCR (N:99 pools; 33 per sampling point) showed end of nursery phase as the highest PRRSv circulation sampling point in growing pigs. Profile results for at weaning; end nursery and mid finisher were: S:P ratio mean: 0.622 / 0.796 / 1.664; stdev: 0.621a / 0.796b / 0.901c ;PRRS Wild Type median: 0%a /100%b / 25%c respectively.

Conclusion
Farms selected for this study were well representative for European conditions in terms of production type and size. Wild type PRRSv prevalence by pig flow was 85%, having the highest circulation at end of nursery. PRRS genotype I was identified in 96% of pig flows. These results highlighted PRRSv presence and circulation in growing pigs as an important epidemiological event that should be considered and addressed in control and eradication programs. Information on virus genetic variability, mortality impact and control practices are discussed in part II.

P148

A COMPARATIVE FIELD STUDY TO ASSESS THE PALATABILITY OF PARACETAMOL (PRACETAM® 40% ORAL SOLUTION FOR PIGS) ON LACTATING SOWS

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Introduction: In order to ensure a correct administration of an oral treatment, palatability is a key factor to control, particularly after farrowing, when sows often exhibit lack of appetite. Paracetamol is widely used in swine production to reduce fever and pain mainly because of its absence of side effects such as haemorrhages or ulcers. This present study aims at assessing the palatability of Pracetam®40% and Pracetam®20% in comparison with placebo when administered to lactating sows.

Material and Method: This contemporary, half-blinded, randomized and controlled study was conducted on a commercial farm. A crossover design was implemented on 30 sows fed with soup meals, one week after farrowing. Immediate voluntary consumption at distribution, occurrence of remaining feed in trough 20 mn after meal, and before the following meal were recorded twice a day. Paracetamol was administered at the recommended dose of 30mg/kg/24h. Each sow received alternatively each treatment (A: Pracetam®40%, B: placebo, C Pracetam®20%). Three rounds of two days were defined, allowing a randomization of the order of treatment (A/B/C, B/C/A, C/A/B). Between each round, included animals received no treatment during two "wash-out" days, during which ingestions were observed to monitor usual appetite. Two ways of distribution were tested alternatively: administered in top feeding with a small amount of feed before the meal, and mixed in soup directly in trough.

Results: Considering immediate acceptance, no significant differences were observed between groups: no reluctance at trough was recorded for placebo (B group) or Pracetam®40% (A group): 0/120 observations, both for B and A, and solely 2/120 for C. 20 min after administration, 90% of animals from both A and B groups and 88.3% in C group had cleared out their trough (non significant difference). Before following meal, only 1.7%, 5% and 5% of troughs with leftovers were observed for A, B, and C respectively (non significant difference). Treatment order had no significant effect on palatability. No impact of distribution type could be observed.

Conclusion: Finally, this study clearly demonstrated a satisfying palatability of Pracetam®40%, as well as for the 20% dosage or for placebo. The acceptance rate remained comparable, regardless of the distribution type.

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PRRSV EXPOSURE DYNAMIC IN GROWING PIGS IN EUROPEAN FARMS PART II

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Introduction
Objectives of the study: to determine PRRSv exposure pattern and virus genetic diversity in growing pigs in European farms; to capture key information about common farm practices on biosecurity, diagnostic monitoring, and control tools and to capture mortality at the moment of sampling

Material and Methods
34 farms were identified across Europe in 13 countries. A cross sectional sampling was implemented. We analyzed 396 PRRS PCR and 50 ORF5 sequences from selected + results. On farm questionnaire provided info on farm characteristics, biosecurity practices, diagnostic and control protocols. Part II includes PRRS sequences from 21 farms in 11 countries (AU, BE, DE, DK, FR, HU, IT, NL, PL, RO, UK) aligned with 5 references (Lelystad; Lena; Porcilis; Amvervac, Ingelvac MLV) getting a genetic distance table and tree. The analysis was based on ORF5 genetic distance considering >3% as a cut off for classifying as different virus giving a nomenclature name specifically structured for this project starting as EPI with a consecutive number for different Wild-Type (WT) viruses identified in genetic distance table. Regression analysis for mortality in nursery and finisher and WT PRRS proportion was run. We analyzed data for descriptive stats on monitoring and control strategies from questionnaire

Results
Sequences distribution: 26% at weaning, 58% end of nursery, 16% mid finisher respectively. The majority of farms had a single variant of WT PRRS circulating in growing pigs. Same clade virus (EPI-2) was identified in farms in Belgium and Germany; also one farm in France and two in Italy shared same clade (EPI-3). A WT PRRS genotype II was observed in Denmark. Vaccine strains were identified in pig-flows with history of vaccination either in sows only or sows & pigs. Mortality mean in nursery was 3.08% +0.026 and 2.89%+0.019 in finisher at moment of sampling in + pig-flows finding a significant association (P value <0.10) between nursery mortality and WT PRRS prevalence at end of nursery but not between finisher mortality and prevalence at mid-finisher (P value >0.10). 70% of farms used MLV as primary tool for PRRS control, out of these, 58% applied mass vaccination, 42% post farrowing/during gestation. Only 29% of these farms vaccinated piglets. From all farms, 32% had previous sequencing information; 68% biosecurity program and 56% monitoring in place. Regarding the semen source question; 62%, 26% and 12% receive semen from external, internal and did not know. For farms receiving semen from external sources, PRRS status was positive in 19% while 17% did not know the boar stud status

Conclusion
Sequencing results confirmed end of nursery as the most important production phase for PRRS circulation; also, it indicated one PRRS variant circulating per pig flow as a common observation. Some farms shared the same virus variant, possibly related to transportation and/or area spread. Mortality impact was associated to the nursery phase, suggesting this as main improvement target for average and batch variability in control programs. Also, this study highlighted the need for better practical understanding of sequencing and semen source status.

P150

COMPARISON OF PERFORMANCE AND CARCASS PARAMETERS IN RESPONSE TO MACROSCOPIC LUNG LESIONS WHILE USING IDENTICAL PRRS AND PCV2 BUT MYCOPLASMA DIFFERENT VACCINES IN PIGS

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Introduction: According to the efforts to further reduce the use of antibiotics in livestock, the question arises whether mild respiratory infections (and associated mild lung lesions) in general lead to impairment of animal health and negatively affect performance and therefore treatment is necessary.
Methods: In a combined piglet producing and fattening farm (200 sows/3-week rhythm) with known mild respiratory infections in fattening, groups of suckling piglets were alternately vaccinated at d 21 of life with a PCV2 (CircoFLEX®) and a PRRS vaccine (Porcilis PRRS®). Three different immunization schedules were applied concerning Mycoplasma vaccines: (group "MFC": Ingelvac MycoFLEX® mixed with the PCV2-Vakzine; group "MFS": Ingelvac MycoFLEX® administered alone at d 21; group "SV": Suvaxyn M. hyo® on d 7 and d 21). About 80 to 90 animals of each group (one replicate/treatment; six groups in total) had been the subject of investigations (laboratory diagnostics in 120 pigs in total; lung lesion scoring of each pig at the slaughterhouse - Annex I to Regulation (EC) no. 854/2004). Carcass characteristics were included. Statistical analyses concerning performance were performed with SAS (One way ANOVA; $p <= 0.05$).
Results: At end of fattening most of the animals were serological positive for Mycoplasma (MFC: 72.5 %; MFS and SV: 100%). Animal losses were within normal range (MFC: 1.04 %; MFS: 2.60%; SV: 2.08%), life daily gains did not differ between the groups (MFC: 605±62; MFS: 604±55; SV: 602±48g). Due to the 3 to 5 d lower age at slaughter, carcass weight in group MFC (95.5±6.95a kg; MFS: 97.7±5.79b kg; SV: 96.9±6.57b kg) as well as index points per kg carcass weight were lower (MFC: 0.964±0,077 a; MFS: 0.997±0,059 b; SV: 0.984±0,064 b). Animals with no lung lesions in group MFC had the numerical highest life daily gains (611 g) whereas in groups MFS und SV animals with mild pulmonary changes (<10 % of lung tissue) had the highest gains (MFS: 613 g; SV: 606 g). In all groups animals with significant pulmonary changes (10-30 % of lung tissue) had the numerical lowest daily gains during life (MFC: 593 g; MFS: 598 g; SV: 588 g). Most of the animals in all groups had no or very mild changes in lung tissue (MFC: 89.1%; MFS: 96.1 %; SV: 94.4 %); only 3 animals had very severe lung lesions (>30 % of lung tissue).
Conclusion: Slight lung lesions must not have a negative impact on performance. Only severe changes in the lung tissues give an indication of infection-related loss of growth and thus on animal health. With regard to the efforts towards a further reduction of antibiotic use, it could be questioned as to when a slight cough should be treated in a herd.

P151

THE BIOSECURITY STATUS OF PIG HERDS DIFFERS AMONG COUNTRIES, IS HIGHER IN LARGER HERDS AND HERDS MANAGED BY UNIVERSITY EDUCATED FARMERS

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Introduction
Reduced and prudent usage of antimicrobials (AM) in livestock production is a hot topic in the media, politics and science nowadays, mainly in relation to public health. Improvement in the biosecurity status of a pig farm is an important factor to improve the overall health status of a farm and subsequently reduce the necessity of AM usage.
Materials & Methods
A cross-sectional study has been conducted in farrow-to-finish pig herds (n= 232) in Belgium, France, Germany and Sweden between 2013 and 2014 to assess the current biosecurity status in these countries and to identify factors associated with the level of biosecurity. The study was part of the European project MINAPIG (www.minapig.eu). The biosecurity status was quantified (score 0-100) by using the risk based scoring tool Biocheck.UGent (www.biocheck.ugent.be).
Results & Discussion
Total biosecurity was lower in Belgium ($\bar{x}$ = 57.8) and France ($\bar{x}$ = 58.6) compared to Sweden ($\bar{x}$= 63.7) and Germany ($\bar{x}$= 63.0). External biosecurity scores were in general higher than internal biosecurity scores. This is maybe due to the fact that it is more convenient to set rules for external visitors compared to implementing routine management changes.
Between countries a significant difference was observed for external biosecurity ($p< 0.01$) and nearly significant for internal biosecurity ($p= 0.06$). The country effect may be related to different habits and approaches towards biosecurity in the different countries. Moreover some biosecurity measures might be easier to implement in some countries (e.g. sparse population density in Sweden).
The number of sows was positively associated with internal biosecurity ($p< 0.01$) and external biosecurity ($p< 0.01$). This may be explained by the fact that larger farms are generally better structured and therefore can more easily implement biosecurity measures.
Furthermore the education level of the farmer also influenced the internal ($p= 0.01$) and external ($p= 0.03$) biosecurity scores in the sense that farms where the main responsible person graduated from university scored on average higher on biosecurity status.
Number of weaned piglets per sow per year and the mortality percentages were not significantly associated with the biosecurity status, indicating that herds with higher biosecurity not necessarily have a higher production. Furthermore weaning age and daily weight gain also showed no significant association to biosecurity.
Conclusion
Improvement in biosecurity status is still possible in the participating countries. Higher biosecurity levels are associated with the number of sows and education level of the farmer.

P152

THE CHANGE IN THE PRODUCTION PARAMETERS IN SWINE HERDS VACCINATING AGAINST PRDC BETWEEN 2011–2014

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Introduction
Porcine Respiratory Disease Complex (PRDC) results from a combination of infectious agents as well as environmental and management stressors and challenges, affecting the health of the pig and resulting in reduced performance, increased mortality and increased medication costs. Vaccination plays an important role in the control of PRDC, but its success also depends on the immune status of the pigs, the housing conditions and other management issues.

Materials and Methods
In March 2011 to October of 2012, we surveyed 52 large-scale pig fattening units in Hungary in terms of environment, management, housing, production parameters and respiratory health status, including vaccinations applied against PRDC, with the Respig Farm Audit Tool™ (MSD AH). The survey included 58,590 sows and 493,878 growing pigs and fatteners which meant more than 25% of the entire Hungarian sow population. In April 2013 to March 2014, the general audit was repeated in 15 swine farms that modified their vaccination strategy after the survey and 16,524 sows and 166,491 growing pigs and fatteners were evaluated again.

Results
In 2011/12, the average number of sows per farm was 1,254, in 2013/14 1,377, while the average finishing capacity was 8,312 and 11,785 pigs, respectively. In the period examined, the slaughter weight decreased from 111.8 kg to 110.4 kg. The post-weaning FCR reduced from 3.0 to 2.75, the post-weaning ADG increased from 612 to 657 g/day. The post-weaning mortality rate, including premature disposal slightly reduced from 8.90% to 8.60%. The animal health cost, consisting of the cost of both medication and vaccination, did not change and amounted to €6.7-6.8/finisher.

Conclusions
The size of the survey gave us a broad and deep scope about technical data of the entire Hungarian swine sector, and we could identify the major changes in the production parameters over 2-2.5 years in those farms that used vaccines against PRDC. It can be concluded that the production parameters were improved and, despite the larger vaccination costs, the animal health costs remained at the same level between 2011/12 and 2013/14 in Hungarian swine herds that continuously monitored and modified their PRDC vaccination programme. The regular check-ups (audits) of the herds might also help to improve the productivity in the pig finishing units.

P153

EFFECTS OF ZNO AND COLISTINE ON ANIMAL HEALTH AND PRODUCTION PARAMETERS IN WEANED PIGLETS

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Introduction: The aim of this study was to identify if ZnO supplementation in weaned piglets has an effect on the piglet's health, production parameters and reduction in the use of antibiotics, in comparison with piglets supplemented with colistin and a negative control group.
Material and methods: This study was conducted on three commercial pig herds during two successive weaning rounds with four groups of each 60 weaners. During the first two weeks of the nursery period, group 1 received colistin (Promycine® 400 IU/mg, premix, VMD, Belgium) in the feed, group 2 colistin (Colistine Eurovet® 400000 IE/ml, Eurovet, Belgium) in the drinking water, group 3 ZnO (Gutal®, 2500 ppm, Huvepharma, Belgium) and group 4 was a negative control group. Pigs were weighted at weaning, at day 14 and at the end of the nursery period to determine daily weight gain (DWG) and feed conversion ratio (FCR). During the entire nursery period, feed intake, mortality and antibiotic use were recorded. On day 7 and 14 after weaning, feces samples were taken for enumeration of total and hemolytic E. coli. Dirty backhands were scored weekly per pen as a measure for diarrhea.
Results: The piglets from the ZnO group showed significantly ($P<0.05$) better DWG during the first two weeks of the nursery period in comparison to the other groups. No statistic significant differences ($P>0.05$) were shown for DWG from day 15 until the end of the nursery period, for the total nursery period and for the FCR. The percentage of mortality/wasting pigs showed no significant difference ($P>0.05$) between the four groups. Total E. coli was significantly lower for the colistin in drinking water group compared to the ZnO and the negative control group ($P<0.05$). The negative control group had the highest percentage (73.5%) of positive samples for hemolytic E. coli ($P<0.05$). The ZnO group scored the lowest number of dirty backhands ($P<0.05$). The antibiotic usage showed clear differences between the two colistin groups compared to the ZnO and the control group ($P<0.05$), due to the use of colistin.
Conclusion: This study documented that ZnO can be used as an alternative for colistin in weaners, as it showed equal or better production results combined with lesser diarrhea and a reduced use of antibiotics.

P154

A LONGITUDINAL FIELD STUDY TO ASSESS THE APPLICABILITY AND ROBUSTNESS OF ORAL FLUID PRRSV RT–QPCR AND ELISA

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Introduction
Detection of Porcine reproductive and respiratory syndrome virus (PRRSV) RNA and antibodies (Ab) in porcine oral fluid by RT-qPCR and ELISA, respectively, has recently gained much attention. Nevertheless, conflicting reports regarding the sensitivity, specificity and robustness of these tests have been published. Here, we evaluated the applicability of porcine oral fluid testing by PRRSV RT-qPCR and ELISA, respectively, in a longitudinal field study involving four different swine farms.
Material and Method
Pen-wise oral fluid and blood from selected individuals, representing 20% of the pen-mates, was sampled at three consecutive time-points. A total of 126 serum and 56 corresponding oral fluid samples were collected and tested for both PRRSV RNA and PRRSV Ab in two different laboratories. The agreement between RT-qPCR and ELISA results obtained from serum and oral fluid in both participating laboratories was assessed, as well as the relative sensitivity and specificity of oral fluid testing in comparison to serum-based tests.
Results
Results from both laboratories were highly agreeing, despite differences in pooling strategy and RT-qPCR protocols between laboratories. In comparison to testing individual blood samples from a fraction of pen-mates, oral fluid RT-qPCR and ELISA were equally sensitive in determining the PRRSV virological and serological status of the pens. While oral fluid RT-qPCR was highly specific as well, several false-positive oral fluid ELISA results were observed.
Conclusion
Our results support the applicability and robustness of oral fluid PRRSV RT-qPCR and ELISA in the studied field situation. We concluded that oral fluid might be a useful matrix to determine the PRRSV virological and serological status of pig herds. However, positive oral fluid ELISA results should still be confirmed by additional blood tests.

P155

PREWEANING PIGLET MORTALITY IN LOOSE HOUSED NORWEGIAN SOW HERDS

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A thorough investigation of preweaning piglet mortality was conducted in loose housed Norwegian piglet producing herds. This study included 5370 piglets and 351 sows in 14 herds; all sows from one batch from each herd. From these, 4914 piglets were liveborn and 456 stillborn (8.4% of totalborn). Preweaning mortality, excluding the stillborns, was 15.3%. All stillborn piglets, and piglets that died before weaning (n=1209, 22.5% of totalborn) were autopsied at The Norwegian Veterinary Institute (NVI). Tissue samples from the liver, heart and lungs were collected for histological investigation. Samples for microbiological examination were obtained when signs of infection were observed
The weaning of piglets occurred at five weeks of age, and the oldest piglet that died was 32 days old. From all 1209 piglets autopsied, 37% died during the first 24 hours (including the stillborn piglets), 12% died at day one, 9% died at day 2, and 42% of the piglets died during days 3 to 32.
The most frequent cause of death in this study, was trauma (n=348, 29%). The second most frequent cause was fully developed piglets that had died during birth (n=165, 14%). Eleven percent (n=132) died due to starvation, and only 7% died of infection (enteritis or other, n=83). Other important autopsy findings were prenatal death (7%), euthanizia (10%), aspiration of meconium (6%), weakborn (4%), anemia (3%), other reasons (4%), and uncertain cause of death (5 %.) The age (median) of piglets that died of trauma was 1 day, ranging from 0-18 days. For piglets dying of starvation, the median age at death was 3 days, ranging from 0-32 days, and for the piglets that died of enteritis, their median age was 7 days with a range from 0-31 days. Escherichia coli was the causative agent in most of the cases with infection. Typing of the E.coli will be performed.
Preliminary statistical multilevel logistic regression analysis showed that the odds of dying of trauma, compared to other causes of death, increases with large batches (P<0.001), and decreases with age of piglet (P<0.001). These analyses will later on account for various management routines.
Further analysis will include characteristics of both sow and management routines to investigate which factors that are associated with various causes of death, with the goal of helping the farmer to reduce the number of piglets dying in the preweaning period.

P156

A TOOL FOR AN OPTIMIZED PARTURITION MANAGEMENT

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Induction of farrowing creates an opportunity for producers to supervise parturition, reduce farrowing duration and stillbirth rates, decrease variability of piglet weaning ages and lactation lengths. Nevertheless, few questions remind concerning the possible side effects of farrowing induction or oxytocin overuse on sows (colostrum production, lactation efficiency, reproductive carrier) and piglets (increasing number of immature and stillborn piglets, decreasing vitality and growth). On the other side, recent studies underlined the positive impact of carbetocine (oxytocin analog with a progressive release and a longer action) on parturition's length and piglets' vitality and the absence of detrimental effect on colostrum production and immunoglobulin G content. In this trial, we propose to test an herd-specific induction protocol combining cloprostenol (Prostaglandine F2β analog) and carbetocine in order to optimize parturition management.

The study was conducted in three farrow-to-finish farms (weaning at 21 days) located in West of France. In each farm, the sows from two consecutive batches have been followed for two farrowing periods. Before farrowing, the average gestation length of the farm was calculated and two groups of sows of the same batch were randomly selected to constitute a control group and an induced group. For the control group, the sows received prostaglandins and/or sergotonine as a post-partum treatment. The induced group was injected with 1mL of cloprostenol one day before the scheduled date for birth and 0.5mL of carbetocine 24 hours later.

We observed for the induced group a lower number of stillborn piglets (0.98 vs 1.17) and immatures (0.11 vs 0.30), a slightly higher rate of liveborn piglets (89.85% vs 89.31%) and an increase of 0.6 piglet reared per sow. Moreover there was no negative impact on the following farrowing with no decrease of fertility or litter size.

The originalty of this study is to evaluate the effect of this protocol on the sows, on their productivity and on their next farrowing without any post-partum treatment. No decrease of the performances have been observed and we noticed a good vitality of the piglets. Interestingly, the rate of cross-fostering toward induced sows was higher which is against the hypothesis that these sows would be less productive or more stressed. These results underline the importance of a well-reasoned herd-specific protocol by taking into account the average gestation for each herd. The use of cloprostenol combined with carbetocine might imply less intervention by the farmer leading to a better management of this work period through a closer surpervision of the parturition.

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THE IMPACT OF STRAW ON GASTRIC HEALTH IN FINISHING PIGS

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Introduction
Ulcerations of the stomach's pars oesophagea (PO) of pigs are a serious health problem, but etiology is not completely clear. Several predisposing factors are described to cause oesophagogastric ulceration (OGU), e.g. overcrowding, genetics or nutrition. Straw is able to improve welfare of pigs housed in barren environments as it enables normal foraging behavior. The aim of the study was to investigate the combined effect of long straw (150g/pig/day) on OGU of finisher pigs on a conventional farm in Austria.
Materials and methods
In total 233 pigs were assigned either to straw (SG, 113/233) or control group (CG, 120/233) and fed with liquid food. Stomachs were collected at the abattoir, opened along the large curvature, washed with water. PO was scored macroscopically as (MS): 0 = normal; 0.5 = mild hyperkeratosis (HK) (< 25 %); 1 = moderate HK (< 50 %); 1.5 = severe HK (> 50 %); 2 = severe HK (> 75 % and erosions); 3 = ulcer. Tissue samples of a defined part of the PO were examined and scored histologically. Histological examination scores (HS) were defined as: 0 = normal; 0.5 = mild hyperkeratosis (HK) (proliferation of stratum (str.) corneum, < 5 cell layers (CL)); 1 = moderate HK (proliferation of str. corneum, 5 - 10 CL), 1.5 = severe HK (proliferation of str. corneum, > 10 CL), 2 = severe HK and erosions (damage under str. spinosum), 3 = ulcer (damage through str. basale).
Results
In total 53 (22 %) stomachs were assessed macroscopically and six (2.6 %) histologically as unaltered. Significant differences of OGU (summarized score 2 and 3) were found (MS: p = 0.034; HS: p = 0.015) between the groups. OGU was found macroscopically in 38/233 (16 %) and 66/233 (28 %) samples by histologic examination. Stomachs with a score of 3 were significantly lower in CG (MS: p = 0.023; HS: p = 0.007) compared to SG.
Conclusion
Our findings reveal the importance of histological examination after visual scoring for detection of early changes of the mucosal surface of the PO, as macroscopic examination alone underestimated the actual prevalence. Furthermore no protective effect of straw for the development of OGU was found, which is in contrast to several other studies. In our study the reason for a higher incidence of OGU in the SG cannot be clearly evaluated. Generally on that farm particle size of food was smaller than recommended and daily weight gain at the time point of slaughter was high. Further studies should focus on the effect of different kind of roughage as well as length and structure of straw.

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EFFECT OF REMOVAL OF IN-FEED ANTIBIOTICS ON PERFORMANCE AND HEALTH INDICATORS OF PIGS DURING THE WEANING STAGE

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Objective: To quantify the effects on health and performance of removing in-feed antimicrobials (AM) in pigs after weaning and using targeted parenteral administration of AM.

Material and Methods: This study was part of a larger trial looking at the effects of removing in-feed AM on indicators of pig performance, health and welfare from weaning to slaughter. The study was carried out on a commercial farrow-to-finish farm (300 sows) positive for PRRS and influenza and with a programme of in-feed AM treatment for meningitis in place (Sulfadiazine-Trimethoprim, Pfizer Ltd., 14.4mg/kg BW/d; for 5 d/wk). Every week for six weeks 140 pigs were weaned at 28 ± 2 d of age, weighed, tagged and sorted into 2 groups of approximately 70 pigs with similar body weight (9.2 ± 0.56kg). In-feed AM were removed from the diet of one group (NONE) and were maintained in the diet of pigs in the other group (ANTIB). At the end of the first weaner stage (i.e. after a period of 4 wks and 4 d) the pigs were weighed and moved to the second stage weaner accommodation. Feed intake of the groups was recorded daily as well as all mortalities and all parenteral administration of antibiotics. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Data were analysed by general linear models using SAS 9.3.

Results: ANTIB pigs showed higher ADFI (61g more; P = 0.051) and ADG (33g more; P = 0.033) than NONE pigs indicating that even when used at therapeutic levels, antibiotics have growth promoting effects. However, the FCR did not differ between the two groups (1.50 ± 0.077; P = 0.484) which shows that the NONE pigs were as efficient as the ANTIB pigs. As expected, removal of in-feed antibiotics increased the number of parenteral treatments (ANTIB 25% vs. NONE 14% of the animals treated; P = 0.040). However, the mortality rate was not different between the two groups (2.0 ± 1.79; P = 0.854). The mean initial body weight of the groups was correlated to the percentage of parenteral treatments (r = 60.7; P = 0.016) and tended to be correlated to mortality (r = 56.3; P = 0.070). This reflects the strong relationship between bodyweight and susceptibility to disease. The reduction in AM used measured as total amount or dose was more than 95%.

Conclusions: Removing in-feed medication during the weaner stage has negative effects on feed intake, weight gain and parenteral treatments but mortality and FCR can be kept at the same levels with a dramatic reduction in the amount of AM used.

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ECONOMIC IMPACTS OF THE VACCINATION WITH ECOPORC SHIGA IN A CLOSED HERD FARM

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Objective: the aim of this study is to determine the economic impacts of edema disease (ED) on an affected herd and the return on investment due to the use of the vaccine Ecoporc Shiga.
Material and methods: the farm is a closed herd farm (300 sows) which previously solved post-weaning E. coli diarrhoea problems via metaphylactic use of antimicrobials and restriction of feed. In November 2013, the first batch was affected with ED. Two to three weeks after weaning 6.9% of the piglets had died from ED. First it was tried to control the disease with antibiotics and changing the feed structure. Despite these measures, the mortality increased even further (13.6 % from weaning to slaughter house) and the daily weight gain (DWG) decreased (from 721 to 707g/day). Finally it was decided to vaccinate the following batches with Ecoporc Shiga after the presence of shigatoxin producing E. coli had been confirmed via microbiology and subsequent PCR analysis. The study is based on the comparison of three different periods:
•before the outbreak of ED (01/2013 to 10/2013 : 10 months)
•during the outbreak ED (from 11/2013 to 06/2014 : 8 months)
•since the start of vaccination (07/2014 to 10/2014 : 4 months)
Results:
•% mortality: from 6.2% before, it increased to 13.6 % during the outbreak and decreased again to 3.1 % since the implantation of the vaccine
•DWG: from 721g before, it decreased to 707g during the outbreak and increased to 739g with the vaccine allowing a heavier weight at slaughter house from 114.9 to 116.4kg.
•Costs of antibiotics: before the outbreak the cost of antibiotics had been already high (1.59 € /piglet), during the disease it increased to 1.61€ but with the vaccination, it decreased to 1.15€
Following these results, 2 calculations of the economic impact of ED and the return of investment of the vaccine were made:
•1. vaccination period vs. outbreak period: The economic impact was 7.36€ per piglet produced. The net gain of the vaccination is 6.9€ per piglet (374 % ROI) due also to the increase in DWG.
•2. vaccination period vs. the period before ED: The net gain of the vaccination is 1.78€ per piglet produced (nearly 100 % of return on investment)
Conclusion: This study shows that the economic impacts of an outbreak of ED are not only due to the increase in mortality, but also due to an increase in therapeutic costs as well as the decrease in DWG. Vaccination with Ecoporc Shiga increases the profitability by the reduction of the losses, the reduction of the use of antibiotics and the increase of the DWG. Then, the return on investment is assured.

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FROM ANTIOBIO–PREVENTION TO METAPHYLAXIS : HOW TO REDUCE ANTIMICRO–BIALS IN LIVESTOCK PRODUCTION ? THERAPEUTIC TRAJECTORIES AND ORGANI–SATIONAL LEARNING ON PIG FARMS.

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Introduction
Reduction in antibiotics has been an issue for a few years in animal health sector and especially in pig industry. In this paper, the authors consider, in comparison to research on human health, that animal health trajectories are motivated by social interactions as much as by medical practice. The authors question the peculiar learnings involved in the therapeutic trajectory of farms with a cut in antibiotherapy.

Material and method
The qualitative study focused on the farms with the higher reduction in antibiotics over the past few years in the Western France. They belong to the main French producers' organizations. 11 farmers, 10 vets and 2 technicians were interviewed on a semi-directive way. The analysis grid included the recent story of the farm, its sanitary status, the health and management practice, the motivations and actions of stakeholders, the economy of the sector. As often as possible, the authors interviewed the vet and technician related to a farm from the panel to confront all positions.

Results
The first results confirmed the wide range of factors involved in the decision making about cuts in antibiotics, among which economics and professional identity. All farms stopped supplementing food with antibiotics during post-weaning or were about to do so. Although a metering pomp revealed useless after a while, it has appeared as an unavoidable step. The farmers praised the lower risk taken, the pomp being available if needed. But they had to learn how to use and maintain the pump, and how to adapt the drug doses. They had to improve their surveillance of animals and to acquire basis of a collective health to react appropriately. They had to dedicate more time to pig care, which supposed thorough changes in the farm's work, especially in crop-livestock farming.

Conclusion
The reduction in antibiotics in specific farms has been made possible through a high degree of social and economic interactions between the farmers, animal health stakeholders and the pig industry. It requires complex technical, cognitive and organizational learnings which lead the farmer from prevention to metaphylaxis. These learnings could not have been completed without the support of vets and technicians all along the decision making and change processes.

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SUCCESSFUL REDUCTION OF ANTIMICROBIALS WITH THE VACCINE ECOPORC SHI–GA

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Introduction
Edema disease (ED) is caused by strains of E. coli that produce Shigatoxin (STEC). Economic consequences of ED are often severe: a steep increase in mortality of piglets combined with a decrease of the average daily weight gain (ADG). The goal of this study is to present an experience of an ED outbreak that was controlled with the prophylactic use of antimicrobials and special feed strategies. In order to reduce the use of antibiotics, and to continue the improvement of ADG, the farm started using the vaccine Ecoporc Shiga. The results compare the technical improvement of both strategies and calculate the gain per piglet of each strategy in comparison with the situation before ED.
Material and methods
The farm is a closed herd with 800 sows. In 2012, with ED, from weaning to slaughterhouse, the mortality rate was 16.7% and the ADG had decreased from 695g to 625g.
In 2013, the implementation of a new feed strategy and a large use of antibiotics, and medication, (from 0.56 € to 1.92 €/piglet), allowed to reduce the losses and to recover an ADG almost at the French average.
In 2014, the use of the vaccine Ecoporc Shiga enabled the successful removal of large part of the medication (-1.56 €/piglet). Moreover, with the vaccine the ADG also improved.
Results
The calculation of the economic benefits takes into account the evolution of the % of losses, the use of different control strategies and the improvement of the ADG. Although the farmer even observed an improvement in the feed-conversion ratio, it could not be added into this calculation.
1.first step : use of antimicrobials and new feed strategy
The mortality decreased from 16.7 % to 6.3 % (-10.4%) while ADG increased to achieve 707 g. Financially, the net gain per piglet due to the use of additional medication (+1.36€ per piglet) is 7.90€ per piglet.
2.second step : vaccination Ecoporc Shiga
The % of mortality stabilized at the French average (6.2%), the ADG increased to 718 g and compared to 2013, the reduction of antibiotics reached -1.55€!
Therefore the parameters from weaning to slaughter house with vaccination compared to the initial situation are:
- -10.50 % mortality
- + 93 g ADG
- -0.19 € antimicrobials/piglet

These improvements lead to a net gain per piglet of 8.48 €
Conclusion
Reduction of antibiotics is one on the main issues to be solved in UE within the next few years, and this case demonstrates that Ecoporc Shiga is an answer for the herds affected with E Coli producing Shigatoxine. In addition being a sustainable solution, it proves the financial interest for the farmer.

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THE USE OF ORAL FLUID ROPES FOR GILT ACCLIMATIZATION

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Introduction
If gilts are brought into a herd they have to run though an acclimatization protocol. Most isolation and acclimatization protocols are designed with PRRSV and other main pathogens in mind. In many cases, this is where the similarity between programs ends. Since so many variables can be different between farms, and even within the same farm over time, it is nearly impossible to apply one protocol across all
systems (1). Pathogen exposure has been a standard procedure of gilt acclimatization protocols, where the intention has been to expose the incoming gilts to the sow farm pathogens and allow them time to develop immunity in a separate facility (2). The idea of the study was to use ropes as used for diagnostics (3) for the exposure of the herd pathogens.

Material and methods
The study was performed in four different farms from 200 to 600 sows. All farms were purchasing gilts. The acclimatization protocols were about six weeks. In the study the exposure to the sow farm pathogens was changed from manure or older sows to cotton ropes, which were chewed by sows in the gestation barn before. Exposure took place in the mid two weeks of acclimatization. Different amounts of ropes and repetitions were used.

Results
Produktion analysis of the farms will be presented with a special focus on return rate of gilts. E.g. the non return rate (cummulative) in farm A improved from 10.3% of sow return to estrus to 6.9% sows return to estrus.

Conclusion and Discussion
It is possible to use cotton ropes in gilt acclimatization protocols for the well directed exposure to the sow farm pathogens. The amount of ropes and repetitions can differ from farm to farm since so many variables can be different between farms. The improvement of return to estrus of gilts could be shown in the study. Farmers like working with the ropes because they are much easier to handle for the aim of exposure than old sows. Using ropes for gilt acclimatization can be an additional tool in the whole acclimatization procedure.

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P163

APPROPRIATE METAPHYLACTYC TREATMENT OF S. SUIS INFECTIONS OF PIGS IN NURSERY WITH VETRIMOXIN LA (LONGOCILLINA LA)

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Introduction
Streptococcus suis belongs to the most frequent bacterial pathogens in pigs raised in industrial farms. Losses of pigs in the nurseries are associated mainly with S. suis serotype 2. The infection causes bronchopneumonia, meningitis or polyarthritis. Frequently seen septicemia, endocarditis or myocarditis may result in sudden death. Clinically ill animals should be treated with the anti-inflammatory product and with the antibiotic. All pigs in a group should be treated when a single pig becomes sick. Amoxicillin is the antibiotic of the first choice in the treatment and control of S. suis infections (Gottschalk 2002). Metaphylactic treatment at weaning is a common practice to reduce the risk of the development of the disease after stress and contamination of comingled piglets. The aim of the study was to compare the efficacy of Vetrimoxin LA (Longocillina LA) containing long acting injectable amoxicillin with Naxcel - a long acting ceftiofur-based product.

Materials and methods
A farrow-to-finish farm with the history of S. suis infections was selected for the trial. About 100 piglets were randomly assigned into three treatment groups after weaning. G1 and G2 group piglets received either Vetrimoxin L.A. or Naxcel in the recommended dosage. Piglets of G3 served as the non-treated control. Colistin and ZnO were used in oral medication for the prevention of PWECD. Any additional treatment was applied only in clinically sick animals. Mortality and emergency treatments were recorded and evaluated per group in the whole post-weaning period.
Results
The total loss in different groups G1, G2 and G3 was 4,95%, 5,94% and 9% respectively. This loss included mortality and forced culling of incurable animals. The clinical observing revealed nervous symptoms and septicemia, in one case (G1) respiratory distress. S. suis strain isolated from dead pigs was susceptible to AMX and CFT.
The additional treatment with injectable antibiotics was required in sick pigs. The overall benefit-to-cost amounts to 1.97€ per pig into trial in the Vetrimoxin LA group and 0.97€ in the Naxcel group in comparison to the control.
Conclusion
The metaphylactic treatment of piglets at weaning with Vetrimoxin LA (Longocillina LA) demonstrated high efficacy in prevention of losses associated with the S. suis infections in post-weaned pigs. This efficacy was higher yet not significantly, than the use of long acting ceftiofur. Vetrimoxin LA appears an appropriate, cost-efficient product for such use.

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ECONOMICS EFFECTS IN PRE–WEANING PHASE OF PORCINE EPIDEMIC DIARRHEA (PED) IN MÉXICO.

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Introduction: Porcine epidemic diarrhoea virus (PEDV), was first identified in the United Kingdom in 1971, and it caused mass epidemics in Europe in the 1970s and 1980s. It has since spread to Asia, where it has been considered endemic since 1982, causing substantial economic losses to pork producers. In México, according to information published by the OIE, the onset of the disease in Mexico was given on July 30, 2013 and pre-confirmation was held on August 8, 2013. Currently, 16 states of México has PED.

Materials and Methods: The study was based on information from 16120 sows in production of farms of México with continuous flow production, production and economic records of the year 2012, 2013 and 2014.
Treatments for comparison economical parameters of breeding área:

- Group I: Animals free of PED virus with subsequent infection
- Group II: Animals infected with PED virus.

Reproductive parameters were compared with T of Student to check statistically significant differences between means.

Results:We found significant differences in the number of weaned piglets and preweaning mortality(p=0.00). T

TABLE 1. Average of weaned piglets per sow per year.

Without PED		With PED	Dif.	Farrows/ sow/year	Looses of weaned piglets /sow /year
Farm 1	10.39	8.69	1.70	2.41	4.11
Farm 2	9.52	7.39	2.14	2.28	4.87
Farm 3	9.66	7.88	1.78	2.13	3.79
Farm 4	9.67	8.69	0.98	2.41	2.37
Average	9.81	8.16	1.65	2.31	3.78

TABLE 2. Economic looses by PED virus in four farms with 16120 sows in production.

Piglets looses/sow/year		Sows in production	Total Piglets Looses	Piglet Price	Economic looses
3.78	16120	60934	29.75 EUR	1,812,786 EUR	

Conclusions and Discussion: The Porcine Epidemic Diarrhea in México has a negative effect in the average of weaned piglets/sow/year generated an important reduced in the economic income because it shows less fat pigs to sell.

P164a

THE « GOING OVERBOARD» SYNDROME (GOS): AN EMERGING PROBLEM IN SWINE HERD MANAGEMENT

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In almost 70 years of combined clinical experience in several countries in swine (GPM) and poultry (JPV) medicine, the two authors have observed on numerous occasions what they call the « going overboard » syndrome. It occurs when, in an effort to do the very best to correct a situation or improve productivity, producers exaggerate in their intervention (hence the expression “going overboard”), exacerbating the problem or creating a new one.
"Over-insemination" is a typical example. Although it has been established that two artificial inseminations (AI) per oestrus are sufficient, in an effort to further improve prolificacy and ensure that AI is performed as close as possible to ovulation, some producers may do 3 and even 4 AI/oestrus. If AI occurs after ovulation, the risk of infection increases; and when it happens, vulvar discharges may be observed. This may lead to a review of AI sanitation
and even the usage of antibiotics during oestrus with the assumption that this is an infectious problem. Another example could be the extreme focus on one biosecurity measure, such as footbaths, while neglecting other basic actions, such as hand washing. In this case, going overboard with one aspect of a biosecurity plan may result in neglecting other aspects, leading to an increased risk of disease outbreak.
We hypothesize that, while producers may be excellent zootechnicians with solid practical knowledge, they are often conditioned to focus more on how to address a given problem without necessarily always digging into why the problem initially occurred.
Kahneman (Nobel laureate in economics) refers to a two-system thinking process: system 1 is fast, automatic and emotional. System 2 is slower but more logical and calculating. Good growers rely daily on system 1 thinking to operate and handle issues. Although it is essential, it may lead to systematic errors in specific situations. This may lead to poor decision making, as described by Hammond et al. (1998) in “Hidden traps in decision making". The purpose of the proposed submission is to present the “going overboard” syndrome using detailed examples and offer ways to approach this growing problem in swine herd management.

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REDUCTION OF SALMONELLA PREVALENCE ON SOWS AND FINISHER PIGS BY USE OF PORCINAT+ IN THE FEED OF LACTATING SOWS AND WEANED PIGLETS

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A mix of organic acids and essential oils protected by a triglyceride coating has been used through feeds in a 280 sows farrow to finish herd infected by Salmonella enterica, with the objective to reduce bacteriological prevalence on sows and serological prevalence on finisher pigs.
Four batches of sows received the micro encapsulated solution at 3 kg/t of feed from one week before farrowing to weaning, and their piglets the same treatment from 6 to 10 weeks of age. The percentage of sows excreting salmonella was 50% before treatment, and 10.4% after. Serological prevalence on 5 months old finisher pigs was 37.5% (mean OD % 45.3) before treatment and 11.7% (mean OD % 20.3) after. Gilts were the population with the highest prevalence before treatment (62.5%) compared to older sows (37.5%).
This method targets sows and piglets and provides an early treatment with a cost three times lower than treatment of finishers. High prevalence in gilts explains the high bacteriological prevalence seen in breeding herds with a high selection pressure.

