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**ECPHM**

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## Welcome Word

Dear colleagues,

As chair of the ESPHM Local Organising Committee it is my honour to be able to welcome you to Prague as delegates, partners, supporters and guests to the 9<sup>th</sup> European Symposium of Porcine Health Management. Once again, this year's symposium is joint collaboration between three parties including the European College of Porcine Health Management, the European Association of Porcine Health Management and for this time, in 2017, the Czech Pig Veterinary Society. It is our absolute pleasure to welcome a record number of more than 1 500 ESPHM delegates to Prague. This proves as a testament to the hard work and dedication of everyone involved in preparing what I'm sure will be a highly memorable event.

For the ESPHM 2017, a total of 395 abstracts were submitted with 50 selected for oral presentations in 10 sessions and 345 accepted poster presentations to be on display throughout the symposium time. The program will be enriched by eight keynote lectures, focusing on antimicrobial resistance at both herd and global levels, new viral diseases along with newly recognised infections with a not well-defined pathogenic effect in pigs and on various approaches for new virus discovery. The significance of optimally balanced composition of the intestinal microbiota for development of the digestion function and general immune functions will also be discussed, as well as the influence of feeding on functionality of the digestive system and on the prevention and control of diseases. Last, but not least we will address swine immunology aimed at measuring and interpreting immune responses and at the development of the new-born immune system. Finally, we will investigate the crucial role of passive maternally derived immunity for piglets.

None of the above would be possible without the scientific contribution of delegates as well as funding of partners and supporters. For this we are extremely grateful. Our thanks are indeed extended to the board members of the College and Association for steering the symposium issues and organisations along the way. Finally, I would like to express my full appreciation to the work of the international scientific committee members who have dedicated their free time to evaluate all the abstract submissions received.

As president of the Czech Pig Veterinary Society, I sincerely hope that ESPHM 2017 will not only enhance cooperation between Western, Central and Eastern European Porcine communities but also strive towards improved international co-operations. I trust you will enjoy the programme and be rewarded with plenty of new scientific input and discussion. At the same time as a proud Czech citizen I hope you can enjoy all that the Czech Republic has to offer. At least to find some time to enjoy the city of hundred spires and the majestic experience only Prague can provide.

I wish you all a successful symposium.

**MVDr. Kateřina Nechvátalová, Ph.D.**

President of the Czech Pig Veterinary Society



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Detailed information on this veterinary medicinal product is available on the website of the European Medicines Agency (<http://www.ema.europa.eu/>).

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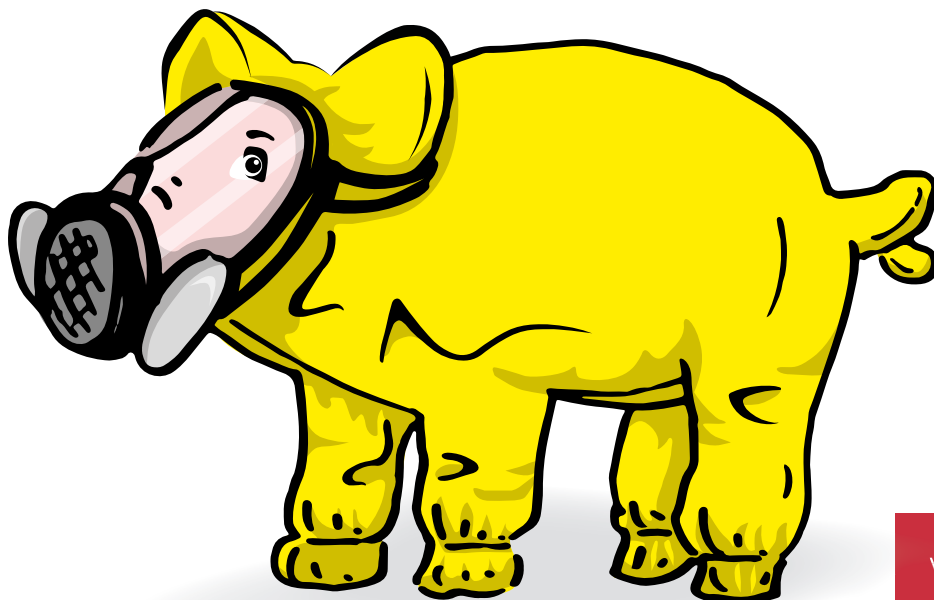


**ERYSENG<sup>®</sup> PARVO. Composition:** Each dose of 2 ml contains: **Active substances:** Inactivated porcine parvovirus, strain NADL-2, RP > 1.15 \*. Inactivated *Erysipelothrix rhusiopathiae*, strain R32E11, ELISA > 3.34 log<sub>2</sub> IE<sub>50%</sub> \*\*. \* RP – relative potency (ELISA). \*\* IE<sub>50%</sub> – Inhibition ELISA 50%. **Adjuvant:** Aluminium hydroxide, DEAE-Dextran, Ginseng. **Indications:** For the active immunisation of female pigs for the protection of progeny against transplacental infection caused by porcine parvovirus. For the active immunisation of male and female pigs to reduce clinical signs of swine erysipelas caused by *Erysipelothrix rhusiopathiae*, serotypes 1 and 2. **Onset of immunity:** Porcine parvovirus: from the beginning of the gestation period. *E. rhusiopathiae*: three weeks after completion of the basic vaccination scheme. **Duration of immunity:** Porcine parvovirus: vaccination provides foetal protection for the duration of gestation. *E. rhusiopathiae*: vaccination protects against swine erysipelas until the time of the recommended revaccination (approximately six months after the basic vaccination scheme). **Interaction with other medicinal products:** Safety and efficacy data are available which demonstrate that this vaccine can be mixed with UNISTRAN<sup>®</sup> PRRS and administered at one injection site. **Shelf life after mixing with UNISTRAN<sup>®</sup> PRRS:** 2 hours. Please refer to the product packaging/leaflets for information about **side effects, precautions, warnings and contra-indications. Marketing Authorisation Holder:** LABORATORIOS HIPRA, S.A. **Local representative:** Hipra UK & Ireland, Ltd. **Marketing Authorization number:** EU/2/14/167/001-007. **Legal category:** UK: POM-V. ROI: POM. Use medicines responsibly. Under veterinary prescription, advice should be sought from Veterinary Prescriber. More information is also available in the medicines' Summary of Product Characteristics (SPC) and at [www.hipra.com](http://www.hipra.com).

**UNISTRAN<sup>®</sup> PRRS. Composition per dose:** Live attenuated Porcine reproductive and respiratory syndrome virus (PRRSV), strain VP-046 BIS 10<sup>3.5</sup>-10<sup>5.5</sup> CCID<sub>50</sub> (cell culture infectious dose). Phosphate buffer solution. **Indications:** **Breeding females:** For active immunisation of breeding females from farms affected with European PRRS virus to reduce reproductive disorders, incidence and duration of viraemia, transplacental virus transmission, virus tissue load and clinical signs associated with infection with strains of PRRS virus. Under laboratory conditions, vaccination of females reduced the negative impact of PRRS virus infection on piglet performance (mortality and weight gain) within the first 28 days of life. **Onset of immunity:** 30 days after vaccination. **Duration of immunity:** 16 weeks demonstrated by challenge. **Pigs from 4 weeks of age:** For active immunisation of pigs from farms affected with European PRRS virus to reduce clinical signs associated with a PRRS virus infection, the incidence and duration of viraemia and the duration of virus shedding by infected animals. Under experimental conditions, it was demonstrated that vaccination reduces the virus tissue load in the lungs. Under experimental conditions, where a PRRSV infection occurred during the fattening period, a reduction in mortality and in the negative effects of infection on daily weight gain was demonstrated. **Onset of immunity:** 4 weeks after vaccination. **Duration of immunity:** 24 weeks. **Interaction with other medicinal products:** Safety and efficacy data are available which demonstrate that this vaccine can be mixed with ERYSENG<sup>®</sup> PARVO and administered at one injection site. **Shelf life after mixing with ERYSENG<sup>®</sup> PARVO:** 2 hours. Please refer to the product packaging/leaflets for information about **side effects, precautions, warnings and contra-indications. Marketing Authorisation Holder:** LABORATORIOS HIPRA, S.A. **Local representative:** Hipra UK & Ireland, Ltd. **Marketing Authorization Number UK:** Vm 17533/4016. IE: VPA 10846/013/001. **Legal category:** UK: POM-V. ROI: POM. Use medicines responsibly. Under veterinary prescription, advice should be sought from Veterinary Prescriber. More information is also available in the medicines' Summary of Product Characteristics (SPC) and at [www.hipra.com](http://www.hipra.com). For more information on PRRS visit: [PRRScontrol.com](http://PRRScontrol.com)



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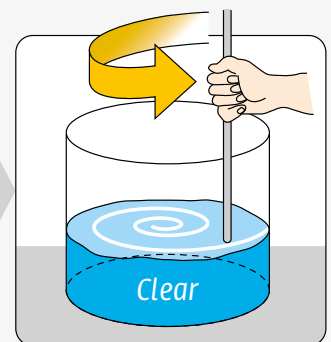
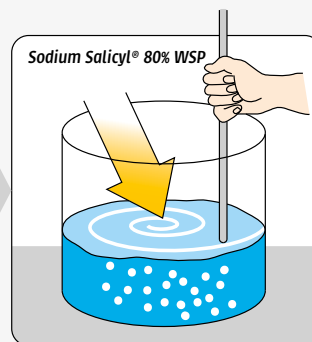
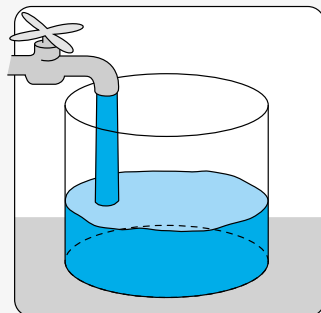
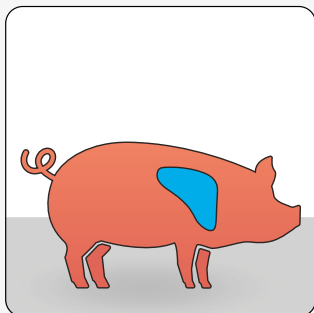
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**References:** [1] Haugegaard, J. et al., 2008; The Pig Journal 61, 69-73, [2] Herschel, H.J. et al., 2012. Proc. 43th AASV Cong. March 2012



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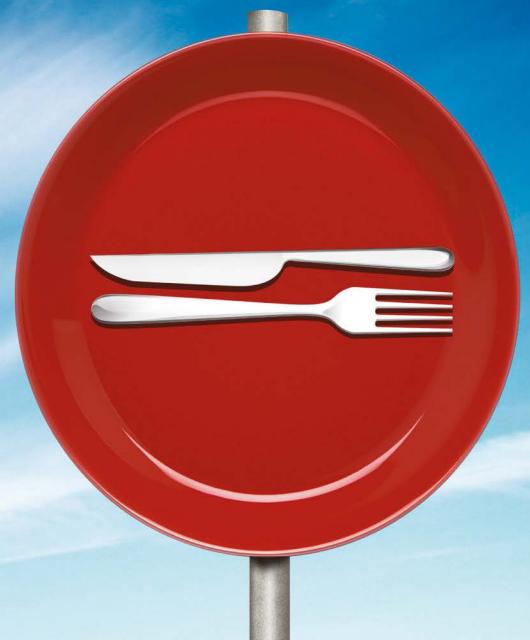