P166

IDENTIFICATION OF GEOGRAPHIC FACTORS ASSOCIATED WITH PORCINE EPIDEMIC DIARRHEA DURING THE 2014 EPIDEMIC IN MIYAZAKI PREFECTURE, JAPAN

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In October 2013, the outbreak of porcine epidemic diarrhea (PED), which was the first case in Japan in 7 years, was reported in Okinawa prefecture, and several cases were found in Miyazaki prefecture on December. The risk factors of the spread has been focused on biosecurity practice or standard operating procedure, but the geographic factors has not been investigated. Therefore, the objective of the present study was to identify the geographic factors that associated with PED occurrence during the 2014 epidemic in Miyazaki prefecture, Japan. Geographical information and farm size in each farm was obtained from the prefecture database containing basic information for livestock producers. The database included 511 pork producers. As the geographic factors, the distance between closest farms and farm density were used in this study. The distance between closest farms was obtained from ArcGIS V10.2 (ESRI, Redlands, California, USA). Farm density was defined as the number of farms within 1, 2, and 3 km radius. The PED case data was obtained from Miyazaki Livestock Hygiene Service Center. Statistical analysis was performed in SAS V9.3 (SAS Institute Inc., Cary, NC, USA). Of the 511 farms located within Miyazaki prefecture, the proportions of farrow-to-finish, farrow-to-wean, and wean-to-finish operations were 45.0, 22.3, and 32.7%, respectively. Average sow inventory in farrow-to-finish and farrow-to-wean operations were 206.5±39.7 and 196.0±58.4, respectively. Mean farm size including both sows and growing pigs in all farms was 1644.4±116.4 pigs. Average distance between closest farms was 0.86±0.06 km, ranging from 0.054 to 15.58 km. Of the 511 farms, 81 (15.9%) were infected by PED virus by September of 2014. Of the 81 farms, 64 (79.0%) were farrow-to-finish or farrow-to-wean operations. No difference between PED virus infected farms and non-infected farms was found for the distance between closest farms (0.76±0.12 vs. 0.88±0.06 km; $P>0.10$). However, there are more farms within 1 km radius containing PED infected farms than those not containing infected farms (2.7±0.3 vs. 2.2±0.1; $P<0.05$). In addition, new cases are more likely to occur within 1 km radius containing infected farms (OR=5.29, 95%CI: 3.14-8.95). In conclusion, PED virus spread in Miyazaki prefecture was related to farm density and distance between farms.

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WILD BOARS – A RESERVOIR FOR PATHOGENIC YERSINIA ENTEROCOLITICA?

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Wild boars are known reservoirs for a number of agents transmissible to domestic animals and humans. In recent years, wild boar population has exploded all over Europe leading to increased hunting activities and consumption of wild boar meat, whereas the impact of transmission particularly of zoonotic pathogens from wildlife to livestock and to humans is still ambiguous.
Pathogenic Y. enterocolitica, in particular strains of bioserotype 4/0:3 and 2/0:9, are the causing agent of Yersiniosis, which is the fourth most frequently reported zoonoses in Europe, associated with human foodborne gastroenteritis.
Domestic pigs are the most important reservoir of these bioserotypes carrying the agent mainly in the tonsils. Altogether, Y. enterocolitica comprises six biotypes (BT) of which five (1B, 2, 3, 4, 5) contain pathogenic strains, characterized by the presence of a virulence plasmid. But to express full virulence, chromosomally encoded factors are required. Although BT 1A lacks the known virulence determinants, gastrointestinal infection in humans due to BT 1A have been reported.
Wild boar tonsils were investigated for Y. enterocolitica, and isolates were characterized by bioserotyping and their virulence profiles using PCR methods, to find a link between wild boars and domestic pigs and to evaluate the risk for consumers of wild boar meat.
Nineteen (17.1%) of the 111 investigated tonsils were positive for Y. enterocolitica. Almost all isolates belonged to BT 1A, two belonged to BT 1B. Plasmid-borne genes (yadA, yopT, and virF) could not be proven. The ystB gene, which is mainly present in BT 1A strains of Y. enterocolitica, was found in all but one isolates (94.7 %). Because both BT 1B strains did not bear any further investigated virulence markers, they were considered to be non-pathogenic variants. Two isolates from BT 1A were identified to carry the ail gene, which was usually used to discriminate pathogenic from non-pathogenic Yersinia strains, but its role as a sufficient virulence marker has already been questioned in literature. Because of the lack of further virulence markers the isolates were considered to be avirulent and a link to enteropathogenic Y. enterocolitica isolates in domestic pigs could not be drawn up. Additionally, phylogenetic analysis based on MLST showed distinct differences between human and wild boar isolates. Therefore, it is assumed that wild boars do not serve as a reservoir for enteropathogenic Y. enterocolitica and the potential risk for wild boar meat consumer getting infected with Y. enterocolitica is comparatively low.

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SCSA AS A MAJOR DRIVER FOR INCREASED SURVIVAL OF SALMONELLA DURING A STRESS RESPONSE

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Introduction
Generally, pigs infected with Salmonella Typhimurium carry the bacterium asymptomatically resulting in so called Salmonella carriers. Recently, we showed that a 24 hour feed withdrawal increased the intestinal Salmonella loads in carrier pigs, which was correlated with increased serum cortisol levels. This stress related recrudescence of a latent infection could be reproduced by a single injection of dexamethasone. We also showed that cortisol promotes intracellular proliferation of Salmonella bacteria in macrophages. The aim of the present study was to identify genes that play a role during stress-induced recrudescence of a Salmonella infection.

Materials and Methods
In vivo expression technology (IVET) was used to identify Salmonella genes that are intracellularly expressed in macrophages after exposure to cortisol. Following IVET, a scsA knock-out mutant (ΔscsA) and a complemented knock-out mutant (ΔscsAc) were constructed. As a control, we electroporated the empty pGV1106 plasmid into the deletion mutant (ΔscsApGV1106). These strains were used in invasion and proliferation assays. Finally, differences in expression patterns between the wild type Salmonella (WT) and ΔscsA strain after cortisol exposure were determined in a medium that reflects the intracellular environment, using microarray analysis.
We then optimized a stress-mouse-model, mimicking the observations we have seen in pigs using DBA/2J mice. Sixteen mice were inoculated with a total of 1 x 10^6 CFU of Salmonella Typhimurium or ΔscsA. At day 14 p.i., eight animals of each group were subcutaneously injected with 100 mg/kg dexamethasone and eight mice were injected with 200 µl HBSS and served as a control group. Twenty-four hours later, all mice were euthanized.

Results and Discussion
Using IVET and intracellular proliferation tests, we identified scsA as a key driver of cortisol induced intracellular replication of Salmonella in porcine macrophages. Deletion of scsA abolished the increase in proliferation, an effect that was restored by the complementation of scsA. Microarray analysis revealed that Salmonella senses cortisol in a scsA dependent way. Finally, we demonstrated the determining role of scsA in vivo using a mouse model.

In conclusion, we showed that Salmonella senses stress conditions both in vitro and in vivo by responding to cortisol. We identified scsA as a major regulator during this process. The bacterium responds to cortisol in a scsA dependent way, with increased intestinal Salmonella loads as a result, which eventually can lead to increased pathogen dispersal.

P169

SUPPLEMENTATION WITH QUATERNARY BENZO(C)PHENANTHRIDINE ALKALOIDS DECREASES SALIVARY CORTISOL AND SALMONELLA SHEDDING IN PIGS AFTER TRANSPORTATION TO THE SLAUGHTERHOUSE

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Salmonellosis is one of the leading foodborne diseases worldwide. Several factors have been identified to increase Salmonella shedding in pigs and the risk of carcass contamination, including transportation stress. Herbal extracts containing quaternary benzo(c)phenanthridine alkaloids (QBA) have shown a wide range of physiological effects such as anti-inflammatory, antimicrobial and immune-modulatory properties. The study was aimed to evaluate the effect of QBA (Sangrovit®, Phytobiotics GmbH, Eltville, Germany) supplementation on salivary cortisol and Salmonella shedding of pigs after transportation to the slaughterhouse. We hypothesized that QBA supplementation would decrease Salmonella shedding by regulating stress response.
Methods: A total of 82 pigs (initial body weight 47.9 ± 7.2 kg) were orally challenged with a cocktail of Salmonella serovars and randomly assigned to 3 treatment groups (day 14; Treatment 1: T1, in-feed QBA for two weeks; Treatment 2: T2, in-feed QBA for two weeks and water soluble QBA for the last three days; Control: CON, non-supplemented). Pigs were transported to the slaughterhouse after two weeks. Saliva and fecal samples were collected from each individual pig at specific times during the study period, and carcass swabs were obtained after evisceration. Salivary cortisol was measured and fecal samples and carcass swabs were analyzed for detection and quantification of Salmonella.
Results: A very high to a moderate positive correlation was found between salivary cortisol and Salmonella shedding after transportation to the slaughterhouse in all groups ($P < 0.05$). Overall, mean salivary cortisol decreased in all groups overtime ($P < 0.0001$). However, after transportation CON group showed a significant increase in salivary cortisol ($P < 0.0001$) and the concentrations in CON were higher as compared to T1 ($P = 0.0002$) and T2 ($P < 0.0001$). Salmonella prevalence decreased significantly after transportation in T2 ($P = 0.05$) and it was higher in CON group as compared to T1 ($P = 0.02$) and T2 ($P = 0.02$). After transportation CON group showed a significant increase in the number of Salmonella shed through the feces ($P = 0.04$), whereas T2 showed a significant decrease in Salmonella shedding ($P = 0.009$) as compared to pre-transport levels. Additionally, T2 shed lower amounts of Salmonella after transportation to the slaughterhouse than T1 ($P = 0.02$) and CON group ($P = 0.08$). The proportion of Salmonella-contaminated carcasses was not different between groups ($P > 0.05$). However, the quantity of Salmonella contaminating the carcasses was higher in CON than in T1 and T2 groups ($P = 0.01$).
Conclusion: This study showed that transportation is a stressful event for pigs resulting in increased Salmonella shedding in conventionally produced pigs. Additionally, regulating stress response due to transportation by adding QBA to the feed and the water of finishing pigs was effective in reducing the proportion of Salmonella-positive pigs as well as the numbers of Salmonella shed through their feces, which may contaminate the carcasses. QBA supplementation may be an effective strategy to reduce stress response in pigs and ameliorate its effects on welfare and the quality and safety of the food products.

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DEVELOPMENT OF A SALMONELLA TYPHIMURIUM CHALLENGE MODEL IN WEANED PIGS: EFFECT OF INOCULATION PERIOD AND BUFFER CAPACITY OF THE FEED.

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Introduction. Salmonellosis is a zoonoses and a public health concern. Salmonella may be transmitted from feed to food in the pork production chain. Therefore, there is a need for dietary prevention and control methods of Salmonella in pig production. Our aim is to develop a Salmonella Typhimurium (S. Typh) challenge model in weaned pigs, to measure the effects of dietary interventions on Salmonella fecal shedding. As a first step, we studied the effects of inoculation period (3 vs. 7 consecutive days) and diet composition (standard vs. high buffer capacity) on fecal Salmonella shedding in challenged weaned piglets. Materials and methods. Four groups of 8 individually housed weaned male piglets were used in a two by two factorial design. Piglets were fed either a standard diet (CON) or a diet with a high buffer capacity (HB) (ACIDB.CAP.PROHASKA pH 3 (mmol/g) 0.60 and 0.78 resp.). After 7 days, pigs were orally inoculated with S. Typh field strain culture (1 ml of 1.09*10^9 cfu/ml; cultured at 37°C in BHI broth) either for 3 or 7 consecutive days starting 7 days post weaning. Rectal fecal samples were taken before (day -6, -4 and 1) and after inoculation (day 3, 7, 10, 14, 17 and 21) for Salmonella quantification. Body temperature and diarrhea incidence was recorded daily, and body weight was measured weekly. Results. No fecal shedding of Salmonella was detected before inoculation. After inoculation, fecal Salmonella shedding peaked at 3.5 log10 cfu/g (Sd 0.64) at day 3, and gradually declined thereafter to 2.3 log10 cfu/g (Sd 0.28) at day 21. Fecal Salmonella shedding was not different between treatments. After inoculation, a short (2 days) 0.5°C increase in body temperature was detected in the inoculated groups, but body temperatures returned to base levels before day 3 in all groups. Diarrhea incidence was similar for all treatments during day 1-7 (63 -73%) and 8-14 (62-81%), but higher for HB-7d (88%) compared to other treatments (62-67%; $P<0.05$) during day 15-21. Average daily growth was similar for all treatments during day 1-14, but lower for HB-7d (460 g/d) compared to CON-3d (640 g/d; $P<0.05$) during day 15-21, whereas other treatments were intermediate. Conclusion. We successfully infected weaned pigs with a S. Typh field strain via an oral inoculation, resulting in quantifiable fecal shedding in all pigs. However, fecal shedding was not significantly affected by inoculation period (3 vs. 7 days) or diet composition (standard vs. higher buffer capacity). It needs further validation whether this Salmonella challenge model is suitable for evaluation of effects of dietary interventions on Salmonella fecal shedding.

P171

TWO NOVEL RAPID TESTS FOR INDIRECT DETECTION OF BOAR TAINT FROM BLOOD OR FROM SWABS OF BLOOD AND OF SALIVA

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Introduction
The renounce of surgical castration requires a reliable detection of boar taint in carcasses. The odour control by meat inspectors at the slaughter line is less accurate than fat androstenone determination with HPLC/GC-MS. These quantitative methods, however, are too cost and time expensive for serial examinations. The goal of this baseline study was firstly to evaluate a competitive rapid test for analysis of testosterone in blood and in saliva as a biomarker for elevated boar taint compounds in fat, and secondly for the same purpose a rapid test which allows an optical control without flourescent reader still intra vitam.
Material and Methods
From females and from castrated, prepubertal, pubertal and adult males 153 samplings were collected consisting each of blood from venipuncture, of a dry and a heparine-moistened swab dipped into this blood and a saliva swab from buccal cavity. With the 1st test, a 2-step competitive assay (primary antibodies against testosterone followed by flourescent secondary antibodies against the primary antibodies), fluorescent signals of testosterone were measured by a novel handheld microarray reader. For the optical control of testosterone content in the 2nd test gold-nano-particles were coated with anti-testosterone-antibodies and then linked with a multimere testosterone-protein-conjugation which changes the colloidale-disperse solution visibly from red to blue due to an altered absorption.
Results
The 1st test takes 20 min. Thereby, flourescent signals from the microarray reader showed for saliva swabs the same significant differences as for blood with regard to gender resp. different male categories. Thanks to the measuring sensitivity this likely applies also to blood swabs according to previous analysis. The 2nd test reacts within 10 min. Preliminary findings of interlacing with gold-nano-particles at different testosterone concentrations are promising, too.
Conclusions
Easy collecting stitch blood or saliva from anesthetized slaughter pigs can be tested for testosterone concentrations with the competitive assay up to the end of the slaughter process. The microarray reader for point-of-care diagnostic allows in addition the parallel determination of further analytes or even antibodies for herd health monitorings. The maximum permissible testosterone content of respective samples just below the approved minimum fat androstenone level for odour perception will be evaluated next in a slaughterhouse study. Regarding the gold-nano-particle assay a smartphone with a photoapp that calibrates for the testosterone threshold value can verify e.g. anti-GnrH vaccinated nonresponders in time before slaughter.

P172

LA–MRSA CC398 IN NORWEGIAN PIG PRODUCTION; EXPERIENCES WITH OUT–BREAKS, SURVEILLANCE AND ERADICATION

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Introduction
Livestock-associated methicillin resistant Staphylococcus aureus (LA-MRSA) of the clonal complex (CC) 398 spa type t034 was first detected from Norwegian pigs in an anonymized abattoir survey in 2011. The following year an anonymized survey demonstrated LA-MRSA CC398 t034 in 1 out of 175 (0.6%) Norwegian pig herds. Both surveys indicated a low prevalence.
In 2013/2014 two apparently unrelated outbreaks of LA-MRSA CC398 t034 were detected in Norwegian pig herds in the Southeastern and Southwestern part of Norway, respectively. The initial detection was an incidental finding in a fattening pig submitted for post-mortem examination at the Norwegian Veterinary Institute. Sampling in the herd of origin, supplying nursery and sow herd revealed LA-MRSA of the same spa-type in all herds. A few months later, a spa-type closely related to t034 was isolated from a hospitalized farm worker with a severe LA-MRSA infection. Contact tracing demonstrated LA-MRSA CC398 in the herd of employment and the supplying sow herd. In both outbreaks LA-MRSA CC398 was demonstrated in several fattening herds that had received pigs from the sow herd. All contact herds were subsequently sampled, to date more than 100 herds.
In 2014, a nationwide LA-MRSA survey was conducted, collecting samples from all sow herds with an inventory of more than 10 sows (986 herds). This survey revealed only one additional LA-MRSA positive herd (CC398 t011). Contact tracing demonstrated the same spa type in one recipient fattening herd.
Material and methods
The Norwegian Food Safety Authority (NFSA) imposed measures to eradicate LA-MRSA including de-population of infected herds, followed by thorough washing, disinfection and negative sampling from the environment before re-stocking. After re-stocking, samples were collected to verify the effect of the eradication.
Results
A total of 26 LA-MRSA CC398 positive herds were identified through the investigations described above and eradication was imposed. Follow-up samplings indicate that 21 herds were successful in eradicating LA-MRSA in the first attempt, whereas three herds were positive and two herds have not yet been sampled. The follow-up testing per-protocol after re-stocking has not yet been completed in all herds. Of the three positive herds, one had re-stocked with LA-MRSA positive pigs and the remaining two lacks an explanation as to why the eradication failed. Two of these later conducted successful re-eradications, whereas the last was still LA-MRSA positive after the second eradication.
Conclusion
The results indicate that LA-MRSA eradication is possible in Norwegian pig production.

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PATHOPIG – A PROJECT TO IMPROVE EARLY DISEASE DETECTION IN SWITZERLAND

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Introduction
In order to improve the early detection of enzootic, exotic and zoonotic diseases in Switzerland the Federal Food Safety and Veterinary Office (FSVO) has launched a project in 2014. With this the FSVO aims to increase the number of post mortem examinations of pigs and to detect changes in disease incidence as soon as possible.

Material and Methods
Pig producers with health problems on farm level are encouraged to call a veterinarian who will investigate the health status of the herd and subsequently select one to three pigs presenting typical symptoms of the health problem. These pigs have to be submitted to one of the seven participating pathology laboratories in Switzerland. At least one of the following criteria must apply for a case: 1.) high morbidity and/or high mortality rate, 2.) unusual clinical signs, 3.) recurrent problems of unknown aetiology resistant to therapy, or 4.) increased use of antimicrobials. A standardised anamnesis protocol has to be filled in by the veterinarian, which then accompanies the selected pig(s) to the laboratory. The FSVO subsidises costs for diagnostics depending on the number of submitted pigs. The pathological diagnosis is made in accredited laboratories with the support of microbiological investigations by subcontractors. The veterinarian receives the results for discussion with the producer and for planning therapy and prevention. The anamnesis protocol, the pathology report and the recommendations are collected in a database of the FSVO. The swine health service (SHS) receives a copy of the recommendations and is responsible for a follow up of their implementation and success. Both is done by a telephone survey with the farmer three to six months later.

Results
In the first nine months of the project period, 417 pigs from 240 farms were examined. In 80% of all cases on farm level an aetiological diagnosis was obtained and in 97% recommendations could be given to the farmer. The follow-up has been finalized in 154 out of the 252 cases. In more than 80% of the cases recommendations had been implemented and in more than 90% problems could be either solved completely or improved considerably.

Conclusions
Major advantages of the project were the improvement of collaboration between diagnostic laboratories and veterinarians and the establishment of new diagnostic tools. State subsidies for post-mortem diagnostics have increased the detection rate of aetiologically relevant pathogens and led to improved treatment and management recommendations in participating pig farms through more informed decisions. The project therefore was extended until the end of 2015.

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VETERINARIANS' PERCEPTION OF ANTIMICROBIAL USAGE IN PIG FARMING IN SIX EUROPEAN COUNTRIES

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Introduction
To reduce the risk of antimicrobial (AM) resistance in humans and animals, it is important that AM are prudently used in livestock farming, such as in pig farming. Veterinarians can significantly contribute to tackling this problem, not only by their prescription practices, but also by supporting pig farmers in health management and in implementing disease prevention measures. However, little is known about veterinarians' perceptions and behaviour regarding AM usage in pig farming and the relation to their pig farming clients.

Method and Materials
We therefore conducted an online survey among veterinarians with expertise in pig medicine from Belgium, Denmark, France, Germany, Sweden and Switzerland (N = 334). The survey was part of the European MINAPIG project (www.minapig.eu). The questionnaire included items about, among others, the risks and benefits of AM usage, veterinarians' willingness and estimated feasibility to reduce the AM usage at their clients' farms, the capability of their pig farmers to reduce their AM usage, social pressure from pig farmers, and the number of completed additional courses. Most items were measured on 6-point Likert scales.

Results
Veterinarians from all six countries were very willing to reduce the AM usage at their clients' pig farms and believed that this aim was very feasible. There were also a few differences between the different countries. For example, Danish veterinarians perceived more benefits but also more risks in AM usage than the veterinarians from the other five countries. Also, Belgian and Swiss veterinarians experienced more pressure from their farmers to prescribe AM than their Swedish, Danish and French colleagues. Regression analysis revealed that veterinarians' willingness to reduce AM usage at their clients' farms was mainly increased by higher estimated feasibility to reduce AM usage, higher perceived capabilities of farmers to reduce AM usage, a better collaboration quality with their clients, and more completed additional courses by the veterinarians.

Conclusion
Thus, to motivate veterinarians to reduce the AM usage at their clients' farms, it seems important to strengthen and extend their skills to advice pig farmers to reduce AM usage at pig farms, as well as to improve skills to support farmers to use AM prudently. In this respect, offering additional coaching can be very effective.

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EVIDENCE OF EXPOSURE OF PIGS TO TOXOPLASMA GONDII AND ASSOCIATED RISK FACTORS IN GREECE

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Introduction
Toxoplasmosis is a worldwide reported zoonotic infection caused by the protozoa Toxoplasma gondii. Pigs may become infected by ingesting feed or water contaminated with cat faeces, by cannibalism, and/or by eating infected rodents. T. gondii infected edible tissues of pigs is a source of infection for humans. The objective of this study was to determine seropositivity of pigs and identify possible risk factors.
Materials and Methods
Blood samples were collected from the anterior vena cava from 10% of the animals of 82 pig farms located in Greek mainland. Information about the geographical place, the size and the biosecurity level of the herd were recorded. Sera were tested for anti-Toxoplasma antibodies employing indirect immunofluorescence antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA).
Results
Toxoplasmosis was detected at 20 of the 82 farms tested (24.39%). In 7 farms toxoplasmosis was detected only in sows' samples, in 4 only in fattening pigs' samples and in 9 farms in both sows' and fattening pigs' samples. Among the categorical data evaluated, the biosecurity level (Odds Ratio=0.13, P<0.01) and the geographical characteristics (Odds Ratio=5.88, P<0.05) significantly affected the presence of toxoplasmosis in the herd. Toxoplasmosis was recorded in significantly higher percentages in mountainous farms compared to lowlands (P<0.05) and in those with fair biosecurity levels than in those with excellent (P<0.05).
A high degree of agreement was observed between the two tests.
Conclusion
The presence of antibodies in pigs is an indirect information on the risk of the infection and an indication of the necessity of biosecurity measures to be taken in order to control infection at least in the areas at greatest risk.

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WHAT IS THE PROFILE OF PIG FARMERS WITH LOW ANTIMICROBIAL USAGE AND HIGH TECHNICAL PERFORMANCE?

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Introduction
As many management factors can impact the antimicrobial usage on a farm, it is difficult to identify individual factors that are consistently and strongly correlated with reduced antimicrobial use. As a first step in that direction, the objective of this study was to describe the profile of pig farmers from 4 European countries with the best technical performances and lowest antimicrobial usage.

Method
A retrospective study was conducted among 231 farrow-to-finish pig farmers from Belgium, France, Germany and Sweden. Farm visits aimed to describe the antimicrobial usage over the past year, the biosecurity level, the technical performance and vaccination schemes. Participants were also asked to complete a questionnaire that investigated their attitudes and perceptions of antimicrobial usage and resistance, including a self-evaluation of their own antimicrobial consumption. We first identified for each country the "top farmers" who met the two following criteria: i) antimicrobial usage was lower than the median usage of the sample and ii) technical performance (expressed as the number of weaned piglets per sow and per year) was higher than the sample median. We then described the top farmers' biosecurity levels, vaccination practices and attitudes/perceptions in comparison with the other farmers from our sample.

Results
In total, 47 farmers met the two selection criteria and were allocated into the top farmers group. Compared to the other 179 farmers, the top farmers had higher external biosecurity levels. In particular, they performed better for biosecurity practices related to animal purchasing policy and they were located in more favourable environments (e.g. low pig density). Top farmers also vaccinated against porcine circovirus (PCV2) more frequently. In addition, they perceived antimicrobials as being very cost-efficient and having quick effects. However, their other attitudes and perceptions, including perceived risks of using antimicrobials and self-evaluation of their antimicrobial usage did not differ from the other farmers. Finally, the proportion of female farmers was higher in the top farmers group.

Conclusion
A limited number of farmers had both low antimicrobial usage and high technical performance. They presented certain characteristics, including farm management practices and attitudes that should further be promoted to encourage antimicrobial reduction without impairing pig farming economical sustainability.

This study was part of the MINAPIG Consortium activities that is working towards the reduction of antimicrobial usage in the pig industry (www.minapig.eu/).

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WHAT IS THE PROFILE OF THE FRENCH VETERINARIANS THAT PERCEIVE A HIGH POTENTIAL FOR ANTIMICROBIAL USAGE REDUCTION IN THE PIG INDUSTRY?

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Introduction
The reduction of antimicrobial usage in the pig industry requires effective collaboration between all stakeholders involved in pig health management. Recent studies have shown that veterinarians have a major role to play in antimicrobial usage reduction. However, to our knowledge, no studies have investigated the veterinarians' profile based on their perceived potential for reducing antimicrobial consumption in the pig industry.

Material and Method
We conducted an online survey among 72 French pig veterinarians that aimed to explore their antimicrobial treatment practices, their opinion on policy measures to reduce antimicrobial usage and their relationships with their pig-farming clients. Most questions were based on Likert scales (scores from 1 to 6), except one question where respondents had to describe on a slider from 0 to 100% the proportion of antimicrobial usage that could be reduced in the French pig industry in the next 5 years without impairing the pig farming economic sustainability. We then compared the profile of the most optimistic veterinarians (the 50% veterinarians who predicted a possible reduction of > 20%) with the most pessimistic ones (who believed possible reduction was equal or less than 20%)

Results
On average, the veterinarians from the optimistic group were significantly younger and had better relationships with their clients, attributing higher scores to items such as "my pig-farming clients adopt all preventive measures I suggest to them" or "it is easy to convince farmers to use alternative methods to antimicrobials". In addition, they were less convinced by the ability of certain policy measures, e.g. benchmarking system between farmers antimicrobial usage, to reduce antimicrobial usage consumption. Finally, they scored higher on items describing prescription behaviours, e.g. "I try to prescribe as few antibiotic treatments as possible". There was no significant difference in their perceived impact of reducing antimicrobial prescription on their revenues, their perceived social pressure to prescribe antimicrobials or their level of continuing education.

Conclusion
French veterinarians who perceived higher potential for reducing antimicrobial consumption in pig farming were young practitioners having good relationships with their clients and good prescription practices. The median estimate of the perceived potential for antimicrobial usage reduction was close to the objective of the French Ministry of Agriculture that aims to reduce antimicrobial usage by 25% between 2012 and 2017.

This study was part of the MINAPIG Consortium activities which aim to reduce antimicrobial usage in the pig industry (www.minapig.eu/).

P178

OCCURENCE OF ANTI-TOXOPLASMA GONDII ANTIBODIES IN SWINES OF NON-TECHNIFIED REARING FARMS

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Introduction
Toxoplasmosis is a zoonosis present worldwide, its protozoal aethiological agent, Toxoplasma gondii, has the abiity to infect several homeothermic animals and mainly human beings. Wild and domestic felines are considered the main host of the parasite for eliminating oocists in the feces, that sporulate in the environment and are able to contaminate water and food resources. The consumption of raw or undercooked meat products containing T. gondii cysts in the tissue, vegetables without washing and using non treated water are risk factors associated to the occurence of human toxoplasmosis. Pigs are an important infection source of T. gondii to humans, in addition, the prevalence of this disease in swine herds varies around the world. Non-technified rearng systems are characterized for bad sanitary conditions of facilities and animal health. Taking into account the importance of toxoplamosis in public health, this study focused on generating data about the prevalence of the disease in swine herds using Direct Agglutination Test (DAT).
Material and Methods
197 swine blood samples were collected from animals of 43 different swine rearing properties. The farms were randomly selected and the samples collected in loco. Afterwards, the serum was separated and tested for the presence of antibodies anti-toxoplasma using the DAT. Also, a questionnaire was applied in 24 of those properties to gather information related to the occurence of possible risk fator situations eg: the presence of cats in the property. The Fisher's exact test was used to correlate the occurence of positive animals with the risk factors at a 95% confidence level.
Results
19 (9.64%) out of 197 swine's serum sample were tested positive for the presence of antibodies anti-Toxoplasma in the DAT. At a herd level 6 (25%) out of 24 had at least one positive animal. The Fisher's exact test showed statistical correlation between the presence of food garden (p value = 0.01) in the farm and the use of non treated water to irrigate the food garden (p value =0.005).
Conclusion
Toxoplasmosis prevalence among pigs reared in non technified rearing farms still high, what could be considered a public health issue once the meat or meat products of these animals meat could be consumed undercooked causing human toxoplasmosis.

P179

DESCRIPTION OF POST-MORTEM LESIONS IN SLAUGHTER PIGS IN BELGIUM BETWEEN 2011 AND 2013.

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Introduction
Slaughterhouse results are a good indication of the general health status of pig herds. They can be used as an important tool to assess the importance of specific diseases (e.g. Mycoplasma) or they can also be used to monitor the level of infection for certain (respiratory) disorders.
The aims of the current study are to describe the prevalence of important lesions in slaughter pigs in Belgium.

Material and Method
A complete set of existing data was gathered from two Belgian pig slaughterhouses from 2011 to 2013. Only fattening pigs originating from Belgian pig farms with quality label were included. Slaughterhouse A was located in the Province of Antwerp and slaughterhouse B in West Flanders. The data contained all Belgian slaughter pigs that were slaughtered in this period. Details about the herd of origin, the number of pigs delivered in each batch and information about the post-mortem findings.
The post mortem findings contain information on livers with white spots and prevalence of livers that were disapproved for white spots. Also information about lungs and the thoracal cavity were present: prevalences of pneumonia, fissures and pleuritis. Fissures are typical lesions and can be considered as "scar tissue", these lesions are considered to be an indication of a previous Mycoplasma-infection.

Results
In total 23 095 batches of Belgian fattening pigs were slaughtered in both slaughterhouses. Details about the post-mortem findings were present for 21 281 batches (92.1%). This made a total of 2.98 million fattening pigs originating from 628 different pig herds. The average (min-max) number of fattening pigs per batch was 140 [25;720].
The yearly overall prevalence of pneumonia reached from 35.8% (highest in 2011 in slaughterhouse B) towards 22.6% (lowest in 2012 in slaughterhouse A). The prevalence of fissures was constantly higher in slaughterhouse A. The prevalence of pleuritis was highest in slaughterhouse B. The prevalence of white spots and disapproved livers tended to decrease during the subsequent years.

Conclusion
The prevalences of pneumonia and pleuritis are in accordance with other publications. Meyns et al. (2011) described a prevalence of 20.8% for pleuritis and 23.9% for pneumonia. Also Maes et al. (2001) described similar levels of pleuritis (16%) and pneumonia (24%).
The average prevalences of white spots, caused by the migration of certain stages of the intestinal parasite, Ascaris suum, are similar with other publications (Ondrejkova et al., 2012).

Further research will investigate temporal variations, correlations between different lesions and associations with management and herd factors.

P180

H3N1, A RARE SWINE INFLUENZA SUBTYPE – A CASE REPORT FROM NORTH RHINE-WESTPHALIA, GERMANY

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Introduction
Three predominant subtypes, H1N1, H1N2, and H3N2, of swine influenza virus (swineFLUAV) are circulating worldwide. A rare subtype in Europe, namely H3N1, has been detected in Italy in a feeder to finisher pig farm recently. We aim to present a case report on the detection of H3N1 in two pig farms in North Rhine-Westphalia, Germany, in 2014.
Material and Methods
Clinical samples
In January 2014, severe respiratory disease was observed in fatteners at two farms (farm A and farm B) which are located 500 m apart. Farm A is a nursery to finisher farm with two locations and in total 1690 heads. Farm B is a finisher farm with 3510 heads. There is no interaction of any kind between the farms. Nasal swabs were taken at both farms about one week apart from acutely ill animals to detect swineFLUAV via PCR. Three weeks later, blood samples were taken from the affected animals to test for seroconversion using HI method.
Challenge trial
In total, 45 influenza naïve pigs were randomly assigned to two vaccination and one placebo group. Thus, there were 15 animals in each group. The three groups were vaccinated with RESPIPORC® FLU3 (consisting of H1N1, H1N2, and H3N2), an autogenous vaccine containing fully inactivated H3N1 virus (FLU-H3N1) or a placebo ,respectively, at day 68 and 89 of life. The placebo was injected at the same time point. Seven days after the second vaccination all three groups were challenged with heterologous H3N1 virus using an aerosol infection model. Following the challenge, each animal was examined for clinical signs and body temperature elevation and nasal swabs and blood samples were taken on a daily basis. Daily weight gain was reported every second day. On days 1, 3, and 9 after challenge five animals were sacrificed to examine the lungs.
Results
swineFLUAV H3N1 could be detected in all nasal swab samples taken at the farms. Typing resulted in subtype H3N1. Seroconversion was detected in 27/30 sampled H3N1 positive animals. Clinical signs were only detected in the placebo group. Also, body temperature and macroscopic lung pathology was significantly different in the vaccinated compared to the placebo group. Viral load was significantly less in vaccinated animals, too.
Conclusion
RESPIPORC® FLU3 protects pigs fully against circulating subtypes in Europe. H3N1 is a rare subtype causing severe respiratory disease in pigs. Its epidemiological relevance still remains to be determined.

P181

THE USE OF ANTEMORTEM TRACHEOBRONCHIAL MUCUS COLLECTION TECHNIQUE FOR MYCOPLASMA HYOPNEUMONIAE

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Introduction
Mycoplasma hyopneumoniae (M. hyo), the causative agent for porcine enzootic pneumonia (EP) and a key component in the porcine respiratory disease complex (PRDC), is a significant disease threat to the swine industry. The focus of this study was to refine antemortem M. hyo detection methods and determine the baseline herd prevalence in the United States pre-weaned pig population.

Materials and methods
Thirty-two weaned pigs demonstrating clinical signs of PRDC from a commercial sow farm were submitted for diagnostic evaluation. A nasal swab and tracheobronchial mucous (TBM) sample were collected antemortem while a deep airway swab, bronchial alveolar lavage (BAL), and lung tissue were collected post-mortem from each pig. To obtain the TBM, a 50 centimeter, 12 gauge, aspiration-type catheter was advanced in the oral cavity until piglet inspiration with further advancement until a voice-change, cough, or wheeze was audible. The catheter was advanced and retracted several times and rotated 360 degrees for TBM collection. The length of the catheter containing the TBM sample was cut off and placed into a conical centrifuge tube containing 1mL of sterile 0.9% sodium chloride. The tube was capped and agitated to facilitate the mucous entering the saline from the catheter tip. In an effort to identify the optimal sample site and PCR extraction method for detection of M. hyo, a real-time PCR using three separate extraction methods (Viral, Total Nucleic Acid (TNA), and High Volume) were run for each sample (32 pigs x 5 samples x 3 extraction methods) for a total of 480 PCRs. This TBM collection technique was then applied to 1759 pre-weaned piglets from PRDC-affected sow farms and 30 pre-weaned piglets exhibiting signs of PRDC.

Results and discussion
Nasal swabs were the least sensitive sample type (6.25% positive), whereas TBMC provided the highest level of sensitivity with 59.3% of the pigs testing M hyo PCR positive. Results showed that sample collection method also had a significant effect on the PCR's Ct value ($p < 0.0001$). Pairwise comparisons again revealed the markedly inferior sensitivity of using nasal swabs and that the least squares mean Ct value for the tracheobronchial mucous collection technique was lowest compared to the other four sample collection methods (Table 1). This suggests the presence of a higher amount of bacterial nucleic acid present in the tracheobronchial mucous requiring fewer rounds of DNA amplification, making this technique an appropriate choice for antemortem diagnosis. In TBM PCR testing of 1759 piglets, 1.1% of litters were positive for M. hyo. In 30 clinically affected piglets, 16.7% tested positive for M. hyo.

Conclusion
TBM collection is an innovative antemortem technique to assess the prevalence of M. hyo in a pig herd. Based on an antemortem diagnosis, a suitable intervention can be selected prior to fulminant herd infection.

P182

POST–WEANING DIARRHOEA IN FRANCE: CHARACTERIZATION OF ESCHERICHIA COLI VIRULENCE FACTORS

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Introduction
Post-weaning diarrhoea (PWD) in pigs remains a major cause of economic losses for the pig industry. PWD is characterized by decreased feed consumption, dehydration, sudden death and mild to severe watery diarrhoea occurring typically in the first weeks after weaning. PWD is caused by enterotoxigenic Escherichia coli (ETEC) harbouring adhesin (F4 and F18) and enterotoxin (LT, STa, STb) genes. Recent data about the prevalence of these virulent factors in ETEC in France is scarce.
The objective of the present study was to determine the prevalence of both adhesins and enterotoxins of ETEC causing PWD in France.
Materials and Methods
A total of 91 farms showing clinical signs of PWD were sampled across France during 2014. Rectal swab samples from 5 diarrheic pigs were collected and submitted to the laboratory (Labocea, Ploufragan, France) within 48 hours of the diarrhoea outbreak. Faecal samples were plated and E. coli colonies isolated following normal laboratory procedures. DNA from 4 colonies per farm was extracted, pooled and tested for the presence of adhesin (F4, F5, F6, F41, F18) and toxin (LT, STa, STb, Stx2e) genes using a multiplex PCR (Casey et al., 2009).
Results
In total, 362 E. coli isolates were obtained from PWD cases in nursery pigs. Fifty per cent of them (n=181) were haemolytic, with 46 of the farms (50.5%) showing at least one haemolytic E. coli isolate.
Regarding adhesin genes detection, F4 was present in 34 out of 91 farms (37.4%) whereas 23 farms were positives for F18 (25.3%). In 9 farms (9.9%), both adhesins (F4 and F18) were found). In 25 farms (27.4%), no adhesins were detected.
Genes for LT, STa, STb and Stx2e were detected in 41 (45.1%), 37 (40.7%), 70 (76.9%) and 18 (19.8%) of the farms respectively. In 17 farms (18.7%), no toxins were detected.
ETEC isolates were identified in 59 of the herds (64.8%). Of these, 57.6 % (34 out of 59) were ETEC-F4, being the most prevalent virotype LT, STb, F4. In 16 herds (27.1%), ETEC-F18 was identified as the cause of diarrhoea, being STa, STb, F18 the type most commonly detected. When both adhesins (F4 and F18) were detected in a farm (15.2% of cases), toxins were detected as well.
STEC (F18 and Stx2e genes) were identified in 4 cases. Three other farms were positives for F18 but negatives for toxins.
Conclusions
ETEC is the most common pathotype involved in PWD cases in France with ETEC-F4 being the most frequent type detected. Since mixed infections can also occur and non-ETEC can be detected, laboratory diagnosis including identification of virulence factors is advised in order to initiate appropriate preventive and control measures.