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# Detailed Programme

Wednesday, 3 May 2017

	Congress Hall	Forum Hall
13:00	<b>Welcome &amp; Symposium Opening</b>	
13:10	<b>Pig and Pork Production in the Czech Republic</b> Ministry of Agriculture of the Czech Republic and Pig Breeder Association of the Czech Republic representatives	
	<b>Keynote Lectures 13:30 – 15:00</b>	
	<b>ONE HEALTH ASPECTS OF ANTIMICROBIAL RESISTANCE</b> Chairs: <b>Andreasen Margit, Palzer Andreas</b>	
13:30	<b>KL01</b> <b>Antimicrobial Resistance from a Global and One Health Perspective</b> <i>Mevius, D.</i>	
14:00	<b>KL02</b> <b>One Health and Antimicrobial Resistance at the Herd Level</b> <i>Gonzalez-Zorn, B.</i>	
14:30	<b>Round Table Discussion</b>	
15:00	<b>COFFEE BREAK / POSTER VIEWING</b>	
	<b>Parallel Sessions 15:30 – 17:30</b>	
	<b>VETERINARY PUBLIC HEALTH &amp; FOOD SAFETY</b> Chair: <b>Grontvedt Carl Andreas, Bureš Jiří</b>	<b>RESIDENT SESSION</b> Chairs: <b>Grosse Beilage Elisabeth, Pedersen Ken</b>
15:30	<b>VPH-OP-01</b> <b>Low Risk for Humans Associated with Use of Pleuromutilins in Pigs</b> <i>Alban, L.; Andreassen, M.; Dahl, J.; Nielsen, E.O.; Ellis-Iversen, J.; Sønksen, U.W.</i>	<b>RES-OP-01</b> <b>Does Dental Health Affect Young Pigs' Performance?</b> <i>Dieste-Pérez, L.; Sparreboom, M.; Allaart, J.; Van Hees, H.; Booji-Vrieling, H.E.; Tobias, T.J.</i>
15:50	<b>VPH-OP-02</b> <b>Effect of Tetracycline Treatment-Dose and Treatment-Mode on Selection of Resistant Coliform Bacteria in Nursery Pigs</b> <i>Olsen, J.E.; Græsbøl, K.; Nielsen, J.P.; Damborg, P.; Herrero-Fresno, A.; Larsen, I.; Folkesson, A.</i>	<b>RES-OP-02</b> <b>Effect of Drinking Water Additives on Growth Performance and Intestinal Microbiota Composition in Weaned Piglets.</b> <i>Mesonero Escuredo, J.A.; Maes, D.; Oudshoorn, A.K.; Davids, M.; Carr, J.; Roubos-van den Hil, P.</i>
16:10	<b>VPH-OP-03</b> <b>The Effect of an Acid Based Feed Additive on the Intestinal Level of ESBL-Producing E. Coli in Feces of Swine</b> <i>Roth, N.; Mayrhofer, S.; Doupovec, B.; Berrios, R.; Waxenecker, F.; Domig, K.</i>	<b>RES-OP-03</b> <b>Increased Prevalence of Hemorrhagic Diathesis in New Born Piglets Due to Thrombocyte-Specific Alloimmune Antibodies</b> <i>Jansen, R.; De Snoeck, S.; Martens, M.; Jennes, M.; Cox, E.</i>
16:30	<b>VPH-OP-04</b> <b>Changes in Hematological, Carcass and Meat Quality Parameters Associated with Liver Milk Spots in Slaughter Pigs</b> <i>Čobanović, N.; Karabasil, N.; Vasilev, D.; Dimitrijević, M.; Teodorović, V.</i>	<b>RES-OP-04</b> <b>Determinants for Mycoplasmal Pneumonia Reproduction Under Experimental Conditions: A Systematic Review and Recursive Partitioning Analysis</b> <i>García-Morante, B.; Segalés, J.; Serrano, E.; Sibila, M.</i>
16:50	<b>VPH-OP-05</b> <b>Toxoplasma Gondii and Parma Ham: No Evidence of Transmission</b> <i>Genchi, M.; Vismarra, A.; Mangia, C.; Faccini, S.; Vicari, N.; Rigamonti, S.; Prati, P.; Marino, A.M.; Fabbri, M.; Kramer, L.</i>	<b>RES-OP-05</b> <b>Effect of Sow Vaccination Against Porcine Circovirus Type 2 on Serological, Virological and Reproductive Parameters in a Subclinical Infection Scenario</b> <i>Oliver-Ferrando, S.; Segalés, J.; López-Soria, S.; Callén, A.; Merdy, O.; Joisel, F.; Sibila, M.</i>
17:10	<b>VPH-OP-06</b> <b>Using Serological Monitoring at Abattoirs and On-Farm Auditing for Identification of Pig Farms with a High Risk for Toxoplasma Gondii</b> <i>Oorborg, D.; Eppink, D.; Klein Koerkamp, M.J.A.; Bouwknegt, M.; Urlings, H.A.P.; Van der Giessen, J.W.P.; Van Asseldonk, M.A.P.M.; Van Wagenberg, C.P.A.; Mul, M.F.; Gonzales, J.L.; Swanenburg, M.; Wisselink, H.J.</i>	<b>RES-OP-06</b> <b>Wasting, Coughing, Arthritis and Central Nervous Symptoms in 8 Weeks Old Piglets Caused by Mycoplasma Hyorhinis In Combination With PRRSV</b> <i>Unterwiesing, C.; Spersger, J.; Voglmayr, T.; Brunthaler, R.; Schwarz, L.</i>
17:40		<b>ECPHM Annual General Meeting</b> Diplomates and residents only (17:40 – 18:30)



## Thursday, 4 May 2017

	Congress Hall	Forum Hall
07:30		<b>EAPHM Annual General Meeting</b> Open for all interested delegates
	<b>Keynote Lectures 08:30 – 10:00</b>	
	<b>NEW VIRUSES = NEW DISEASES?</b> Chairs: Marco Enric, Celer Vladimír	
08:30	<b>KL04</b> <b>Approaches for New Virus Discovery: Solving or Creating Problems?</b> <u>Stadejek, T.</u>	
09:00	<b>KL03</b> <b>It's Raining Viruses... What Does It Mean?</b> <u>Segalés, J.</u>	
09:30	<b>Round Table Discussion</b>	
10:00	<b>COFFEE BREAK / POSTER VIEWING</b>	
	<b>Parallel Sessions 10:30 – 12:30</b>	
	<b>VIRAL DISEASES</b> Chairs: Martelli Paolo, Václavek Petr	<b>HERD HEALTH MANAGEMENT &amp; ECONOMY</b> Chairs: Rose Nicolas, Janssen Rick
10:30	<b>VVD-OP-01</b> <b>Comparison of PRRSV RNA Detection by PCR in Different Sample Types</b> <u>Opriessnig, T.; Jinghui, F.; Gerber, P.; Eppink, L.; Wang, C.</u>	<b>HHM-OP-01</b> <b>Leveraging Data Across a Large Geographic Area to Increase Context and Understanding of PRRSV Transmission</b> <u>Lowe, J.; Lowe, E.</u>
10:50	<b>VVD-OP-02</b> <b>Efficacy of an Attenuated PRRSV-1 Vaccine in Pigs upon Challenge with a Highly Pathogenic EU PRRSV Strain</b> <u>Canelli, E.; Catella, A.; Ferrari, L.; De Angelis, E.; Ogno, G.; Bonilauri, P.; Guazzetti, S.; Sandri, G.; Martelli, P.</u>	<b>HHM-OP-02</b> <b>Understanding the Culture of Antimicrobial Use Behaviours in Agriculture: A Mixed Methods Study of UK Pig Farmers</b> <u>Coyne, L.; Latham, S.; Williams, N.; Smith, R.; Pearson, R.; Donald, I.; Dawson, S.; Pinchbeck, G.</u>
11:10	<b>VVD-OP-03</b> <b>Evaluation of the Effect of Maternal Immunity on Neonatal Piglet Vaccination Protocols for PRRS</b> <u>Dee, S.; Nerem, J.; Hanson, D.; Philips, R.; Wetzell, T.; Schmalig, E.; Edler, R.</u>	<b>HHM-OP-03</b> <b>The Effects of Amoxicillin Use in Newborn Piglets on Their Umbilical Problems, Other Treatments and Resistance of Intestinal Coliforms</b> <u>Heinonen, M.; Yun, J.; Hänninen, M.L.; Oliviero, C.; Olkkola, S.</u>
11:30	<b>VVD-OP-04</b> <b>Recombination Between a Field and a Vaccine Strain of PRRSV Detected by Routine ORF5 Sequencing</b> <u>Steinriegl, A.; Revilla-Fernandez, S.; Entenfellner, F.; Schmolli, F.</u>	<b>HHM-OP-04</b> <b>Stability of Antimicrobials in Contact with Disinfectants in Drinking Water for Pigs</b> <u>Hemonic, A.; Pupin, P.; Jacob, C.; Leorat, J.; Maris, P.; Correge, I.</u>
11:50	<b>VVD-OP-05</b> <b>Atypical Porcine Pestivirus Association to Congenital Tremor in the Last Two Decades in Spain</b> <u>Canturri, A.; Domingo, M.; Muñoz-González, S.; Pérez-Simó, M.; Bohórquez, J.A.; Rosell, R.; Cabezón, D.; Segalés, J.; Ganges, L.</u>	<b>HHM-OP-05</b> <b>Sow Vaccination Against Porcine Circovirus Type 2 At Different Physiological Stages: Effects On Reproductive Parameters And Passive Immunity In Piglets</b> <u>López-Soria, S.; Sibila, M.; Pleguezuelos, P.; Cuadrado, R.; López-Jiménez, R.; Pérez, D.; Huerta, E.; Llorens, A.; Segalés, J.</u>
12:10	<b>VVD-OP-06</b> <b>Detection of Human Pandemic Influenza A Viruses H1N1/2009 in Swine Herds in the Netherlands in 2016</b> <u>Koenders-van Gog, K.; Henritzi, D.; van der Wolf, P.; Veldhuis, H.; Biermann, J.; Kateman, L.; Struik, D.; Wacheck, S.; Harder, T.</u>	<b>HHM-OP-06</b> <b>Sow Mortality Due to Liver Lobe Torsions (Four Cases)</b> <u>Geudeke, T.; Van Garderen, E.; Vos, J.; Greydanus, S.; Dijkman, R.; Junker, K.; Houben, M.; Franssen, P.; Duinhof, T.</u>