P183

SEASONAL VARIATION IN PREVALENCE OF DIFFERENT RESPIRATORY PATHOGENS DURING POST–WEANING AND FATTENING PERIOD IN BELGIAN AND DUTCH PIG HERDS USING A TRACHEO–BRONCHIAL SWAB TECHNIQUE

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Introduction - Besides Mycoplasma hyopneumoniae (M.hyo), many other viruses and bacteria can be present, provoking the disease complex known as PRDC. Recently, a new sampling technique has been developed and validated for the detection of M.hyo in pigs using PCR, namely the tracheo-bronchial swab (TBS) technique. The aim of the present study was to obtain data on the variation in distribution of different pathogens involved in PRDC in closed pig herds in Belgium and the Netherlands using the TBS technique during the different seasons of the year.
Materials and methods - Four hundred and twelve pig farms were sampled using the TBS technique from 1st January 2012 till 30th June 2014. In every herd, at least 30 coughing piglets were sampled in at least two age groups (3-5, 6-11 and 12-20 weeks of age). TBS were collected as previously described and analyzed using mPCR and/or dPCR (IVD GmbH, Hannover, Germany). PCR results were reported as negative or positive for the presence of PRCV, PRRSV (EU, US or EU/US), SIV, PCMV, PCV2, App and Hps. Results were categorized and analyzed according to the season of sampling (S1, winter; S2, spring; S3, summer; S4, autumn).
Results - In all age group, a different prevalence is obtained depending on the season. In piglets of 3-5 weeks of age, SIV has the highest prevalence during spring (S2, 32.3%), followed by PRRSV-EU (12.5%) and M.hyo (10.4%). The 2nd highest prevalence for these 3 pathogens was during winter (S1). During summer months, overall prevalence of all examined pathogens were at their lowest level. At the end of the nursery period (piglets of 6-11 weeks of age), the highest prevalence were observed during winter with SIV at 20.0%, PRRSV-EU at 29.2% and M.hyo at 16.1%. In this age category, PCV-2 also started to play a role in PRDC pathology with prevalence of > 10.0% in all seasons. In fattening pigs, prevalences for M.hyo, PCV-2 and PRRSV-EU did not differ a lot among season, except for SIV that was much lower (3.2%) during spring as compared to the other 3 seasons (15.0 to 18.6%).
Conclusion
The present study clearly shows that different viral pathogens responsible for PRDC may be present during the post-weaning and fattening period. Following analysis of seasonal variation, it can be concluded that depending on the pathogen, a clear variation in seasonal impact in the PRDC is present. This is in accordance other studies showing a clear seasonal variation in M.hyo prevalence in Spain.

P184

PREVALENCE OF VIRULENCE FACTORS IN ESCHERICHIA COLI ISOLATED FROM PIGS WITH POST–WEANING DIARRHOEA IN BELGIUM AND THE NETHERLANDS

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Introduction
Post-weaning diarrhoea (PWD) is a major cause of economic losses for the pig industry due mainly to increased mortality rates and poor weight gain in surviving piglets. PWD is usually caused by some strains of E. coli, known as enterotoxigenic E. coli (ETEC), with the ability to produce one or several enterotoxins and attach to intestinal cells. The most common adhesins found on ETEC from PWD in pigs are fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are heat-labile toxin (LT), heat-stable toxin a (STa), and heat-stable toxin b (STb).
The objective of this study was to investigate the prevalence of virulence factors in E. coli isolated from pigs with PWD in Belgium and The Netherlands.
Materials and methods
A total of 88 pig herds in Belgium and the Netherlands showing typical clinical signs of PWD were selected for the study in 2014. Seventy piglets (3-5 weeks of age) from 70 Belgian farms were submitted to the laboratory (DGZ, Belgium) and faecal samples obtained during post mortem examination. In addition, rectal swabs were obtained from diarrheic pigs (n=5 per farm) in 18 Dutch farms. In both cases, samples were taken within 48 hours of the start of a clinical PWD outbreak and used for E. coli diagnostics according to the routine procedures of the laboratory. The presence of adhesin (F4, F5, F6, F18 and F41) and toxin (LT, STa, STb, Stx2e) genes in haemolytic E. coli isolates was investigated by multiplex PCR (IZSLER, Italy).
Results
In total, 123 E. coli isolates were obtained from 88 farms. The overall prevalence of adhesin and toxin genes was: F4 (50.4%), F5 (0.8%), F6 (0.8%), F18 (43.1%), F41 (0.0%), LT (20.3%), STa (25.2%), STb (35.8%) and Stx2e (4.9%). Only 1.6% of the isolates had mixed F4+F18 adhesins present.
A total of 39.02% of isolates carried both adhesin and enterotoxin genes, being classified as ETEC. These ETEC isolates were recovered from 36.36% of the initial PWD outbreaks, of which 68.8% were ETEC-F4 and 31.2% ETEC-F18. No mixed infections of ETEC-F4 and ETEC-F18 were detected. The ETEC virotypes most frequently detected were F4, STb, LT (25.0%) and F4, STa, STb (18.7%). In 51 herds (57.9%) the isolates harboured only fimbrial or toxin genes in this assay. The role of these strains in the development of diarrhoea needs further investigations.
Conclusion
Among ETEC isolates involved in cases of PWD, ETEC-F4 is more widespread than ETEC-F18 in Belgium and Dutch farms. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of different E. coli isolates in PWD outbreaks and initiate appropriate preventive and control measures.

P185

FIELD INVESTIGATION OF LAWSONIA INTRACELLULARIS VERTICAL TRANSMISSION FROM SOWS TO PIGLETS AND IMPACT OF ANTIBIOTIC TREATMENT OR VACCINATION ON THIS TRANSMISSION

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Introduction. Little information is available about vertical transmission of Lawsonia intracellularis (Li) from sows to piglets. Swine practitioners may ask themselves if such a vertical transmission exists, and if an antibiotic treatment or a vaccination can have an impact on it. This study represents a field approach to these issues.

Materials and methods. This field study was implemented in 2012 in a farrow to finish farm, conducting in 7 batches of 40 sows, weaning piglets at 4 weeks of age (woa), with a history of early Li infection in flat-deck, confirmed by serologically positive piglets at 10 woa. 6 sows and 2 piglets per sow from 6 successive batches were included; cross-fostering was not allowed in included litters; sows from batches 1 and 2 (B1N and B2N) received no antibiotics and were not vaccinated against Li; sows from batches 3 and 4 (B3A and B4A) received oral Tylosin (Compomix VT®, 10 mg/kg body weight) during 10 days, 5 days before and 5 days after expected date of farrowing; sows from batches 5 and 6 (B5V and B6V) were orally vaccinated with Enterisol® Ileitis, 5 and 3 weeks before farrowing. Excretion at 1 week after farrowing (sows) and at 1 and 4 weeks of age (piglets) was assessed using a quantitative PCR (qPCR) developed in Denmark. Serological status of sows (1 week after farrowing) and piglets (1 and 4 weeks of age) was assessed using a Li ELISA (BioScreen).

Conclusion. In this particular farm, vertical transmission appeared to be rare. We found positive but not quantifiable faeces (in both sows and piglets) after farrowing in only one batch (B3A). This rare excretion did not allow us to conclude on the impact of antibiotic treatment or vaccination on vertical transmission. However, it would be very interesting to investigate this vertical transmission from sows to piglets in more farms, to increase our knowledge about Li epidemiology.

P186

ANTIMICROBIAL SUSCEPTIBILITY OF NON-ENTEROTOXIGENIC ESCHERICHIA COLI ISOLATES FROM NURSERY PIGS IN FRANCE

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Introduction
Antimicrobials are usually required for treatment of post weaning diarrhoea (PWD), a frequent disease in nursery pigs. Due to the occurrence of resistance to one or more classes of antimicrobials (Luppi et al., 2013), monitoring E. coli antimicrobial susceptibility is recommended.
This study aims to investigate the antimicrobial susceptibility of non-enterotoxigenic isolates of E. coli from nursery pigs in France.

Materials and Methods
A total of 111 E. coli isolates recovered from nursery pigs with diarrhoea were included in this study. Isolates originated from 29 farms across France sampled in 2014 in which diarrhoea by ETEC had been excluded by PCR (Casey et al., 2009). 86.5% of the isolates were no-haemolytic.
Antimicrobial susceptibility testing was performed by disc diffusion method following the recommendations of the Antibiogram Committee of the French Microbiology Society and as listed in the French AFNOR Standard NF U47-107 (Labocea, France). The following antimicrobials were tested: neomycin (NEO), gentamycin (GEN), apramycin (APR), amoxicillin (AMX), amoxicillin-clavulanic acid (AMC), ceftiofur (CFT), enrofloxacin (ENR) and spectinomycin (SPT). Colistin (CST) was tested by E-test.

Results
E. coli resistance to SPT was detected in 40.54% of the isolates. NEO, APR and GEN resistant isolates were detected in 16.22%, 14.41% and 11.71% of the cases respectively. 33.33% of the isolates were resistant to AMX and only 0.90% to its combination with clavulanic acid (AMC). ENR resistant isolates were confirmed in 7.21% of the cases. In addition, 7.21%, 2.70% and 0.90% of the isolates had intermediate susceptibility to AMC, GEN and NEO respectively.
Regarding CST, 11% of the isolates were classified as having decreased susceptibility (MIC>2mg/L). Using an MIC breakpoint of ≥8 mg/L, 4% of the isolates were considered resistant to CST. For CST, a MIC90=4 mg/L and a MIC50=0.125 mg/L (range: 0.062-8 mg/L) was shown. Fourteen isolates (12.61%) presented resistance to 4 or more of the antimicrobials tested.
The percentage of AMX, APR and NEO resistant isolates was lower in non-ETEC isolates than in ETEC isolates (Gin et al., 2015), with a difference of 25.64%, 24.05% and 20.96% respectively. For CST, a 13% difference in resistant isolates (≥8 mg/L) was observed.

Conclusions
This study shows that antimicrobial resistance to CST, AMX, APR and NEO is more widespread in ETEC than in non-ETEC isolates. As previously reported (Kempf et al., 2013), acquired resistance in E. coli is most frequently encountered among pathogenic isolates, particularly in piglets suffering from diarrhoea.

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PREVALENCE OF VIRULENCE FACTORS IN ESCHERICHIA COLI ISOLATED FROM PIGS WITH POST–WEANING DIARRHOEA IN GERMANY.

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Introduction
Post-weaning diarrhoea (PWD) is a major cause of economic losses to the pig industry from both mortality and reduced growth rates (Fairbrother et al., 2005). PWD is caused by some strains of E. coli, known as enterotoxigenic E. coli (ETEC), with the ability to produce one or several enterotoxins, including heat-labile toxin (LT), heat-stable toxin a (STa) and heat-stable toxin b (STb), and attach to intestinal cells. The adhesins most commonly found in ETEC causing PWD in pigs are fimbriae F4 (formerly K88) and F18. In spite of its importance, recent data on the prevalence of virulence factors of ETEC causing PWD is scarce.
The objective of the present study was to investigate the prevalence of ETEC virulence factors involved in cases of PWD in Germany.
Materials and Methods
A total of 99 samples from German farms (n=17) showing clinical signs of PWD (sudden death, watery diarrhoea, decreased feed consumption, dehydration and depression) in nursery pigs were included in the study. Rectal swab samples from 5 to 10 pigs with diarrhea were collected within 48 hours of the start of the outbreak and submitted to the laboratory for E. coli diagnostics (Giessen University, Gerrmany). DNA was extracted from E. coli isolates and the presence of adhesins (F4, F5, F6, F18 and F41), intimin (eae) and toxins (LT, STa, STb, Stx2e) was tested by PCR.
Results
E. coli was identified in 92 samples (94.8%) by culture and biochemical characterization. One to four different isolates per sample were tested by PCR (n=178). 75.4% of the isolates were positive for at least one virulence factor. Genes for STb, STa, LT and Stx2e were detected in 48.88%, 15.17%, 12.92% and 3.37% of the isolates. Regarding adhesin genes, F4 was the most prevalent (16.85%), followed by F18 (11.80%) and aea (2.25%). No F5, F6 or F41 genes were detected. Thirty-nine isolates containing at least one toxin and one adhesin were classified into pathotypes, 32 of them (82%) as ETEC. From these, 90% were ETEC-F4 and 10% ETEC-F18. The most common virotype among ETEC isolates was LT, STb, F4 (n=20). E.coli isolates containing the gene for F18 adhesin but not expressing toxins were a common finding (n=15).
ETEC-F4 was identified as the cause of diarrhoea in 47% of the cases of PWD outbreaks in nursery pigs, whereas ETEC-F18 was involved in only 6% of the cases.
Conclusion
This study shows that ETEC-F4 is an important pathogen in Germany, being the most prevalent cause of PWD in nursery pigs. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of different E. coli isolates in PWD outbreaks and initiate appropriate preventive and control measures.

P188

PREVALENCE OF VIRULENCE FACTORS ASSOCIATED WITH POST WEANING DIARRHOEA (PWD) IN PIGS IN ITALY

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Introduction
Post weaning diarrhoea (PWD) caused by enterotoxigenic Escherichia coli (ETEC) is globally distributed disease. PWD has a great economic impact on pig production, leading to reduction on performance and increased mortality. Porcine ETEC strains typically express F4 (K88) or F18 fimbria and heat-labile (LT) and/or heat-stable (STa, STb) enterotoxins.
This study investigates the prevalence of virulence factors of ETEC isolated from PWD cases in Italy.

Materials and methods
In total, 159 E. coli isolates from 84 herds located in Northern Italy were obtained from nursery pigs with PWD from 2012 to 2014 using standardized bacteriological methods from diagnostic samples (rectal swabs, faeces and small intestine). To evaluate the prevalence of virulence factors, a multiplex PCR (Casey and Bosworth, 2009) for F4 (K88), F5 (K99), F6 (987P), F18, F41, STa, STb, LT and Stx2e genes was performed on E. coli isolates. When more than one isolate per herd resulted in the same virotype being detected, it was counted only once for prevalence calculations. E.coli strains were classified as ETEC when isolates carried both fimbrial and toxin genes.

Results
In 98.8% of the cases, only one strain of E. coli was involved in the PWD outbreak. In addition, haemolytic activity was detected in 95.24% of the isolates.
The prevalence of fimbriae genes was: F4 (54.76%), F18 (33.33%) and F6 (1.19%). In 5 cases, the strains isolated harboured more than one fimbrial gene: F4+F18 (4.76%) and F5+F41 (1.19%). The prevalence of toxin genes was: LT (55.95%), STa (63.10%), STb (71.43%) and STx2e (9.52%).
ETEC isolates were detected in 68 of the herds (80.95%), with 98.83% of them being haemolytic. In 16 herds (19.05%) the isolates were negative for fimbrial or toxin genes in this assay. The role of these strains in the development of diarrhoea needs further investigations.
57.57% of ETEC isolates (39 out of 68) were ETEC-F4, whilst 36.76% (25 out of 68) were classified as ETEC-F18. In 4 cases (5.88%), ETEC carrying F4 and F18 genes were detected. The two most common ETEC virotypes were F4, STb, LT (25%) and F4, STa, STb, LT (22.06%).

Conclusion
This study confirms that ETEC-F4 is most commonly associated with PWD in Italy, similarly to the situation previously described from 2002 to 2012 (Luppi et al., 2014), resulting more prevalent than ETEC-F18. The most prevalent virotype was F4, STb, LT.
Information about the prevalence of ETEC in cases of PWD is relevant when measures of control of the disease such as vaccination must be taken. Alternative approaches to antibiotic therapy are needed, since very high rates of antimicrobial resistance are reported in ETEC isolated from cases of PWD.

P189

DIVERSITY OF THE GASTRIC MICROBIOTA IN HELICOBACTER SUIS–INFECTED AND H. SUIS–NEGATIVE SLAUGHTERHOUSE PIGS

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Introduction.
Helicobacter suis infection is an important cause of gastric disease in pigs and humans. In H. suis-infected humans, gastritis, peptic ulceration and gastric MALT lymphoma have been described to develop. In pigs, H. suis infection has been shown to cause chronic gastritis and decreased daily weight gain. In addition, infection has been associated with ulceration of the pars oesophagea of the stomach, which is covered by a stratified squamous epithelium, in contrast to the glandular epithelium which is found in all other regions of the stomach. Pigs and pork are considered to be the main source of infection for humans. H. suis is found in the majority of pigs worldwide, but little is known on the presence of other microorganisms in the stomach of these animals. In addition, the presence of H. suis itself or the changes H. suis infection induces may affect the composition of the porcine gastric microbiota.
In this study, we aimed at analyzing the porcine gastric microbiota and at investigating differences in the gastric microbiota between H. suis-positive and H. suis-negative pigs.
Materials and methods.
Quantitative PCR was performed on pooled samples of biopsies taken from the 4 different regions of the stomach from slaughterhouse pigs to determine H. suis positivity. Subsequently, 6 H. suis-positive and 6 H. suis-negative animals were selected for further analysis. After amplification of 16S rRNA genes from the bacterial population, sequencing was performed using the Genome Sequencer Junior System (Roche 454 Life Sciences).
Results and conclusions.
Metagenomic analysis revealed that, although the microbiota was diverse and differed between animals, the most frequently detected bacteria were Fusobacterium spp. (including a hitherto uncharacterized new species), Lactobacillus spp., Campylobacter spp. and Escherichia coli. The number of E. coli bacteria was higher in the stomach of H. suis-positive pigs compared to H. suis-negative pigs. In vitro experiments showed that growth of E. coli was stimulated when co-incubated with H. suis and that E. coli was able to suppress the growth of H. suis. Additional experiments are currently being performed to characterize the newly identified Fusobacterium species and to elucidate its potential role in porcine gastric disease.

P190

EFFICACY OF VACCINATION AGAINST M. HYOPNEUMONIAE WITH A ONE–SHOT VACCINE ADMINISTERED SIMULTANEOUSLY WITH ECOPORC SHIGA® UNDER FIELD CONDITIONS

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Introduction
The majority of vaccinations for piglets are performed during the suckling period due to an early onset of most infections and the duration for establishment of immunity. Since enzootic pneumonia (EP) occurs worldwide, 80% of piglets are vaccinated against Mycoplasma hyopneumoniae (M hyo). Some of the commercially available vaccines are designed to be administered on the 3rd day of life and according to individual infection dynamics on farm level, early vaccination can be useful and necessary, not only against M hyo but also against edema disease (ED). The aim of this study was to examine the efficacy of Stellamune®One against EP caused by M hyo after one shot vaccination compared to the effect of an additional and simultaneous administration of a vaccine against ED (ECOPORC SHIGA®) in the first week of life (4th-7th day of life) under field conditions.
Materials and Methods
The study was performed in 3 consecutive batches in a farrow-to-finish farm in southern Germany between Dec 2013 and Aug 2014 with a known history of EP but unsuspicious regarding ED. The piglets were randomly assigned to one control group (CG; n=135) and two M hyo vaccination groups VG1 and VG2, receiving the vaccines based on vaccination schedule: VG1 (n=135) Stellamune®One, i.m.; VG2 (n=135) ECOPORC SHIGA® + Stellamune®One, i.m.
The efficacy of the M hyo vaccination was determined by comparing performance parameters such as bodyweight at the end of finishing as well as average daily weight gain (ADG) between day 84 and the end of finishing (day 168). Furthermore the percentage of physically retarded animals was evaluated and lung lesions were scored at slaughter.
Results
Bodyweights at the end of finishing did not differ significantly between VG1 (86.88kg) and VG2 (86.35kg) but weights of CG (84.15kg) were numerically lower without differing significantly. The ADG for the pigs belonging to VG1 and VG2 was 766.0g and 762.7g respectively and 730.0g for the pigs from the CG. Pigs of VG1 achieved significant higher ADG than CG ($p<0.025$). In VG1, 4.6% and in VG2, 5.4% of pigs were physically retarded in contrast to CG with 10.7% ($p>0.025$). Lung lesion scores of VG1 and VG2 were 5.7 and 6.8 respectively and 7.7 for the pigs of CG ($p>0.025$).
Conclusion
The data of present study showed that the concurrent vaccination with ECOPORC SHIGA® and Stellamune®One leads to similar results concerning ADG, bodyweight and percentage of physically retarded animals compared to a single vaccination with Stellamune®One. Lung lesion score of pigs vaccinated simultaneously (VG2) exceeded that of VG1 and was lower than that of CG, not vaccinated against M hyo, without significant differences.

P191

ANTIMICROBIAL SUSCEPTIBILITY MONITORING OF RESPIRATORY AND ENTERIC PATHOGENS ISOLATED FROM DISEASED PIGS ACROSS EUROPE BETWEEN 2009 AND 2012

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Introduction: VetPath is an ongoing pan-European resistance monitoring program for veterinary pathogens isolated from diseased but not yet treated cattle, pig and poultry. Antimicrobial susceptibilities of pathogens isolated from pigs suffering from respiratory, enteric infections or meningitis cases are presented here. Methods: Lung/nasal, rectal/fecal or CNS samples were collected from animals with acute clinical signs, not recently treated with antibiotics, in 8 EU countries. Actinobacillus pleuropneumoniae (Ap), Pasteurella multocida (Pm), Bordetella bronchiseptica (Bb), Streptococcus suis (Ss), Haemophilus parasuis (Hp) and Escherichia coli (Ec) were isolated (one isolate/outbreak/farm) by standard methods. Susceptibility to 17 antibiotics was determined in a central laboratory by broth microdilution as per CLSI standards where appropriate. Results were interpreted using CLSI approved breakpoints (VET01-S2, 2013) where available. Results: Overall 859 isolates were recovered. Resistance of Ap (n=157) to amoxicillin/clavulanic acid (AMC), ceftiofur, tiamulin and tulathromycin was absent. Ap showed high susceptibility (97-99%) to enrofloxacin, florfenicol and tilmicosin. In contrast, resistance of Ap to tetracycline (T) amounted to 23.6%. All 152 Pm isolates were fully susceptible to AMC, ceftiofur, enrofloxacin and tulathromycin. Tilmicosin and T resistance was 2.0 and 20.4%, respectively. Clinical resistance of Bb isolates (n=118) against tulathromycin was absent and low resistance against AMC (0.8%) and florfenicol (5.1%) was found. Ss isolates (n=151) showed high susceptibility (96-100%) to enrofloxacin, ceftiofur, florfenicol and AMC, but resistance to T amounted to 88.1%. In the case of Hp isolates (n=68) low MIC90 values (0.06-2.0µg/ml) were found for amoxicillin, AMC, the fluoroquinolones, florfenicol, tiamulin, tilmicosin and trimethoprim/sulfamethoxazole (TMS). Higher MIC90 values (8-16µg/ml) were identified for spectinomycin, lincomycin and tylosin. Ec isolates (n=213) showed high susceptibility (89-90%) to gentamicin and AMC. In contrast, very high levels of resistance were found for T (75.6%) and TMS (62.4%). The enro-, dano- and marbofloxacin MIC90 values were 2-4µg/ml. Conclusions: Except for T versus Ss, Pm and Ap and T and TMS versus Ec, the results show an absence or low prevalence of antimicrobial resistance among the major respiratory and enteric pathogens isolated from diseased but non-treated pigs across the EU. The results are similar to those generated in the previous (2002-2006) VetPath project.

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CORRELATIONS BETWEEN FAECAL LAWSONIA INTRACELLULARIS EXCRETION AND INDIVIDUAL PERFORMANCE PARAMETERS IN VACCINATED PIGLETS OR PIGS WITH OR WITHOUT CLINICAL SIGNS OF INFECTION

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Introduction: In piglets with a clinically obvious Lawsonia intracellularis (L.i.) infection increasing numbers of L.i. in pig faeces showed a strong negative correlation with average daily weight gains (ADWG). This study tested the hypothesis that the known correlations between performance and qPCR results are also valid for clinically inapparent and vaccinated animals.
Methods: In 3 consecutive trials a total of 27 potentially naturally L.i. infected pigs (bw: 19.0 ± 1.50 kg; 9 pigs/group) were allotted to 1 of 3 groups: not vaccinated, without clinical findings = VAC–CF–; with clinical findings (soft to liquid faeces) = VAC–CF+; vaccinated (Enterisol®Ileitis; as suckling piglet) = VAC+. Pigs were housed individually and fed the diet for 10 d to determine faeces DM content, performance parameters (feed intake, daily gains=ADWG, etc.). The L.i. excretion was analysed in an aliquot of faeces from the collection period (5d) by qPCR. Statistical analyses were performed by one-way ANOVA (procedure GLM; p <= 0.05). Correlations are given by means of the Pearson (normal distributed, PCC) or Spearman correlation coefficient (not normally distributed, SCC).
Results: Faecal shedding of L.i. was found in all groups (25 of 27 animals) with the highest numbers of genome equivalents (GE) seen in group VAC–CF+ (lg GE 7.70 ±1.65; VAC–CF–=lg GE 5.83 ±2.35; VAC+=lg GE 6.00 ±2.89). Animals in group VAC–CF+ had the significantly lowest dry mater content (DM) in the faeces (211 ±19.8 g/kga; VAC–CF–= 245 ±16.8 g/kgb; VAC+=236 ±18.4 g/kgb) and showed the significant lowest ADWG (785 ±137 ga; VAC–CF–= 857 ±86.3 gab; VAC+=894 ±73.4 gb). In group VAC–CF+ animals, there were moderate (feed intake-SCC: r=-0.35) or clear linear negative correlations for the analysed performance parameters (ADWG-PCC: r=-0.58) and DM content of faeces (DM: PCC=-0.56). In VAC–CF– pigs only poor or rather no correlations could be seen (feed intake-SCC: r=0.06; ADWG-PCC; r=0.01; DM-PCC: r=-0.15). In vaccinated animals there was a moderate linear positive correlation between feed intake and amount of excretion (SCC: r=0.49) and between ADWG and faecal L.i. shedding (PCC: r=0.43) but rather no correlation between lg GE L.i. in faeces and DM content of faeces (PCC: r=0.17).
Conclusion: While we found in this study a negative correlation between performance and L.i. counts in faeces in non-vaccinated, clinically apparent animals, confirming the results of a previous study, we did not find the same correlation in vaccinated, clinically healthy animals. To our knowledge this is the first time that the correlation between L.i. counts in feces as measured by qPCR and performance of vaccinated animals was evaluated.

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IMPORTANCE OF MYCOPLASMA HYORHINIS IN DEEP AIRWAYS BACTERIAL CO-INFECTIONS IN FATTENING PIGS: RESULTS OF A CASE CONTROL STUDY.

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Importance of Mycoplasma hyorhinis in deep airways bacterial co-infections in fattening pigs: results of a case control study.
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Introduction
The porcine respiratory disease complex is a major issue today in pig production. Controlling the disease and thus limiting its economic impact in farms remains difficult as it is multifactorial and implies numerous infectious agents whose respective roles are hard to determine. The objective of this case control study was to investigate if the detected presence of Mycoplasma hyorhinis was a risk factor for observed respiratory clinical signs in field. It also enabled to evaluate the feasibility of trachea-bronchial washing as a routine practice.

Material and Method
Nineteen farms located in the main pig production area in France ('Grand Ouest') were included in the study. Ten were identified by their veterinarian as having major respiratory problems difficult to control. Nine others on the contrary were considered as control farms. Twenty pigs were selected in each case farm and ten in each control farm; all in the second half of the finishing stage. Half of the pigs purposively selected in the case farms presented cough. Each pig was sampled twice, first by by tracheo-bronchial swabbing (probe of 53cm length) and second by trachea-bronchial washing (probe of 90cm length). The samples were tested individually by PCR method for the detection of Mycoplasma hyorhinis and Mycoplasma hyopneumoniae. Statistical analysis was performed with Excel.

Results
Whatever the sampling method we found a significant difference in the frequency of detection of Mycoplasma hyorhinis ($p<0.01$) between pigs coughing or not; this difference ($p<0.01$) appeared for the detection of Mycoplasma hyopneumoniae and for the simultaneous detection of both Mycoplasma. None of the pigs was infected by Mycoplasma hyorhinis alone). The difference was higher with washes. There was no difference in the frequency of detection of Mycoplasma hyopneumoniae alone (without Mycoplasma hyorhinis).

Conclusion
Tracheo-bronchial samples revealed easy to perform and useful for the diagnosis of respiratory diseases in pigs. Results also suggested that the presence of Mycoplasma hyorhinis associated to that of Mycoplasma hyopneumoniae could increase the risk of observing respiratory clinical signs especially cough. However, performances of diagnostic tests in field should be evaluated and may give insights on the relationship between detecting the presence of either of the mycoplasmas concerned and observing clinical signs.

P194

INDIVIDUAL INJECTION THERAPY VS. ORAL BATCH-TREATMENT WITH OXYTETRACYCLINE IN LAWSONIA INTRACELLULARIS INFECTED NURSERY PIGS

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Introduction. Several countries have implemented strategies to reduce antimicrobial consumption due to general concerns of selection of resistant bacteria. One way to reduce AC is to treat only diseased pigs by individual injection rather than to treat all pigs in a nursery compartment by water medication. The objective of this study was to evaluate individual vs. batch-wise oxytetracycline (OTC) treatment of enteritis caused by Lawsonia intracellularis (LI) in nursery pigs.

Materials and Methods. A randomized clinical field trial was conducted in four Danish pig herds with outbreaks of diarrhoea caused by LI in 24 nursery compartments. Two treatment strategies were applied at compartment level with 10 mg OTC per kg for five days: 1. Injection treatment of individual pigs with clinical diarrhoea and 2. Oral batch treatment administered through medicated drinking water for all pigs in a nursery compartment. Faeces was sampled from 15 randomly selected pigs in each compartment before and two days after end of treatment, and LI in faeces was determined by qPCR. The result was dichotomized above and below the detection limit of 100 LI bacteria per gram faeces.

Results. Data from 340 pigs were included for analysis. Individual treatment and oral batch treatment included 174 and 166 pigs and 12 compartments each. Prevalence of LI positive pigs before treatment ranged between 21 and 50 % in the four herds. Injection treatment of pigs with clinical diarrhoea resulted in medication of 54 % of the pigs. Oral batch treatment through drinking water resulted in medication of 100 % of the pigs. Individual injection treatment resulted in 52 % pigs shedding LI after medication whereas oral batch treatment resulted in 17 % pigs shedding LI after medication ($p<0.0001$). In the individually injected pigs 41 % were still shedding LI after treatment, while 66 % of the non-injected pigs from the same compartments were shedding LI after treatment ($p <0.01$).

Discussion and Conclusion. Oral water treatment with OTC of all pigs in a nursery compartment was more effective in reducing faecal shedding of LI compared to a similar OCT dose used for injection of pigs with clinical diarrhoea (17 vs. 52 %). A higher LI prevalence in injected (41 %) vs. orally treated pigs (17 %) indicate that either OTC administered by injection is less efficacious than orally administered OCT, or that injection treatment of only a proportion of pigs in a compartment is not effectively stopping the spread of LI. The results indicate that injection treatment as a strategy in order to reduce AC, even though extremely work-demanding, may lead to insufficient treatment of LI enteritis in nursery pigs.

P195

CASE REPORT: CONTROL OF MYCOPLASMA HYOPNEUMONIAE IN A HERD USING AIVLOSIN®

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Introduction: This case report describes a strategy to control Mycoplasma hyopneumoniae (Mh) infection in a farrow-to-finish herd with a high level of Mh circulation in spite of piglet's vaccination. Material and Method: The case happened in a 500 commercial sow herd in the center of France. Piglets were vaccinated against Mh at 6 weeks of age. Replacement gilts are Mh-negative and vaccinated into the quarantine. In March 2013, Influenza and Mh were identified after an outbreak of cough on all physiologic stages. Influenza vaccination of sows and treatments on pigs allowed a reduction of the symptoms but coughing were still observed on 100 day-old pigs and on 1st and 2nd parity sows. In January 2014, Mh diagnosis (PCR and ELISA) was done. Early infection on piglets was diagnosed (46% positive piglets by PCR at 28 days of age) and a seroconversion was observed at 10 weeks of age on finishers. A treatment with Aivlosin® (2,125 mg tylvalosin/kg BW) was given to all sows for 10 days and followed up by a treatment 7 days before farrowing. Moreover a treatment of 7 days on 70 day-old pigs was done with Aivlosin® (same dose) and vaccination was maintained. Results: Medication with Aivlosin® stopped cough on sows and pigs in nursery but remained on 120-140 day-old finishers. In September 2014, analyses indicated that 28 day-old piglets from non-treated sows were Mh negative. Finisher's Mh infection seemed to be delayed at 15 weeks of age. FCR and ADG were improved after the medication. 9 months after the 1st treatment, medication on sows has been stopped without impact on clinical signs and a blitz Mh vaccination followed up by a booster 3 weeks before farrowing has been settled up. Conclusion: Aivlosin® and Mh vaccination is effective in the Mh control in cases of early infection.

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ERADICATION OF MYCOPLASMA HYOPNEUMONIAE IN A HERD USING AIVLOSIN®

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Introduction: the objective of this project was to eradicate Mycoplasma hyopneumoniae (Mh) from a 360 sow farm, with antibiotic treatment in the breeding animals and offsprings and without depopulation. Tylvalosin (Aivlosin® 42.5 mg/g medicated premix for pigs) was selected as the key antibiotic to perform this Mh eradication program, as this antibiotic shows mycoplasmacidal activity and because it has proved effective in other Mh eradication protocols. Material and Method: This is a commercial breeding herd in the North-West of France with weaning at 28 days of age. Replacement gilts come from one of fattening sites (self-renewal) which is Mh positive but from the purchase of free Mh gilts as well. The farm was populate in 2001 and was infected in 2003. Piglets and gilts are vaccinated against Mh. The protocol consists in stopping self-renewal and sale of piglets, a mass Mh vaccination of all animals and medicating all gilts, sows and boars with 2.125 mg tylvalosin per kg bw and per day, during 28 days. Suckling piglets receive from D0 and every 10 days 2.5 mg tulathromycin per kg bw. After this, Mh vaccines are stopped and a new flock of replacement gilts comes into the quarantine. A serological monitoring is performed every 6 weeks on non-vaccinated gilts introduced after the end of the protocol and a quantitative Real-Time PCR is done on weaned piglets from these gilts and from sows present at the time of the protocol. Results: 2 years after the eradication protocol, all tested samples have been negative. Conclusion: the farm is now considered as Mh free. This protocol using a treatment with Aivlosin® has proved effective in the attempt to eradicate Mh.

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EFFICACY OF TULATHROMYCIN (DRAXXIN® 25 MG/ML) FOR THE TREATMENT OF SWINE RESPIRATORY DISEASE ASSOCIATED WITH SEVERAL MAJOR RESPIRATORY PATHOGENS – EU FIELD STUDIES

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Introduction
Objective was to evaluate the efficacy of tulathromycin (Draxxin® 25 mg/ml) vs tildipirosin (Zuprevo®) for the treatment of Swine Respiratory Disease (SRD). Draxxin® 25 mg/ml has shown to be bio-equivalent to Draxxin® 100 mg/ml and was recently approved in Europe. The sole discriminator between both products is the active substance concentration, with the lower concentration being more convenient for use in young animals.

Materials and Methods
Two single site studies were run, one in Germany and one in Spain. In both countries, 192 piglets aged 4-6 weeks were enrolled. At study inclusion (Day 0), pigs had moderate (score 2) or severe (score 3) clinical signs of SRD (depression, dyspnoea, coughing and sneezing). The nature of the disease was assessed by broncho-alveolar lavage (BAL) from 10-15% of piglets enrolled. Each animal was randomly allocated in a 1:1 ratio to either T01 (tildipirosin 4 mg/kg BW) or T02 (tulathromycin 2.5 mg/kg BW). On Days 1 to 14, inclusive, and on Day 21, clinical observations were performed. The primary efficacy variable was the clinical cure (SRD score ≤ 1) rate on Day 14 with the objective to demonstrate non-inferiority of tulathromycin compared to tildipirosin.
Results
Several major respiratory pathogens have been recovered: Bordetella bronchiseptica (83%), Haemophilus parasuis (79%), Streptococcus suis (40%) and Pasteurella multocida (23%).
Clinical signs decreased rapidly after treatment in both groups. On Day 3, the observed difference between T01 and T02 (16.3%) was statistically significant in Germany ($P = 0.0181$), with less animals in T02 showing clinical signs. In Spain, there were no significant differences between treatment groups.
In both studies, treatment with tulathromycin was shown to be non-inferior to treatment with tildipirosin based on percentage of clinical cure on Day 14. In Germany and Spain, respectively, the clinical cure rate was 93.4% and 100.0% in T01, compared to 93.7% and 98.9% in T02.
Relapse rates on Day 21 were 2.4% (T01) compared to 4.5% (T02) in Germany. In Spain, one T02 animal relapsed by Day 21 versus none of the animals in T01. There were no significant differences between treatment groups.

Conclusion
Tulathromycin (Draxxin 25 mg/mL) was shown to be non-inferior to tildipirosin (Zuprevo) for the treatment of SRD associated with several major respiratory pathogens. Clinical signs decreased rapidly after treatment, there were no SRD-related mortalities and relapse rates were low.

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EFFICACY OF TYLVALOSIN (AIVLOSIN®) TO CONTROL SYMPTOMS OF MYCOPLASMA HYOPNEUMONIAE IN FATTENING PIGS

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Introduction
French studies showed that lesions due to enzootic pneumonia are detected in 69.1% and 72% of pigs by the end of fattening and that this disease is responsible for major economic losses.
This clinical study shows that targeted antibiotic treatment with tylvalosine (Aivlosin®) can reduce the dynamics of Mycoplasma hyopneumoniae infection in farms with immunised pigs.

Materials and Methods
The study was conducted in a farrow to finish French farm with 150 sows. All piglets are systematically immunised with a one-dose vaccine against mycoplasma at the age of 21 days.
In September 2012, the breeder noticed breathing difficulties, slow-growing animals (GMQ=713g, IC=3.24) and an increase in fattening losses (5.2%).
Serological tests showed mycoplasma in the fattening unit and this was confirmed by histological analysis. No other respiratory pathogen was detected in the tests.

Results
All animals of more than 10 weeks of age were administered tylvalosine at 2.125 mg/kg live weight, daily for 7 days in November 2012. In July 2013, new samples tended to confirm a relationship between the observed clinical improvement and reduction of the pressure due to infection with Mycoplasma hyopneumoniae. This was further confirmed by improved technical results (GMQ=854 ; IC=2.76 ; loss rate = 3.6%).

Conclusions and Discussion
This study shows that targeted antibiotic treatment is sometimes necessary to control the spread of mycoplasma in an infected unit, even if the pigs are immunised. This case history illustrates the clinical usefulness of tylvalosine in the treatment of lung infection with Mycoplasma hyopneumoniae.

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COMBINATION OF FEED ADDITIVES TO REDUCE ANTIBIOTICS USE IN POST WEANING

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The antibiotic consumption in the French swine sector is predominantly for piglets during post weaning and frequently based on polypeptides and tetracycline to solve digestive disorders.
Indeed, according to various studies:
- Digestive disorders in post weaning represents 69% of nDD/a (Number of Daily Dose per Animal) and 69% nCD/a (Number of Course dose per Animal) (INAPORC panel, 2014).
- ALEA (Animal Level of Exposure to Antimicrobials) for polypeptides and tetracycline is respectively 0.294 and 0.275 for a cumulated ALEA of 0.991 (ANMV report, 2012).
- The antibioresistance diagnosis is focused at 51% on piglets. Among the piglets' antibioresistance diagnosis, the digestive disorders represent 54% of the samples and among this 54%, E.coli represents 88% of the bacteria investigated (RESAPATH report, 2012).
Therefore, propose alternative solution to reduce antibiotic use in recently weaned piglet is needed. Neovia developed 2 feed additives: B-SAFE and POWERJET. B-SAFE is an activated copper on specific clay and POWERJET is a plant extracts combination. These 2 products demonstrated that their mode of action allows the modulation of intestinal microbiota and intestinal inflammation.

In R&D post weaning facilities, 144 piglets are allocated in 3 groups: negative control, positive control (120 ppm of colistin + 800 ppm of chlortetracycline in prestarter feed and non-medicated starter feed) and tested group (400 g/T of B-SAFE HC + 200 g/T of POWERJET HC in prestarter and starter feed). Prestarter feed is distributed from 20 to 43 days old and starter feed from 44 to 69 days old. A deliberately downgraded environment is set up (no cleaning, density x 1.5 and 15°C temperature) in order to create digestive challenge.

At 69 days old, positive control group and tested group have a LW (Live Weight) respectively improved by +1.7 kg and +0.5 kg. For the positive control, the DFI (Daily Feed Intake) is 6.4% higher and FCR (Feed Conversion Ratio) is 1.1% lower compared to negative control. For the tested group, the DFI is 1.9% higher and FCR is 2.6% lower compared to negative control. In the negative control 48% of piglets have diarrhea symptom when the prevalence is respectively 8% and 25% for the positive control and for the tested group. In the negative control 27% of piglets receive an individual medication when no piglet is treated in the positive control and 8% of piglets are treated in the tested group.

With an experimental design downgrading the environment in order to challenge the piglet digestive process, the association of B-SAFE and POWERJET allows a better result compared to non-medicated feed and a lower result compare to medicated feed.

P200

ROSEN'S ASSESSMENT TO OBJECTIVELY EVALUATE B-SAFE FEED ADDITIVE IN PRE STARTER AND STARTER PERIOD

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The number and diversity of growth promoting feed additives available for animal feed have intensely increased in the last decades. In the meantime, improvement of technical and economical performances remains a major objective for farmers. Therefore, it is necessary to define which additive is reliable and evaluate objectively its benefits on zootechnical performances and economical return on investment.