## Thursday, 4 May 2017

	Congress Hall	Forum Hall
12:30	LUNCH BREAK / POSTER VIEWING	
	<b>Keynote Lectures 13:30 – 15:00</b>	
	<b>GASTRO-INTESTINAL DISORDERS</b> Chairs: Šperling Daniel, Wierchoslawski Karol	
13:30	KL05 <b>Microbiota &amp; diseases</b> <u>Zentek, J.</u>	
14:00	KL06 <b>What Can I Do in Case of Gastrointestinal Disorders?</b> <u>Schafzahl, W.</u>	
14:30	<b>Round Table Discussion</b>	
15:00	EAPHM, Peter Hogedal Award & JPHM presentation	
	<b>Parallel Sessions 15:20 – 16:20</b>	
	<b>BACTERIAL DISEASES I</b> Chairs: Duran Oliver, Bernardy Jan	<b>WELFARE &amp; NUTRITION</b> Chairs: Hennig Pauka Isabel, Heinonen Mari
15:20	<b>BBD-OP-01</b> <b>Enterotoxins of F4+ Escherichia Coli Induce IL17 in the Pig Small Intestine and Enhance Colonization with F18ac+ Verotoxigenic E. Coli</b> <u>Cox, E.; Luo, Y.; Atef Yekta, M.; Loos, M.; Coddens, A.; Arnouts, S.; Wim, V.D.B.; Lundberg, U.; Devriendt, B.</u>	<b>AWN-OP-01</b> <b>Access to Chewable Materials Increases Piglet Activity During Lactation Period</b> <u>Swan, K.M.; Telkänranta, H.; Munsterhjelm, C.; Peltoniemi, O.; Valros, A.</u>
15:40	<b>BBD-OP-02</b> <b>Association Between Isolation of Enterotoxigenic E. Coli and Numbers of E. Coli F18 Genes in Faeces in Nursery Pigs</b> <u>Weber, N.; Nielsen, J.P.; Pedersen, K.S.</u>	<b>AWN-OP-02</b> <b>Meat Inspection Data Highlight Health Problems in Free-Range Production Systems.</b> <u>Kongsted, H.; Tind Sørensen, J.</u>
16:00	<b>BBD-OP-03</b> <b>Detection of Salmonella Antibodies in the Saliva of Pigs from Salmonella Typhimurium-Vaccinated and Unvaccinated Herds</b> <u>De Lucia, A.; Rabie, A.; Smith, R.P.; Davies, R.; Bianco, C.; Ostanello, F.; Martelli, F.</u>	<b>AWN-OP-03</b> <b>Behavioral Analysis of Piglets Treated with Metacam® Thirty Minutes Before and at Time of Castration.</b> <u>Law, J.; Cunningham, G.;</u>
16:20	COFFEE BREAK / POSTER VIEWING (16:20 – 16:40)	



## Thursday, 4 May 2017

	Congress Hall	Forum Hall
	<b>Parallel Sessions 16:40 – 18:00</b>	
	<b>BACTERIAL DISEASES II</b> Chairs: Maes Dominiek, Spiru Daniel	<b>MISCELLANEOUS</b> Chairs: van Leengoed Leo, Malášek Jiří
16:40	<b>BBD-OP-04</b> <b>A Lawsonia Intracellularis Fecal Quantitative Polymerase Chain Reaction Assay and the Correlation to Pig Performance in Lawsonia Intracellularis Challenged Pigs.</b> Seate, J.; Tubbs, R.; Gauger, P.; Scheidt, A.; Edler, R.; Doolittle, K.; Playter, S.	<b>MIS-OP-01</b> <b>Butorphanol Induces Anxiety-Like Behaviour and Distress in Piglets</b> Cap, V.H.; Abass Mossa, M.; Hug, P.J.; Kümmerten, D.; Hug, C.; Bettschart-Wolfensberger, R.
17:00	<b>BBD-OP-05</b> <b>Effect of Pre-Farrowing Sow Vaccination Against Mycoplasma Hyopneumoniae on Offspring Colonization and Lung Lesions</b> Arsenakis, I.; Michiels, A.; Boyen, F.; Haesebrouck, F.; Maes, D.	<b>MIS-OP-02</b> <b>Short- and Long-Term Effects of Early Metaphylactic Use of Ceftiofur on the Faecal Microbiota in Suckling and Growing Pigs</b> Ruczizka, U.; Unterweger, C.; Metzler-Zebeli, B.U.; Mann, E.; Schwarz, L.; Knecht, C.; Krauss, I.; Mayerhofer, S.; Hennig-Pauka, I.
17:20	<b>BBD-OP-06</b> <b>Use of Molecular Characterization Tools to Investigate M. Hyopneumoniae Outbreaks</b> Anderson, A.; Fano, E.; Pieters, M.; Amanda, S.; Dalquist, L.	<b>MIS-OP-03</b> <b>Urolithiasis in Finishing Pigs</b> Vrielinck, J.; Van Poucke, A.; Janssens, G.; Maes, D.
17:40	<b>BBD-OP-07</b> <b>Prevalence and Serotype Distribution of Streptococcus Suis in Clinically Ill Pigs and in Healthy-Carrier Pigs at Different Stage of Production</b> Farzan, V.; Arndt, E.; MacInnes, J.; Friendship, R.	<b>MIS-OP-04</b> <b>Closing the Innovation Gap – How to Tune Research to Farmers Needs</b> De Smet, S.; Van Gansbeke, S.; Millet, S.