One impartial way to assess a feed additive is to submit the product results to the Rosen evaluation (Rosen G.D. 2004.). Indeed, the model contains 7 criteria to know if a growth promoting product can be considered as an efficient feed additive or not. The 7 criteria evaluate the reliability of the data, performance improvement and reproductibility of the effects.
B-SAFE is a natural growth promoter based on a patented technology: activated copper exchanged on specific clay. Thus, B-SAFE modulates the intestinal microbiota and secures the digestive process in order to improve daily weight gain (DWG) and the feed conversion ratio (FCR).

For piglet in pre starter and starter period, B-SAFE achieves the majors Rosen's criteria. Indeed, B-SAFE dossier contains:
- 27 trials carried out in R&D center and commercial farm (>30 expected)
- Only 1 trial without negative control (<5 expected)
- One scientific publication on the mode of action is in progress (>1 publication expected)
- B-SAFE improves performances in 78% of case (>70% expected)
- The coefficient of variation of performances improvement is 106% ([100 ; 200] expected)
- The dosage maximizing the return on investment is known (dose response curve established)
- The model predicting the product response under specific conditions is in construction
B-SAFE allows an average increase of +5.0% on DWG and an average decrease of -1.9% on FCR. B-SAFE gains are more predictable and more repeatable at the end of post weaning (starter period) because sanitary troubles are most of the time due to digestive disorders at this moment. B-SAFE gains are more changeable and more variable at the beginning of post weaning (prestarter period) because sanitary troubles are most of the time multifactorial at this moment. Indeed, performances can be impacted by digestive disorders but also by immunity deficit, feed intake drop off, etc.

Thanks to the Rosen's analysis, B-SAFE proved that it is an efficient and reliable natural growth promoter feed additive. The large number of trials set up all over the world also demonstrates that B-SAFE is a cost effective solution in various situations.

P201

A BRACHYSPIRA HYODYSENTERIAE INFECTION MODEL: EFFECT OF BODY WEIGHT AND INOCULATION STRAIN

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Dysentery, caused by Brachyspira hyodysenteriae (B. hyo), causes economic losses and animal welfare problems at pig farms and is associated with high antibiotic use. Therefore, there is a need for alternative interventions against B. hyo infection. In order to evaluate effects of these interventions, a suitable B. hyo challenge model in pigs needs to be developed. As a first step, we studied the effects of pig weight (50kg vs. 10kg) and B. hyo strain (ATCC B204 vs. field strain) on clinical signs and fecal shedding in orally challenged pigs. Material and Methods. Male pigs were assigned to 3 groups: A50 (ATCC B204 strain, 50kg, N=4); F50 (field strain, 50kg, N=4); F10 (field strain, 10kg, N=8). For both B. hyo strains, each pig was inoculated daily for 6 consecutive days with ≥2.8*10^9 CFU B. hyo (anaerobically cultured for 2 days at 37 °C in BHI broth to ≥2.8*107 cfu/ml) and one mashed full SBA plate of cultured B. hyo. Mixed broth and plate were voluntarily ingested from 50 ml syringes (without needles) by all pigs. Rectal fecal samples were taken before (day (D) -4 and 0) and after inoculation (D10, 14, 17 and 21) for B. hyo detection by qPCR. Fecal quality (0 = normal; 1 = soft, but formed; 2 = watery diarrhea; 3 = diarrhea with blood and/or slime) was recorded daily for each pig. At the end of the trial (D24), or earlier due to severe discomfort, pigs were euthanized. Results. Before inoculation until D13 after inoculation, feces quality was normal (score 0 or 1) in all pigs. Feces quality suddenly changed from normal to score 3 in 3/4 pigs of A50 (at D14 (euthanized(†) at D15), 18 and 24 resp.), in 1/4 pigs of F50 (at D14; († at D14) and in 3/8 pigs of F10 (at D15 († at D15), D16 and D16 resp.). Fecal samples before inoculation were all negative for B. hyo. For A50, fecal samples were positive for B. hyo in 0/4 pigs on D10, 1/4 pigs on D14, 1/3 pigs on D17 and 2/3 pigs on D21. For F50, positive PCR results were found in 1/4 pigs on D10, 3/4 on D14, 3/3 on D17 and D21. For F10, fecal samples were positive for B. hyo in 3/8 pigs on D10, 6/8 on D14, 5/7 on D17, 7/7 on D21. Ct-values were not significantly different between groups. Conclusions. We successfully orally infected pigs with ATCC strain B204 resulting in both dysentery and fecal shedding in 75% of the 50kg pigs. The B. hyo field strain resulted in dysentery in 25% of the 50kg pigs and 37.5% of the 10kg pigs, and in fecal shedding in all 50kg and 10kg pigs, although not at the same time. Results indicate a similar colonization capacity, but lower pathogenicity of the field strain compared to the ATCC B204 strain, and a comparable response in 10kg and 50kg pigs for the field strain.

P202

SEROTYPING OF ERYSIPELOTHRIX RHUSIOPATHIAE ISOLATES FROM PIGS IN ENGLAND AND WALES FROM 1987 TO 2012

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Introduction
Porcine erysipelas (PE) caused by Erysipelothrix rhusiopathiae (ER) is present worldwide. Despite the availability of commercially available vaccines, PE is still considered economically important. ER is also a zoonosis, although mainly considered an occupational health risk. In 2011-2012, anecdotal reports from veterinary surgeons in England reported an increase in PE outbreaks in young breeding pigs that should have been fully vaccinated. The objective of this study was to serotype ER isolates archived at the Animal and Plant Health Agency (APHA) to investigate whether this related to emergence of a particular serotype.

Methods
Protein extracts from 98 ER isolates ER were submitted to Iowa State University for serotyping. These isolates were from pig submissions to APHA diagnosed as PE outbreaks.
Results
From the 98 submitted ER isolates, 46 were serotyped. The protein extracts of the untyped isolates were of insufficient quantity or quality. Of those serotyped successfully, 27 were ER serotype 2, 12 were serotype 1a, three were serotype 1b and four were serotype 11. Serotype 1b was identified for the first time in 2009 (one isolate) and again in 2012 (two isolates). In 2011- 2012, four of the eight isolates identified were serotype 2, with two each of serotypes 1a and 1b. No serotype 11 isolates were identified after 2008.

Discussion
This work allowed us to assess the serotypes of disease-associated ER isolates from pigs in England and Wales for the first time in at least 25 years, and emphasises how useful it is to retain pathogen isolates from disease outbreaks. Although a substantial number of isolates still require typing, the predominant serotype identified overall was serotype 2, with serotype 1 (a and b) accounting for the majority of other serotypes identified. Serotype 2 remained the most common identified serotype in 2011-2012 while there was an apparent trend for more isolates of serotype 1a or 1b since 2004. It is not yet possible to say whether this represents a real change, and further serotyping is planned. Commercially available vaccines claim protection against serotypes 1 and 2. These results suggest that serotype change may not account for suspected vaccine failures in the field. No further reports of PE in supposedly vaccinated animals have been received. Cases of suspected lack of vaccine efficacy should be investigated to assess compliance with the vaccination programme and recommendations for vaccine storage and administration. Where suspected lack of vaccine efficacy is identified, this should be reported (https://www.vmd.defra.gov.uk/adversereactionreporting/) and serotyping of the ER isolate may be indicated.

P203

EFFICACY OF AN ALTERNATIVE TREATMENT SCHEME WITH TILMOVET® IN PIGS

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Introduction
Tilmicosin (TMS) is semi-synthetic broad-spectrum macrolide, currently approved for veterinary use in pigs, poultry and cattle. TMS maintains high concentrations in the lung tissue several days after the treatment has ended1. This is probably why practioners often use an alternative treatment scheme: 5 days medication-2 days off medication (pause interval)-5 days medication. This seems mainly be driven in their aim to reduce the consumption of antibiotics. The current study aimed to evaluate the efficacy of this treatment scheme. For this reason the concentrations of TMS were measured at several timepoints during the treatment scheme and if the concentrations of TMS during the 2 day pause interval are sufficient to prevent occurrence of clinical symptoms and lesions after inoculation with Actinobacillus pleuropneumoniae (App) serotype 2.

Materials and Methods
Nine groups of 4 SPF pigs (Danube White), equal number of each sex (20.5-25.0 kg), 10-12 weeks of age, were used. All pigs were infected with App serotype 2. Group I didn't receive any medication (control group). Five days before the challenge, group II-IX received orally 16 mg TMS per kg bodyweight as Tilmovet®, following the scheme: 5-day treatment, 2-day pause and 5-day treatment. At each time point one group of the treated animals was euthanized and necropsied to assess concentrations of TMS using HPLC method for determination in plasma and lungs of pigs. Efficacy of the medication was evaluated by differences in pathological (App gross lesions) and microbiological (App reisolation) parameters between Group I (control) and group IX at the last time point. The results of the examination were determined according to t-test of Student-Fisher.

Results
No statistically differences of TMS concentrations in lung and plasma were found on day 5 of both treatments and 24 h and 48 h after the two treatments. There was a statistically significant difference in TMS concentration in plasma and lungs on the 72nd hour after the second treatment.
A statistically significant difference was remarked in App gross lesions between group I (control) and group IX. App could not be reisolated in group IX.

Conclusions
During the double 5 days treatment scheme with a 2 days pause interval, the lung and plasma concentrations stayed the same for 12 days. Only 72 hours after the last treatment the concentrations of TMS started to decrease in plasma and lung. Pigs inoculated with App in the 2 days pause interval, did not develop any clinical signs or lesions. This alternative treatment scheme can considered as efficient in the treatment of respiratory disease.

P204

CLINICAL EFFECT OF AN ALTERNATIVE TREATMENT SCHEME OF TILMOVET® IN WATER TO DEMONSTRATE POST ANTIBIOTIC EFFECT.

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Introduction
Tilmovet® (tilmicosin) is a semisynthetic macrolide registered for respiratory disease in pigs associated with Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae (APP) and Pasteurella multocida registered in Denmark and other countries. Tilmicosin possesses unique pharmacokinetic properties such as accumulation in for example lung macrophages and maintainance of the same concentrations in the lung tissue 48 hours after the treatment has ended (Post-antibiotic effect). This prolonged post antibiotic effect was investigated in clinical practice by comparing a full 12 day treatment program of Tilmovet® 250 mg/ml solution at 16 mg/ kg bodyweight (BW) (= control group) to a 5 day treatment program of Tilmovet® 250 mg/ml solution at 16 mg/ kg BW, 2 days no treatment (2 days placebo) and 5 days additional treatment with Tilmovet® 250 mg/ml solution at 16 mg/ kg BW. Furthermore the potential antiviral and anti-inflammatory effects of tilmicosin are taken into consideration. The study was performed as randomized single blinded study with a positive control group.
Materials and methods
804 weaned pigs at 4 weeks of age, originating from a 1600 sow farm declared positive for influenza, APP serotype 2 and Mycoplasma hyopneumoniae, were included . The watersupply in one stable was divided in 2 separate strings and equipped with Dosatrons. 399 pigs were randomly assigned to the exposure group (5/2/5) in one side of the stable and 405 pigs were assigned to the control group in the other side. The herd manager medicated daily both groups in the water after relocation to the nursery (Day 1) and was blinded as to treatment. All pigs were housed in the same room, and received standard dry feed without medication, except Zinc oxide for 14 days from Day 1.60 pigs in each group were ear tagged and individually weighed at day 0 and day 12. Mean weight at start was 6.78 kg in the exposure group and 6.94 kg in the control group (p=0.34).
Results
Weight at day 12 was 8.93 kg in the exposure group and 9.22 kg in the control group (p=0.33). There was no statistical difference between both groups. 1 month after starting the trial, the data were assessed before exporting pigs.
Mortality in the control group and the exposure group was respectively 8 and 11 (P=0.64). Daily gain was 268 g/day in the control group and 277 g/day in the exposure group (P=0.23). Pigs receiving individual treatment in hospital pens were 43 in the control group and 33 in the exposure group (P=0.37).
Conclusions
Overall no significant differences in weight gain, treatments or mortality was found between both groups, confirming the postantibiotic effect of Tilmovet® after 5 days treatment at 16 mg/kg BW and demonstrating the clinical effect of a 5/2/5 treatment scheme.

P205

SURVEY OF THE US SWINE INDUSTRY ON MYCOPLASMA HYOPNEUMONIAE ACCLIMATION OF GILTS

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Introduction
Properly acclimatizing gilts to Mycoplasma hyopneumoniae (M.hyo) constitutes an important element of managing the Infection Chain™ and providing optimal PRDC control for the entire downstream herd. Improper acclimatization may result in replacement gilts that are either naïve or M.hyo bacteria shedders entering an M.hyo positive sow herd. This could result in sub-populations, leading to M.hyo colonization of the suckling pigs, which has a negative impact on pig performance. A comprehensive survey was designed to learn more about conditions, procedures, methods, goals and expectations of the M.hyo gilt acclimation protocols in the U.S. swine industry. We developed a list of opportunities and a gap analysis to assist customers to develop more effective acclimation protocols.
Materials and Methods
The survey covered different factors related to farm demographics and structure/design of health protocols: source status, age at exposure, gilt flow, exposure procedure, exposure validation, timing, final status verification, vaccination, other intervention(s) and sow herd M. hyo stability. Criteria to be included in the survey were that the herd/flow must have M.hyo positive sow herd and that they were focused on M.hyo control.
Results
Respondents represented 963,750 sows (n=30 flows) in diverse U.S. swine production geographies with a range of sow herd/flow size of 1,150-215,000 (Mean = 32,125). The main risk factors for gilt entry were identified: a) 55% of respondents receive naïve gilts that enter positive herds, b) 41% of respondents (500,000 sows) have >50% replacement rate, c) 60 % do not attempt to acclimate to the strain specific to their herd, d) over 53% do no acclimate gilts < 20 weeks of age and e) only 20% validate M.hyo (serology, clinical signs) exposure and recovery (end of shedding). All of "e" had doubts about interpretation of their validation steps. We identified information associated to exposure: a) M.hyo vaccination is used in 93% of gilt flows, however, timing is not based on the infection dynamics, b) <10% use lung homogenate to acclimate, c) young pigs are not used frequently to acclimate, d) 34% use cull sows to acclimate with no consistent protocol and e) 72% use continuous flow acclimation. with no consistent protocol. In general, the majority of the respondents were not confident in their methods, nor a clear definition of sow herd stability (sampling protocol, clinical signs).
Conclusions
Since risk factors, such as receiving negative or unknown shedding status gilts and high replacement rates were identified in this survey, proper and improved acclimation protocols are necessary to achieve consistent M.hyo sow herd stability. However, there were many uncomfortable with their exposure and validation of recovery methods. Some opportunities identified were: 1) early and quick exposure protocols (age, method, timing) for the group are needed and 2) best practices for validation via diagnostics (animal, sample type, quantity, test, frequency) of gilt exposure, shedding, recovery (cool-down) and Gilt/P1 stability need to be developed.

P206

ASSESSING MYCOPLASMA HYOPNEUMONIAE PREVALENCE AT WEANING AS AN INDICATOR OF SOW HERD STABILITY

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Introduction
Sow herd stability constitutes an important element of managing the Infection Chain™ and providing optimal Mycoplasma hyopnuemoniae (M.hyo) control for the entire downstream herd (whole herd/systematic approach). The slow spread of the agent and the ability of long term shedding can result in sub-populations within the sow herds, perpetuating the infection process and potentially leading to sow herd instability. The final consequence of these is colonization of the suckling pigs, which has been shown to have a negative impact on pig performance. This continuous infection cycle has health and economic impacts; therefore, understanding sow herd stability is essential to the systematic control approach for M.hyo. There is not a clear definition of Mhyo sow herd stability; therefore, a large scale study was designed to assess: a) prevalence at weaning as an indicator of sow herd stability, b) frequency of sow herd instability, and c) variability between/ within farms.
Materials and Methods
In order to asses M.hyo sow herd stability and understand the variability between/within farm(s) for M.hyo stability, 21 sow herds (several systems and flows throughout the United States) were selected based on downstream clinical evidence of PRDC and suspicion of M.hyo instability in the sow herd. Thirty due-to-wean, poor doing pigs, were selected. Either bronchial or oropharyngeal samples (flocked swabs, Copan, USA, Amies transport media) were collected from each pig. Three sampling points were performed in every sow herd, one every 3-4 weeks (90 total samples per farm). Individual samples (n=1,890) were tested for M. hyo by qPCR (HMC, Ames, IA).
Results
In this study, 52% (n=11/21) of the farms tested M.hyo PCR positive, 64% (n= 7/11) of those had one of three positive sampling points, 9% (n= 1/11) showed two of three positive sampling points and 27% (n= 3/11) showed all positive sampling points. Also, 38% (n=8/21) of the tested farms showed a prevalence >10 % in at least one of the samplings. The total prevalence, including all farms, was 7.0 %.
Conclusions
Variability in M.hyo prevalence at weaning within a farm over time and variability between farms was documented in this study. Multiple sampling points over time are necessary to asses this. Over half of the sow herds evaluated in this study showed evidence of M.hyo instability indicating that those herds' downstream M.hyo control may not be as desired due to the transmission to piglets during the lactation phase. Further studies clarifying definition/classification criteria and ongoing risk factors of the gilts role in sow herd instability are ongoing. Sow herd stability needs to be a target of investigation if downstream PRDC and M.hyo are diagnosed.

P207

INFLUENCE OF PHYSICOCHEMICAL CONDITIONS ON THE IN VITRO GROWTH OF BRACHYSPIRA HYODYSENTERIAE

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Introduction
Swine dysentery, caused by Brachyspira hyodysenteriae (B. hyodysenteriae), is a multifactorial disease, with different factors influencing colonization, transmission and disease. Diet is one of the factors with a major influence on the clinical outcome of swine dysentery. The influence of diet might be related to an effect on the intestinal microbiota or to an effect on physicochemical conditions in the gut. The aim of the present study was to evaluate the influence of physicochemical conditions (pH and viscosity) on the in vitro growth of B. hyodysenteriae.
Materials and Methods
Growth of B. hyodysenteriae was monitored in anaerobic broth with different pH. Immediately before inoculation, blank samples were obtained as blank-value for optical density and pH measurement. After inoculation with B. hyodysenteriae ATCC strain B78, the different flasks were incubated anaerobically at 38°C. At fixed time points, the optical density was measured as an indicator of bacterial growth.
During growth of B. hyodysenteriae, a decline of pH was observed. Therefore, in a second experiment, growth of B. hyodysenteriae was followed at a constant pH. A batch fermentor model with computer-regulated pH control (New Brunswick Bioflo 110 system) was optimized for growth of B hyodysenteriae. Growth of B. hyodysenteriae ATCC strain B78 in the fermentation vessels was compared at a constant pH level of 6 or 7 (feasible pH in the large intestine).
In a last experiment, different concentrations of carboxymethylcellulose (CMC) were used to adjust the viscosity of anaerobic broth. After measuring viscosity with a viscometer (Brookfield, DVII+Pro), the broth was inoculated and growth was followed during several days, by determination of the optical density.
Results
The best growth of B. hyodysenteriae strain B78 in broth was observed at neutral or slightly alkaline pH. Slower growth and lower final bacterial concentrations were observed at pH 6 and 9. At pH 5 and pH 10, optical density did not change during incubation. The fermentor model confirmed that at a constant pH of 6, B. hyodysenteriae strain B78 grew slower and reached lower maximal yields compared to pH 7.
Viscosity of the broth supplemented with CMC varied between 1.36 and 166.5 cP. These variations in viscosity had no clear influence on growth of B. hyodysenteriae strain B78.
Conclusion
In vitro results demonstrate that optimal growth of B. hyodysenteriae strain B78 occurs in a pH neutral environment. Alkaline and acidic conditions reduce or even prevent growth. This suggests that diets, lowering intestinal pH, might have a protective effect on the development of swine dysentery.

P208

ANALYSIS OF PROTEIN FRACTIONS IN SERA FROM SEVERELY DISEASED PIGS AFTER INTRATRACHEAL INFECTION WITH HAEMOPHILUS PARASUIS (HPS) SEROVAR 5

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Introduction:
A former study detected protein fraction alterations in nursery pigs within 7 days following infection with HPS serovar 5 (10^8 CFU). The aim of this study was to analyze protein fractions by serum gel electrophoresis from piglets infected with 5 times the infection dose used in the former study.

Materials and Method:
Eighteen 3-week-old piglets were randomly allocated to a non-infected (HPS-NEG) or infected group (HPS-POS) with each group containing 9 piglets. After 10 days of acclimation, piglets were either intratracheally infected with 5x10^8 CFU HPS or they received sterile saline. Blood samples collected on the day prior to infection (d-1), day 3 p.i. (d3) and at study end/day of euthanasia (d14) were analyzed by photometric methods. Total protein, albumin, α1-globulin, α2-globulin, β-globulin, and γ-globulin were differentiated by serum gel electrophoresis in the lab of the Clinic for Swine and Small Ruminants, University of Veterinary Medicine Hanover.

Results:
A subgroup, HPS-DEATH, was formed since four animals from the HPS-POS group died prior to study termination (≤d4 p.i.). Mean total protein of the HPS-DEATH, HPS-POS, and HPS-NEG groups on d3 were 49.6±4.1 g/L, 51.9±3.2 g/L, and 51.7±3.5 g/L, respectively. Albumin concentration was significantly lower ($p<0.05$) in the HPS-DEATH group (21.0±0.7 g/L) than those of the HPS-POS group (25.2±1.57 g/L) and HPS-NEG group (27.5±2.37 g/L) on d3. α1-globulin and ß-globulin concentrations were lower in the HPS-DEATH group than the HPS-NEG group on d3. α2-globulin concentration was significantly higher ($p<0.01$) in the HPS-DEATH group (17.1±2.4 g/L) compared to the HPS-NEG group (11.4±1.4 g/L). α2-globulin concentration was higher in the HPS-DEATH group than the HPS-POS group (13.7±2.7 g/L; $p=0.08$). Significant differences in protein fractions were not observed between HPS-POS and HPS-NEG groups on d-1 and d14.

Conclusion:
Significant alterations in protein fractions were observed in sera from piglets infected intratracheally with a high dose of HPS serovar 5 (5x10^8 CFU). Changes in protein fractions were mainly seen in sera from the HPS-DEATH group on d3 p.i.. Based on the study previously mentioned, differences in protein fractions are present even up to d7p.i.. Peracute deaths in piglets after HPS infection are widely documented in literature. A mortality rate of 100% was seen in piglets from the HPS-DEATH group correlating with the significant changes in protein fractions. This study demonstrates that changes in protein fractions reflect immune responses and also the severity of disease.

P209

ANALYSIS OF PROTEIN FRACTIONS IN SERA FROM WEANED PIGLETS INFECTED INTRATRACHEALLY WITH HAEMOPHILUS PARASUIS (HPS) SEROVAR 5

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Introduction:
Infections with HPS affect piglets worldwide leading to substantial economic losses in pig production. As clinical signs already allow the suspicion of HPS involvement in diseased piglets, we hypothesized that blood protein fractions might be useful diagnostic markers to detect inflammation, such as caused by HPS.

Materials and Method:
Serum samples from 12 non-infected (HPS-NEG) and 12 piglets experimentally infected with HPS (HPS-POS) were analyzed during the course of infection. Piglets were weaned at 3 weeks of age and randomly allocated to the different treatment groups. After 10 days of acclimation, piglets were either intratracheally infected with HPS serovar 5 (10^8 CFU) or received physiological saline instead. Blood samples were collected seven days prior to HPS infection (d-7), on the day of infection (d0) and on the day of euthanasia (d7). Total protein was analyzed by photometric methods. The protein fractions were differentiated by serum gel electrophoresis in the lab of the Clinic for Swine and Small Ruminants, University of Veterinary Medicine Hanover.

Results:
HPS infection resulted in significantly lower albumin concentrations in HPS-POS piglets at d7 (18.6 g/L vs. 20.2 g/L ($p<0.01$)). Furthermore, α1-globulin (3.3% vs. 2.7% ($p<0.01$); 1.5 g/L vs. 1.2 g/L ($p<0.01$)) and γ-globulin (11.3% vs. 9.9% ($p<0.05$)) concentrations of HPS-POS piglets were higher than values of HPS-NEG at the end of the experiment.

Conclusion:
Obtained results are consistent with the conducted HPS infection and piglets´ subsequent immune response: the increase in HPS-POS piglets' γ-globulin concentrations reflected their active antibody production against the inserted pathogen. Based on the significant increase of α1-globulins (positive acute phase proteins) and significantly lower albumin concentrations (negative acute phase protein) in HPS-POS piglets compared to HPS-NEGs, inflammatory processes due to successful HPS infection can be assumed.
In conclusion, within 7 days after intratracheal HPS serovar 5 infection, significant changes in protein fractions (albumin (g/L), α1-globulins (% and g/L) and γ–globulins (%)) could be demonstrated in comparison to sera of non-infected animals by means of serum gel electrophoresis.

P210

TREPONEMA SPP. IN EAR NECROSIS OF COMMON PIGLETS

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Introduction
Ear necrosis in pigs is a noticeably and frequently found disease in pig farms. Historically, Treponema spp. have been described as saprophytes, which benefit from the special conditions in skin lesions and the poor immune response in necrotic material. Recent studies have shown that treponemes may have an important role in ulcers and ear necrosis. Especially one treponemal species is frequently found in necrotic skin lesions, i.e. T. pedis. This species is involved in the development and/or chronicity of bovine digital dermatitis. Previous data supports the concept of Treponema sp. having an active role in the pathogenesis of ear necrosis in pigs. In particular, a recent study provided evidence for the presence of T. pedis in porcine ear necroses. These findings led us to address this issue.
Materials and methods
With the owners´ consent, we have collected necrotic material of ear lesions (19/23) and shoulder ulcers (4/23) from a total of 23 pigs from 3 different farms, which had a long-standing history of ear necrosis in piglets and/or ulcers in sows. Following DNA extraction by using a DNeasy Blood and Tissue Kit, PCR compatibility of DNA isolates was confirmed by routine β-actin PCR. Presence of treponemal DNA was assessed by conventional PCR using primer pairs 5'/3' TPed for specific amplification of Treponema pedis ssp. nov. DNA and universal Treponema primers 5'/3' TT. Confirmedly Treponema-positive bovine DNA, equine sarcoid DNA and sterile water were included in all reactions as positive, negative, and no template controls. The identity of TT-PCR amplification products was determined by sequencing and subsequent BLAST analysis.
Results
T. pedis PCR scored positive for 7/23 DNA isolates (one shoulder ulcer (SU), six ear necrosis (EN) samples). In three distinct samples, TT PCR revealed amplicon sequences similar to other Treponema sp., i.e. T. berlinense (2 EN) and uncultured Treponema clone EMP_K5 (1 SU). In sum, treponemes were detected in 10/23 samples. Interestingly, TT PCR also revealed the presence of Fusobacterium necrophorum in 5 (4 EN, 1 SU), and a sequence similar to F. periodonticum in one swab (1 EN). F. necrophorum is also known to be involved in footrot, especially in ovine species. Sequences similar to different unclassified gut bacteria were detected from 9/23 swab DNA isolates, thus probably originating from skin contaminations by faeces.
Conclusion
In conclusion, treponemes and other bacteria, which are known to cause necrosis, e.g. F. necrophorum, should be taken into account when evaluating chronicity of porcine ear necroses in pigs.

P211

EFFICACY OF TILDIPIROSIN TO CONTROL H.PARASUIS INFECTION IN NURSERY

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Introduction
The efficacy of Zuprevo™ (tildipirosin) 40 mg/ml in the control of H.parasuis infection has been demonstrated under several conditions. Zuprevo™'s bactericidal effect is an essential feature in the face of bacterial infections combined with immunosupressive viruses such as PRRS. The aim of this trial was to prove the efficacy and profitability of Zuprevo™ 40 mg/ml in the control of H.parasuis infection.
Materials and Methods
The trial was carried out in a continuous flow nursery with a total capacity of 8900 piglets, with 1500 piglets entering the facility on a weekly basis. The piglets came from a PRRS positive but stable sow farm, where S.suis and mainly H.parasuis infections were routinely detected in the nursery. Because mortality could not be reduced from 2-3%, it was decided to initiate a specific treatment with Zuprevo™ to improve productive parameters. The nursery was organized in batches of 330 piglets. The animals were weighed in groups of 30 when entering the nursery and 28 days later. The trial was divided in two sub-trials. Subtrial 1: half of the animals per batch were treated with 0,5ml of Zuprevo™ (1485 piglets) and the other half remained as untreated controls (1487 piglets). Sub-trial 2: all piglets per batch were treated with 0.5 ml of Zuprevo™ (659 piglets) and were compared with untreated batches (1312 piglets).
The parameters analyzed and compared between groups were: Weight Increase during the 28 days of the trial, ADWG and Mortality rate. Tests used were ANOVA and U de Mann Whitney.
Results
Subtrial 1: mortality was significantly lower in the treated group compared to control (Zuprevo™ 1,55% vs Control 2,83%; $p<0,05$). No differences were found in ADWG (Zuprevo™ 272g/d vs Control 266g/d) or weight increase (Zuprevo™ 7,59kg vs Control 7,48kg)
Subtrial 2: mortality was not significantly different between groups (Zuprevo™ 1,82% vs Conrol 2,05%). Nevertheless, ADWG was higher in Zuprevo group (328 g/sd vs 277g/d; $p<0,005$). In addition, weight increase during the study period was also 1,33 kg higher in the Zuprevo™ group (9,2kg vs 7,87 kg; $p<0,005$).
Conclusion
The improved productivity parameters obtained when all piglets in a batch were treated with Zuprevo 40mg/ml® suggest that it might be insufficient to treat only part of the animals in a batch in high infectious pressure scenarios. In addition, the return on investment (ROI) was higher when treating the whole batch with tildipirosin than when only applying a partial treatment (ROI 2,3 Subtrial 1 vs ROI 5,1 Subtrial 2).

P212

SIX MONTHS DURATION OF IMMUNITY OF HYOGEN® VACCINE IN FATTENING PIGS AGAINST MYCOPLASMA HYOPNEUMONIAE

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Introduction
Mycoplasma (M.) hyopneumoniae is a major infectious agent in swine, worldwide responsible for persistent dry cough, retarded growth rate and reduced performances. Prevention is possible by vaccination. The objective of the trial was to test the six month duration of immunity of Hyogen® vaccine.
Materials and methods
Forty, three-week-old piglets, seronegative to M. hyopneumoniae were randomly assigned into two groups of twenty animals each. In the first group, piglets were vaccinated intramuscularly with Hyogen® at 3 weeks of age, while in the second one the animals were injected with placebo. The challenge was carried out 181 days after the vaccination with a 2x108 CCU/animal dose of a virulent M. hyopneumoniae strain and repeated on the following day. After a 28-day observation period, the animals were euthanized. The following parameters were investigated: lung lesions (scored using the method of the European pharmacopoeia: 2448), humoral and cellular immune (CMI) responses, mortality and clinical signs.
The following laboratory methods were applied: standard histology, ELISA, Porcine IFNβ ELISpot kit and Taqman qPCR.
Results
Mortality and clinical signs: one animal died 25 days post-challenge in the control group. The observed typical severe lesions, confirmed by PCR, indicated M. hyopneumoniae as the causative agent.
In general, the clinical signs observed during the post-challenge period were very rare and light. Therefore these data were not statistically analysed.
Serology: All the study animals were seronegative before vaccination; 80% of the vaccinated became seropositive 23 days post-vaccination and 70% of them remained seropositive till the 181st day after vaccination. The control animals remained sero-negative during the whole study.
CMI: the vaccinated group exhibited a significantly higher frequency of M. hyopneumoniae-specific IFNβ secreting cells ($p=0.0057$).
Lung lesion score: the average lung score of the vaccinated group was significantly lower (2.80), whereas in the placebo group, it was 40.10 (Wilcoxon ranksum test, $p=0.0001$). M. hyopneumoniae was confirmed as causative agent of the lung lesions by histology and qPCR.
Conclusion
In this study, six months after vaccination with Hyogen® vaccine, we observed a significantly increased cell-mediated immunity as well as a significant protection against lung lesions caused by Mycoplasma hyopneumoniae. Vaccination take was confirmed by serology on the vaccinated animals and the serological positivity was still high, even six month after vaccination.

Hyogen® is a trade mark of Ceva Santé Animale

P213

COMPARISON OF EIGHT LUNG LESION SCORING SYSTEMS USED FOR THE ASSESSMENT OF MYCOPLASMA HYOPNEUMONIAE INDUCED PNEUMONIA

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Introduction
Lung lesions observed in pigs infected with Mycoplasma hyopneumoniae (Mhyo) consist of cranioventral pulmonary consolidation (CVPC) areas. Several lung scoring methods are currently in place for the evaluation of CVPC extension. However, information on the association between such scoring methods is scarce in the literature. The aim of this study was to compare lung lesion scoring systems used for CVPC evaluation by means of a Mhyo experimental infection.
Material and Methods
Ninety-eight 6 week-old Mhyo seronegative pigs were endotracheally inoculated with Mhyo fresh culture. Animals were euthanized at either 21 or 28 DPI and CVPC assessment was carried out using 8 different scoring systems published in the literature (Goodwin et al. 1969; Goodwin and Whittlestone 1973; Hannan et al. 1982; Madec and Kobisch et al. 1982; Morrison et al. 1985; Straw et al. 1986; Christensen et al. 1999; Sibila et al. 2014). For the analysis of the different scoring methods, only pigs affected by CVPC were used (n=76), considering the individual pig as the experimental unit. In order to evaluate the association between different scoring methods, a linear regression analysis was performed.
Results
A significant correlation ($p<0.05$) between all lung lesion scoring systems was observed and their coefficients of determination (r2) were very high (from r2=0.824 to r2=0.989) for all comparisons, except the image analysis (r2=0.7). Additionally, the correlation between each scoring method with average daily weight gain (ADWG) was evaluated. A significant association ($p<0.05$) between ADWG and all lung scoring methods was observed; however, the r2 was very low (between 0.04-0.09).
Conclusion
The present study provides useful information to find a universal method to assess the extent of CVPC and to compare (even retrospectively) lesions evaluated using different scoring systems.

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P214

A SURVEY OF BRACHYSPIRA INFECTION IN FRENCH FARMS CONCERNED WITH DIARRHEA IN FATTENING PIGS : FIRST RESULTS.

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Introduction :
The anaerobic Gram-negative spirochaete Brachyspira is known to infect the large intestine of many species, including pigs. Two swine diseases are due to these bacteria: swine dysentery (B. hyodysenteriae) and intestinal spirochaetosis (B. pilosicoli). The first causes severe mucohaemorhagic diarrhea and mortality while the second typically causes chronic diarrhea, poor growth and heterogeneity. Recently, additional Brachyspira species have been suspected to have a pathogenic potential (B. intermedia, B. murdochii) and a new pathogenic species has been identified: B. hampsonii.
The object of the study was to evaluate prevalence of Brachyspira infection in French conventional farms concerned with diarrhea in fatteners after 8 weeks of age, and describe the farms in terms of housing, biosecurity status, mortality and growth performance.

Material and Method:
Veterinarians operating in Reseau Cristal Network were asked to identify cases, sample 9 diarrheic pigs per farm using rectal swabs, and complete information on the farm. Swabs were sent to Resalab, pooled by three, and tested with a Real Time PCR kit identifying B. intermedia, B. pilosicoli, and B. hyodysenteriae (Brachyspira@CeeramTools for pilosicoli and intermedia, LSI for hyodysenteriae).

Results:
From June to November 2014, 10 veterinarians identified and sampled 6 to 9 pigs affected by diarrhea in 18 case farms. 6 farms showed negative results for B. pilosicoli, hyodysenteriae, and intermedia. 5 farms were positive for B. pilosicoli: one was also positive for hyodysenteriae and intermedia, two showed mixed infection with B. intermedia. One farm was positive for B. hyodysenteriae and B. intermedia, and 4 farms showed positive (2/4) or suspect (2/4) results for B. intermedia only.

Conclusion:
The preliminary results of this survey show that although Brachyspira infection are said to be rare in France, they may be frequently implicated in cases of diarrhea in fatteners. B. intermedia was the only Brachyspira identified in 4 cases of diarrhea, further investigation is needed in these cases to see if there could be a confusion with B. hampsonii, as cross reactions in PCR may occur, or if other agents or farm factors may explain the diarrhea.

P215

ASSESSMENT OF THE EFFICACY OF MYCOPLASMA HYOPNEUMONIAE CONTROL STRATEGIES UNDER SPANISH FIELD CONDITIONS.

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Introduction
Mycoplasma hyopneumoniae (M. hyo) causes enzootic pneumonia (EP), a chronic respiratory disease in pigs responsible for major economic losses worldwide. Different strategies for controlling EP are available, with vaccination being widely applied worldwide. With timing of vaccination against M. hyo being highly relevant, early vaccination has the advantage that immunity can be induced before pigs become infected, and that less pathogens are present that can interfere with the immune response (Maes et al., 2008).
This study aims to evaluate the efficacy of different M. hyo control strategies in Spain by comparing them to the vaccination of pigs with Stellamune® Once (Elanco AH).
Materials and Methods
From 2011 to 2014, a total of 83,189 lungs from 279 pig farms were assessed in 16 different abattoirs distributed all over Spain. A blind evaluation of EP-like lesions following a 0-5 scoring method was performed. Subsequently, lung lesion scores were matched with vaccination strategies as reported by the attending veterinarians: (1) Stellamune® Uno [Elanco AH], n=40,043; (2) Suvaxyn Mh One [Zoetis], n=15,174; (3) Ingelvac MycoFLEX [Boehringer Ingelheim Ltd], n=16,783; (4) Porcilis M Hyo [Merck], n=11,089.
EP-like lesion distribution of pigs vaccinated with Stellamune® Uno was compared with the other control strategies using the Tukey test (JMP v.11) at a significance level of 0.05. In addition, maximum lung lesions (EP-like score >4) by control strategy were compared between batches of pigs.
Results
The percentage of lungs affected by EP-like lesions in the Stellamune® Uno vaccinated group (44.32%) was significantly lower ($p<0.05$) than pigs vaccinated with Ingelvac Mycoflex (55.63%) and Suvaxyn MhOne (53.62%) , no differences were observed between Stellamune® Uno and Porcilis M Hyo (26.37%).When the percentage of maximum lung lesions was studied, pigs vaccinated with Stellamune® Uno (2.82%) presented a significantly reduction in the percentage of pigs affected compared to pigs vaccinated with Porcilis M Hyo (16,18%), Ingelvac Mycoflex (5,77%) and Suvaxyn Mh One (4,36%) respectively.
Conclusion
Vaccination of pigs with Stellamune® Uno was efficacious in controlling lung lesions caused by M. hyo. When compared to other control strategies, fewer pigs were affected by EP-like lesions when vaccinated with Stellamune® Uno and lesions, when present, were less severe.

P216

DESCRIPTION OF MYCOPLASMA HYOPNEUMONIAE MLVA–TYPES IN SUCCESSIVE BATCHES OF SLAUGHTER PIGS FROM TEN PIG FARMS

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Introduction
It has not been clearly shown whether Mycoplasma hyopneumoniae (Mhyo) MLVA-types are persisting within the populations of a herd. Since Mhyo has a small genome and cannot rely on stress genes, genotypical variation might be the answer to survive in a hostile environment. The aim of this study was to describe the circulation of Mhyo MLVA-types in three successive batches of ten pig farms.
Materials and methods
Ten single-site farrow-to-finish pig herds (>100 sows) and practicing vaccination of piglets against Mhyo were randomly selected. Within each herd, three batches of slaughter (pigs 1-2 months in between the batches) were investigated. From each batch, BAL fluid was collected from 20 pigs. BAL fluid was tested by nPCR (Stärk et al., 1998) and positive samples were submitted to MLVA analysis (Vranckx et al., 2011) to obtain the peak length of the amplicated variable number of tandem repeats (VNTR) and thus the number of different MLVA-types per batch and per farm. An MLVA-type was defined as an MLVA-variant when at least one locus mutation was different from another variant.
Results
In total, 135 different Mhyo MLVA-types were found with on average 14 different MLVA-types and a minimum and a maximum of respectively 6 (herd B) and 23 MLVA-types (herd F). MLVA-type 2, 42, 45, 59, 61, 77, 78 and 117 were found in each of the three sampling periods in farm C, F, F, E, E, H, H and A, respectively. All farms showed MLVA-types which were found in at least 2 out of 3 sampling periods, except for farm B. Most MLVA-types were found in 2 consecutive sampling points. Five MLVA-types were found in the first and the third sampling point. Herd E had the highest number of MLVA-types (7) found in 2 consecutive sampling periods. Some animals were infected with at least 2 or 3 Mhyo MLVA-types. In total, 16, 15, 4, 36, 11, 13, 6 and 1 animals carrying two MLVA-types were found in farm A, C, D, E, F, G, H, and I respectively. In two pigs of farm F, triple MLVA-types were obtained. In farm A, C, E, and G, one sample carrying triple MLVA-types was detected.
Conclusion
This study documented, using a MLVA analysis, that a high inter and intra farm variability in Mhyo exists. The same MLVA-types were circulating in all three batches in 50% of the farms and in at least 2 of the 3 batches within a herd in almost all the farms. The study also showed that pigs can be infected simultaneously with 2 or 3 Mhyo MLVA-types. Since the MLVA technique can only detect a maximum of three MLVA-types in one sample, it cannot be excluded that some animals were infected with >3 MLVA-types at the same time.

P217

IMPROVEMENT OF THE APPLICABILITY OF A COMMERCIAL SWINE ERYSIPELAS ELISA TEST FOR VACCINATION COMPLIANCE PURPOSE

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Introduction
The utility of serologic tests for evaluation of Erysipelas infection and protection conferred by vaccines has been a controversial issue. Furthermore, the variability and validity of current commercial ELISA tests makes difficult its interpretation and limits its applicability in the field. However, they might be of interest to detect the eventual serological response to vaccination in order to determine the vaccination compliance. Indeed, vets usually check sera from vaccinated animals in order to test vaccination compliance or immunological response. Therefore, the aim of this trial was to assess the serological response to PARVORUVAX, a PPV and Erysipelas combo vaccine, with an Erysipelothrix rhusiopathiae (E.r.) ELISA test and to evaluate its reliability according to the interpretation criteria.
Material and methods
Twenty nine gilts and sows of a farrow-to-finish farm were selected and classified according to its age and vaccination status in three groups: 10 randomly selected unvaccinated gilts (NV group) from the gilt pool, 10 pregnant gilts that had been vaccinated twice with PARVORUVAX before mating (BV), and 9 pregnant multiparous (4th to 6th parity) sows that have received several vaccinations with the same vaccine (MV). Blood samples were taken and tested for E.r. antibodies by an indirect ELISA (Ingezim Mal Rojo, Ingenasa). The optical density readings were statistically analysed by logistic regression (LR) to evaluate the sensitivity (Se) and specificity (Sp) of the test according to two criteria: known vaccination status or classification according to the test interpretation guidelines. ROC curves were designed for each model.
Results and discussion
The Se and Sp for detection of seroconversion after vaccination following the guidelines of the test were 52.6% and 100% resp. whereas they were 100% and 100% when the test recommended threshold limit (0.294) was corrected to 0.100 according to our LR model. As a consequence, the area under the curve (AUC) changed from 0.763 to 1.0. Furthermore, our data indicate the variance increased according to the number of vaccinations received: nil, 2 or >2, indicating the need to take parity into consideration for a correct interpretation of test results.
Conclusion
Erysipelas serological tests have been developped to evaluate infection. According to our results, it is advisable to reconsider the threshold of positivity recommended for this test in order to maximize its sensitivity and specificity for compliance purposes.

P218

PATHOLOGICAL AND BACTERIOLOGICAL CHARACTERISATION OF NEONATAL PORCINE DIARRHOEA (NPD) WITH UNCERTAIN AETIOLOGY

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Introduction
Problems with NPD of uncertain aetiology are reported from several countries. This study aimed to investigate pathology and bacteriology in pigs from affected herds.
Materials and Methods
Fifty cases and 19 controls were selected from 10 herds. The pigs were <1 week old and not treated with antibiotics. The pigs were blood sampled, necropsied and the small and large intestine (SI, LI) sampled for histopathology and culture of E. coli, C. perfringens and C. difficile; the latter only from the LI. Serum γ-globulin was determined by gel electrophoresis and results analysed by a generalized mixed model including diarrhoea, age and herd. E. coli (276 isolates) were examined by PCR for LT, STa, STb, EAST-1, F4, F5, F6, F18, F41 and AIDA. C. perfringens (152 isolates) were examined by PCR for major toxins and β2-toxin. Enteroadherent cocci demonstrated by microscopy had previously been identified as Enterococcus (E.) hirae by 16SrRNA- gene analysis, MALDI-TOF MS and PCR. Associations between diarrhoea, pathology and microbiology were evaluated by Fisher's exact test.
Results
There was no difference in γ-globulin concentration between cases and controls (mean ± SD, 21.8±7.7 vs 19.8±9.1 g/L, p=0.71). Several diarrhoeic pigs had a dilated SI (60%) and/or LI (58%) and 38% had a hyperaemic SI. Mesocolonic oedema was noted in both cases and controls (44% vs 53%). Microscopically, SI colonization by E. hirae was noted in 18 diarrhoeic pigs from six herds. Damaged epithelium (n=13) or villous atrophy (n=5) in the SI were only observed in case pigs. Epithelial damage was more common in pigs colonized by E. hirae compared to uncolonized pigs (56% vs 6%, p<0.01). Lesions in the LI were scarce. Growth of C. perfringens did not differ between cases and controls (76% vs 68%, p=0.55). All isolates were type A and all but one were β2-toxin positive. Growth of C. perfringens was similar in pigs with and without epithelial lesions in the SI (54% vs 78%, p=0.09). In pigs ≥ 2 days old, C. difficile was more common in controls (100% vs 50%, p<0.01). Growth of C. difficile was similar in pigs with and without mesocolonic oedema (56% vs 46%, p=0.47). Isolates of E. coli positive for STa:F5:F41 were found in two case pigs and STb:EAST-1:AIDA positive isolates in two cases and one control.
Conclusion
The results demonstrate occurrence of NPD unrelated to ETEC and C. perfringens type C. In six herds, diarrhoea and pathological findings were associated with SI colonization by E. hirae. In the remaining four herds pathological and bacteriological findings were inconclusive. No association between C. perfringens type A or C. difficile and diarrhoea could be demonstrated.

P219

HAEMOTROPIC MYCOPLASMAS – A REASON FOR SUDDEN DEATH IN SOWS?

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Introduction
Haemotropic mycoplasmas are bacteria affecting red blood cells and they are described as infectious agents for several species like dogs, cats, ruminants and pigs. In most cases the infection with haemotropic mycoplasmas is latent and does not lead to clinical signs of disease. In pigs the infection with Mycoplasma (former: Eperythrozoon) suis is an multifactorial disease and can be the cause of anaemia. Typical symptoms are seen in immunosuppressed animals with a hemolytic anaemia as the most important one beside cyanosis, pyrexia, weakness, weight loss and wasting. The disease affects mainly piglets after weaning and during the fattening period.

Materials and Methods
On a commercial farrowing to weaning farm with 300 sows cases of sudden death in sows without clinical symptoms appeared. This changed to a clinical picture with cyanosis, abortion, ataxia, blindness, conjunctivitis and hemorrhagic skin alterations. Nearly all diseased sows died within 24 to 72 hours after the onset of clinical symptoms. Over all within two months 38 sows died. Surviving sows showed a very long recovery period with weakness, low feed intake and recurring cyanosis. A necropsy and histological examination was performed of 13 sows, blood samples for PCR and serology as well as feed samples were taken.

Results
Within the macroscopic examination of the sows no specific alterations were found. The histological examination showed lymphohistiocytic inflammation in nearly all body tissues. The PCR and serological examinations were negative for all ordinary swine diseases like for example PCV-2, PRRSV, Influenza, M. suis, Brucella ssp., Leptospira ssp., Enterovirus, African and Classical Swine Fever as well as the results of a toxicological analysis of tissue and feed. Only the results of a metagenome analysis lead to the detection of a high number of mycoplasma DNA in the diseased sows in comparison to clinically healthy sows or pigs from other farms. Sequence analysis revealed that this mycoplasma species has most similarities with Mycoplasma turicensis which is closely related to M. haemofelis and M. haemocanis causing haemolytic anaemia in dogs and cats. So far an infection with haemotropic Mycoplasmas from dogs or cats was not described in pigs.

Conclusion
As the detected Mycoplasma sp. related to M. turicensis was the only detected pathogen within the diseased and perished sows it has to be discussed if it might be the reason for the clinical outbreak and the sudden deaths. But as it is a very newly described species of Mycoplasma and its pathogenesis is only poorly understood this cannot be proofed so far.

P220

THE USE OF ORAL FLUID FROM PIGS FOR THE DIAGNOSIS OF ATROPHIC RHINITIS

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Atrophic rhinitis (AR) is an important upper respiratory disease in swine, causing production losses and trade limitations. The presumptive clinic-pathological diagnosis of AR is confirmed by demonstration of its causative agents, Bordetella bronchiseptica (Bb) and toxigenic strains of Pasteurella multocida (PMT), by means of culture or PCR from nasal or tonsil swabs or biopsies. Given that pig oral fluid (OF) is being increasingly used as a diagnostic specimen in pigs, the objective of this research was to evaluate its potential use as a sample for the diagnosis and surveillance of AR, coupled with PCR testing.

The study was performed in a 1700-sow multi-site pig herd suffering from AR (previously diagnosed clinically). For comparison purposes, pen OF and individual nasal swabs (NS) were collected and tested in parallel. A total of 8 pens comprising 139 pigs 14-18 weeks of age (16- 18 pigs/pen) were selected. Firstly, presentation of one rope per pen and OF sampling was done in all pens. Then, NS from all pigs placed in those pens were taken, while coughing and sneezing were recorded. Presence of Bb and/or PMT in OF and NS were determined by RT qPCR.

At pen level, 5 out of 8 (63%), 4/8 (50%) and 2/8 (25%) OF were positive by RT qPCR to Bb, PMT or Bb+PMT, respectively. Similarly though relatively low percentages of positive OF samples were obtained with NS: 57/139 (41%), 20/139 (14%) and 7/139 (5%). The level of agreement of the results obtained from both sampling methods was measured at pen level. On basis that a pen is positive if it contains at least one positive result, using NS 8 pens resulted positive to Bb (100%), 7 to PMT (88%) and 7 to Bb+PMT (88%). The proportion of agreement between the two sampling techniques at pen level, measured as kappa, was 0.63 (95% C.I. 0.24 to 1) for Bb and 0.18 for PMT (95% C.I. -0.42 to 0.78).

These results showed a substantial agreement between the two sampling methods for Bb, and slight agreement for PMT, probably influenced by the prevalence assessed. In the event of Bb all those pens with a higher prevalence of 12% were detected, while for PMT the positive ropes were not consistently related to the positive proportion observed within pen by NS. Even if these results could be confirmed in other farms, ages and pen sizes, it seems that OF sampling from pigs could be in a level with classical NS sampling for PMT and especially for Bb detection at farm level, being a suitable approach for increasing cost effectiveness of AR surveillance in swine herds.

P221

IN VITRO ACTIVITY OF PHYTOGENICS AGAINST LAWSONIA INTRACELLULARIS

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Introduction
Lawsonia intracellularis (LI) is the causative pathogen of porcine proliferative enteropathy (commonly called ileitis). Various forms of this disease have been described. While the chronic and subclinical forms cause diarrhea and impaired growth performance in younger pigs, the acute form can cause sudden mortality in older pigs. Conventional microbiological methods like agar plate or micro dilution assays cannot be used for culture and research of LI.
Phytogenics are plant-derived materials used as feed additives in animal production with the aim of exerting a beneficial influence on health and growth.
The objective was to screen phytogenic samples for their activity against LI using two complementary assays.
Material and Method
LI were reconstituted from a live vaccine (Boehringer Ingelheim, Germany), filtered and washed to remove debris.
For the viability assay, LI were incubated with phytogenic samples, stained with fluorescent dyes and analyzed via flow cytometry. Bacteria were distinguished from debris in a FSC/SSC-plot. Live and dead bacteria were distinguished by their fluorescence and gated in a green/red fluorescence plot to determine viability rates.
For the host cell assay, LI were co-cultured with McCoy mouse fibroblast cells in 96 well plates in the presence of samples. After 5 d the cells were fixed and intracellular bacteria were stained with a primary anti-LI and a secondary fluorescein-conjugated antibody. The fluorescence of intracellular bacteria was measured with a microplate reader.
In both assays untreated and sample-treated LI were compared to assess the relative inhibition by phytogenics and antibiotics.
Results
In both assays, several phytogenic samples showed a dose-dependent antibacterial activity. Among them, silymarin and a chestnut extract performed best. Among the used antibiotics, tylosin tartrate was able to inhibit LI in the host cell assay, but showed no activity in the viability assay.
Conclusion
Phytogenics and antibiotics were able to impair LI viability and infectivity as determined by two in vitro assays. Since the viability assay shows direct bactericidal activity of sample and the host cell assay shows effects on intracellular proliferation, the combined results of these assays suggest possible modes of action of active substances .

P222

GUT PORCINE MICROBIOTA MODIFICATIONS UPON INFECTION WITH ATTENUATED OR VIRULENT SALMONELLA TYPHIMURIUM STRAINS

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Pork has been considered the source of about 70% of human cases of salmonellosis in Italy. On that account, there is an urgent need for new methods for Salmonella control in pigs. The intestinal microbiota is an efficient barrier against pathogen colonization and plays a role in the progression of salmonellosis. The aim of this study was to investigate the relation between Salmonella Typhimurium virulence and gut microbiota in infected pigs.

Materials and Methods
Bacterial cultures Virulent S. Typhimurium ATCC14028 and its isogenic attenuated mutant strain denominated Salmonella Typhimurium ΔznuABC were used throughout the study.

Experimental design
Thirty-one weaned piglets, 20-25 days old, were divided in a group of 9 (Group A) and two groups of 11 (Groups B and C). Group A was used as control, Groups B and C were orally infected with 2×109 CFU of S. Typhimurium znuABC (Group B) or 2×109 CFU of S. Typhimurium ATCC 14028 (Group C). Faecal samples were collected at different time points (T0, challenge; T2 two days and T4, 12 days after infection).
16SRNA Metagenomics Sequencing
Extracted DNA was used to amplify by PCR the hypervariable V3-V4 regions of the 16SRNA [3]. PCR amplicons underwent sequencing library prep (Illumina, US). All the libraries were normalized and pooled by 24 prior to sequencing on Illumina MiSeq using a 2x250 paired-end setting.
Bioinformatics and statistical analysis
The Lederhosen pipeline was used to create theo perational taxonomic unit (OTU) table for each sample. Kruskal-Wallis Test was used to test significant differences among the groups. Benjamini-Hochberg False Discovery Rate (FDR) method was applied to correct for multiple testing. Statistical significance was set at $P<0.05$.

Results
Piglets infected either with fully virulent or attenuated S. Typhimurium shed bacteria through faeces with different patterns in the two groups. Metagenomics analyses revealed no significant differences among the three groups at T0. At T2, 7 phyla,112 families and 404 genera resulted differentially present among the three groups. Notably, the hierarchical clustering of the most represented genera displayed a perfect clusterization of each single sample into its belonging study group.

Discussion and conclusions
At T2 and T4, pigs belonging to different groups clustered together; this difference was more evident at T4, when the excretion of Salmonella was significantly lower in group B than in group C. Salmonella infection caused a shift of porcine gut microbiota composition. The extent of the shift was correlated to the virulence, since the effect was more pronounced in S. Typhimurium ATCC14028 infected pigs than in S.Typhimurium ΔznuABC infected animals.

P223

BACTERIAL CO-INFECTION OF PIGS WITH PORCINE CIRCOVIRUS-2 IN LATVIA

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Introduction. Porcine circovirus-2 (PCV2) infection is considered to be worldwide distributed throughout domestic pig population, and it is the primary agent of Postweanig Multisystemic Wasting Syndrome (PMWS), which has become a high economic loss for the swine industry in many countries. PMWS manifested with wasting and immunity decreasing of piglets, therefore often is added bacterial infection. The aim of study was to investigate bacterial co-infection in lymph nodes of pigs with postweanig multisystemic wasting syndrome in Latvian farms.
Materials and Methods. Five farms were investigated with 5-15 weeks-old 42 piglet's carcases with pathological features like PMWS. All tested piglets were injected with pseudorabies virus, and porcine reproductive and respiratory syndrome virus vaccination. Pig necropsy was performed during 12 hours after pigs' death, and samples of lymph nodes lnn. inguinales superficiales, lnn. jejunales, and lnn. tracheobronchales were collected for the bacteriological investigation, histological test and immunohistochemistry to the PCV2 antigen detection (Ingenasa 36A9).
Results. PMWS was confirmed only in 38 % of cases with PCV2 antigen, and specific histological lesions in lymph nodes. Those included 11.9% of piglets, where bacteria in the lymph nodes was not found, and other 26.1% piglets with PCV2 antigen with detected bacterial infection in the lymph nodes. Should be noted, that one animal was detected from one to four species of bacteria in the lymph nodes. Most frequently bacteria in piglet's lymph nodes were Streptococcus spp. – 29.4%, which include St. agalactiae, St. pyogenes and hemolytic C group Streptococcus spp. Comparatively was often detected Staphylococcus spp. (23.5 %) and Salmonella spp. (23.5 %). Only in 11.8% of cases was found enteropathogenic Escherichia coli. In two pigs lymph nodes with PCV2 detected non pathogenic bacteria Citrobacter freundii (n=1) and Corynebacterium spp. (n=1).
Conclusion. Streptococcus spp., Staphylococcus spp., Salmonella spp. and Escherichia coli are common infection of pig population, as well as common in the environment; therefore piglets with immune deficiency from PMWS often get these bacteria. Considering that PMWS is immune deficiency disease, it may also explain the presence of non-pathogenic infection in the lymph nodes. However the samples were taken shortly after pigs' death, non pathogen bacteria in lymph nodes could be postmortem infections.

P224

COMPARISON OF THE EFFICACY OF HYOGEN® AND COGLAPIX® IN THE PREVENTION OF ENZOOTIC PNEUMONIA AND PLEUROPNEUMONIA WITH THE COMPETITOR VACCINES

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Introduction
Enzootic pneumonia(EP) due to M. hyopneumoniae(M.h.) and porcine pleuropneumonia (PP) due to A. pleuropneumoniae (A.p.) belong to the most prevalent respiratory infectious diseases in commercial pigs. Even if both M.h. and A.p. are primary pathogens, they are often associated together mainly in heavy pigs. M.h. is known to contribute to the more severe course of A.p. infection. Active protection against both pathogens has to be developed in order to prevent losses cause by those infections. The objective of the study was to compare Hyogen® (M.h. vaccine, Ceva) and Coglapix® (A.p. vaccine, Ceva) with competitor vaccines based on relevant indicators of efficacy which are: mortality, growth performance and lung lesion score in slaughter pigs.
Material and methods
Conventional two site farm was selected for the trial. In total 9600 pigs of 6 batches were vaccinated with Hyogen® with Imuvant TM at three weeks of age and with Coglapix® at 9 and 12 weeks of age (G1). Six batches consisting of 8400 pigs were vaccinated with competitor one-shot M.h. vaccine contaning Amphigen as the adjuvant and competitor A.p. toxoid+OMP vaccine in the same protocol (G2). PRRSv was present on the farm, sows are vaccinated 3 times a year. PCV2 was vaccinated at 3 weeks of age. Medication schemes based mostly on florphenicol were the same for all batches.
Lung lesions in 100 lungs per each batch were assessed and scores calculated according to the Ceva Lung Program methodology (Krejci 2013).
Results
The mortality from 60 days to slaughter was 3,37% in G2 while 7,4% in G2. The ADG in the same period was 0,64kg in G1 and 0,62kg in G2 ($p<0.05$). The FCR in G1 was 2,69kg/kg and 2,76kg/kg in G2. The average % of M.h.-like lesions was 23% in G1 and 51% in G2 ($p<0.05$). The average incidence of A.p.-like lesions and corresponding APPI index was 8% (APPI=0,26) in G1 and 21% (APPI=0,58 in G2). Higher M.h.-like lesions corresponded to the lower ADG (p=0,0007), while APPI was positively correlated to mortality (p=0,0003).
Conclusion
Pigs vaccinated with Hyogen® and Coglapix® exhibited more solid protection against the infections of M.h. and A.p., than competitor vaccines measured by the development of corresponding lung lesions. Higher protection resulted in better performance results in terms of mortality, feed efficiency and growth rate. Lung lesion scoring at the slaughterhouse confirmed its relevancy in assessing the efficacy of the preventive measures applied in pigs.

P225

PCV–2 AND M.HYO VACCINES COMBINATIONS EFFICACY COMPARISON IN RUSSIA

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Introduction: Vaccination against PCV-2 and M.hyo are widely used in Russia today. As all the vaccines are different by their composition, it is very important for veterinaries to choose vaccines combination with proper efficacy.
The main purpose of this trial is to compare the efficacy of different vaccines combinations against PCV-2 and M.hyo on a big farm with 14500 sows.
Materials and methods: 12 groups of piglets (1050-1080 piglets in each) were vaccinated on a farm with combination of PCV-2 vaccine and Hyogen®. 14 other groups were vaccinated with PCV-2 vaccine and commercial M.hyo vaccine (encrypted by us as Myco-GMbH). 16 groups were vaccinated with local PCV-2 vaccine and commercial M.hyo vaccine (encrypted by us as MycoSA).
One-shot vaccination scheme was implemented for all vaccines in accordance with SPC.
Vaccines efficacy comparison was made by performance parameters recording in all vaccinated groups during fattening period. Totally, there have been about 45 000 piglets in this trial.
Results: Hyogen® vaccine has demonstrated the highest safety and average daily gain result at fattening with any PCV-2 vaccine. The safety in the Hyogen groups was 98,32% in comparison to 98% in both other two vaccines.
Hyogen® vaccine has also demonstrated the highest efficacy in comparison with two other M.hyo vaccines. ADG in piglets vaccinated with Hyogen® was higher for 14 and 34 g in comparison with another M.hyo vaccines (937g, 923g and 903 g respectively).
Conclusions: One-shot vaccination with Hyogen® can be widely used for effective M.hyo prevention in big swine productions. This vaccine can be successfully combined with any PCV-2 vaccine.

P226

STUDY OF THE DIVERSITY OF THE ACTINOBACILLUS PLEUROPNEUMONIAE STRAINS ISOLATED FROM CLINICAL CASES IN FRANCE

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Introduction: In the context of limiting antimicrobial consumption in France, the preventive approach to disease control is emphasized. This is particularly the case in porcine pleuropneumonia, for which important amounts of antibiotics are used for prevention, treatment as well as for metaphylaxis. In this context, a better epidemiological knowledge of the strains would allow improving prevention of the disease that, in France, is due mainly to serotype 2 and serogroup 9-11.
Material and methods: Seventy-two strains of Actinobacillus pleuropneumoniae isolated from lesions of porcine pleuropneumonia in different areas in France from 2008 to 2014 were selected for a study of genetic linkage mapping. The study also included twelve reference strains of each serotype from 1 to 12. The serotyping of these strains was first implemented by using immune sera with the rapid plate agglutination method. The comparison of the strains was carried out with the ERIC-PCR method (Enterobacterial Repetitive Intergenic Consensus by Polymerase Chain Reaction), a technique which has already been described for the differentiation of other Actinobacillus species.
Results: Forty-seven of the 72 strains belonged to serotype 2 and the other twenty-five to serogroup 9-11. The genetic profile of the French strains enabled to identify four groups within serotype 2 and thirteen groups within serogroup 9-11. This technique also allowed differentiating the reference strains belonging to serogroups 1 to 12 from the others, except in the case of serotype 8, which showed a profile that was similar to two other strains of serogroup 9-11.
Conclusion: The selected strains showed an important genetic diversity within each serological group studied. This diversity should be taken into account for the choice of the appropriate means for controlling porcine pleuropneumonia.

P227

SALMONELLA ON BELGIAN BREEDING AND REARING FARMS

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The aim of the study was to gain insight in the prevalence of Salmonella on breeding and rearing farms and to define a practical sampling method for assigning qualifications to herds by bacteriological sampling. The effect of pooling as well as the usefulness of the biocheck in relation to Salmonella in pigs was evaluated.
40 breeding and 9 rearing farms were sampled monthly during 6 months. On each sampling round 2 pair of hand swabs and overshoes were taken in the farrowing-, insemination- and gestation units.. In the fattening unit 2 pairs of hand swabs were taken. In the rearing farms, the same number of samples was taken spread over the different compartments. Overshoes and swabs were pooled per 2 pairs. In the last sampling round, only overshoes were taken. Per unit or compartment 2 pairs of overshoes were pooled and 2 pairs were examined separately. In the fattening unit the association between serology and bacteriology was studied. Therefore the serological results of blood samples taken during the project for the Belgian Salmonella Action Plan were taken into consideration. On each herd a questionnaire about biosecurity (Biocheck) was completed and the standard use of antibiotics and acids was registered.
Among the 2177 analyses, Salmonella spp. were isolated 334 times in 36 herds. This isan overall proportion of positive samples of 15.34% (IC95%: 11.53-20.13) and a herd prevalence of 73.47% (IC95%: 59.33-84). The medium within herd prevalence was 29%. The 3 most frequently isolated serotypes were S. Derby, S. Typhimurium including monophasic variants of S. Typhimurium strains and S. Livingstone.62% of the herds had a changing status over the different consecutive sampling rounds. The probability for a herd to have 2 or 3 consecutive positive statuses was 0.31 and 0.24.
Statistically more positive samples were found using overshoes compared to hand swabs (Odds Ratio: 2.45 [IC95%:1.77-3.38]). No statistically significant difference was found between pooled and non-pooled overshoes. The correlations between the results of the 4 sampling units are small to moderate. The highest correlation was observed between the insemination and gestation units (rho=0.54).
There was no significant relationship between the standard use of acids and antibiotics or the results of the biocheck and the Salmonella status.. In conclusion, these results indicate a high prevalence of Salmonella on the Belgian breeding and rearing farms. The most optimal way to determine the Salmonella status is by taking 12 pools of 2 pairs of overshoes spread over all the different units. At least 3 consecutive sampling results are needed to get a clear view of the Salmonella status.

P228

EFFECTS OF EARLY VACCINATION AGAINST GNRF (IMPROVACTM) ON BOAR TAINT AND GROWTH PERFORMANCE OF MALE PIGS RAISED UNDER COMMERCIAL FIELD CONDITIONS IN GREECES

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Introduction
The aim of this study was to evaluate safety (in terms of detecting possible adverse clinical effects attributable to vaccination), efficacy, and effects on growth performance of a gonadotropin releasing factor analog-diphtheria toxoid conjugate (commercially distributed as Improvac; Zoetis) in male pigs raised in a commercial Greek farm.
Materials and Methods
A total of 1,230 male pigs was enrolled in 16 weekly batches and allocated to 3 groups: barrows (castrated on the next day after birth [study Day 0]), pigs vaccinated with the above-mentioned product and intact boars. Vaccinated pigs were injected subcutaneously with 2 mL of the anti-gonadotropin releasing factor (GnRF) vaccine at 9 to 11 wk of age (60-78 d) and 15 to 17 wk of age (102-120 d) and slaughtered at 22 to 25 wk of age (152-176 d).
Results
No clinical abnormalities or adverse events attributable to vaccination occurred. Mean BW of vaccinated pigs was 6% greater compared with barrows at slaughter ($P < 0.0001$). The vaccinated pigs had greater ADG than barrows from castration to slaughter (8%). In detail, a lower ADG from first to second vaccination (-12%; $P < 0.0001$) and a 27% greater ADG from second vaccination to slaughter ($P < 0.0001$) were observed. The ADG of vaccinated pigs and intact boars was not significantly different throughout the study, except from first to second vaccination (boars greater; $P = 0.0059$) and second vaccination to slaughter (vaccinated pigs greater; $P = 0.0390$). FCR of barrows was 11 and 8% greater compared with vaccinated pigs ($P = 0.0005$) and boars ($P = 0.0062$) from first to second vaccination but was 23 to 26% lower compared with vaccinated pigs ($P < 0.0001$) and intact boars ($P < 0.0001$) from first vaccination to slaughter and 7 to 9.5% lower from the second vaccination to slaughter ($P = 0.0029$ and $P = 0.0003$ for vaccinated and intact boars, respectively). At slaughter, the belly fat androstenone concentration of all vaccinated pigs and 64% of intact boars was below 200 ng/g. Belly fat skatole concentration was below 20 ng/g in samples from all groups.
Conclusion
In conclusion, vaccination against GnRF using the GnRF analog-diphtheria toxoid conjugate tested did not induce adverse clinical effects, proved effective, and compared with physical castration, resulted in greater BW at slaughter and improved ADG and FCR from first vaccination to slaughter.

P229

PRESENCE AND SIGNIFICANCE OF PROSTAGLANDINS (PGF2A) IN PLACENTAS OF ABORTED PIGLETS.

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Introduction
In the Netherlands it is mandatory that pregnant sows are loose housed from four days after insemination. However, from practice indications are received that in certain sow herds the number of early disruptions of pregnancy increases sharply when sows are loose housed in so called stable groups during early gestation. In some cases these early abortions seem to occur as outbreaks resulting in percentages of non-pregnant sows within groups as high as 50 per cent or more. However, typically no obvious additional disease symptoms are reported and in adjacent groups pregnancy rates can be much higher. Possible explanations for this phenomenon are chronic stress due to continuing competition for feed or the release of pheromones by aborting sows within the same group. Another concept is that oral intake of aborted placentas by susceptible group members results in the uptake of significant amounts of prostaglandin F2a (PGF2a) causing disruption of pregnancy. GD Animal Health, Deventer (Netherlands) conducted a study, commissioned by the Dutch pig industry (NVV / LTO), to investigate the presence and significance of PGF2a in placentas of aborted piglets.

Material & Method
Placentas of 16 aborted litters, which were regularly submitted for post mortem examination to the histopathology department of GD Animal Health, were tested for PGF2a using a commercially available ELISA test (Abcam ab133041 – PGF2 alpha ELISA Kit, Cambridge, UK). The piglets were aborted at various stages of gestation.

Results
In placentas form all 16 examined placentas, PGF2a was detected in concentrations of up to 8.8 mg / kg wet weight. However, the measured PGF2a concentrations varied hugely, mainly depending on the method of preparation and upgrading of the tested tissue material.

Discussion
Further validation and standardization of the test procedure is necessary to make it operational and consistent in practice. However, the provisionally measured PGF2a concentrations were within the range of quantities that can result in abortion in sows. In addition, from literature it can be concluded that oral ingestion of PGF2a likely results in biologically active concentrations in the body.

Conclusion
It cannot be excluded that the uptake of aborted placentas by pregnant sows can in turn lead to disruption of pregnancy. Yet, it can neither be confirmed based on the present results, since this study was limited to the detection of PGF2a in placental tissue of aborted piglets. To prove whether the consumption of such material by pregnant sows can result in abortion, additional research is needed.

P230

THE INFLUENCE OF LIANOL® FERTI GIVEN AROUND WEANING ON THE REPRODUCTION PARAMETERS OF GILTS AND SOWS.

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Introduction:
Previous research demonstrated a positive effect of Lianol®, a complementary feed based on fermented potato protein, on plasma insulin-like growth factor-1 (IGF-1) levels in pigs. IGF-I is involved in many metabolic processes including fertility. Therefore it can be expected that Lianol® can affect fertility parameters in sow production.

The objective of this research was to evaluate the effect of this new complementary feed stuff on fertility parameters and litter size in gilts and sows.

Material and methods:
This research was part of a Master of Science thesis in agricultural and bio-science. The trial was performed on a Hungarian sow farm. 4 groups with 100 animals each were selected. Group 1 was the control group for the gilts and was synchronised with Regumate®. The gilts in group 2 received 10 grams of Lianol® Ferti for 5 days starting just after ending the Regumate®. Group 3 was the control group for sows and group 4 received 10 grams of Lianol® Ferti per sow per day starting three days before till 2 days after weaning.
The control parameters were insemination %, farrowing %, total number of born piglets and total number of life born piglets.

Results:
The farrowing % increased by 3 % and 4 % respectively for sows and gilts treated with Lianol® Ferti. Lianol® Ferti increased the pregnancy % by 7 % for the gilts. Lianol® Ferti significantly increased the total number of born piglets with 0.8 piglets versus the control. The number of live born piglets for the Lianol® Ferti treated animals was significantly increased with 0.82 piglets in gilts and numerically with 0.31 piglets in sows. Lianol® Ferti also significantly improved litter uniformity.

Conclusions:
Supplementation with Lianol® Ferti around weaning improved the pregnancy %, farrowing % and the number of live born piglets. Consequently the production figure was improved. If we project these results on a standard reproduction farm, the treatment with Lianol® Ferti would result in an extra profit of around 4000 euro per 100 animals present.

P231

SINGLE FIXED TIME INSEMINATION IN A COMMERCIAL FARM USING PORCEPTAL: A CASE REPORT

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Introduction
Buserelin, a GnRH analogue, is able to induce an LH release to synchronize ovulation. The aim of this study was to demonstrate that a single fixed time insemination protocol following buserelin injection (Porceptal®, MSD Animal Health) generates similar reproductive performance as the one observed in the conventional management with multiple inseminations.
Materials and Methods
The trial was conducted in a conventional farm of 460 sows located in the north-east of Spain. The farm worked in a weekly batch system and had a weaning average age of 26 days. Six batches, with a total of 82 multiparous sows were included in the study, 41 in the Porceptal group (P) and 41 in the Control group (C). The sows were paired and randomly assigned to any of the two groups based on parity, body condition score, lactation length and piglets weaned at previous lactation. From weaning to the moment of Porceptal® injection, the sows, regardless of treatment group, were managed the same. The same boars and same personnel were used to inseminate and check heat in the two groups. Sows of the Porceptal® group were injected with 2.5ml of Porceptal® i.m 88h post-weaning. About 31-32 hours later sows were checked for heat, and if positive, were inseminated with one dose of commercial semen. No heat detection was done after this one and only insemination. Sows of the control group were inseminated 1st time when first standing heat and every 24h (maximum 3 AI) if heat was still detected. Fertility and prolificacy results were compared via Pearson Chi Square Test and ANOVA.
Results
Sows of Group P received 1 dose of semen per sow, while sows of Group C received an average of 3 doses per sow.
For Group P, 85,4% of the sows were in heat at the time of IA. Six sows were not in heat: 2 were detected 2 days later and inseminated in the conventional way, and 4 were in heat more than 1.5 months later and were excluded from the study. In group C, 97% of the sows were in heat 4 to 6 days after weaning. No statistical differences were detected between groups in terms of farrowing rate (80% Group P and 81,6% Group P, p=1). In regards to prolificacy, no differences were found either (Total Born 14,26 (P) vs 14,45 (C); Born Alive 13,04 (P) vs 13,61 (C); Born Dead 1,2 (P) vs 0,83 (C)). Gestational length was significantly different, with 0,915 days less is the Porceptal® group (115,19 P vs 116,06 C, p>0,03).
Conclusion
The single fixed time insemination protocol with Porceptal® was demonstrated to be useful for industrialized farms, reducing the cost of semen and labour while mantaining reproductive performance at the same level of conventional management.

P232

INCIDENCES OF RETAINED PLACENTA AND THEIR EFFECT ON SUBSEQUENT FERTILITY

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The sow has changed greatly over the last years with increasing litter size and birth duration. These developments appear to be associated and can potentially lead to secondary uterine inertia. The lack of uterine contractions can complicate placenta expulsion. The aim of the study was to investigate if the birth duration has an effect on the expulsion of the placenta and the occurrence of retained placenta. According to the literature, retained placenta occurs rarely in sows and expulsion of the fetal membranes and placentas usually starts after the birth of the last piglet and is completed within 4 hours, latest within 12 hours. Subsequently, it was investigated if the duration of placenta expulsion has an effect on the next pregnancy rate.
At birth, we recorded 149 sows for the following farrowing traits: piglet expulsion duration (PiD, time interval between birth of first and last piglet), placenta expulsion duration (PlD, time interval between expulsion of first and last placenta), last piglet last placenta duration (LPLPD, time between expulsion of last placenta relative to the last piglet), last piglet first placenta duration (LPFPD, time between expulsion of first placenta relative to the last piglet), and primary placental retention (PPR, if no placenta was expelled at all or if LPLPD < 0 minutes). For studying the effect of PiD on PlD, LPLPD, and LPFPD, a linear regression model was used. For studying the effect of PiD on PPR, a logistic regression model was used.
The PiD was 411±250min (mean±SD) with tending significant effect on PlD (p=0,086) and significant effects on LPLPD (p=0,003) and FPLPD (p=0,001). With increasing PiD, the PlD increased, and LPLPD and FPLPD decreased. In 55% of the sows the LPLPD was < 4 hours, in 39% 4-12 hours, and in 6% > 12 hours. There was a highly significant effect of PiD on PPR (p=0.001). In 3% of the sows no placenta was expelled (PiD 660±177min) with the last piglet or afterwards and in another 3% of the sows no placenta was expelled at all (PiD 1008±275min). For further analyses, sows with PPR were given a PlD value of 0min and a logistic regression model was used for effects of PlD and LPLPD on the subsequent pregnancy rate (PREG vs. NotPREG). PlD was 272 ± 233min (PREG) vs. 153 ± 178 min (NotPREG, p=0,031). LPLPD was 268 ± 228 min (PREG) vs. 169 ± 172 min (NotPREG, p=0,061).
The results show, that retained placenta occurs more frequently than reported and that long LPLPIs are common in sows nowadays. Long LPLPIs seem not to be a problem for the subsequent fertility, whereas short PlDs and LPLPDs appear to reduce fertility.

P233

THE EFFECTS OF LIVE YEAST SACCHAROMYCES CEREVISIAE SUPPLEMENTATION ON SOWS AND SUCKLING PIGLETS' PERFORMANCE AND HEALTH STATUS DURING TWO SUCCESSIVE REPRODUCTION CYCLES

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Introduction
The aim of the study was to determine the effect of live yeast Saccharomyces cerevisiae supplementation in sow diets, on their performance, colostrum antibodies, milk composition, as well as on the performance of their litters during 2 consecutive reproductive cycles (RC).

Materials and Methods
The Actisaf Sc47, a feed additive of live yeasts S. cerevisiae, was tested. The study was carried out in a commercial farrow-to-finish farm with a capacity of 800 sows. Seventy one healthy primi- and multiparous sows were divided into 2 groups and fed as follows for 2 consecutive reproductive cycles (RC): a) Negative Control group= sows received basal gestation and lactation feed, b) Actisaf group = sows received the same basal lactation feed plus the test product as top dressing (1.1010 CFU/kg of complete feed) for 14 days pre-farrowing and basal gestation feed plus Actisaf (5.109 CFU/kg of complete feed) for the whole lactation period. Parameters such as sows' body weight, back fat, colostrum antibodies (IgA, IgM, IgG), milk composition, litter characteristics, morbidity and mortality were investigated.

Results
Results showed significant improvement of Actisaf-supplemented sows' mean back fat loss (mm) (6.0 in 1st RC, 5.3 in 2nd RC) compared to NC (7.1 in 1st RC, 6.4 in 2nd RC). In the 2nd RC, mean litter BW (kg) at farrowing and at weaning were 17.5 and 93.3 in the Actisaf group and 16.1 and 83.1 in the NC group, respectively (p=0.032 and 0.003, respectively), while mean litter size at weaning was 12.7 in the yeast-supplemented group and 11.7 in the NC group (p=0.004). Similarly better average daily gain was seen in litters from yeast-fed sows during their 2nd RC compared to NC (p=0.005). No differences were observed on colostrum antibodies' levels and milk composition.

Conclusion
S. cerevisiae supplementation to sows for 14 days pre-farrowing and the lactation period for 2 consecutive RC could lead to significant reduction of back fat loss in sows in both RC and improvement of litter performance, especially in the 2nd RC.

P234

EFFECT OF A FIXED TIME INSEMINATION PROTOCOL ON REDUCTION OF

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Introduction
GnRH analogues are synthetic peptides that allow ovulation induction, resulting in feasibility of single fixed time insemination protocol (FTI). FTI improves work planning on farms and helps improve profitability. The objective of this study was to use Porceptal® (MSD Animal Health), GnRH analogue, in multiparous sows, for improving effectiveness in inducing ovulation.

Material y Methods
This trial was conducted in a 2,200 sow farm in Spain (Lerida). Historical results from this farm were: 92 % fertility, 87% farrowing rate and an average of 27 piglets weaned / productive sow and year. Weaning takes place two days a week (Wednesday and Thursday morning). Randomization of two groups, Porceptal (P) and Control (C) was done in 47 of the weaned sows in a week. Porceptal group was treated with the product 85 h after weaning and inseminated 32 h later. Control group followed the farm standard insemination protocol. Both groups used post-cervical artificial insemination.

Results
P group had only 1 IA and Group C an average of 2.5 IA / sow ($p < 0.001$). For sows in group P, only semen from one boar was needed, while for Group C sows, 3 boars were used. At insemination time, 89% of P group sows were in heat and all the sows in heat became pregnant. Remaining 11 % of P group sows not in heat were inseminated following conventional protocol. Total Born in group P were 12.94 vs 12.93 in C, and 11.67 vs 11.46 in Live Born ($p > 0.05$). While wean to estrus interval was similar (5 days for P group and 5.5 days in C), main difference was found in wean to fertile interval (5 days for P and 8.1 days for C). For this reason, farrow to farrow interval varied from 141 days for P to 144 for C. Using all this data, the calculation of live born piglets per sow and per year (LB-PSY) for each group equals 30,2 piglets for P group [(365/ 141= 2,59) x 11,67 (LB) = 30,2], and 29,05 piglets for C group [(365/144= 2,535) x 11,46 (LB)= 29,05].