Friday, 5 May 2017

	Congress Hall	Forum Hall
	<b>Keynote Lectures 08:30 – 10:00</b>	
	<b>IMMUNOLOGY FROM THEORY TO PRACTICE</b> Chairs: Nechvátalová Kateřina, Nathues Heiko	
08:30	<b>KL07</b> <b>Measuring and Interpreting the Immune Response</b> Borghetti, P.; Saleri, R.; <u>Martelli, P.</u>	
09:00	<b>KL08</b> <b>The Immune System in New Born Piglets &amp; Passive Immunity</b> Nechvátalová, K.; Krejčí, J.; Bernardy, J.; Ondráčková, P.; Štěpánová, H.; Hlavová, K.; Levá, L.; Kudláčková, H.; <u>Faldyna, M.</u>	
09:30	<b>Round Table Discussion</b>	
10:00	<b>COFFEE BREAK / POSTER VIEWING</b>	
	<b>Parallel Sessions 10:30 – 12:30</b>	
	<b>IMMUNOLOGY &amp; VACCINOLOGY</b> Chairs: Kristensen Charlotte, Eggen Alex	<b>REPRODUCTION</b> Chairs: Kauffold Johannes, Peltoniemi Olli
10:30	<b>IMM-OP-05</b> <b>Vaccination Against Post-Weaning Diarrhoea with Coliprotec® F4: Production Results from a Large Scale Farm in Spain</b> <u>Sánchez Uribe, P.</u> ; Lamrani, Á.; Núñez, P.; Hidalgo, Á.; Martínez, J.; Oliva, J.E.; Márquez, G.; Nadeau, É.	<b>REP-OP-01</b> <b>Prolonged Farrowing May Increase Weight Loss During Lactation</b> Björkman, S.; Oliviero, C.; <u>Peltoniemi, O.</u>
10:50	<b>IMM-OP-02</b> <b>Study of the Efficacy of an Autogenous Vaccine Directed Against Arthritis Due to Trueperella Pyogenes</b> <u>Marchand, D.</u> ; Pommelet, C.; Guibe, L.; Millemann, Y.	<b>REP-OP-02</b> <b>Relationship Between Age at First Farrowing and Longevity in Swedish Sow Herds</b> <u>Eliasson-Selling, L.</u> ; Lundeheim, N.
11:10	<b>IMM-OP-03</b> <b>Differentiation of Porcine Post-infection and Post-Vaccination Antibodies After Salmonella Typhimurium Infection and Vaccination with Salmonella Typhimurium-Based Inactivated Vaccine</b> <u>Gebauer, J.</u> ; Kudlackova, H.; Tesarik, R.; Faldyna, M.; Matiasovic, J.	<b>REP-OP-03</b> <b>Infrared Thermography and Ultrasound as Possible Tools for Investigating Udder Health in Sows</b> Spiegel, S.; Spiegel, F.; Wendt, M.; Luepke, M.; <u>von Altröck, A.</u>
11:30	<b>IMM-OP-04</b> <b>Strategic Piglet Vaccination as a Tool to Eliminate PRRS Virus Recirculation in a Commercial Nursery</b> Laza, C.; Puig, R.; Marcos, M.; Menjón, R.; <u>Jiménez, M.</u>	<b>REP-OP-04</b> <b>Use of an ANTI-GNRF Vaccine to Suppress Estrus in Iberian Gilts Reared in Commercial Extensive Conditions in Spain</b> <u>Pall Dalmau, A.</u> ; Palliser, J.; Rodriguez, V.; Hernandez, J.; Romero, A.; Sierra, M.A.; Velarde, A.; Isera
11:50	<b>IMM-OP-01</b> <b>Conserved HA-Peptides as Multivalent Vaccine Against Influenza Viruses</b> <u>Sisteré-Oró, M.</u> ; Vergara-Alert, J.; G. Zabala, J.; Pina-Pedrero, S.; Córdoba, L.; Martínez-Pulgarín, S.; M. Escribano, J.; Darji, A.	<b>REP-OP-05</b> <b>Reduced Fertility in a Boar Due to Multicystic Degeneration of the Bulbourethral Glands</b> <u>Grahofer, A.</u> ; Nathues, H.; Gurtner, C.
12:10	<b>IMM-OP-06</b> <b>Good Vaccination Practices: It All Starts with Good Refrigerator Temperature</b> <u>Vangroenweghe, E.</u> ; Kelderman, K.; Vanhooydonck, K.	<b>REP-OP-06</b> <b>Optimization of Castration Under Inhalation Anaesthesia with Isoflurane Using Parenteral Butorphanol, Meloxicam or Intratesticular Lidocaine in 7 – 14 Days Old Piglets</b> <u>Hug, P.J.</u> ; Čáp, V.H.; Honegger, J.; Schwarz, A.; Schüpbach-Regula, G.; Bettschart-Wolfensberger, R.
12:30	<b>Closing ceremony / ESPHM 2018 in Barcelona (12:30 – 13:00)</b>	





# Opening lecture

## PIG PRODUCTION IN THE CZECH REPUBLIC

**Jan Stibal, Pavel Kerber\***

*Pig Breeders Association of the Czech Republic*

Pig production in the Czech Republic went through many changes during the last 25 years. Historically we produced approximately same volume of pork as we consumed and foreign trade had no important role. Because of historical development we had relatively big companies often specialized to pig and/or chicken production. Many of them operate without land and without crop production. Unfortunately, they were not able to utilize advantage of the size and their efficiency and productivity was much lower than efficiency and productivity of their counterparts in old EU. Additionally, these companies had no access to direct payment because we introduced system Single payment system based on size of agricultural land. Also, slaughtering and processing sector was quite weak – focused on domestic market with no ambition to export.

Pork has a very strong position in the Czech Republic. Every inhabitant consumed 83 kilograms of meat per year and more than half of that volume is pork. Moreover, pork consumption is very firm. While inhabitants in western countries eat less and less pork, in the Czech Republic are figures at (more or less) same level more than 15 years. It makes excellent space for pig producers. Unfortunately, consumers and processors behaviour caused that since our entrance to EU importance of foreign trade has been bigger and bigger. Imports of meat rose every year and nowadays we are producing half of needed meat (220 thousand tons of slaughter weight). Approximately same volume is imported from abroad. These factors lead to quick drop of pig population and domestic production.