Discussion and Conclusions
Both groups had similar results in fertility and prolificacy. However, there was a significant difference in wean to fertile interval, minimizing the number of non-productive days with the FTI program, and therefore increasing farm profitability. The use of a single semen dose compared to 2.5 in control also reduces cost in each production cycle and fewer boars are needed in the FTI program. Both aspects enable workforce to be more efficient by having better grouping at farrowing, leading to a greater homogeneity of litters and improved management in the following production phases.

P235

TOLERANCE OF A CONCENTRATED PARACETAMOL SOLUTION (PRACETAM® 40% ORAL SOLUTION FOR PIGS) ON REPRODUCTIVE FUNCTION IN SOWS

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Introduction: Paracetamol is frequently administered to treat fever in swine because of its safety and the absence of undesirable effects (haemorrhages, ulcers) often observed with classical non steroidal anti-inflammatory drugs such as aspirin. The objective of this trial was to assess the safety of treatment with Pracetam 40% oral solution on the reproductive function in gestating and lactating sows.

Materials and Methods: A GCP VICH complying field trial has been performed in a commercial swine farm: 150 sows were included in the trial. They were randomly allocated in two groups, one treated and one untreated. Five batches of 30 sows were divided into two groups of 15 sows each.
Sows received treatment at five different periods of the reproductive cycle: before insemination (from the day after weaning, before insemination), during early, mid and end pregnancy, and during lactation. The treated groups were orally treated with drinking water containing PRACETAM®40% oral solution at three times the daily recommended dose (i.e. 90 mg/kg/day) during 5 consecutive days, the untreated group received the same drinking water without the product. In all, 15 sows were treated and 15 were not treated, for each of the five reproduction periods considered. All piglets from the observed sows in batches 2, 3, 4 and 5 have also been included in the study.
Animals were monitored for criteria related to reproduction, depending on their stage of reproduction such as: interval until first heat and until first AI (artificial insemination), pregnancy after first AI, number and date of abortions, number of births: number and weight of piglets born alive, piglet mortality: number of piglets born dead and number and type of malformations, number of weaned piglets per sow and early lactation interruption rate.

Results: No relevant statistical or clinical difference was observed between Paracetamol treated group and the untreated group in all the parameters followed, at all stages of reproduction.

Conclusion: The results give evidence that the administration of PRACETAM®40% oral solution to sows, at three time the recommended dose, does not have any negative effect on reproduction. PRACETAM®40% oral solution can be safely used in sows at all stages of their reproductive cycle gestating and lactating.

P236

ALTRESYN® EFFICIENTLY SYNCHRONIZES ESTRUS IN SOWS AFTER WEANING AND INCREASES THE LITTER SIZE

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Introduction
In modern pig farming there is often a need to manipulate the time of estrus in sows after weaning (e.g. loss of litters due to PED, completing the batches, second litter drop, etc.). The most convenient way to synchronize estrus of sows with other batch-mates is delaying heat by using synthetic progestagen - altrenogest. Altrenogest has an inhibitory effect on the pituitary gland which suppresses the release of gonadotrophins during the treatment period. After withdrawal of the treatment the inhibitory effect stops, and gonadotrophins stimulate the rapid and synchronous growth of a new follicular wave resulting in ovulation. The aim of this study was to demonstrate the efficacy of Altresyn® (Ceva) on the synchronisation of oestrus of sows after weaning.
Materials and methods
Sows were randomly allocated to 3 treatment and 3 control groups with 30 individuals in each group. Treated sows received Altresyn® 5ml for 3, 10 and 18 consecutive days, respectively, starting 1 day before weaning. Heat detection was carried out regularly in the mornings and in the evenings in the presence of a teaser boar. After detection of the first standing reflex, the sows were inseminated twice (12 hours apart). Between Days 28 to 34 after insemination, the sows were tested for pregnancy by means of ultrasonography.
Results
In treated group the total 94,4% of sows and in control group 95,6 of sows exhibited estrus. There were no statistically significant differences among the treated and non-treated groups (P>0.05). In the treated groups (Altresyn®) the mean interval between treatments and detection of oestrus was 4.2 to 4.6 days. Pregnancy rate was 90.6% in the Altresyn® group and 89.6% in the control group (p>0,05). The N° of total born piglets per litter was in Altresyn® group 13,3 and in the control group 12,8 (p>0,05) with 0,4-0,7 more piglets were born alive in the treated groups (0,4 in Group 1T, 0,6 in Group 2T and 0,7 in Group 3T, respectively) than in the control groups.

Conclusions
This study demonstrated that Altresyn® synchronized efficiently the estrus of sows after the withdrawal of the treatment. There was no difference between different treatment protocols. Altersyn® didn't influence negatively the incidence of oestrus after treatment neither the reproductive performance, on contrary the litter size was higher in treated sows, yet the difference was not statistically significant.

P237

IMPACT OF SYNCHRONIZATION OF GILTS WITH ALTRESYN® ON REPRODUCTIVE PERFORMANCE

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Introduction
Synchronization of estrus in gilts also in weekly managed systems improves the flow of sows within different compartments of breeding unit. Planned number of gilts in heat for each particular batch allows having the right number of gilts at the appropriate age served every week, which avoids gilts getting too old at first mating. (Think 2014). Lower average age results in decreased cost per gilt in terms of housing and feeding expenses on the farm. The aim of this study was to evaluate the impact of gilt synchronization on other production parameters such as longevity, number of piglets per litter depending on age at first service and also overall the sow mortality.
Materials and methods
The conventional 1170 sow farm with the weekly farrowing cycle started to practice the synchronization of gilts using Altresyn® in 2012. Gilts after observed minimum first estrus were treated 18 days with Altresyn®. The criteria for gilts at first service were as follows: weight 135-150kg, optimal age 220-250 days, back fat minimum 14-15mm. Number of piglets per litter was compared between 6110 farrowings prior the use of Altresyn on the farms and 3930 litters of sows treated with Altresyn before their first mating. Sows were divided into three groups according to their age at first mating: G1=210-239 days, G2=240-269 days and G3=270-330 days. Sow mortality was compared in the time before gilt synchronization and after.
Results
Number of live born piglets per litter prior to Altresyn use was in first parity (P1) sows of G1=14,0 ; G2=14,8 and G3=14,8. In P1 sows after Altresyn it was: G1=15,3 ; G2 15,3 and G3 15,5. The litter size slightly increased, particularly in the youngest age category (by 0,8 live born piglet per litter). The total average number of litters per life of sows before Altresyn G1=3,9/61,6 ; G2=3,7/58,8 and G3=3,7/58,8. In sows after Altresyn it was G1=4,1/66,2 ; G2=3,9/65,6 and G3=4,2/70,2.
The sow mortality in the 30 months period before gilt synchronization was implemented reached 10,6%, while in the 18 months after Altresyn started to be used in gilts the sow mortality dropped to 6,8%. The reason is that sows at risk could have been culled always in a planned way, because synchronized gilts allowed planned replacement.
Conclusion
Synchronization of gilts with Altresyn improves the age distribution of gilts at first mating without compromising their reproduction potency and longevity. On contrary gilts treated with Altresyn had bigger litters mainly the youngest ones. The reasons might be higher prolificacy due to altrenogest inhibition (Soede 2007) and the fact that gilts are never served at first heat anymore which allows planned matings with systematic flushing 8-10 days before expected breeding. Improved management of the sow herd also resulted in lower sow mortality.

P238

INVESTIGATION OF THE EFFICACY OF A ONE–SHOT–VACCINE AGAINST MY–COPLASMA HYOPNEUMONIAE COMPARED TO A TWO–SHOT–VACCINE IN PIGS IN A FIELD TRIAL.

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Introduction: Vaccination against Mycoplasma (M.) hyopneumoniae with inactivated vaccines is one of the most employed methods to control clinical signs of Enzootic Pneumonia1. This investigation was aiming to compare the efficacy of two commercial vaccines applied in a one-shot or a two-shot protocol.
Material and Method: The study was conducted in a closed 1-site farm in Austria, where presence of M. hyopneumoniae was confirmed in weaned piglets already at 5 weeks of age. The general health status of the pigs at the study farm was considered high. Vaccination against M. hyopneumoniae with a two-shot vaccine was implemented for several years. A total of 271 piglets from two consecutive farrowing batches were assigned to two treatment groups distributed equally according to their weight and gender. Group MF received Ingelvac MycoFLEX® (Fa. Boehringer Ingelheim) once intramuscularly around day 30 (29 - 33) of life. Group SM was vaccinated intramuscularly with Stellamune® Mycoplasma (Fa. Elanco), receiving the first dose around day 9 (8 - 12) and the second dose around day 30 (29 - 33). Pigs were individually weighed four times and the average daily weight gain was calculated for 255 pigs during suckling, growing and fattening period. Additionally, lung lesions were scored for 226 pigs and mortality was recorded. Serum samples of 16 pigs per group were tested for antibodies against M. hyopneumoniae in order to confirm exposure of the study groups to the pathogen.
Results: In this study no significant differences were found between both treatment groups with regards to average daily weight gain, lung lesions and mortality. Exposure to M. hyopneumoniae was confirmed by seroconversion. Piglets in MF group showed no vaccine induced seroconversion, while a maximum of 25 % of the sampling animals of the SM group seroconverted after vaccination.
Conclusion: In the present study no difference of efficacy was shown between Ingelvac MycoFLEX® applied in a one-shot and Stellamune® Mycoplasma applied in a two-shot protocol. Vaccine induced seroconversion was no predictor for vaccine efficacy.
1Maes et al. 2008: Vet Microbiol 126, 297-309

P239

IMPACT OF CORTICOÏD TREATMENTS CONCURRENTLY INJECTED WITH PCV-2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES IN WEANERS

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Introduction. Dexamethasone is widely used in pig practice. Immunosuppression is frequently mentioned in the contraindication section of Summary of Product Characteristics. Yet, few papers have been published on the topic, regarding pigs [1].
Objective. The objective of this trial was to investigate the impact of injections of dexamethasone salts (phosphate and acetate) at the time of 2 usual piglet vaccinations (M hyo and PCV-2) in field conditions.
Materials and Methods. A farrow-to-finish operation with 160 sows was selected by the practitioner. Management of sows is done in 4 batches running every 5 weeks. 60 piglets randomly selected from 20 litters have been allocated to 3 treatment groups at the age of 19 days. Ear tags with three different colours are applied the same day. Following weaning and transfer to the subsequent unit, piglets are vaccinated against PCV-2 and Mycoplasma hyopneumoniae using 2 separate syringes (Porcilis® PCV and Suvaxyn® M hyo Mono) at the age of 28 days. The control group receives 0.5 ml of isotonic glucose i.m., while the Solution group receives 0.5 ml of Dexamethasone phosphate i.m. and the Suspension group, 0.5 ml of Dexamethasone acetate i.m. Bleeding occurs on three dates at the age of 19 days (allocation), 49 days (3 weeks post-vaccination) and 84 days (8 weeks post-vaccination). The blood are delivered to the diagnostic laboratory a few hours after collection and sera are centrifugated then processed with kits (DAKO Mycoplasma hyopneumoniae ELISA and SERELISA PCV-2 Synbiotics).
Statistical analysis is performed with SYSTAT (Wilkinson, L., SYSTAT: The system for statistics. Evanston, IL: SYSTAT, Inc.), version 12.0 for Windows.
Results. The amount of corticoids injected was 3 to 6 times the recommended dosage of the label. Before treatment all 3 groups were comparable for M hyo or PCV-2 antibodies (p= 0.799 and 0.601 respectively), with a high level of maternal antibodies. At 7 weeks of age no statistical difference was noted for both vaccines, with a huge decrease of maternally derived antibodies. At 12 weeks of age no difference was observed for PCV-2. For M hyo, there was no statistically significant difference in prevalence, despite the antibodies in the Control group were significantly higher than in both treatment groups. No circulation of wild PCV-2 had occurred yet, but some circulation of M hyo may have started.
Conclusion: the injection of dexamethasone salts, at the time of piglet vaccination against PCV-2 or M hyo, did not influence the serological immunologic response, even at 3 to 6 times the normal dosage.
Reference K. P. Flaming, B. L. Goff, D. E. Frank, J. A. Roth, Comp Haematol lnt (1994) 4:218-225

P240

AN ANTI-IDIOTYPIC ANTIBODY-DERIVED KILLER PEPTIDE (KP) STIMULATES BOTH INNATE AND ADAPTIVE IMMUNE CELLS IN PIG PBMC

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Introduction
A killer peptide (KP) derived from the anti-idiotypic antibody representing the internal image of a yeast (Pichia anomala) killer toxin mediates antimicrobial effects in human fungal and viral infection models in mice, rats and dogs, and interact/modulate immune cells in mice. KP binds to murine dendritic cells (DC) by SIGN-R1, MHC-II and CD16 and up-regulates the CD8α, CD80 and CD4 expression; it also binds to macrophages and sustains CD4+ T cell proliferation. The aim of the study was to evaluate the effects of KP on porcine immune cells in order to obtain basic knowledge useful for investigations in cellular models of pig viral infection.

Materials and methods
Swine PBMC, isolated from conventional animals (N = 10), were stimulated in vitro with KP (10, 20, 40 µg/ml) for 24, 48 and 72h. The effects of KP were evaluated using flow cytometry by quantifying the cellular response (%) and marker expression increase (MFI: mean fluorescence intensity) in both innate [monocytes and natural killer (NK) cells] and adaptive (T lymphocytes) immune cell subsets. Staining for SCW3, CD14, CD3, CD16, CD4, CD8α, and CD8β was performed.

Results
Stimulation with KP induced a shift in CD14 expression of SWC3+CD14+ cells mainly at 24 h characterized by a significant CD14+low cell decrease and a CD14+high cell increase ($p<0.05$).
CD3-CD16+ NK cells showed a strong dose-dependent increase at 24 h, 48 h and 72 h. The most intense response as percentage and expression (MFI) was observed after 24 hours of treatment.
CD3+CD8β+ cells [(cytotoxic T lymphocytes (CTL)], showed an increase in percentages and MFI at all time points ($p<0.05$), with the most intense response at 24 h.
CD4+ and especially CD8α+ cells showed a dose-dependent increase at all time points ($p<0.05$). Particularly, the decrease of CD4+CD8α- cells was associated with the increase of the CD4+CD8α+ fraction ($p<0.05$), also showing cells with high CD8α expression (CD8α+high).

Conclusions
KP could have a role in inducing the increase of CD14 expression in differentiating SWC3+CD14+ monocytes.
The most intense CD16 expression/up-regulation in NK cells testifies that KP may have a role in inducing NK cell differentiation/activation.
Considering the effect on CD4 and CD8α/CD8β T cell subsets, KP may sustain the proliferation and differentiation/activation of both regulatory and cytotoxic T cells. Specifically, it is worth nothing that CD4+CD8α+ memory T helper cells and double positive (DP) CTL are included. Thus, influencing the response of CTL (CD4-CD8α+high and CD3+CD8β+ cells), producers of IFN-γ, perforins and granzymes, KP may trigger the Th1 response.

P241

EFFICACY OF TWO ONE–SHOT MYCOPLASMA HYOPNEUMONIAE VACCINATION STRATEGIES UNDER FIELD CONDITIONS

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Introduction
Mycoplasma hyopneumoniae (M.hyo) is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs causing major economic losses to the pig industry worldwide. Early vaccination against M.hyo has the advantage that immunity can be induced before pigs become infected; also, there are less pathogens present at time of vaccination that can interfere with the immune response. This study compares the efficacy of early vaccination with Stellamune® Once with another one-shot M.hyo vaccine administered at weaning.
Materials and Methods
This study was conducted in a 300-sow farrow-to-finish farm with a history of EP. Piglets born to sows from 8 consecutive batches of production were randomly allocated into two treatment groups and vaccinated following manufacturer's recommendations as follows: Stellamune group (n=500 pigs) was vaccinated with a single dose of Stellamune® Once (Elanco AH) at 7 days of age (average sow parity = 2.9). Group B (n=500 pigs) was vaccinated with Ingelvac MycoFLEX® (Boehringer Ingelheim Ltd) at weaning (average sow parity = 3).
Ten blood samples were collected per treatment group at 4, 9 and 23 weeks of age for M.hyo antibody determination by ELISA test (Oxoid). Average daily weight gain (ADWG) and EP-like lesions at slaughter (Sanchez-Vazquez et al., 2011) were used to compare the efficacy of both vaccination strategies.
Results
M.hyo infection was confirmed by serology during the fattening period, with 20 /100% (Stellamune group) and 10 /90% (Group B) of positive pigs at 9 /23 weeks of age.
Pigs vaccinated with Stellamune® Once performed better than pigs in group B (713.8 g/d and 694.4 g/d, respectively), gaining 19.4 g/d more from wean to finish ($p=0.001$). Average weight in both groups was similar at weaning. However, Stellamune® Once vaccinated pigs were 2.7 kg heavier ($p<0.05$) by the end of the finishing period.
A total of 377 lungs (n=172, Stellamune group; n=205, group B) were investigated in two different visits to the abattoir. Stellamune group had a lower percentage ($p<0.05$) of lungs with EP-like lesions (score >0) than group B (34.3% and 53.6%, respectively). Similarly, average EP-like lesions were significantly increased ($p<0.05$) in group B when compared to the pigs vaccinated with Stellamune® Once (4.5 and 2.4, respectively).
Conclusion
Early vaccination with Stellamune® Once was more efficacious in controlling EP and lung lesions associated with M.hyo than a vaccination regime at weaning with another vaccine. In addition, ADWG was significantly improved (19.4 g/d, wean to finish) and pigs were on average 2.7 kg heavier at the end of the finishing period when vaccinated with Stellamune® Once.

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CONCURRENT VACCINATION AGAINST MYCOPLASMA HYOPNEUMONIAE AND EDEMA DISEASE IN PIGLETS EFFECTUATE SIMILAR SEROLOGICAL RESPONSE UNDER FIELD CONDITIONS

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Introduction
E.coli edema disease (ED) and enzootic pneumonia (EP) caused by Mycoplasma hyopneumoniae (M hyo) are causative for economic losses in the swine industry. Commercially available vaccines are administered in the EU from the 3rd or 4th day of life, respectively. Considering individual infection dynamics on farms, it can be useful and necessary to do simultaneous vaccination during the suckling period. The aim of this study was to evaluate the immune response after a single vaccination against M hyo (Stellamune®One) compared to an additional and simultaneous administration with a vaccine against ED (ECOPORC SHIGA®) in the first week of life (4th-7th day of life) under field conditions.
Materials and Methods
The study was performed in 3 consecutive batches in a farrow-to-finish farm in southern Germany between Dec 2013 and Aug 2014 with a known history of EP but unsuspicious regarding ED. The piglets were randomly assigned to 3 vaccination-groups: VG1 (n=135) Stellamune®One, i.m.; VG2 (n=135) ECOPORC SHIGA® + Stellamune®One, i.m.; VG3 (n=135): ECOPORC SHIGA®, i.m.
Every four weeks, blood samples were collected from 25% of the study animals. Sampling was initiated at the 1st week of life until 24th week of life using a commercial ELISA-Kit ("Mycoplasma Hyopneumoniae Antibody Test Kit", IDEXX Laboratories, USA). In order to measure antibodies, induced by vaccination with ECOPORC SHIGA®, an inhouse SNT-Test (IDT Biologika GmbH, Dessau) was used in weeks 0, 4 and 8.
Results
The mean antibody values against M hyo (in SP-Ratio) of VG2 were significantly higher than of VG3 at 8th, 12th, 16th and 20th week of life. VG1 and VG2 did not differ significantly. The percentage of pigs being seropositive (cut off: 0.4) in week8 was 24.2% of VG1, 18.8% of VG2 and 0% of VG3 ($p<0.025$). In week12 42.2% of VG1, 37.5% of VG2 and 13.3% of VG3 and in week16 75.8% of pigs of VG1, 65.6% of VG2 and 36.7% of VG3 seroconverted.
Concerning the development of antibodies referring to ECOPORC SHIGA®,
neutralizing antibodies were shown in week4 in 100% of pigs of VG2 and 85.7% of VG3, respectively. By week8 96.4% of VG2 and 96.9% of VG3 had developed antibodies. Neutralizing antibodies were not detected in VG1 ($p<0.025$).
Conclusion
The present study shows that a concurrent vaccination with Stellamune®One and ECOPORC SHIGA® did not affect the development of antibodies against each vaccine antigen in comparison to a single administration. Simultaneously vaccinated pigs achieve similar humoral immune response in comparison to single vaccinated pigs by developing equal values of neutralizing antibodies (ECOPORC SHIGA®) and equal values of antibodies concerning M hyo.

P243

ASSESSMENT OF SAFETY AND IMMUNE RESPONSES IN GILTS AFTER INTRADERMAL APPLICATION OF PORCILIS® PRRS AT TWO DIFFERENT APPLICATION SITES UNDER FIELD CONDITIONS

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Introduction
The study purpose was to evaluate the safety and immune responses of intradermal application of Porcilis® PRRS to gilts in two different application sites under field conditions.

Materials and Methods
Gilts seronegative for porcine reproductive and respiratory syndrome virus (PRRSV) were vaccinated intradermally with a live attenuated PRRS Type 1 virus vaccine (Porcilis® PRRS) in the neck (group 2, n=17) or perianal region (group 3, n=17). Group 1 pigs (n=10) served as non-vaccinated controls. Porcilis® PRRS was administered intradermally at a dose of 0,2ml using a needle free device (IDAL). Vaccinated pigs and non-vaccinated control pigs were maintained in separate barns with separate air spaces. Pigs were monitored for a period of 28 days post vaccination (dpv). An individual examination for clinical signs including assessment of rectal temperature, general health, appetite, body condition score, behavior, respiratory signs and digestion was performed daily, starting the day before vaccination. Injection sites were monitored for presence or absence of reactions including redness, swelling, consistency, necrosis and pain during palpation on day 0 prior to vaccination, 4h post-vaccination and subsequently on a daily basis. Blood samples were collected on -7, 0, 7, 14, 21 and 28 dpv for detection of virus-specific antibodies by a commercial ELISA kit (PRRS X3, IDEXX, Switzerland AG, Liebefeld-Bern) and for PRRSV-specific IFN-β secreting cells by ELISpot. On 28 dpv all pigs from groups 2 and 3 were euthanized and submitted to necropsy with subsequent microscopic examinations of the application site.

Results
No significant differences were found in frequency and degree of clinical signs between both vaccination groups. On 9, 10, 11, 12 and 18 dpv the quality (diameter and consistency) of injection site reactions was significantly higher in pigs vaccinated intradermally in the neck compared to pigs vaccinated in the perianal region. All pigs were negative for PRRSV-specific antibodies at the start of the study. PRRSV-specific antibodies were present in the serum of all vaccinated animals from 14 dpv onwards. All control pigs remained ELISA-negative throughout the study. Immune response was evident in both vaccinated groups in terms of PRRSV-specific IFN-γ producing cells from 7 dpv on and no differences were found between both vaccinated groups.

Conclusion
The results of this study indicate that the perianal region is an alternative application site for the intradermal application of Porcilis® PRRS to gilts. Furthermore the intradermal application induced a comparable immune response in groups 2 and 3 vaccinated either in the neck or perianal region.

P244

POSITIVE IMPACT OF PIGLET PCV2 VACCINATION ON FATTENING PIG PERFOR-MANCE IN A SUBCLINICALLY INFECTED FARM

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Introduction
PCV2 is not only involved in PMWS (Post Weaning Multi-systemic Syndrome) or PCVD-systemic disease (PCVD-SD) but also in a large array of pathological presentations including subclinical infections (PCVD-SI). PCV2 vaccination has been largely used as an efficient tool against mortality, lesions, viremia, excretion and for the improvement of growth. Nevertheless, although it is an important driver of profitability, and mainly due to practical constraints, very few controlled trials have looked at feed conversion efficiency. In this study we tested the efficacy of PCV2 piglet vaccination in addition to sow vaccination, in a herd with a subclinical PCV2 circulation, on growth performance and feed conversion efficiency in the fattening period.

Material and Methods
Sows used to be vaccinated against PCV2 for at least 4 years. At 6 weeks of age, 168 piglets from the same farrowing batch were allocated into 84 pairs of similar piglets according to sex, weight, parity of their dam and size of their litter and in which one piglet was vaccinated (CIRCOVAC®, 0.5 ml, IM) and one was given a placebo (saline, 0.5 ml IM). Both groups (84 vaccinated and 84 placebo) were exposed at the end of the fattening period to the natural circulation of PCV2, as evidenced by PCV2 antibody seroconversion from the 17th week of life. Individual bodyweights, feed consumption and slaughterhouse data were registered. Furthermore, clinical score, mortality and lung lesions were also individually recorded.

Results and discussion
PCV2 vaccination in piglets at 6 weeks of age had no impact on growth performance during the post-weaning period. During the fattening period, the average daily weight gain was not different between groups, as well as feed consumption. However, the Feed Conversion Ratio (FCR) was significantly ($p<0.01$) decreased in the vaccinated group by 0.10 (2.67 against 2.77). These results may be explained by the very high growth performance during fattening in the two groups (917 g/d). Mortality was very low in the two groups. The other criteria (clinical signs, lung lesions and carcass traits) were not found significantly different.

Conclusion
In this high performing farm with a subclinical circulation of PCV2 in the fattening period, piglet PCV2 vaccination on top of sow vaccination in comparison to PCV2 vaccination in sows only induced a significant FCR improvement during the fattening period.

P245

MONITORING OF PCV2 MATERNAL DERIVED ANTIBODIE LEVELS IN 3 WEEK OLD PIGLETS IN 6 SPANISH COMMERCIAL FARMS

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Introduction
Protective immune response in face of high levels of MDA has been demonstrated for Ingelvac CircoFLEX(1,2) and no restrictions apply with regard to MDA levels at vaccination(3). However, for other PCV2 vaccines, the benefits of vaccination in pigs with high levels of mAb have not been demonstrated or the vaccines must to be administered a second time(4,5). Typically, antibody levels >8-10 log2 are classified as 'high'(6). The objective of this study was to evaluate the prevalence of levels of mAb at weaning in piglets deriving from non-vaccinated sows.
Materials and Methods
The study was conducted on 6 commercial farms located in Spain. Five two-site and one multisite production system were selected. Overall 270 blood samples, 45 samples per sow herd were collected as follows. Five piglets (3 weeks old) from 3 sows of each of the following three parity groups: parity 1-2, 3-4 and 5-6. Sows and piglets were unvaccinated against PCV2 and cross-fostered piglets were excluded.
The serum samples were analyzed for PCV2 antibodies with the Serelisa®PCV2 AbMonoBlockingELISA(Synbiotics Corp). The results are expressed as log2 titers with titers of 10 or above classified as 'high'.
Results
Figure 1 reveals that, in total, 68% of the piglets showed high MDA levels (>10 log2) and piglets with high MDA levels were present in each of the sampled farms (ranging from 36% in farm F to 89% in farm E). Only in one of the 6 farms (farm F) less than 50% of the piglets had high titer levels.
All parity groups (across herds) had a prevalence of high titers in piglets of more than 50%. While 61% of piglets from younger sows had high levels of MDA, 72% of pigs from parity 3&4 and 73% of the pigs from the older sows displayed those.
Conclusions and Discussion
This study indicates that in Spanish commercial sow herds the majority of piglets deriving from non-vaccinated sows have high levels of MDA at the time of weaning. Across all farms and parity groups 68% of the piglets had a titer of 10 or above. For some vaccines (including a 2-ds product) a possible interference with high levels of maternal antibodies is suggested based on the product information or field data. In contrast, Ingelvac CircoFLEX®, a single dose ORF2 based subunit vaccine, has demonstrated to provide solid and consistent protection independent of maternal antibody titres(1, 2).

P246

CASE REPORT: MONITORING OF PCV2 MATERNAL DERIVED ANTIBODIE LEVELS IN 3 WEEK OLD PIGLETS IN 29 SPANISH COMMERCIAL FARMS

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Introduction
Prevention programs on young piglets must consider the presence of maternal derived antibodies at the time of vaccination. However, the efficacy of Ingelvac CircoFLEX®, a one shot PCV2 vaccine, has been repeatedly demonstrated independent of the antibodies levels.
MDA levels can easily be measured in the piglet serum by ELISA technics. Typically, antibody levels >10 log2 are classified as 'high' (6).
A previous study showed that 68% of 3 weeks old piglets born of non-vaccinated sows had high MDA titers in 6 Spanish commercial farms.
The objective of this study was to evaluate the level of maternal PCV2 antibodies at weaning in piglets derived from non-vaccinated sows in a larger number of farms in the same country.

Materials and Methods
The study was conducted on 29 commercial farms located in five different regions of Spain as follows: Aragon (A), Cataluña (B), Murcia (C), North of Castilla y León (D) and South of Castilla y León (E) A combination of farrow-to-finish, two-sites and multisite production systems were selected. Overall 8055 blood samples, 45 samples per sow herd were collected as follows. Five piglets (3 weeks of age) from 3 sows of each of the following three parity groups: parity 1-2, parity 3-4 and parity 5-6.
Sows and piglets were unvaccinated against PCV2 at the time of sampling and cross-fostered piglets were excluded. Piglets were vaccinated against PCV2 after the blood sampling.
The serum samples were analyzed for PCV2 antibodies with the Serelisa® PCV2 Ab Mono Blocking ELISA (Synbiotics Corp). The results are expressed as log2 titers with titers of 10 or above classified as 'high'.
Results
In total, 58% of the piglets showed high MDA levels (>10 log2) and piglets with high MDA levels were present in each of the sampled farms (ranging from 5% farm to 93%). In 18 of the 29 farms more than 50% of the piglets had high titer levels.
All parity groups (across herds) had a prevalence of high titers in piglets of more than 50%. While 61% of piglets from younger sows (parity 1&2) had high levels of MDA, 58% of pigs from parity 3&4 and 56% of the pigs from the older sows (parity 5&6) displayed those.

Conclusions
High MDA levels were commonly observed in 3 week old piglets in Spanish farms.

P247

COMPARATIVE EFFICACY OF MYCOPLASMA HYOPNEUMONIAE VACCINES; COMBINED INGELVAC CIRCOFLEX® AND INGELVAC MYCOFLEX® VACCINATION VERSUS CONCOMITANT INGELVAC CIRCOFLEX® AND OTHER M. HYOPNEUMONIAE VACCINES

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Introduction
This paper reports 5 separate field studies that were performed in order to compare the efficacy of combined Ingelvac MycoFLEX® and Ingelvac CircoFLEX® vaccination (FLEX®Combo) versus concomitant vaccination with Ingelvac CircoFLEX® and other M. hyopneumoniae vaccines, as prescribed by the herd veterinarians.

Materials and Methods
Studies were conducted on 5 separate M. hyopneumoniae-positive production systems; 3 all-in/all-out straw-based nursery and finishing units (A-C) and 2 single-site continuous flow units (D, E). Basic study design was the same on all units. Pigs on the day of weaning (28d old) were selected by eye in descending size order and allocated alternately to treatment groups. Ear tags were inserted and pigs were vaccinated intramuscularly with either Treatment 1: a single intramuscular injection of a 2ml mixture of Ingelvac CircoFLEX® (1ml) and Ingelvac MycoFLEX® (1ml) (FLEX®Combo) or Treatment 2: 1ml of Ingelvac CircoFLEX® and another M. hyopneumoniae vaccine administered (dose as per datasheet) concurrently on separate sides of the neck (off licence use under herd veterinarian instruction). Competitor vaccine X was used in studies A-C, vaccine Y in study D and vaccine Z in study E. Treatment groups were co-mingled throughout. The primary outcome measure was wean-to-slaughter average daily gain (ADG). Statistical analysis was performed using Statistica® Release 8 (Statsoft); analyses included t-test (ADG) and Chi-square (mortality/culls).

Results
Serology confirmed all farms to be M. hyopneumoniae positive and all farms showed seroconversion to swine influenza A and/or porcine respiratory and reproductive syndrome. There was evidence of seroconversion to Actinobacillus pleuropneumoniae in both continuous flow units. No marked clinical issues were seen during the studies, apart from study C where swine influenza infection occurred close to slaughter age. In four out of five studies lower mortality rates were recorded in the FLEX®Combo groups whilst ADG values were comparable between treatment groups in all but one study, where the FLEX®Combo group performed significantly better. This resulted in Margins over Food and Medicine in favour of FLEX®Combo in four out of five studies.

Conclusions and Discussion
These results demonstrate the efficacy of Ingelvac MycoFLEX®, when used in combination with Ingelvac CircoFLEX® (FLEX®Combo), producing ADG values comparable to those obtained with other market leading M. hyopneumoniae vaccines. Margin over food and medicine per pig produced was higher for FLEX®Combo treated pigs in four out of the five studies.

P248

IMUVANTTM – A NOVEL ADJUVANT; EFFICACY AND SAFETY PROPERTIES IN HYOGEN® VACCINE

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Introduction
We evaluated and compared the safety and potency of several adjuvants: Carbomer 974, DDA, Squalene, Montanide ISA 206, competitors' swine vaccine adjuvants and Imuvant, a proprietary Ceva adjuvant used in Hyogen, a vaccine for the prevention of swine mycoplasma pneumonia. Based on our data, only Imuvant was both immune-potentiating and safe using a model antigen.
The selected adjuvant, Imuvant, consists of mineral oil and Escherichia coli J5 non-toxic LPS, which significantly boosts both the cellular and the humoral immune responses to the vaccine antigen. Our aim was to assess the boosting effect of the J5 component of Imuvant on the efficacy and evaluate the safety using Hyogen vaccine in 3-week-old piglets.
Material and methods
Efficacy
Forty-five (45), Mycoplasma hyopneumoniae (M.h.) sero-negative piglets were divided into 3 groups of 15 and then vaccinated with the following vaccines: (i) M.h. bacterin adjuvanted with Imuvant (containing J5), (ii) M.h. bacterin adjuvanted with mineral oil only, with no J5 added and (iii) Placebo. Eighteen (18) days after vaccination, the animals were challenged with a virulent M.h. strain. After 28 days, the animals were slaughtered and subjected to lung scoring according to the PhEur. Tracheal swab samples were also taken for qPCR to determine the level of colonization of the lungs by M. h..
Safety
Three lots of Hyogen vaccine were injected into 3-week-old seronegative piglets under placebo control. In addition to monitoring the systemic and local reactions to the vaccines, as well as measuring body temperature increase at several time-points, aspartate aminotransferase (AST) and creatine kinase (CK) blood biochemical tests were also performed as being fine indicators of possible tissue damage due to vaccination.
Results and conclusion
Based on the evaluation of lung lesion 28 days post M.h. challenge, the efficacy study has revealed the clear advantage of the Imuvant formula containing J5 over the non-J5 containing one. The formula containing no J5 also reduced the lung scores but statistical significance could not be demonstrated against the placebo group. The qPCR titres confirmed the same trend in the reduction of tracheal colonisation by the challenge strain.
The assessment of the safety study revealed no major systemic or local reaction to the vaccines and the rectal temperatures remained in the normal range. The AST and CK analysis indicated no tissue damage due to the vaccination.
This study confirmed the excellent efficacy and safety of Imuvant adjuvant in Hyogen vaccine.

P249

EVALUATION OF THE IMMUNE RESPONSE TO PCV2 VACCINATION IN PRRSV VIREMIC AND NON VIREMIC PIGS

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Introduction
Interaction of PRRSV with other pathogens tends to reduce the efficiency of the immune response and increase the severity of associated diseases. This study aims at evaluating the humoral and cell mediated immune response to PCV2 vaccination in piglets infected by PRRSV at the time of vaccination compared with non-viremic pigs.
Material and methods
In a PRRSV unstable 1000-sow herd, 200 pigs were divided into two groups at four weeks of age (weaning). Group A (n=100) was vaccinated and Group B was non-vaccinated. Blood samples were collected at vaccination and 4 weeks apart. Retrospectively, in both groups the animals were regrouped based on the detection of PRRSV infection in serum (viremia) at vaccination, so that 4 subgroups (20 pigs each, randomly selected) were considered. The AMI (Antibody Mediated Immunity) to PCV2 was evaluated by ELISA serology and IPMA test, and the CMI (Cell Mediated Immunity) by ELISpot technique. All statistical analyses was done using the SAS system V.9.1.3 (SAS institute Inc., Cary, NC, USA). For all analyses, the individual pig was considered as the experimental unit. The significance level (α) was set at 0.05. Differences between groups for PCV2 ELISA S/P, IPMA, IFN-γ SC at each sampling time were compared using an ANOVA test for normally distributed variables and Kruskall–Wallis test for non-normally distributed parameters.
Results and discussion
PRRSV viremic pigs at PCV2 vaccination have a lower ELISA S/P ratio ($p<0.05$) as compared to non-viremic pigs in both subgroups (vaccinated and non-vaccinated) as a consequence of a reduced amount of maternally derived antibody caused by reduced colostrum intake. These differences are not confirmed with IPMA serology. In fact, no differences can be detected in the different groups at vaccination. However, in vaccinated animals even if no seroconversion can be detected, the decline of MDA was reduced as compared to un-vaccinated and a constant level of IPMA antibodies is maintained. Conversely, the cell mediated immune response measured as number of PCV2 specific IFN- SC in vaccinated and PRRS viremic animals seems to be compromised, as demonstrated by the lack of any increase of cells after vaccination in comparison with the non-viremic pigs.
Conclusions
In this study, PRRSV infection did not interfere with the antibody response to PCV2 vaccination but strongly affects the induction of the cell mediated immune response. Based on these evidences, it can be assumed that PRRSV infection directly compromises the cellular components of the early immune response, reducing the number and the activation of the memory T cells specific to PCV2 induced by vaccination.

P250

FIELD EVALUATION OF SEPARATE AND MIXED USE VACCINATION AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME AND HAEMOPHILUS PARASUIS IN NURSERY PIGS

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Introduction: The aim of the study was to test a separated and mixed use vaccination against the porcine reproductive and respiratory syndrome (PRRS) and Haemophilus (H.) parasuis. Material and Methods: The study was conducted in a 1200 head nursery farm positive for PRRSV and Glässer´s disease. A total of 360 piglets were randomized at 26 days of age into three groups. Group A was vaccinated against H. parasuis (Porcilis®Glässer) and PRRS (Porcilis®PRRS) separately, group B with Porcilis®Glässer and Porcilis®PRRS mixed before use, group C was the unvaccinated control group. The compatibility was evaluated by the measurement of the body temperature and a palpation score of the injection site 0, 4, 24 and 72 hours after vaccination. During the nursery and the fattening period the average daily weight gain (ADWG) and the number of runts and losses was evaluated. Additionally blood samples were taken every 2 weeks during the nursery period to perform an HPS oppA-ELISA. First group A was compared to the control group to evaluate the effect of the vaccination, than the group A was compared with group B. Results: No significant difference could be measured regarding the body temperature between group A and group C, while piglets of group B had a significantly higher body temperature 4 and 72h post-vaccination than those of group A. The palpation score was significantly higher in group A at 4 and 24 h compared to the unvaccinated control group. ADWG was significantly higher only during the fattening in the vaccinated groups. The number of losses during the nursery period was significantly higher in group C. Although the number of positive animals in the oppA-ELISA was not different between groups, HPS vaccinated pigs seemed to react earlier. No ELISA positive pigs in the vaccinated groups developed Glässer´s disease. Conclusion: Mixed use vaccination had no negative influence on the effectiveness but was slightly more reactive than a separate vaccination. In this study, the vaccination with Porcilis®Glässer might have influenced the results in the HPS oppA-ELISA.

P251

VACCINATION IN ORDER TO PREVENT PLEUROPNEUMONIA CAUSED BY A NON TYPABLE ACTINOBACILLUS PLEUROPNEUMONIAE STRAIN IN HUNGARY

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Introduction
Actinobacillus pleuropneumoniae is a facultative pathogenic bacterium that causes haemorrhagic-necrotic pneumonia and fibrinous pleuritis in growing pigs throughout the world. On the basis of nicotinamide adenine dinucleotide (NAD) requirement A. pleuropneumoniae biotype 1 (NAD-dependent) and biotype 2 (NAD-independent) can be distinguished, and 15 serotypes can be differentiated. Indirect haemagglutination test is one of the most sensitive and specific serotyping methods.

Material and Method
The examinations were done in a Hungarian swine herd, where acute pleuropneumonia occurred in pigs in spite of using a commercial pentavalent bacterin vaccine.
A total of 13 acute pneumonic lung samples were transported to the laboratory of the Department of Microbiology and Infectious Diseases and inoculated on Tryptone Soy Agar (TSA) containing 10% sheep blood. NAD demand was examined by cross inoculation of the plates with a Staphylococcus aureus strain. Cultures were incubated in aerobic environment at 37°C. After replacing the bacterin vaccine with a toxoid one containing ApxI, ApxII, ApxIII and outer membrane protein, 14 lung samples were collected at the slaughter house for bacteriological examinations.
The isolated A. pleuropneumoniae strains were serotyped using the indirect haemagglutination test. Hyperimmune sera were produced in rabbits against the 15 type strains of A. pleuropneumoniae.