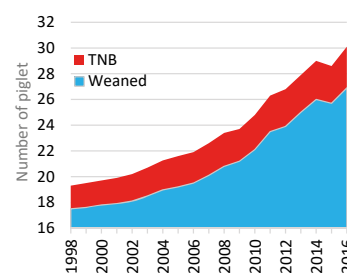
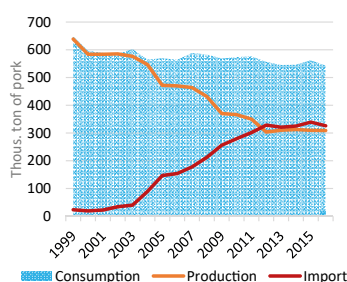
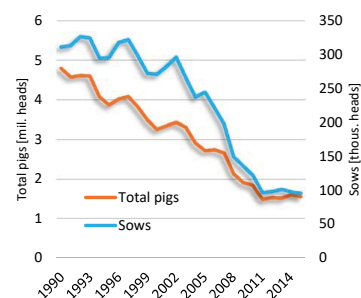
Very important role in these process plays also pig and pork prices. Our neighbour is Germany – one of most important pig producer in Europe. Our pig prices follow from German ones. They are not same as in Germany but only derived from it. Our price has usually same (more or less) tendency but it is much lower. While other countries dependent on import of pork have higher prices than average of EU, Czech prices are between prices of pure exporters – Netherland, Belgium or France. From economical point of view, it does not make sense.

During time farmers in the Czech Republic improved all aspects of production. They changed genetics, technologies, feeding and of course know-how of management. One of very important matter which affects productivity is health status of herds. Mainly for sow farms it is crucial factor. The Czech Republic is free of seriously dangerous diseases like Aujeszky disease, African or Classical swine fever. From economical point of view there are lot of other important diseases: PRRS, MHYO, APP, E. coli and lot of others. To improve health status there has been prepared subsidy program which supports repopulation of sow herds. More than two third of herds undergone repopulation so they dramatically improved health.

As a result of these (and many others) changes farmers has been able to improve production of piglets. While in 2004 when we entered the EU, there was on average 18,5 weaned piglets per sow and year in 2016 it was 26,9 weaned piglets. Increase by more than 8 piglets during twelve years is quite considerable. Moreover, it is just national average. Best farms are much far. They reached 34,5 weaned per sow and year so they are fully competitive with farmers from western Europe.

Second very positive effect of improved health is possibility to decrease consumption of antimicrobials. In the Czech Republic, we just started to build up quality scheme which will be based mainly on lowering of consumption of antimicrobials.

Probably as a result of improved productivity and deep changes in structure, numbers of pig have been stabilised. During last three years, despite European pig crisis, our stock decreased only very slightly. Pig production plays important role in value added chain and also in employment in rural areas. Despite big drop of pig population, it is still very important part of agriculture and it is necessary at least maintain (or rather increase) present volume of production.





# Keynote Lectures



## Keynote lecture I.

### Dik Mevius

Wednesday, 3 May 2017, 13:30 – 14:00, Congress Hall

#### Antimicrobial Resistance from a Global and One Health Perspective



Prof Dik Mevius, DVM, PhD, veterinary microbiologist is an expert in the field of molecular epidemiology of antimicrobial resistance. At the Wageningen Bioveterinary Research in Lelystad, the Netherlands, he is responsible for the Dutch national reference laboratory on antimicrobial resistance (AMR) in animals. He coordinates numerous research projects on molecular epidemiology of AMR. Since 2007 he holds a Chair on antimicrobial resistance at Utrecht University. The current research of his group is focused on Extended Spectrum Beta-Lactamases, the plasmid mediated colistin-resistance gene (*mcr-1*), plasmids and other mobile genetic elements involved in the epidemiology of resistance genes and isolates and the association between AMR in bacteria in animals and in humans.

Prof. Mevius has from 1996 onwards been a member of various scientific advisory or expert working groups on antimicrobial resistance of CVMP, EFSA, DG-SANCO and the Dutch Ministries of Health Welfare and Sports (Health Council) and Economic Affairs (Raad voor Dieraangelegenheden) and several ad hoc Dutch Expert Advisory panels on AMR.

## Keynote lecture II.

### Bruno Gonzalez Zorn

Wednesday, 3 May 2017, 14:00 – 14:00, Congress Hall

#### One Health and Antimicrobial Resistance at the Herd Level



Prof. Bruno Gonzalez-Zorn, DVM, PhD is Professor of Animal Health at the Veterinary Faculty in the Complutense University in Madrid, and Adjunct Professor of Biotechnology at the University for Development Studies in Ghana. He gained his DVM in 1996 studying in Spain and Germany and his European PhD in 2001. After his Postdoc at the Pasteur Institute in Paris he received a Ramon y Cajal tenure-track contract from the Spanish Ministry of Science to return to Spain. In 2011 he was awarded the National Microbiology Award, the biannual Jaime Ferran Award from the Spanish Society for Microbiology. Currently he leads a group of young researchers working on molecular microbiology and the ecology of antimicrobial resistance in Madrid. His research interests focus on the role the ecology of antimicrobial resistance, including humans, animals, food and the environment, focusing his research on genomics from a One Health perspective. He is part of numerous Committees on Antimicrobial Resistance at National and International Institutions. He is the President of the Molecular Microbiology Group of the Spanish Society for Microbiology.



## Keynote lecture III.

### Joaquim Segalés

Thursday, 4 May 2017, 09:00–09:30, Congress Hall

#### It's Raining Viruses... What Does It Mean?

**Academic degrees:** DVM (1991), PhD (1996), Dipl. European College of Veterinary Pathologists (ECVP, 2000) and Dipl. European College of Porcine Health Management (ECPHM, 2004, founding member)

**Current position:** Director and researcher of the *Centre de Recerca en Sanitat Animal* (CRESA) and Associate Professor at the Veterinary School of the *Universitat Autònoma de Barcelona* (main subjects: pathology and swine clinics). Current Past-President of the ECPHM, for which was Vice-president (2010–13) and President (2013–16).

**Veterinary service activity:** Diagnostician at the Pathology Department of the Veterinary School of Barcelona since 1996. Responsible for the pathological diagnostic activity in swine (1996–2012).

**Research activity:** Involved in research of swine diseases since 1993, mainly infectious diseases (including infections by porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus, porcine circovirus type 2 (PCV2), swine hepatitis E virus, swine Torque teno sus viruses (TTSuV), *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis* and *Mycoplasma hyopneumoniae*). He has co-authored more than 250 articles in international peer-reviewed journals. He recently started working on MERS (Middle East Respiratory Syndrome)-coronavirus infection animal models.



## Keynote lecture IV.

### Tomasz Stadejek

Thursday, 4 May 2017, 08:30–9:00, Congress Hall

#### Approaches for New Virus Discovery: Solving or Creating Problems?

Professor Dr. Tomasz Stadejek graduated from the Veterinary Faculty of the University of Life Sciences in Lublin, Poland in 1990. From 1991 to 2011 he worked at the Department of Swine Diseases of the National Veterinary Research Institute in Pulawy, Poland. He obtained there his Ph.D. in 1996 and D.Sc. in 2002. In 2007 Prof. Stadejek was nominated by the World Organization for Animal Health (O.I.E.) as an expert for PRRS, and in 2007–2011 he was the head of the O.I.E. Reference Laboratory for PRRS. He is a member of Arterivirus Study Group of the International Committee for Taxonomy of Viruses. In 2008 he obtained diploma of the European College of Porcine Health Management and from 2011 to 2013 he was a board member of the secretary of the college. **Since 2012 he is full professor of the Faculty of Veterinary Medicine at the Warsaw University of Life Sciences, at the Department of Pathology and Veterinary Diagnostic. Current research is focused on diagnostics and epidemiology of viral diseases of swine.** He is a co-author of more than 60 scientific papers published in peer reviewed journals.





## Keynote lecture V.

### Jürgen Zentek

Thursday, 4 May 2017, 13:30 – 14:00, Congress Hall

#### Microbiota & diseases

Prof. Zentek received his degree as veterinarian in 1985 from the Veterinary school of Hannover. He received his doctoral degree in 1987 and the degree as specialist in animal nutrition and dietetics in 1993. The habilitation (venia legendi at the Hannover Vet School) was finalized in 1994. He had an extraordinary professorship at the Hannover vet school from 1999–2001. After one year as research scientist in Bristol/UK, school of veterinary science, he was appointed on the chair of clinical nutrition at the Veterinary University of Vienna where he became the head of the institute of nutrition. In 2005, he was appointed as professor at the Institute of Animal Nutrition, Freie Universität Berlin. The main research interests cover the role of nutrition for the intestinal microbiota and immunity of the gastrointestinal tract. Prevention of health disorders in domestic animals is one of the main topics of the institute with a specific focus on gastrointestinal diseases. Prof. Zentek had directed a collaborative research group on the mode of action of probiotics in pigs and has been involved in many European and national research projects on feed and food safety.



## Keynote lecture VI.

### Wolfgang Schafzahl

Thursday, 4 May 2017, 14:00 – 14:30, Congress Hall

#### What Can I Do in Case of Gastrointestinal Disorders?

##### Work experience

Dates: 1988 to present: occupation or position held: Veterinarian

Main activities and responsibilities: Swine veterinarian, manager of a veterinary clinic, international swine health and management consultant

##### Education and training

Dates: April 1998: title of qualification awarded: *Specialist in swine medicine*

Name and type of organisation providing education and training: Austrian chamber of veterinarians

Dates: September 1994: title of qualification awarded: *Specialist in animal nutrition and dietetics*

Name and type of organisation providing education and training: Austrian chamber of veterinarians

Dates: 1986 to 1988: title of qualification awarded: *Dr. med. vet. (Doctor of veterinary medicine)*

Principal subjects/occupational skills covered: Veterinary medicine (Doctoral Programme)

##### Topic of the thesis:

Seroepidemiological survey about the incidences of particular swine diseases in the district of a veterinary practitioner

Name and type of organisation providing education and training: University of Veterinary Medicine, Vienna, Austria

Level in national or international classification: ISCED 7





## Keynote lecture VII.

**Paolo Martelli**

Friday, 5 May 2017, 08:30–09:00, Congress Hall

### Measuring and Interpreting the Immune Response



Paolo Martelli (DVM) graduated in Veterinary Medicine with honour in 1984 and since 2002 he is Full Professor of Internal Medicine (Veterinary Clinical Medicine) at the University of Parma – Department of Veterinary Sciences. Paolo Martelli was Head of the Department for 8 years and Vice Rector of the University of Parma for three years. He is Founding Father (2004) and Diplomat of the European College of Porcine Health Management (ECPHM). Currently, Paolo Martelli is President of the ECPHM (2016–2019). He was President of the International Symposium on Emerging and re-emerging Pig Diseases (Rome – Italy, 2003) and of the 6<sup>th</sup> European Symposium on Porcine Health Management (Sorrento – Italy, 2014). He was chair of the Scientific Committee of the 24<sup>th</sup> IPVS/8<sup>th</sup> ESPHM – Dublin 2016. He acted also as President of the Italian Pig Veterinary Society (1995–2001 and 2007–2013).

He has in charge the teaching of Physical examination and Internal Medicine - Clinics of Large Animals. The research activity focuses on porcine health management.

## Keynote lecture VIII.

**Martin Faldyna**

Friday, 5 May 2017, 09:00–09:30, Congress Hall

### The Immune System in New Born Piglets & Passive Immunity



Martin Faldyna, DVM, Ph.D. graduated in veterinary medicine at the University of Veterinary Medicine and Pharmaceutical Sciences Brno, Czech Republic in 1994. Since 1994 he is an employee of Veterinary Research Institute, Brno, Czech Republic. From 2001, when he defended his Ph.D. thesis in veterinary microbiology and immunology, dr. Faldyna became head of Immunology Department of Veterinary Research Institute. He is also involved in teaching of veterinary immunology as assistant professor at Institute of Infectious Diseases and Microbiology of the University of Veterinary Medicine and Pharmaceutical Sciences Brno. Current research activities cover clinical and anti-infectious immunity in farm and pet animals, incl. host-pathogen interaction mainly on swine model, flow cytometry and postnatal development of immune system. He is a (co)author of more than 100 scientific papers published in peer reviewed journals.





## KL01 - ANTIMICROBIAL RESISTANCE FROM A GLOBAL AND ONE HEALTH PERSPECTIVE

***D. Mevius***<sup>1,2</sup>

<sup>1</sup> *Head of the National Reference Laboratory on Antimicrobial Resistance in Animals, Wageningen Bioveterinary Research - Lelystad, Dept. of Bacteriology and Epidemiology, The Netherlands*

<sup>2</sup> *Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Utrecht The Netherlands.*