Results
Six A. pleuropneumoniae strains of biotype 1 were isolated from the lesions at the beginning of the examinations. These animals were clinically sick and died in the farm. The isolated strains were considered non-typable as they could not be assigned any serotypes.
In the animals vaccinated with the toxoid vaccine, no clinical signs like coughing, sneezing were observed, and no animals died because of pleuropneumonia caused by A. pleuropneumoniae in the farm. Fourteen of these vaccinated pigs did have chronic lesions at slaughter. From the pneumonic lesions, 14 A. pleuropneumoniae strains were isolated that could not be assigned any A. pleuropneumoniae serotypes.

Conclusion
The toxoid vaccine proved to be effective in the prevention of acute pleuropneumonia (there were no clinical signs and mortality) and chronic lesions were seen at slaughter caused by this untypable A. pleuropneumoniae strain.

P252

SEROLOGICAL RESPONSE OF APP UNSUSPICIOUS GILTS TO VACCINATION WITH PORCILIS® APP

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Introduction: Actinobacillus pleuropneumoniae (APP) is one of the most important respiratory pathogens in swine, especially fattening pigs and most often enters the herd via introduction of subclinically infected animals. Because of this, farms purchasing piglets and gilts are at a high risk for APP infection. Due to antibiotic reduction plans in swine production, the vaccination against APP becomes more and more important. A frequent question in this context is if APP vaccination of piglets and gilts is done properly. Reliable serological data to compare the current ELISA-tests in vaccinated pigs are not available in the moment. Material and methods: On an APP non-suspect farm (depop-repop in 2008 with APP non-suspect gilts, monitored over six years), 20 gilts were randomly chosen for investigation and divided into two groups. Ten gilts were vaccinated with Porcilis® APP and ten unvaccinated served as a control group. All animals were kept under the same conditions. Sampling took place for all 20 gilts routinely for diagnostic reasons. Nasal swabs were taken to exclude APP colonization without serological reaction by PCR testing. First blood samples were taken at 140 days of age (day 0). Vaccination with Porcilis® APP was done twice as recommended by the manufacturer (day 0 and 4 weeks later). Subsequently, the next samples were collected at 3 and 6 weeks after the second vaccination. For serological testing several routine tests were used: ID-screen APP screening indirect ELISA (ID Vet, France), IDEXX APP APX IV-ELISA (IDEXX Switzerland), in-house APX 2-ELISA (IVD GmbH, Germany). The APP-PCR was done according to Schaller et al. 2001.
Results: As expected all pigs showed negative serological and PCR results before vaccination of Porcilis® APP. The control group remained negative throughout the investigations whereas vaccinated pigs showed positive ELISA results in different frequency and quality following vaccination. The PCR controls to exclude APP infection or colonization were negative results for all animals.
Conclusion: This study confirmed seroconversion in Porcilis® APP vaccinated pigs compared to a control group. This demonstrates the potential of this vaccine to introduce a serological response in several commercial available ELISA tests. The appearance of even non-protective antibodies can confirm if whether pigs and gilts are vaccinated; at least for formerly APP naïve animals. Further studies are necessary to confirm this observation e.g. for animals with unknown APP status.

P253

ASSESSING THE CONVENIENCE OF MYCOPLASMA HYOPNEUMONIAE VACCINATION WITH STELLAMUNE® ONE AT 3 DAYS OF AGE IN COMMERCIAL PIG FARMS

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Introduction
Mycoplasma hyopneumoniae (M.hyo) causes enzootic pneumonia (EP), a chronic respiratory disease in pigs responsible for major economic losses worldwide. Early vaccination against M.hyo has the advantage that immunity can be induced before pigs become infected and that less pathogens are present that can interfere with the immune response (Maes et al., 2008). Stellamune® One has been recently approved in Europe to be used in piglets from 3 days of age.
This study aims to evaluate the convenience of administering Stellamune® One, measured as time required for vaccination, during processing at 3 days of age compared to vaccination of suckling piglets at 7 and 14 days of age.
Materials and Methods
A 2,000-sow farm located in Belgium was selected for the study. In total, 740 piglets born to 60 sows were included. Three experimental groups were created according to the timing of vaccination (3, 7 and 14 days) and litters from 20 sows included at random in each group. Piglets in group 1 (n=257) were vaccinated with Stellamune® One (Elanco AH) at 3 days of age at the end of the processing. Piglets in group 2 and 3 (n=245 and n=238) were vaccinated at 7 and 14 days of age with the same vaccine.
Average duration of vaccination (seconds/piglet) was calculated by dividing the total duration of vaccination per litter by the number of pigs in the litter. A group of 3 farm workers performed the intervention, with the same operator vaccinating piglets in the 3 study groups. The vaccination technique was similar across groups. Mean vaccination times were compared across groups with a one way ANOVA at a significance level of 0.05.
Results
In group 1 (3 d of age), average duration of vaccination at the end of the processing was 2.64 s/piglet (SD 0.36). For piglets vaccinated at 7 days of age, it increased significantly ($p<0.05$) up to 4.89 s/piglet (SD 0.78). For pigs in group 3 (14 d of age) the mean vaccination time was 6.04 s/piglet (SD 0.98), significantly higher ($p<0.05$) than groups 1 and 2.
In this farm, vaccination of piglets at 3 days of age allowed a reduction of 3.4 s/piglet when compared to 14 days of age. Under the conditions of this study, vaccinating pigs at 3 days of age was more convenient, meaning labour savings of up to 140 hours/year (17.6 working days) for a team of 3 farm workers.
Conclusion
Vaccination of pigs at 3 days of age with Stellamune® One during the processing requires less time than vaccination at 7 and 14 days of age (2.25 and 3.4 seconds/piglet respectively), being more convenient. In addition, handling of younger, lighter pigs improves the ergonomics of the intervention, with additional benefits for farm workers.

P254

PCV–BACUCHECK™, A NEW TOOL FOR EVALUATING PCV2 VACCINATION COMPLIANCE

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Introduction
Although PCV2 vaccines have drastically reduced the economic impact of the disease, outbreaks still occur as a result of improper implementation of vaccination programs. Therefore, a reliable serological monitoring tool for vaccine compliance can be key to a vaccination program's success. PCV-BacuCheck™, an ELISA that detects and measures antibodies against Baculovirus markers and PCV2, is an easy-to-use and value-added herd monitoring test that verifies PCV-vaccinated piglets versus non-vaccinated piglets. The abstract summarizes the results from field samples originating from multiple countries that were tested in the PCV-BacuCheck™ ELISA.
Material and Methods
The antigen in MSD PCV vaccines is a structural capsid protein encoded by ORF2 of the PCV2 genome. The protein is produced in insect cells with a Baculovirus expression system. The Baculovirus antigen in the PCV-BacuCheck ELISA is based on components of the Baculovirus expression system and the PCV is based on the BioChek PCV2 ELISA. The PCV-BacuCheck™ test is applied to serum samples collected 4 to 7 weeks post vaccination and from piglets not older than 10 weeks of age. A positive result is based on the combination of an average Baculovirus S/P ratio equal or greater than 0.3 with 8 out 10 pigs positive and an average PCV S/P ratio of equal or greater than 0.5. A total of 716 samples were tested from 7 different countries and 40 submissions.

Results
From a total of 716 pigs vaccinated with Porcilis PCV, only 53 samples tested negative. The negative samples originated from 5 different submissions: one was suspected to have a vaccine compliance issue; insufficient background about vaccination procedures was available to make a conclusion from 2 submissions; 2 submissions were suspected to have vaccinated with less than a full dose.

Conclusion
Vaccination against PCV2 is a proven tool in reducing clinical signs and negative impact on various production parameters. As vaccines are costly, implementing proper vaccination strategies is important and having access to a compliance test can support their success. The results support that PCV-BacuCheck™ ELISA is a reliable tool to confirm PCV vaccination status of a herd based on 10 samples collected between 4-7 weeks post-vaccination and from pigs not older than 10 weeks of age. Interpretation of the PCV-BacuCheck results combined with a vaccination quality review can be followed by remedial action in the event of vaccination procedure failure.

P255

EFFICACY OF A NEW PCV2 AND M. HYOPNEUMONIAE COMBINATION VACCINE:PCV2 CHALLENGE STUDIES

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Introduction
PCV2 was first identified as the causative agent of "Postweaning Multisystemic Wasting Syndrome", but is also involved in a number of other disease syndromes which have collectively been named Porcine Circovirus Diseases (PCVD). Vaccination against PCV2 is widely practiced in the pig industry, but from a convenience and animal welfare perspective a ready-to-use one dose combination product against PCV2 and M. hyopneumoniae infection, another major pathogen of finishing pigs, would be a preferred option. The objective of the present studies was to evaluate the efficacy of a new one dose combination product - Porcilis® PCV M Hyo - against PCV2 experimental challenge infection.
Material and Methods
Onset of immunity and duration of immunity were determined in experimental challenge studies. In each experiment, 3 week old pigs were randomly divided into two groups of 15 animals (vaccine and control) at the time of vaccination. At the time of vaccination, serum samples were taken and at the time of challenge as well as 2 and 3 weeks thereafter serum, fecal and nasal swab samples were collected and examined for PCV2 antibodies by ELISA and PCV2 nucleic acid by quantitative PCR (qPCR). In the DOI study, blood samples for serology were also taken at regular intervals between vaccination and challenge. Three weeks after challenge, the pigs were necropsied and the presence of the challenge virus was quantified by qPCR in inguinal and mesenteric lymph nodes, lung and tonsil. For the viral load in serum and swabs, the Area under the Curve (AUC) was calculated and differences in the viral load in serum, swabs, lung and lymphoid tissues between vaccinates and controls were analyzed by the Kruskal-Wallis Test.
Results
In both challenge experiments, mean viral loads were in general in the order of 2-3 log10 lower in the vaccinated pigs for the different samples tested, and the differences between the groups were all statistically significant ($p<0.05$). Vaccination also resulted in a clear antibody response against PCV2 after vaccination, whereas the control group remained serologically negative after the decline of maternal antibody titers until the time of challenge. Following challenge, vaccinates developed an anamnestic response and the animals in the control group started to seroconvert.
Conclusion
The experimental challenge studies indicate that the onset of immunity occurs as early as 2 weeks post vaccination and duration of immunity lasts for 22 weeks. Accordingly, a single vaccination of animals at 3 weeks of age may protect fattening pigs against PCVD infections during the complete production life cycle.

P256

EFFICACY OF A NEW PCV2 AND M. HYOPNEUMONIAE COMBINATION VACCINE: FIELD STUDIES

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Introduction
PCV2 and M. hyopneumoniae (M hyo) are the two most prevalent pathogens in finishing pigs. PCV2 causes the disease complex named "Porcine Circovirus Diseases" (PCVD). At the moment, subclinical PCV2 infection, characterized by poor growth performance in apparently healthy pigs, is the major presentation of PCVD. M hyo is the causative agent of enzootic pneumonia and is, together with PCV2 and other pathogens, implicated in the Porcine Respiratory Disease Complex. Vaccination against PCV2 and M hyo is standard practice in the pig industry, but a convenient ready-to-use one dose combination product has not been available in Europe until now. Here, the efficacy in the field of such a new combination vaccine - Porcilis® PCV M Hyo - is described.
Material and Methods
Ten studies were performed according to a controlled, randomized and blinded design in herds with a PCV2 and/or M hyo infection. Three week old piglets were allocated randomly, within litters, to one of two groups of ±300 piglets each. The pigs in one group were vaccinated with Porcilis® PCV M Hyo and the pigs in the control group were injected with PBS. The primary efficacy parameters were PCV2 viremia, lung lesions at slaughter and average daily weight gain (ADWG) during finishing. Secondary parameters were overall ADWG during the study, mortality, morbidity, pleurisy lesions and PCV2 shedding. The pigs were weighed at vaccination, at transfer to the finishing unit and before slaughter. The lungs were examined at slaughter to score the severity of typical M hyo lesions. The level of PCV2 was tested by quantitative PCR (qPCR) in serum samples from ±30 pigs per group approximately every 4 weeks and in rectal and/or nasal swabs taken at the same time points in 8 of the studies. Differences were analyzed statistically.
Results
A significant reduction in PCV2 viremia was observed in all 10 studies and the reduction in lung lesions was statistically significant in 5 of the 7 farms with a substantial M hyo infection. The reduction in lung lesions varied between 6 and 75% (overall mean: 31%), and the increase in ADWG during finishing ranged between 5 and 65 g/day on the individual farms (mean: 26 g/day). The ADWG improvement was significant in 5 of the studies. The lack of significance in the remaining studies may be the result of the relatively small number of study pigs as well as potential other confounding factors, such as a late onset of infection. The secondary efficacy parameters improved in almost all studies, but the differences were not always statistically significant.
Conclusion
These 10 studies support that Porcilis® PCV M Hyo is efficacious against PCV2 and/or M hyo infections under field conditions.

P257

SAFETY OF A NEW PCV2 AND M. HYOPNEUMONIAE COMBINATION VACCINE: LABORATORY AND FIELD STUDIES

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Introduction
The majority of piglets in the pig industry are vaccinated against PCV2 and M. hyopneumoniae, the two most prevalent pathogens in finishing pigs. At the moment, most vaccines in the market are single products that require one or two vaccinations or have to be mixed before injection. A safe and efficacious ready-to-use one dose combination product would therefore be desirable from a practical and an animal welfare perspective. Here, the safety of such a new combination vaccine - Porcilis® PCV M Hyo - is described.
Material and Methods
The safety studies were done according to the Ph.Eur in 17-24 day old piglets. In the laboratory study 2 groups (Porcilis® PCV M Hyo or PBS control) of 12 SPF piglets were used and the field study was done in 3 farms (at least 28 pigs per group). The health of the piglets was checked daily and rectal temperatures were measured for 4 days post-vaccination (dpv). In addition, the injection site was palpated for local reactions for 14 days. In the laboratory study, all piglets were sacrificed to examine the injection site and in the field study animals were weighed before vaccination and at the end of the study (21 dpv). Furthermore, in the 10 field efficacy studies that were performed with the vaccine (total: ± 3100 pigs per group) safety parameters (general health/local reactions/average daily weight gain (ADWG) until the end of the nursery period) were recorded. The ADWG was compared using a mixed model ANOVA.
Results
In the laboratory study, none of the animals developed local or systemic reactions and no macroscopic abnormalities were observed at the injection site at necropsy. A transient rise in temperature ($p<0.05$), which was well below the limit allowed by the Ph.Eur, occurred 4h post vaccination. In the field safety study, treatment resulted in local reactions with a maximum diameter of 1 cm in the vaccinates and 0.3 cm in the controls (observed at 4h post vaccination only). The numbers of piglets with a deviation from the normal general health were similar in both groups (vaccinates: 6%; controls: 5%). A difference in rectal temperature ($p<0.05$) was again only measured 4h post vaccination. ADWG was not different between groups during the observation period. In the field efficacy studies, local reactions were observed in approximately 1% of the pigs in both groups (max. size: 2 cm) with a maximum duration of one day. A deviation from the normal general health was found in 3% of the vaccinates and 1% of the controls. There were no significant differences in ADWG during the nursery phase.
Conclusion
The data support that the new Porcilis® PCV M Hyo vaccine can be administered safely to 3 week old piglets.

P258

EFFICACY OF A NEW PCV2 AND M. HYOPNEUMONIAE COMBINATION VACCINE: M. HYOPNEUMONIAE CHALLENGE STUDIES

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Introduction
M. hyopneumoniae is a respiratory pathogen and is the primary agent of enzootic pneumonia (EP), a chronic disease in pig herds. M. hyopneumoniae in association with other bacterial and viral agents, such as PCV2, is also implicated in the Porcine Respiratory Disease Complex (PRDC). Vaccines against M. hyopneumoniae are routinely used in the pig industry, but for the convenience of the user and to reduce the number of injections given to piglets, a ready-to-use one dose combination product with PCV2 would be highly desirable. The objective of the present studies was to evaluate the efficacy of a new one dose combination product - Porcilis® PCV M Hyo - against M. hyopneumoniae laboratory challenge.
Material and Methods
Onset of immunity (OOI) and duration of immunity (DOI) were determined in experimental challenge studies in accordance with Ph.Eur monograph 2448 for EP vaccines. In each experiment, 3 week old pigs from an SPF herd were randomly divided in two groups (vaccine and control) at the time of vaccination; OOI: 19 animals per group, DOI: 40 pigs per group. Blood samples were taken just before vaccination and just prior to challenge. In the DOI study, blood samples were taken at regular intervals between vaccination and challenge.
M. hyopneumoniae challenge was performed intratracheally on two consecutive days with a culture of a Danish field isolate (provided by Dr N. Friis, National Veterinary Laboratory, Copenhagen) at 7 or 24 weeks of age. Three weeks after challenge, the pigs were necropsied to evaluate lung lesions which were scored according to Goodwin; the maximum score is 55. The differences in lesion scores were analyzed by the Wilcoxon Rank Sum test.
Results
A serological response was seen in the vaccinated groups in both challenge studies and the control animals remained seronegative until challenge. However, almost all the control animals responded serologically to the challenge infection. At necropsy three weeks post challenge, the median M. hyopneumoniae lung lesion scores in the control group were 9.2 and 10.0 in the OOI and DOI study, respectively. Median lesion scores of the vaccinated groups ware 77% (OOI study) and 50% (DOI study) lower than in the control groups ($p<0.05$).
Conclusion
The experimental challenge studies indicate that immunity against M. hyopneumoniae was provided for 21 weeks post-vaccination with Porcilis® PCV M Hyo. This means that a single vaccination of animals at 3 weeks of age can protect fattening pigs against EP until the end of the finishing period.

P259

FIELD EFFICACY OF THREE PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINE COMBINATIONS UNDER SPANISH FIELD CONDITIONS

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Introduction
The objective of this study was to investigate the efficacy of three combinations of vaccines against PCV2 and Mycoplasma hyopneumoniae (M hyo) under field conditions.
Material and methods
This study was performed in a farrow-to-finish farm located in Huesca Spain. Signs related to M hyo infection during fattening, and associated with lung lesions at slaughter were present before the start of the trial.A group of 532 healthy 3-week-old piglets was included in the study and allocated to the following treatment groups using randomization according to initial live weight and gender:
- T1 (n=169): CIRCOVAC + STELLAMUNE UNO
- T2 (n=169) : PORCILIS PCV + M+PAC
- T3 (n=169) vaccinated with CIRCOFLEX + MYCOFLEX
- Controls (n=25) kept unvaccinated.

Vaccines were administered at the same time but separately in T1 and T2 and mixed prior to administration in T3. Pigs were housed grouped by gender and apparent size. Pigs from the different groups were co-mingled in the fattening pens only.
Individual live weight was recorded at 3, 11 and 23 weeks of age (slaughter age). Mortality, treatments as well as lungs scores in at least 60% pigs per group were recorded. Serologies were performed to assess PCV2 and M hyo passages.
Results
At slaughter age, weights of the vaccinated pigs (T1, T2 or T3) were +3.4 to +4.9 kg higher than weights of non-vaccinated pigs ($P<0.05$). Average daily gain from weaning-to-slaughter was +23 to +33 g/day higher than in controls ($P<0.05$). At 24 weeks of age, the weight of the pigs in groups T1 and T2 were +0.8 and +1.5 kg higher than those in T3 ($p>0.05$). During fattening, growth of the pigs vaccinated with T3 was not statistically different than that of the non-vaccinated pigs (724 g/d vs. 700 g/d, $P>0.05$) contrary to the other vaccinated group (735 g/d and 740 g/d for T1 and T2 resp.). Throughout the study, mortality and treatment rates in group T1 (5.3% and 36%, respectively) were numerically lower compared to the other vaccinated groups (both 7.7% and 42%). At the abattoir, there were numerically less lesions in the lungs of the pigs vaccinated with T1 and T2 than those vaccinated with T3 ($P>0.05$). PCV2 and M hyo natural challenge were confirmed serologically. Circulation of SIV and PRRSv was also evidenced by additional investigations.
Discussion and conclusion
Under the conditions of the study, only separated administrations of PCV2 and Myco hyo vaccines provided improved results on ADWG against both a field PCV2 and M hyo challenge contrary to the other combination tested.

P260

FEEDBACK PROGRAMMES ARE AN ESSENTIAL PART OF ADULT HERD IMMUNISATION PROGRAMMES

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Over the years, feedback programmes have been restricted by veterinarians because of fears of moving pathogens and the drive for all-in/all-out appears to contradict deliberate faecal movement. These fears have substance, when pathogen movement between farming units (inter-unit) is considered, as each farming unit will have a different megagenomic pattern. However, an intra-farm unit this fear needs to be rationalised. It is a vital component of healthy pig production that the adult females are immune to all (or as many as possible) pathogens (real and opportunistic) that are currently on the farm. While man continues to develop vaccines, our current primitive state of knowledge of pathogens means that we do not have a full armory. The use of a structured feedback programme can supplement this lack of science.

Different farm units are defined when there is a physical separate locations for breeding, nursery and finishing, this would be considered to be three different farm units. Whereas a farrow to finish farm would be considered one farm unit.

Design of an effective feedback programme
There are three components of a feedback programme:
Reproductive – using placenta, stillborn and mummified piglets – to gilts pre breeding
Parvovirus, Porcine Circovirus, Teschovirus,

Intestinal – using piglet and nursery faeces – to sows pre-farrow to enhance colostrum
Clostridia spp, Escherichia coli, Pasteurella multocida, Porince Epidemic Diarrhoa, Rotovirus, Staphylococcus hyicus, Streptococcus suis, Transmissible Gastroenteritis Virus.

Respiratory – using rope in the nursery – to gilts pre breeding
Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Haemophilus parasuis, Mycoplasma spp, Pasteurella multocida, Porcine Circovirus and Porcine Reproductive and Respiratory Syndrome.

Summary note
If feedback was not available, for legal reasons, the control of pathogens such as Porcine Epidemic Diarrhoea would be extremely difficult and result in the death

P261

A FIELD TRIAL WITH PORCILIS PCV2 M HYO IN HUNGARY

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Introduction
PCV2 and M. hyopneumoniae (M hyo) are the two most prevalent pathogens in finishing pigs. Vaccination against PCV2 and M hyo is standard practice in the pig industry, but a convenient ready-to-use one dose combination product has not been available up till now. Here, a study to assess the safety and efficacy of such a new combination vaccine - Porcilis® PCV M Hyo –under field condition, is presented.
Material and Methods
The study was designed as a controlled, randomized and blinded field trial and conducted in a farrow-to-finish herd in Hungary with confirmed PCV2 and M hyo associated diseases. Three week old piglets were allocated randomly, within litters, to one of two groups of ±300 piglets each. The test group was vaccinated with Porcilis® PCV M Hyo and the control group was injected with PBS as placebo. The primary efficacy parameters were PCV2 viremia, lung lesions at slaughter and the average daily weight gain (ADWG) during finishing. Secondary parameters were overall ADWG during the study, mortality, morbidity, pleurisy lesions and PCV2 shedding. The pigs were weighed at vaccination, at transfer to the finishing unit, and before slaughter. The study animals were fattened on separate finishing units (A and B). The severity of typical M hyo lesions in the lungs was scored at slaughter according to the method of Goodwin and Whittlestone. The PCV2 virus load was tested by qPCR in serum samples taken from ±60 pigs per group every 3-4 weeks and in rectal and/or nasal swabs taken at the same time points.
Results
A significant reduction in PCV2 viremia was observed and also the reduction in lung lesions from 16.1 in the control group to 4.1 in the vaccinated pigs was statistically significant. The increase in ADWG during finishing was 65 g/day in finishing unit A and 6 g/day in unit B. Also the ADWG during the nursery phase and the overall weight gain was improved in the Porcilis PCV M Hyo group. The severity of pleurisy and shedding of PCV2 was significantly reduced in the vaccinated pigs, but no differences were seen in mortality and morbidity. No vaccine related local or systemic reactions were observed.
Conclusion
Porcilis® PCV M Hyo proved to be safe and efficacious against PCV2 and/or M hyo infections under field conditions.

P262

PRACTICAL EXPERIENCE AFTER USING IDAL AS AN ALTERNATIVE TO TRADITIONAL INTRAMUSCULAR VACCINATION IN PIGS

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Introduction
IDAL (IntraDermal Application of Liquids) is a device for applying vaccines intradermally (ID).
The device was marketed in DK early 2013 and information of practical handling and customer perception was missing. This report concludes an investigation about the practical use of IDAL on farms.
Materials and Methods
IDAL was introduced in 33 farms to vaccinate with either Porcilis® M Hyo ID ONCE or Porcilis® PRRS.
Vaccinations were mainly given to suckling piglets from 14 days of age and piglets at or after weaning. In all cases, an MSD AH field advisor demonstrated the technical handling of IDAL.
After 6 months, farms were revisited and a questionnaire administered with emphasis on advantage and disadvantage of the device compared to intramuscular injection of vaccines. The questionnaire focused only on practicalities, perception and satisfaction of using the device.
Further, assessment of the biosecurity issue in relation to sending IDAL for service was started with the Danish SPF-company, as this might pose a risk of cross-contamination across devices in the event that devices are mixed up during service. Henke Sass Wolf (HSW) was visited with Quality Auditors from Danish SPF-company and all procedures regarding IDAL service were audited.

Results
A total of 33 herds were included with 30 vaccinating against M. hyo and 7 against PRRS. During the test period, 12 of the farms decided to stop using IDAL. The remaining 21 farms continued using IDAL. Although all 33 farms expressed a wish that the IDAL was lighter, only 1 farm stopped usage for this reason. All 21 farms expressed satisfaction with IDAL compared to IM vaccination. Most of the 12 farms that discontinued IDAL would like to continue if certain issues are solved. When used in suckling piglets, the time reduction is limited. Time reduction is significant in weaners – up to 50%, including preparing and cleaning of the IDAL.
As a result of the QA visit from SPF auditors, a certificate of approval was issued and it was agreed to assess the procedure via a yearly QA visit.
Conclusions
IDAL was well received as an alternative to IM vaccination in most farms. In suckling piglets, the time spent on vaccination was not reduced due to other causes, but vaccination of weaners went faster and almost reduced the time by half. Twenty-one (21) farms continued to use IDAL. Only one farm stopped due to technical issues. The remaining 11 farms switched back to IM vaccination for reasons not related to the technical performance. Biosecurity of the current procedure was evaluated to be acceptable to fulfill Danish SPF-demands. IDAL is a fast and safe alternative to IM-vaccination.

P263

PIGLET'S STRATEGIC VACCINATION: A TOOL TO CONTROL PRRS INFECTION IN THE NURSERY

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Introduction
The capability of some PRRS MLV vaccines to reduce viremia and viral shedding has been demonstrated in previous experimental trials. The objective of this trial was to evaluate temporary strategic piglet vaccination as an effective tool to reduce PRRS viral transmission in the nursery.
Materials and Methods
The trial was done in a closed herd of 350 sows located in the province of Barcelona (Spain). The farm was PRRS positive but classified as stable. Gilts were vaccinated and revaccinated before entering the farm, and the sows were herd vaccinated every 4 months (Porcilis® PRRS). Piglets were vaccinated against M. hyopneumoniae at 15 days of age, and against PCV2 just before weaning at 3 weeks of age.
In March 2013, some problems were detected, such as anorexia, retarded growth, respiratory disease, Glässer's Disease, etc. Mortality in the nursery increased significantly, reaching values of 7-10%. Serology and PCR of 6 to 9 week old animals confirmed involvement of PRRSv. In the sows, no clinical signs were detected, but stability was confirmed through negative PCR of 3 week's piglet's blood samples. A Salmonella outbreak at late nursery complicated the situation, and PRRS control measures were not initiated until the end of May, when it was decided to vaccinate 14 day old piglets with Porcilis® PRRS, intradermally with the IDAL device, over a period of 12 weeks.
After vaccination, the success of the strategy was determined by measuring the absence of PRRSv in nursery. Mortality data of pre- and post-vaccination batches were also compared (ANOVA and Tukey Test).
Results
The samples from the first non-vaccinated piglets after a vaccination period of 12 weeks confirmed that PRRS virus was not circulating in the nursery. Non-vaccinated piglets of 5, 7 and 9 weeks of age were bled, and serology (Idexx) and PCR were done and were negative. Mortality rate was reduced by 70% comparing the results of the non-vaccinated batches to the PRRS vaccinated batches (from 6,7% to 1,9%, $p<0,01$). In addition, respiratory disease and medication costs reduced, and growth rate improved. Considering only the reduced mortality, the investment of strategic piglet vaccination was returned after the first 12 weeks of improved mortality results.
Conclusion
Strategic piglet vaccination is an effective tool to control viral transmission in the nursery, being therefore a valid alternative to a sanitary depopulation of the facilities. Besides the epidemiological effect, the improved productive parameters ensure the profitability of the vaccination.

P264

TOWARDS AN EXPLORATION OF PRRSV VACCINE EFFICIENCY IN SILICO

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Introduction:
Vaccination is the main control strategy against the Porcine Respiratory and Reproductive Syndrome virus (PRRSv), a major concern of the swine industry. However, none commercial vaccine is efficient to eradicate the infection mainly because they only confer a partial protection of the host. Moreover, PRRSv exhibits a high virulence variability among strains and current vaccines provide only a partial cross-protection. Consequently, improving vaccine efficiency at the host level is still a major challenge for PRRS control. The immune mechanisms involved in vaccination and determining the host protection are not yet fully identified and seem variable among PRRSv strains. Considering this variability context and the complexity of immune mechanisms, we chose a modelling approach to tackle this issue. We focused on modified-live vaccines, as they are assumed to be the more efficient against PRRSv.

Materials and Methods:
From the literature review of PRRSv immune dynamics, we developed an original model of the PRRSv within-host dynamics representing the immune mechanisms at the between-cell scale. This model describes the evolution over time of (i) the within-host viral load and (ii) the major immune components (involved in the PRRSv-target cell interactions, the innate response, the adaptive response orientation and the cytokine regulations). We used this model to simulate a PRRSv infection of a vaccinated pig (i.e. starting with memory response, 2 levels of memory activation has been tested) considering both PRRSv virulence (3 levels: low, reference and high) and exposure (3 intensities x 2 durations) variabilities.

Results:
Our results exhibited a clear vaccination efficiency to reduce the infection duration, whatever the strain virulence and the exposure, but no efficiency on either the infection severity (characterised by the area under the curve of the viral titer) or the viral peak.
The higher the memory activation level and the virulence level, the higher the vaccine efficiency. The innate mechanisms (phagocytosis, cell infection, viral replication) were not involved in the vaccination efficiency. The adaptive response orientation associated with the infection duration decreases exhibited a high variability depending on virulence and exposure level. High vaccine efficiency was globally associated with high levels of regulatory response. Finally, the higher the cytolysis and neutralisation activities the higher the infection duration decreases due to vaccination.

Conclusion:
We proposed an original and adapted method to explore the efficiency of vaccination strategies. Our results provide insights to improve the vaccination efficiency, in particular regrading the adaptive response orientation towards the regulatory. As we here considered a full cross-protection between viral strains, this model has to be adapted to take into account the genetic heterogeneity observed in the field.

P265

EFFICACY OF A NOVEL E. COLI/CLOSTRIDIUM VACCINE: INDUCTION OF PROTECTIVE ANTIBODIES

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Introduction
Sows and gilts are routinely vaccinated to protect their offspring against neonatal diarrhea caused by Enteropathogenic E. coli (ETEC). Because C. perfringens is also often involved in neonatal disease, combination vaccines have been developed against ETEC and Clostridium spp.. For these vaccines, it is crucial that sufficient vaccine-induced antibodies against the virulence factors of both organisms reach the progeny via the colostrum. In earlier challenge studies with E. coli strains expressing the virulence factors F4ab, F4ac, F5, F6 and LT, titer levels in ELISA were related to the reductions in mortality and severe diarrhea, and minimum protective titers were determined. Also for C. perfringens type C it has previously been shown that vaccination can induce antibodies in colostrum that protect against hemorrhagic enteritis. In the present study, the functionality of the colostrum antibodies after vaccination with Porcilis® ColiClos was further characterized.
Materials and Methods
Pregnant sows and gilts (n=24) were vaccinated twice with Porcilis® ColiClos or with a placebo (n=4). All animals were vaccinated at 6 and 2 weeks before farrowing and colostrum samples were taken. The samples were tested by ELISA for antibodies against the ETEC virulence factors F4ab, F4ac, F5, F6 and LT and the C. perfringens β-toxin that are included in the vaccine. The functionality of these antibodies was determined by haemagglutination inhibition (fimbrial antigens), neutralization of the cytopathic effect on Vero cells (LT) and inhibition of mouse toxicity (β-toxin).
Results
The ELISA antibody levels correlated with haemagglutination inhibition titers and LT and β-toxin neutralization titers. The inhibition/neutralization titers against the E. coli antigens in colostrum samples were all ≥ 7 log2 and they contained 20-50 IU/ml of anti β-toxin antibodies. No antibodies that reacted in the functional assays were found in the placebo control group.
Conclusions
The new vaccine Porcilis® ColiClos induces protective antibodies in colostrum that inhibit the binding activity of the E. coli F4ab, F4ac, F5 and F6 fimbriae and neutralize E. coli LT and the C. perfringens β-toxin.

P267

EFFECT OF PRRS INFECTION ON PCV2 VACCINATION EFFICACY AND MEASURES TO CONTROL THE NEGATIVE IMPACT

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Introduction
Common questions in modern pig production are how PRRSv recirculation impacts PCV2 vaccination, what the impact is on productivity parameters or what actions can be taken to prevent these situations. The objective of this study is to describe the interaction between PRRS and PCV2 and to demonstrate that PRRS control is key in PCV2 vaccination efficacy.
Materials and Methods
The study was conducted in a 3000 sow farm (site 1+2), PRRS positive but stable. In 2014, production rates of the fattening units began to deteriorate and clinical signs compatible with PCV2 infection were observed. Piglets were vaccinated against PCV2 in the nurseries, but it was detected that PRRSv was recirculating in nurseries, mainly at 8 weeks of age (serology and PCR positive results). In order to control PRRSv recirculation, it was decided to implement a strategic and temporal PRRS vaccination in 14 day old piglets (Porcilis® PRRS via intradermal). A total of 22 097 piglets were vaccinated over a 12 week period. Production data of the fattening units such as mortality, Average Daily Gain (ADG) and medication costs were recorded, and the results of the vaccinated batches (from 21/7/14 to 30/9/14) were compared with previous non-vaccinated animals (from 23/4/14 to 20/7/14). Control of the PRRSv infection in nurseries was evaluated by serology.
Results
Samples obtained from the first non-vaccinated piglets after 12 weeks of vaccination confirmed that PRRSv no longer circulated in the nursery. Non-vaccinated 3, 6 and 9 week old piglets were bled for serology and PCR and were confirmed negative. With respect to production data, the results of the non-vaccinated batches were not as good presumably because of the reduced PCV2 vaccine efficacy linked to a PRRS infection. Production parameters were significantly improved during and after PRRS vaccination, with similar results as prior to PRRSv infection. ADG of the fatteners improved from 652,2g/day in the non-vaccinated batches to 690,5g/d in the vaccinated animals (p<0,001). Medication costs were not significantly different (2,31vs 2,42€ /pig, p=0,709). Mortality was significantly improved from 6,8% in the non-vaccinated animals to 2,9% in the PRRS vaccinated animals (p<0,001).
Conclusions
PRRSv recirculation in the nursery may affect the efficacy of PCV2 vaccination. Strategic temporary intradermal PRRS vaccination of 14 day old piglets with Porcilis® PRRS over a 12 week period helped to control PRRS infection in nurseries and resulted in a clear improvement in production parameters during the fattening period.

P268

EFFICACY OF A NOVEL ONE SHOT COMBINATION VACCINE AGAINST SIMULTANEOUS ENZOOTIC PNEUMONIA (MYCOPLASMA HYOPNEUMONIAE) AND SUBCLINICAL PORCINE CIRCOVIRUS (PCV2) INFECTION IN THE PRESENCE OF AVERAGE MATERNALLY DERIVED PCV2 IMMUNITY IN PIGS

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Introduction
The primary objective of the study was to assess the efficacy of a ready-to-use combination vaccine consisting of a PCV2 subunit and inactivated Mycoplasma hyopneumoniae (M. hyo) strain J [Porcilis PCV M Hyo (Intervet International BV, The Netherlands)] against M. hyo clinical and PCV2 subclinical infection, under field conditions. Serological examination at 3 weeks of age showed that PCV2 maternally derived antibodies were at the level of 9.38 log2 which is considered as average (level≥10 log2 considered as high).
Material and Methods
The study was performed according to a controlled, randomised and blinded design in a Greek swine unit with active Enzootic pneumonia (EP) and subclinical PCV2 infection. In total, 625 healthy three week old suckling piglets were allocated randomly, within litters, to one of two groups. First group piglets were intramuscularly vaccinated with the test product and the 2nd group animals (control) were injected with buffered saline, at 3 weeks of age. Vaccination efficacy investigation was primarily based on alterations in lung lesions at slaughter (LLS) and average daily weight gain (ADWG) during finishing.
Results
No local or systemic safety reactions were observed due to vaccination. LLS and ADWG values were significantly better in the vaccinated group when compared with controls. In detail, mean LLS was 17.1 for the control animals and 10.6 for vaccinated pigs. Similarly, pigs immunized in face of MDA had 34 grams better ADWG when compared with non-vaccinated pigs, for the total trial period. Additionally, significant reduction of PCV2 viraemia was observed in vaccinated animals when compared with the controls (0.35 vs 2.20 mean log10 DNA copies PCV2 /per µl DNA extract, respectively).
Conclusion
Although maternally derived immunity can interfere with priming of piglets' immune response, the results from this study suggested that the test product is safe and effective as one-shot administration, against concurrent clinical M. hyo and subclinical PCV2 infection in 3 week old pigs with average maternal antibody levels.

P269

EVALUATION OF THE VACCINATION AGAINST CLOSTRIDIUM PERFRINGENS TYPE A – A CASE REPORT FROM GERMANY

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Introduction: Regarding pig production, the most important isolates of Clostridium (C.) perfringens are type A and C, which primarily affect suckling piglets during the first three days of life. Suckling piglets infected with C. perfringens type C may show a bloody diarrhea, as production of toxins will cause necrotic lesions in the intestine. Often however, piglets may die suddenly without any clinical signs. Piglets with type A infections usually show a milder, watery diarrhea and the mortality rate is lower, but sudden deaths can occur as well. When piglets do survive the disease, they usually scour and remain runts for the rest of their lives. Over the past years there has been a growing incidence in Germany regarding C. perfringens type A as the major cause of diarrhea in suckling pigs. Before 2012, the only option to protect the suckling piglets against C. perfringens type A had been to vaccinate their dams with autogenous vaccines. Now pig producers can use a registered vaccine (CLOSTRIPORC A) for sows, that will protect piglets effectively against C. perfringens type A α- and β2-toxins.
Material and Methods: The production data of nine farms with a total of 4080 sows were compared from before and after vaccination with CLOSTRIPORC A. All nine farms had been identified as C. perfringens type A α- and β2-toxin positive in samples of diarrhea from suckling piglets. Mortality rate of piglets, weaned piglets per sow per year and weight of piglets at weaning were monitored during a period of six months before and after vaccination. The results are presented as an average of all nine farms.
Results: Vaccinating the sows helped to reduce the mortality rate of suckling piglets from 11% to 7.5%. Before starting the vaccination program farmers weaned 23.9 piglets per sow per year. This average could be increased to 25.1 piglets per sow per year. The average weight at weaning of piglets was increased by 250g (from 7.1kg to 7.35kg).
Conclusion: If diarrhea in suckling piglets is caused by Clostridium perfringens type A as the major pathogen, vaccinating the sows is the ideal solution.

P270

VACCINATION OF PIGLETS WITH A GENOTYPE 1 MODIFIED LIVE PRRS VIRUS VACCINE DELAYS TRANSMISSION BETWEEN PIGS AND DECREASES VIRAL LOAD IN A ONE-TO-ONE TRANSMISSION EXPERIMENT.

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INTRODUCTION
Control of porcine reproductive and respiratory syndrome (PRRS) relies on four pillars: early diagnosis and monitoring, biosecurity, herd management and immunization. Any intervention leading to a decrease of the likelihood of transmission within or between farms is positive for the purpose of controlling the infection. The present study was designed to assess the transmission of genotype 1 PRRSV by contact on a one-to-one basis to and from vaccinated pigs.
MATERIAL AND METHODS
Fourty-seven 3-week-old PRRSV-naïve piglets were divided in two groups: vaccinated animals (V, n=22; vaccinated IM with dose of a commercial modified live vaccine (MLV) (PORCILIS PRRS® MSD Animal Health) and unvaccinated animals (NV, n=25). On the 30th day post-vaccination (dpv), 19 NV pigs were separated and inoculated IN with 2 ml of a genotype 1 PRRSV isolate (strain 3267, 93.4% of global nucleotide similarity to the vaccine) containing 105.5 TCID50 ml-1 (from now on designated as "seeder" pigs (S)). After 48h, S pigs were mixed with V and NV pigs on a one-to-one basis (1S:1V, n=13 and 1S:1NV, n=6). This was designated as contact phase 1. Once a V animal was detected as viremic following contact with a S pig, the infected V was transferred (in less than 24 h) to a new pen where it was left in contact with a new V pig (reserved from the initial stock, n=9) for at least 14 days. Blood samples were taken at 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21 days post-contact (dpc) with the S pigs. The newly 1 infected-V: 1V groups were also bled every 2-3 days after the introduction of the infected V. Quantification of PRRSV in sera was done by one step Taqman RT-PCR (ORF7) the same day of collection of the samples. For discriminating vaccine from challenge virus, the first positive sample by qRT-PCR of all V pigs was sequenced (ORF5).
RESULTS
All S were viremic by 2 days post-inoculation (dpi) and remained so at least for the following 17 days with 70% of them still viremic at 21 dpi. All NV pigs (6/6) became viremic after contact with S pigs (5.5±2 days after contact) with an average duration of viremia of 15.5±2 days. Regarding V pigs exposed to an S, although all (13/13) became finally infected, the first detection of viremia occurred at 12.8±3 dpc, namely one week later compared to NV and the average duration of viremia was reduced by more than 50% (6.6±5 days) compared to NV. Transmission from vaccinated-infected pigs occurred but the mean length of viremia was only 4.3±3 days for newly infected V contacts.
CONCLUSION
In the present study, vaccination of piglets significantly reduced infectious pressure and slowed transmission although did not fully prevent transmission of PRRSV.

P271

AN INTRAMUSCULARLY ADMINISTERED SALMONELLA TYPHIMURIUM ATTENUATED VACCINE (S.TYPHIMURIUM ΔZNUABC) INDUCES PROTECTION VERSUS S. TYPHIMURIUM INFECTION IN PIGLETS.

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INTRODUCTION:
Salmonella enterica serovar Typhimurium (S.Typhimurium) is the major cause of human salmonellosis due to consumption of pork products.
Vaccination represents a complementary solution to control Salmonella spp. in positive farms. Recently, we had demonstrated that an orally administered mutant strain of S. Typhimurium (S.Typhimurium ΔznuABC) was protective versus salmonellosis in mice and pigs.
Here, we have assessed if S.Typhimurium ΔznuABC, intramuscularly administered, is also safe and effective in piglets.
MATERIALS AND METHODS:
Twenty-five piglets were divided in 5 groups. Attenuated S. Typhimurium ΔznuABC strain was intramuscularly administered at concentration of 104 CFU in group A and orally at 5x107 and 5x105 CFU in group B and C, respectively. Group D was the control group and group E was orally challenged with 4x108 CFU of wild type S.Typhimurium at 6 weeks after vaccination of other piglets. Afterwards all groups were allocated in the same pen for two weeks to favor contact among uninfected and shedder animals (seeder model).
Feces were collected once a week after vaccination and after contact to estimate the bacteria shedding. Four weeks after animals contact, samples of ileocecal lymph nodes, ileum, caecum, and colon, were collected during necropsy and then were submitted to microbiological and histological analyses.
RESULTS:
Attenuated S.Typhimurium ΔznuABC is safe because no clinical signs of salmonellosis was recorded after parenteral and oral vaccination. In addition, it is isolated only in feces of orally vaccinated animals for a limited period of time. The attenuated strain is effective because it reduces fecal shedding, organs lesion and colonization of wild type S. Typhimurium in vaccinated groups. In particular, gross lesions, ileum wall thickening and reactivity of ileocecal lymph nodes, were recorded in group D and C. Histologically, villi conglutination and necrosis were associated to vasal congestion and lymph nodes depletion in group D. Similar lesions were observed in group C, that was the most affected of the vaccinated groups.
DISCUSSION:
In conclusion, intramuscular and high dose oral administration of the S.Typhimurium ΔznuABC mutant strain is protective against infection with a virulent S.Typhimurium strain. Vaccination is a method suggested to decrease Salmonella sp. prevalence in swine farms and to prevent its dissemination through the pork production chain.

P272

EVALUATION OF THE PORCINE CIRCOVIRUS TYPE 2 (PCV2) INFECTION DYNAMICS OF DIFFERENT CIRCOVAC® VACCINATION PROGRAMS IN PIGLETS SUFFERING FROM A NATURALLY OCCURRING PCV2–SUBCLINICAL INFECTION

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Introduction
Porcine circovirus type 2 (PCV2) is one of the most prevalent viruses that cause great economic losses in the worldwide pig industry. The widespread use of vaccines has reduced the presence of clinical outbreaks associated to this virus. Therefore, nowadays, PCV2 vaccines have shown to maintain production performance under PCV2 subclinical infection scenarios. PCV2 vaccination in piglets is mostly recommended from 3 weeks of age (woa) onwards. The objective of this study was to determine if the age at vaccination could influence the serological and virological outcome of PCV2 vaccination in a subclinical infection scenario.
Material and methods
At 2 woa, 56 piglets born from non-PCV2 vaccinated sows were bled, weighted and allocated to 4 treatment groups of 14 pigs balanced by S/P ELISA value, bodyweight and sex. The pigs from groups 1, 2 and 3 were vaccinated with a single dose of CIRCOVAC® vaccine 0.5 mL IM at 3, 6 or 10 woa, respectively, and pigs from group 4 were kept unvaccinated. Animals were allocated at weaning (3 woa) into pens according to the treatment group. The pigs were bled and monitored for their clinical status, presence of PCV2 antibodies (Ingezim Circo IgG 11.PCV.K1®, Ingenasa) and PCV2 viral load (LSI VetMAX™ Porcine Circovirus Type 2-Quantification, Life Technologies) in serum samples at 2, 6, 10, 14, 18 and 25 woa.
Results
No clinical sign evoking a PCVD was observed during the course of the study. The serological and virological results confirmed the circulation of the virus. The animals vaccinated at 3 and 6 woa showed a significant ($p<0.05$) increase in ELISA S/P values after vaccination. In the group vaccinated at 10 woa, it was not possible to distinguish the antibody response derived from vaccination to the one due infection. The number of viremic animals was clearly decreased in pigs vaccinated at 3 and 6 woa as compared to the control group ($p<0.05$ at 14 and 18 woa). On average, the pigs vaccinated at 3 and 6 woa experienced a lower viremia than those left unvaccinated or vaccinated at 10 woa (AUC, $p<0.05$). PCV2 viremia in this last group was only numerically decreased as compared to the unvaccinated group.
Conclusion
In this study, it was concluded that PCV2 vaccination at 3 or 6 woa offered similar virological and serological results. In contrast, vaccination at 10 woa was probably too late as it occurred after or concomitantly to a PCV2 infection. Therefore, age at PCV2 vaccination should be adapted according to the viral infection dynamics.
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P273

A COMPARISON OF THE GROWTH RATE OF PORCILIS PCV VACCINATED PIGS WITH THAT OF CONTROLS IN A PCV CLINICALLY FREE HERD

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INTRODUCTION
The objective of the study was to compare the growth rates of porcine circovirus (PCV2) vaccinated pigs with that of controls in a 300 sow unit, selling pigs at 100kg bodyweight, in Ireland. The herd in question had a history of clinical freedom from PCV2 infection, as evidenced by the absence of an elevated incidence of clinically wasting pigs, a mortality rate of approximately 3% and a growth rate of approximately 650 g per day from weaning to sale.

MATERIALS AND METHODS
At 21-32 days of age, prior to weaning, 50% of the piglets in each litter were randomly allocated to the treatment group (Porcilis PCV, MSD AH, Milton Keynes, UK) and 50% to the control group, balanced for gender, tagged, and weighed, and the details recorded. A total of approximately 420 piglets were included in the study. PCV2 serological monitoring involved sampling pigs at 4,8,12, and 20-24 weeks of age, then testing for PCV2 antibodies and virus isolation. Subsequent recording involved recording the mortality details and final weights of pigs at or shortly before slaughter.

RESULTS
The vaccinates grew faster at a rate of 689g per day compared with 666g in the control group and the total weight gained per pig was higher in the vaccinates at 89.3kg as compared with 85.7 in the control group. A total mortality of 12 pigs was recorded, 5 in the vaccinates and 7 in the controls.
PCV2 antibody titres in piglets at four weeks of age were high and variable and deemed typical of those associated with maternal antibodies. Almost all pigs in the 20-24 week old pigs had positive titres to PCV2 which suggested that they had been exposed to a PCV2 virus challenge, yet only two pigs showed detectable levels of PCV2 virus in the serum. The results of clinical, serological and virus isolation tests suggest that, whilst the pigs were previously exposed to PCV2 virus, the level of circulating virus in the pigs was low. This could explain the lack of clinically detectable PCV2 disease. The study design did not permit monitoring of feed conversion efficiency.

CONCLUSION
In conclusion, there was evidence of exposure to PCV2 virus since almost all pigs tested had seroconverted to PCV2 virus and as a result were sero-positive at slaughter. The results suggest that sub-clinical PCV2 disease has a negative impact on growth in the absence of clinical symptoms of PCV2 infection in the herd.
The results of this study suggest that vaccination of pigs with Porcilis PCV vaccine in a pig herd that has a history of clinical freedom from PCV2 disease can prove beneficial as a result of improved growth rates.

P274

EVALUATION OF AN ERYSIPELOTHRIX RHUSIOPATHIAE EXPERIMENTAL INFECTION IN PIGS VACCINATED WITH ERYSENG®.

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Objective: The aim of this study was to demonstrate the efficacy of ERYSENG® after a challenge with two different E. rhusiopathiae virulent strains, corresponding to serotype 1 and serotype 2.

Materials and methods: Sixteen eight-week-old pigs, clinically healthy and free from antibodies against E. rhusiopathiae, were randomly assigned to group A (n=10) or group B (n=6). Animals from group A were vaccinated twice intramuscularly (2 ml/dose, at 8 and 11 weeks of age) with ERYSENG®. Animals from group B (control) received PBS following the same schedule as group A. Serum samples were obtained on days 0, 21, 28, 35, 42, 49 and serum antibodies to E. rhusiopathiae (IgG) were titrated using a commercially available ELISA assay. On day 42, all the animals were challenged by separate intradermal injections at the flank, with a 106 cfu/dose of pathogenic E. rhusiopathiae BRP (Biological reference preparation), batch 1, belonging to serotypes 1 and 2. Body temperature and diameter of skin lesions at the challenge injection site were recorded until the end of the trial (day 49). Temperatures were compared between groups using the Student's t-test ($p<0.05$).

Results: All the animals from the vaccinated group (A) were seropositive from day 28 of the study and remained seropositive throughout the trial (49 days). On the contrary, the animals from group B (control group) remained seronegative and only some of them seroconverted after the challenge. From day 43 to day 48 there was a statistically higher mean body temperature in the control group (B) compared to the vaccinated group (A). Moreover, no statistically significant differences were detected between baseline temperature and the temperatures observed post-challenge in the vaccinated group (A). All the vaccinated pigs remained healthy after the challenge with two virulent strains of E. rhusiopathiae corresponding to serotype 1 and serotype 2. In contrast, diamond skin lesions were observed at the challenge injection sites in all the control pigs.

Conclusions: ERYSENG® administered by intramuscular route prevents a rise in body temperature and the clinical signs produced by experimental infection with virulent E. rhusiopathiae strains corresponding to serotype 1 and serotype 2.

P275

SAFETY OF THE MIXED ADMINISTRATION OF ERYSENG® PARVO AND UNISTRAIN® PRRS IN GILTS

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Introduction: The aim of this study was to assess the safety of the mixed administration of UNISTRAIN® PRRS and ERYSENG® PARVO through one overdose of UNISTRAIN® PRRS with the repeated administration of ERYSENG® PARVO in gilts.

Materials and Methods: Thirty-one six-month-old gilts, clinically healthy and free from antibodies against PPV, E. rhusiopathiae and PRRSV, were randomly assigned to group 1 (n=20) and group 2 (n=11). Animals in group 1 were vaccinated intramuscularly three times two weeks apart: with one dose of ERYSENG® PARVO (2 ml/gilt, on day 0), with the combination of ERYSENG® PARVO and UNISTRAIN® PRRS (2 ml/gilt, association of one dose of ERYSENG® PARVO and an overdose of UNISTRAIN® PRRS, on day 14) and with one dose of ERYSENG® PARVO (2 ml/gilt, on day 28, two weeks before mating). Animals in group 2 (non-vaccinated) received PBS following the same schedule as group 1. The main variable observed and compared to the control group was the possible negative impact on gestation and on the offspring (analysed with Mann-Whitney $p>0.05$). Body temperatures were recorded daily from day -1 until day 40, and 2, 4 and 6 hours after each vaccination, and were assessed using the ANOVA test ($p<0.05$). General clinical signs were evaluated daily from day -1 until day 40.

Results: After the vaccinations, all the gilt temperatures were within the physiological range. Regarding the increase of temperatures, no significant differences were observed between groups (the maximum increase was 0.31 °C in group 1). General clinical signs derived from the vaccination were not broadly observed. The only systemic reaction was a mild depression observed in one vaccinated gilt 6 hours after the mixed administration. Regarding the reproductive performance, there were no abortions or any alterations to the normal gestational progress. The farrowing parameters from the vaccinated group (total number of piglets born, number of piglets born alive, weak piglets at birth, number of stillborn piglets and number of the mummifies) were not statistically different compared to the control gilts.

Conclusions: The safety of the mixed administration of ERYSENG® PARVO and UNISTRAIN® PRRS is confirmed concerning all the safety parameters evaluated (local reactions, general clinical signs and body temperature). The mixed administration of ERYSENG® PARVO and UNISTRAIN® PRRS did not have any negative effects on reproductive parameters.

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EVALUATION OF AN ERYSIPELOTHRIX RHUSIOPATHIAE EXPERIMENTAL INFECTION IN GILTS VACCINATED WITH THE MIXED ADMINISTRATION OF ERYSENG® PARVO AND UNISTRAIN® PRRS

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Objective: The aim of this study was to evaluate the efficacy against swine erysipelas of the mixed administration of ERYSENG® PARVO and UNISTRAIN® PRRS in gilts after a challenge with two different pathogenic swine E. rhusiopathiae strains.

Materials and methods: Twenty six-month-old gilts, clinically healthy and free from antibodies against PPV, E. rhusiopathiae and PRRSV, were randomly assigned to group 1 (n=10) and group 2 (n=10). Animals in group 1 were immunised intramuscularly with ERYSENG® PARVO (2 ml/dose) and revaccinated three weeks apart with the combination of ERYSENG® PARVO and UNISTRAIN® PRRS (2 ml/dose). Animals in group 2 received PBS following the same schedule as group 1. Blood samples were obtained on days 0, 21, 35 and 41 to compare the humoral immune responses between groups. Serum antibodies to E. rhusiopathiae (IgG) were titrated using a commercially available ELISA assay, and titres between groups were compared using an ANOVA 1F test ($p<0.05$). On day 42, both groups were challenged with separate dorsal and intradermal injections of a 106 cfu/dose of pathogenic E. rhusiopathiae BRP belonging to serovars 1 and 2, and their body temperature and the diameter of skin erythema at the injection site were recorded until the end of the trial (day 48). Temperatures between groups were compared by means of an ANOVA 1F test ($p<0.05$) and skin lesions were compared using a chi-square test.

Results: E. rhusiopathiae-specific ELISA antibody titres in group 1 exceeded the cut-off value from day 21 until the end of the trial. The mean antibody titre from group 1 was statistically significant from day 21 until the end of the trial. No seroconversion was detected in any of the animals in group 2. On days 42-44 and 46-48 of the study, the mean body temperature results of the animals from group 1 showed statistically significant differences compared to group 2, reaching a peak of 40.21 °C in group 2. The percentage of animals that displayed typical skin lesions after the challenge with serovars 1 and 2, respectively, were: group 1 10/10 and group 2 100/100. Statistically significant differences were observed in the appearance of typical skin lesions after infection between groups 1 and 2 for both serovars.

Conclusions: The humoral immunity elicited by the mixed administration of ERYSENG® PARVO and UNISTRAIN® PRRS enabled animals to manage the experimental infection, showing a reduction of fever and skin lesions after a challenge with virulent E. rhusiopathiae strains.

P277

DURATION OF THE PROTECTIVE IMMUNITY IN PIGLETS AGAINST ATROPHIC RHINITIS AFTER VACCINATION OF GILTS USING RHINISENG® UNDER FIELD CONDITIONS

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Introduction: The aim of this study was to evaluate the duration of passive immunity against progressive atrophic rhinitis in piglets of gilts vaccinated with RHINISENG®.

Materials and Methods: This field trial was performed in a 120 sow one-site farm in France, with a total of 20 piglets from nulliparous sows. For the protocol of RHINISENG®, gilts received two doses in quarantine, the animals were vaccinated twice intramuscularly 6 and 3 weeks prior to mating and a third injection 3 weeks before farrowing. RHINISENG® was administered intramuscularly with a 2 ml dose. Serum samples were collected from piglets at 15, 70, 105, 140 and 175 days of age. Antibody levels against PMT were quantified with a commercial ELISA (Oxoid).

Results: At 15 days of age, 100% of piglets analyzed were positive to PMT. At 70 days of age, on entering the fattening unit 100% of animals were still positive. At 105 days 60% of the animals remained positive against PMT, 10% were doubtful results and 30% were negative. At 140 and 175 days of age 100% of the animals were negative to PMT.

Conclusions: The study revealed that the immunization of gilts with three doses of RHINISENG® prior to farrowing induced a robust humoral response that results in the provision of a high and homogeneous concentration of colostral antibodies in piglets. The duration of passive immunity against PMT exceeded 70 days of age in all animals and it began to decrease towards 105 days of age in piglets coming from gilts vaccinated with RHINISENG®.

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COMPARATIVE SAFETY AND EFFICACY STUDY OF TWO ATROPHIC RHINITIS VACCINES IN GILTS

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Introduction: The aim of this study was to evaluate and compare the efficacy and the safety of two commercially available vaccines for the prevention of atrophic rhinitis (AR) in swine by comparing the serological response and the body temperature increase after vaccination.
RHINISENG® is a vaccine for preventing progressive and non-progressive AR in swine and it contains Hipramune®-Gd as adjuvant. The other commercial vaccine used in this study, is based on dl-α-tocopherol only protects against progressive AR.

Materials and Methods: Eighteen eight-month-old gilts, clinically healthy and free from antibodies against P. multocida toxin (PMT) were randomly assigned to 3 groups of 6 animals each. Groups 1 and 2 were vaccinated twice intramuscularly 3 weeks apart prior to farrowing under field conditions. Group 1 was vaccinated with RHINISENG® (2ml/dose per animal) and group 2 was vaccinated with the vaccine based on dl-α-tocopherol (2ml/dose per animal). Group 3 received a 2 ml dose of PBS twice as a placebo.
Body temperatures of gilts were evaluated one day before and at 0, 6, 24 and 48 hours after each injection in the three different groups. Blood was drawn on study at day 0 (just before vaccination), 21 days later (just before re-vaccination) and at day 42 (just before farrowing). Antibody levels against PMT were quantified with a commercial ELISA (Oxoid). Both variable observed was assessed using ANOVA test ($p<0.05$).

Results: The mean body temperature increase from 0 to 6 hours after vaccination in the RHINISENG® group was around 0.5°C; in the group 2 presented an increase of more than 1°C ($p<0.05$). At 6 hours after injection significant differences were also detected between the group 2 and the groups 1 and 3. No significant differences were detected between the group RHINISENG® and the group 3. Twenty-four hours after vaccination the temperatures had returned to the baseline in all groups.
The mean anti-PMT antibody titers (log3) 21 days after vaccination was statistically significant higher in the RHINISENG® group than groups 2 and 3, which had not seroconverted. Just before farrowing, the anti-PMT antibody titers in the RHINISENG® group doubled the one provided by the other commercial vaccine ($p<0.05$). The control group remained negative throughout the study.

Conclusions: The results obtained from the RHINISENG® group demonstrate better safety and higher humoral response results than the competitor under field conditions, as shown by a lower body temperature increase and a higher and more homogeneous serological response after vaccination.

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VACCINATION WITH THE MIXED ADMINISTRATION OF UNISTRAIN® PRRS AND ERYSENG® PARVO IN GILTS CLINICALLY PROTECTS AGAINST A HETEROLOGOUS PRRSV INFECTION

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Introduction: The clinical protection of the mixed administration of UNISTRAIN® PRRS and ERYSENG® PARVO against the Porcine Reproductive Respiratory Syndrome (PRRS) in gilts was assessed in this study. The reproductive performance after a heterologous challenge was the main efficacy parameter.
Materials and Methods: Twenty six-month-old gilts, clinically healthy and free from antibodies against PPV, E. rhusiopathiae and PRRS were randomly assigned to vaccinated group (n=10) and control group (n=10). Animals in vaccinated group were vaccinated following the recommended protocol; they were immunised intramuscularly with ERYSENG® PARVO (2ml/dose) and revaccinated three weeks apart with the combination of UNISTRAIN® PRRS and ERYSENG® PARVO (2ml/dose, the freeze-dried tablet of UNISTRAIN® PRRS was reconstituted with ERYSENG® PARVO). Vaccination and revaccination were done seven and four weeks before mating, respectively. Animals in control group received PBS using the same strategy as vaccinated group. At ninety days of gestation, all gilts were inoculated intranasaly with 1 ml culture PAM lysate containing 106.39 CCID/ml of a pathogenic type I PRRSV strain. Gilts were examined daily after challenge until 28 days after farrowing. The reproductive parameters were analysed using non-parametric Mann-Whitney ($p<0.05$).
Results: In the vaccinated group, there were no abortions or premature farrowing (less than 111 days of gestation) and the gestation length was the optimum for the right foetal development (115.1 days in vaccinated vs. 113.3 days in control group). Consequently, vaccinated compared to control group significantly reduced the number of weak piglets (0.9 ± 0.99 vs. 2.4 ± 1.58) and the presence of mummies (0.2 ± 0.42 vs. 1.8 ± 1.93). Although statistical differences were not observed, in the vaccinated group there was also an improvement of the piglets born alive/sow (11.3 vs. 9.1) and a drop in the total stillborn piglets/sow (1.4 vs. 1.9),
Conclusion: The mixed administration of UNISTRAIN® PRRS and ERYSENG® PARVO reduced significantly the number of weak piglets and the presence of mummies in vaccinated gilts after a heterologous PRRSV challenge. So the use of the vaccine mixture clinically protected gilts from a heterologous PRRSV infection.

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THE MIXED ADMINISTRATION OF UNISTRAIN® PRRS AND ERYSENG® PARVO IN GILTS REDUCES VIRAEMIA AND VERTICAL/HORIZONTAL TRANSMISSION AFTER A HETE-ROLOGOUS PRRSV CHALLENGE

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Introduction: The aim of this study was to demonstrate that vaccinated gilts with the mixed administration of UNISTRAIN® PRRS and ERYSENG® PARVO control better viraemia than non-vaccinated gilts after a heterologous challenge.
Materials and Methods: Twenty six-month-old gilts, clinically healthy and free from antibodies against PPV, E. rhusiopathiae and PRRS were randomly assigned to vaccinated group (n=10) and control group (n=10). Animals in vaccinated group were vaccinated following the recommended protocol; they were immunised intramuscularly with ERYSENG® PARVO (2ml/dose) and revaccinated three weeks apart with the combination of UNISTRAIN® PRRS and ERYSENG® PARVO (2ml/dose, the freeze-dried tablet of UNISTRAIN® PRRS was reconstituted with ERYSENG® PARVO). Vaccination and revaccination were done seven and four weeks before mating, respectively. Animals in control group received PBS using the same strategy as vaccinated group. At ninety days of gestation, all gilts were inoculated intranasaly with 1 ml culture PAM lysate containing 106.39 CCID/ml of the pathogenic PRRS strain. Sera samples were obtained prior to challenge, post challenge (on 93, 100 and 105 days of gestation) and post-partum (on 0, 7 and 28 days of lactation). Evolution of viraemia after challenge and vertical and horizontal transmission to piglets were determined by RT-PCR and assessed using the $\chi 2$/Fisher ($p<0.05$). Length of viraemia was assessed using Mann-Whitney ($p<0.05$).
Results: All animals were negative to virus in sera after vaccination until challenge. After challenge significant differences appeared in the evolution of viraemia between vaccinated and control animals (20% in vaccinated vs. 100% positive gilts in non-vaccinated group). Regarding the length of the viraemia after challenge significant differences were observed (2.5 ± 1.7 days in vaccinated vs. 16.9 ± 2.7 in control group). Furthermore, vaccination significantly inhibited the vertical transmission of the heterologous PRRSV to piglets detected at birth (6.3 % positive piglets born from vaccinated vs. 65.9 % from control group) and the horizontal transmission assessed at 28 days post-partum (0 % positive piglets from vaccinated vs. 51.5 % from control group).
Conclusion: Vaccination with the mixed administration of UNISTRAIN® PRRS and ERYSENG® PARVO reduced significantly the percentage of viraemic gilts, the duration of viraemia and the vertical and horizontal transmission to their piglets after a heterologous PRRS infection.

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BETTER PERFORMANCE OF PIGLETS BORN FROM GILTS VACCINATED WITH THE MIXED USE OF UNISTRAIN® PRRS AND ERYSENG® PARVO AND CHALLENGED WITH A HETEROLOGOUS PRRSV STRAIN

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Introduction: The efficacy of the mixed administration of UNISTRAIN® PRRS and ERYSENG® PARVO against the Porcine Reproductive Respiratory Syndrome (PRRS) was evaluated in this study assessing piglet performance during lactation after a heterologous PRRSV challenge.
Materials and Methods: Twenty six-month-old gilts, clinically healthy and free from antibodies against PPV, E. rhusiopathiae and PRRS were randomly assigned to vaccinated group (n=10) and control group (n=10). Animals in vaccinated group were vaccinated following the recommended protocol; they were immunised intramuscularly with ERYSENG® PARVO (2ml/dose) and revaccinated three weeks apart with the combination of UNISTRAIN® PRRS and ERYSENG® PARVO (2ml/dose, the freeze-dried tablet of UNISTRAIN® PRRS was reconstituted with ERYSENG® PARVO). Vaccination and revaccination were done seven and four weeks before mating, respectively. Animals in control group received PBS using the same strategy as vaccinated group. At ninety days of gestation, all gilts were inoculated intranasaly with 1 ml culture PAM lysate containing 106.39 CCID/ml of the pathogenic PRRS strain. After farrowing, the born piglets were examined until weaning (28 days of age). Percentage of weaned piglets, piglet weight and average daily gain (ADG) were assessed to evaluate piglet performance. Piglets were weighted at day 0 and 28 postpartum and Mann-Whitney ($p<0.05$) was used to analyse data. ANOVA was used to analyse weaned piglets record.
Results: The percentage of weaned piglets was significantly increased in the vaccinated group (93.81 % vs. 74.73 % in non-vaccinated group). The mean piglet weight was significantly higher in vaccinated gilts at partum (1.36 ± 0.07 kg vs. 1.22 ± 0.04 kg) and at weaning time (6.64 ± 0.54 kg vs. 5.91 ± 0.43 kg in non-vaccinated group) compared to the no vaccinated group. The average daily gain (ADG; g/piglet/day) was also significantly better in the vaccinated gilts than in control group (187.96 ± 16.64 vs. 167.40 ± 16.85).
Conclusion: Piglets from vaccinated gilts were weaned heavier and grew faster than piglets from control gilts. After gilt infection, global piglet performance was significantly better in piglets born from vaccinated gilts with the mixed administration of UNISTRAIN® PRRS and ERYSENG® PARVO.

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IMPROVING REPRODUCTIVE, NURSERY AND FATTENING PERFORMANCE IN 11.700 SOWS AFTER IMPLEMENTING UNISTRAIN® PRRS VACCINATION

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Introduction: One of the largest pig companies in Italy (11.700 sows) had been experiencing PRRS reproductive problems since 2011 despite being vaccinating with a MLV commercial available vaccine (EU strain). In the present study reproductive, nursery and fattening records are summarized after switching to another MLV commercial available vaccine (EU strain, UNISTRAIN® PRRS) in 2013.
Material and methods: 7 PRRS positive sow-herds with recurrent clinical PRRS outbreaks despite vaccinating with a MLV EU commercial available vaccine were used in this trial. A total of 11.700 productive sows are distributed in 7 different herds (6 commercial sow farms and one GP farm producing the future breeders for the other 6 commercial farms). Each sow farm has its own nursery facilities, but not fatteners present. All gilts are vaccinated with MLV vaccine at arrival and monitored by serology before the first insemination. Due to poor reproductive performance during 2011 and 2012, in September 2013 was decided to replace the vaccine to UNISTRAIN® PRRS and the following vaccination protocol was implemented: 3 mass vaccinations per year and 3 doses/gilt before the first mating. To compare the efficacy of both vaccines reproductive, nursery and fattening performance of the 9 following months after changing the vaccine were compared with the same period of the year before.
Results: At reproductive level, farrowing rate, percentage of abortions, total piglets born alive/sow/year and number of stillbirth improved when using UNISTRAIN® PRRS (77.14 vs. 86.68; 3.7 vs. 1.9; 27.15 vs. 28.82; and 7.84 vs. 7.15, respectively). Regarding piglet performance in farrowing rooms mortality, average body weight at weaning and number of weaned piglets/sow/year were also improved (11.79 vs. 10.26; 6.34 vs. 6.58; and 23.68 vs. 25.78; respectively). Besides, mortality in both nursery and fattening units also decreased (4.48 vs. 3.73; and 3.84 vs. 3.52, respectively). Average daily gain improved (701 vs. 712 g/day).
Discussion: When talking about PRRS vaccination, one of the most important challenges for any vaccine strain is their ability to confer heterologous protection in front of new field strains. The present study is a good example to demonstrated that in front the same field challenge, the strain contained into UNISTRAIN® PRRS confers better heterologous protections than another EU strain MLV vaccine.

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EVALUATING THE IN-USE STABILITY OF COGLAPIX BY TESTING ITS EFFICACY AGAINST SEROTYPE 9 ACTINOBACILLUS PLEUROPNEUMONIAE

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Porcine pleuropneumonia caused by Actinobacillus pleuropneumoniae (A.p.) is a highly contagious respiratory disease, characterized by rapid onset, short course, high morbidity and mortality. The disease occurs worldwide with varying incidence and severity. Controlling the disease is difficult, but vaccination can provide efficient protection by decreasing the prevalence and extension of pneumonia and pleuritis, and reduce consequent weight loss of pigs.
Coglapix vaccine contains inactivated serotype 1 and 2 A.p. strains and their RTX toxoids (ApxI, ApxII, and ApxIII) in order to provide protection against a broad range of A.p. serotypes.
In order to avoid excessive vaccine wastage and reduce sum vaccination costs we investigated the efficacy of Coglapix using a vaccine vial having been partly used and then the rest of the vaccine kept refrigerated (+2-8oC) for a week ("open-vial Coglapix").
Six weeks old pigs were vaccinated either with unopened Coglapix and "open-vial Coglapix" in a prime-boost regime, with three weeks interval. Six weeks after prime vaccination the vaccinated and the control groups were challenged with an A.p. serotype 9 strain in an aerosol chamber by applying approximately 108 CCU/pig of the bacterium suspension. Clinical signs were monitored daily throughout the 7 days post-challenge (pch) observation period. Humoral immune-responses to Coglapix® was measured by ELISA (APX II: in-house method of Ceva-Phylaxia, Budapest; ApxIV: Idexx CHEKIT APP-ApxIV kit). All animals were euthanized and subjected to post mortem examination on D7 pch. Post-mortem lesions in the lungs (lung lesion score, LLS) and on the pleura were evaluated, and were compared with each other and with un-vaccinated challenged and un-vaccinated non-challenged groups of pigs. Serological results, body weight gain, and LLS data were analysed by ANOVA. Differences were considered significant at $p<0.05$.
There was no significant difference between the "normal regime", using new bottle of vaccine for each vaccination and the "open-vial Coglapix" vaccination regime, concerning (i) humoral immune-response to RTX toxins and (ii) the calculated vaccine efficacy against challenge with A.p. 9. The lung lesion scores of both vaccinated groups were significantly lower, than in the control pigs.
In conclusion, the results obtained confirmed the in-use stability of Coglapix at least for a week if the partially used vaccine kept refrigerated at +2-8oC .

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EFFICACY OF COGLAPIX IN PREVENTION OF PLEUROPNEUMONIA AND SEROPROFILING AS A TOOL FOR A.P. MONITORING

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Introduction: Actinobacillus pleuropneumoniae – is a primary cause of pleuropneumonia, infectious swine disease characterized with respiratory tract lesions, wide range of pathogen circulating serotypes and different forms of clinical signs(1).
Today commercial test kit IDEXX APP-ApxIV Ab is considered as a universal diagnostic tool for ApxIV toxin antibodies detection. This toxin is a marker for infectious A.pp process of all pathogen serotypes.
The purpose of this trial is to learn post infectious antibodies dynamics before and after A.pp vaccination on swine farm in Russia.

Materials and methods: trial was made on a farm with 3200 sows. A.pp was registered as acute outbreak in pigs in the age of 130-135 days. Mortality rate in fattening period was 7,5%. Diagnosis was confirmed by necropsy and ELISA test data (IDEXX APP-ApxIV Ab Test).
In the beginning blood serum monitoring have been implemented during the whole life of piglets with 2 weeks interval in order to learn the dynamics of post infectious antibodies.
Further such analysis was made after two-shot vaccination with commercial vaccine Coglapix® in the age of 50 and 70 days to monitor decrease percentage of piglets with post infectious antibodies in different time of vaccination.

Results: This farm has high rate of infection in sows and consequently piglets have post infectious colostrum immunity that gradually decreases and achieves its minimum after 9 week of life. Further, during 7 weeks researched blood samples were negative. Starting from 19-th week we can see impetuous growth of seropositive animals from 0 to 60%, and starting from 21-st week and till the slaughter – 100% of piglets have high level of post infectious antibodies.
After vaccination number of piglets with clinical signs of respiratory disease at fattening has significantly decreased, mortality rate has decreased from 7,5 to 1,9%, and ADG has increased per 50 g/day in comparison with non-vaccination period.
Conclusions: Serological monitoring with IDEXX APP-ApxIV Ab Test is a very usefull tool for correct and timely diagnosis confirmation for A.p. It helps as well to determine the terms of colostrum immunity decrease in piglets and critical periods of disease outbreaks. Such analysis helps to develop the strategy and optimal vaccination scheme, and control vaccination results on serological level.
Coglapix® vaccine has demonstrated high efficacy and long post vaccination immunity in pigs till the slaughter.

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EVALUATION OF THREE ADJUVANTS FOR VACCINE FORMULATIONS AGAINST STREPTOCOCCUS SUIS INFECTIONS IN A MOUSE MODEL.

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Streptococcus suis is associated with a wide variety of diseases in pigs and it is considered an emerging zoonotic agent for people in contact with diseased pigs or their products. Various approaches to develop an effective vaccine have been carried out and currently the focus has shifted towards proteins as vaccine candidate agents, mainly to surface proteins. An adequate adjuvant selection is an important step for vaccine development, since may influence the magnitude and the quality of the adaptive response in the host. In this work three adjuvants have been evaluated firstly for using in vaccine formulations against S. suis infection in mice. Thirty-two 4-week-old female CD1 mice were randomly assigned to four groups and were treated with aluminum hydroxide (ALOH), incomplete Freund's adjuvant (FIA), oil-in-water adjuvant (OW), and PBS. After one week, they were challenged intraperitoneally with a lethal dose of S. suis type 2 (cps+mrp+ef+sly+). The control animals died within 48 hours postinfection, whereas the number of dead animals previously treated was significantly lower ($p\beta$ 0.001). The protection rate of ALOH was 71.13%, whereas in FIA and OW groups were 70.81% and 54.07%, respectively, after 48h of challenge. Clinical CNS disease appeared in three animals belonging to FIA group, and two animals of the OW group and one in ALOH group. The histopathological analysis revealed a severe lymphoid depletion in spleen in animals from PBS, FIA and OW group, but not in ALOH group suggesting that this migration would be capable of inducing a Th2 response. A significant reduction in the number of bacteria at 7 dpi in ALOH and FIA (0-75 CFU/ml) groups was obtained. Finally, the antibody titers in animals belonging to OW group presenting the highest titters, although showed the lowest reduction in bacterial counts in most of the examined organs. To sum up, the three adjuvants used in this study showed a protective effect against S. suis infection in mice. Aluminum hydroxide has revealed as the best adjuvant in this model of S. suis infection.

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THE CELL WALL RECOMBINANT PROTEIN SURFACE-ANCHORED DNA-NUCLEASE PROTECTS MOUSE AGAINST STREPTOCOCCUS SUIS.

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Streptococcus suis is a major Gram-positive swine pathogen, which has raised in the last years a great public concern. Protein-based subunit vaccines, using several surface-exposed proteins of S. suis, are been investigated. A common candidate to the majority of the serotypes with clinical significance is the objective of the actual researches worldwide. In previous studies, we selected a new vaccine candidate, a cell-associated DNase (112 kDa), encoded by the SsnA gene, involved in the virulence of S. suis, present in most of the serotypes and clinical isolates analysed, which was highly immunogenic, accessible to antibodies. This protein was assayed in an in vivo murine model, using a protocol based on clinical, histopathological, bacterial kinetics and immune response (antibody titles against the recombinant fragment protein) against S. suis serotype 2 in infected animals. Twenty-four 4-week-old female CD1 were used and four groups of six animals each were established. Mice of group 1 were injected intraperitoneally twice at a 2-weeks interval, with 100µL of aluminum hydroxide (ALOH) mixed with 20µg of rSsnA protein diluted in 100µL of PBS (1:1). Animals of group 2, with 100µL of ALOH mixed with an equal volume of PBS and animals of group 3 (PBS) and 4 (control) were injected with PBS. One week after the second injection, the animals in groups 1-3 were intraperitoneally infected with S. suis serotype 2(cps+mrp+ef+sly+). Group 4 was inoculated with PBS. We showed that the recombinant SsnA protein combined with ALOH as adjuvant allow a significant decrease of clinical and lesional features in animals, faster elimination of the bacteria from organs and a highest humoral response against S. suis after 3 days post-infection. The results showed that this combination (rSsnA+AlOH) could be a good vaccine formulation against S. suis, although further studies are necessary to evaluate their use for swine and human species.

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ACTINOBACILLUS PLEUROPNEUMONIAE AUTOGENOUS VACCINATION IN FATTENING PIGS: INFLUENCE OF THE ADJUVANT, THE ANTIGENIC FORMULATION AND THE VACCINATION PROTOCOL ON THE SEROLOGICAL ANSWER AND THE LESIONS OF PLEUROPNEUMONIAE IN THE SLAUGHTERHOUSE.

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Introduction: Vaccination for the prevention of pleuropneumoniae due to Actinobacillus pleuropneumoniae (APP) in pigs is of increasing relevance with the reduction of antibiotics consumption. Significant global vaccination schedules have led to efforts to simplify the farmer's labor while maintaining the efficacy of this preventive treatment. The aim of this study was to compare autogenous vaccines with different adjuvants, with or without a booster. Material and methods: 252 healthy 9-week-old pigs, serologically APP serogroup 3-6-8 negative and infected with APP serotype 2, were divided in three groups. Each group was vaccinated through a deep intra-muscular route with an autogenous vaccine containing two strains: APP biovar 1 serovar 2 isolated in the farm, and APP biovar 1 serogroup 3-6-8. Group 1 (126 animals) was injected with a primer at 12 weeks and a booster at 16 weeks of age, of a vaccine with an Aluminium hydroxide adjuvant. Group 2 (63 animals) received 2 injections at 12 and 16 weeks of age of a vaccine with HPE, a specific oil in water adjuvant from Biovac. Group 3 (63 animals) received a single injection of MONOVAC, a vaccine with HPE adjuvant in a specific formulation at 16 weeks of age. In each group, ten animals were identified with an ear-tag. At 12 and 16 weeks, five of each group were blood sampled and at 19, 23 and 26 weeks, ten of each group were sampled. Their 3-6-8 LPS antibodies were measured using an ELISA test. Pleuropneumoniae lesions were registered at the slaughterhouse for 223 animals (112, 58 and 53 for Groups 1, 2 and 3 respectively). Statistics: Chi-2 test was used to compare the antibodies and the pleuropneumoniae lesions among the groups. Results: at 23 weeks of age, the percentage of ELISA negative APP 3-6-8 sera was statistically higher between Group 1, vaccinated with the vaccine with aluminium hydroxide adjuvant and groups 2 (p 0.0029) and 3 (p 0.0034) with oil adjuvant. There was no statistical difference (p 0.6392) between Group 2, which received a primer and a booster of the vaccine with oil adjuvant and Group 3, which received the one shot vaccine with oil adjuvant, MONOVAC. The percentage of lesions at the slaughterhouse for Group 1 was statistically higher (p 0.047) than Groups 2 plus 3 (oiled-adjuvanted vaccines). Conclusion: MONOVAC, an oil-adjuvanted autogenous vaccine administered in one single injection showed equally good results as the oil-adjuvanted vaccine administered in two injections and better results than the aluminium hydroxide adjuvant vaccine on the serological answer. The percentage of pleuropneumoniae lesions is higher with alumine-adjuvanted vaccines than with oiled-adjuvanted ones.

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