Infectious diseases, as well as non-communicable diseases and in humans, animals, and plants, are inherent to life on Earth. During human history, much has been achieved in the control of diseases, leading to significant advances in human health and agricultural productivity. What we have gained with effective disease control and prevention has been of great benefit both to the developed and the developing world. However, new diseases continue to emerge and these diseases are major stumbling blocks to prosperity. Antimicrobial resistance (AMR) is not a disease as such, but its rapid evolution in health care in the past decades greatly affects the efficacy of antimicrobial treatment of patients infected with multi-drug resistant (MDR) organisms. Therefore, AMR is currently considered to be one of the major public health threats for the near future. The environment is the natural reservoir of resistance genes. Soil bacteria, especially bacteria belonging to the family of Actinomycetes are known to produce antibiotic resistance proteins to protect themselves from antibiotics they produce themselves. Possibly antibiotics produced by these bacteria exert selective pressure on other microorganisms in the same habitat as well. The existence of these resistance genes, as well as precursor proteins that originally have alternative biochemical functions but can easily change into resistance proteins in the case of selective pressure, are considered the source of resistance in human and animal bacterial populations. The existence of natural resistance genes is demonstrated by the presence of low frequencies of resistance genes in *E. coli* isolated prior to 1950, before antibiotics were widely used, and the presence of antibiotic resistance in natural, remote environments. The use of antimicrobial drugs in human and animal healthcare, as well as additional applications such as aquaculture, crop protection, animal feed additives and food conservation has resulted in the widespread development of resistance not only in bacteria in humans and animals, but also in the environmental reservoir. Consequently, MDR organisms such as VRE, MRSA, ESBL-producers, and carbapenemase-producers have emerged in patients from the 1960s onwards, posing increasing threats to health care. All these MDR-organisms of importance for humans have a partial source and separate evolution in livestock which results in ongoing debate about antibiotic use in livestock, upscaling of farms sizes, farm densities in densely populated country regions and food-safety. AMR is therefore truly a one health problem, of which the relationships between animal, environmental and human reservoirs is evident. However, these relationships are extremely complex and indirect. This will be presented from the perspective of an expert veterinary microbiologist from a country with low antibiotic use and effective infection control and AMR-problems in humans and high but decreasing antibiotic use but still substantial AMR-problems in livestock.



## KL02 - ONE HEALTH AND ANTIMICROBIAL RESISTANCE AT THE HERD LEVEL

### **B. Gonzalez-Zorn**<sup>1,2</sup>

<sup>1</sup> Department of Animal Health, Veterinary Faculty, Madrid

<sup>2</sup> Health Surveillance Center VISAVET, Madrid

Antimicrobial resistance (AMR) is, today, one of the most important health threats for humanity. This has been stated by the WHO and the UN in the last year. Traditionally, the fight against AMR was faced by antimicrobial prescribers, medical and animal doctors, in a non-coordinated manner.

Today we know, that bacteria and AMR-genes move between different compartments including the environment, animals, humans and food, and that the battle against AMR needs a common harmonised effort of all professionals working in these fields.

In this sense, Veterinarians all over the world need to take measures at a farm level, that mostly include management solutions that can reduce use of antimicrobials. Reduction of prescription of antibiotics is urgently needed. The difficulty and interrelation with Public Health has become specially clear in the case of colistin. Colistin was banned for human parenteral use in the early 50s due to its nephrotoxicity. Many alternative effective antibiotics were then available for clinical use. However, due to the high-level resistance of most of the nosocomial pathogens and the lack of therapeutic alternatives, colistin has become in the past years a last resort antibiotic in Hospitals, used to save human lives in Intensive Care Units all over the world. Further, novel mobile colistin resistance genes are being identified in farms, the community and hospitals. In addition to colistin, in different countries, the focus on the reduction of antibiotics is set on different antimicrobial molecules and families. Harmonization of the reduction of specific antimicrobial families in different countries may be a good strategy to reduce world wide the impact of AMR in pigs on human health.

The global approach, One Health, represents the very basis of the actions against AMR in Europe and world-wide. In this context, the measures to be taken in AMR in pig production, do not only have to have an animal health rationale, but rather a One Health approach, aimed at reducing the antibiotic selective pressure to, ultimately, save human lives and preserve the efficacy of our current antimicrobial armament.



## KL03 - IT'S RAINING VIRUSES... WHAT DOES IT MEAN?

**J. Segalés<sup>1</sup>**

<sup>1</sup> *Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona and Centre de Recerca en Sanitat Animal (CRESA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*

Emerging infectious diseases (EID) can be defined as those which incidence is increasing following its first introduction into a new host population or in an existing one as a result of long-term changes in its underlying epidemiology. This concept can also include those diseases linked to pathogens expanding into an area in which it was not previously reported, or due to pathogens that changed significantly its clinico-pathological presentation. During last 10 years, much emphasis has been focused on human EID caused by pathogens of animal origin. All these zoonotic threats and events have emphasized the need for a “One Health” approach, which integrates communication, collaboration and coordination between public health, animal health and other communities at multiple levels to prevent, detect and control emerging or re-emerging infectious diseases at the animal–human–environment interface.

The number of novel conditions in swine included under the concept of emerging and re-emerging diseases has increased importantly during last 20-30 years. Most of them are infectious diseases, being those of viral origin of great importance. Their transmissibility and maintenance into a population is favoured by a number of phenomena, including intensive rearing practices and globalized/international trading.

Besides those novel or re-emerging pathogens able to cause overt disease, there are a number of newly discovered viruses for which no evidence of associated disease does exist. For example, from 1985 to 2010, novel pathogen species were identified at an average annual rate of 3 in pigs (including all types of pathogens). The advent of modern diagnostic and research methodologies, sometimes without the need of previous knowledge about the putative pathogen (i.e., high throughput sequencing), has increased significantly the number of microorganisms that are infecting animals. In consequence, a complex scenario with novel infectious agents which importance is rather unknown is being faced nowadays by researchers and veterinarians.

The objective of the present review is to discuss about new swine diseases or novel presentations of already known diseases, as well as newly recognized infections with a not well-defined pathogenic effect in pigs. Such scenario implies to play with certainties and uncertainties, since last 30 years taught us about:

The emergence of global diseases for which there is still not a clear definitive solution (i.e., porcine reproductive and respiratory syndrome, PRRS)

The emergence of global diseases for which the pathogen existed long before, but overt disease was only recognized recently (i.e., porcine circovirus type 2-systemic disease, PCV2-SD)

The emergence of global diseases for which the pathogen has apparently varied in virulence (i.e., porcine epidemic diarrhoea, PED)

The recognition of putative novel viruses for old diseases (i.e., atypical porcine pestivirus as cause of congenital tremors type AII)

The discovery of viruses that were not novel but considered potential causes of zoonosis (i.e., hepatitis E virus)

The discovery of viruses that were not novel with unknown outcome related with its infection, although considered to be harmless (i.e., torque teno sus viruses)

The list of new recognitions, identifications and discoveries is much longer and will definitively increase in the future. Moreover, who can predict the impact of these new viruses? Are the today's considered harmless viruses the potential pathogenic agents of tomorrow? Are some of these agents part of the „normal“ virome of the pig? Are we able to stop the entrance of some of these agents in our country or region? Do we really know if these agents are already in our region or country? Are we selecting pig genetics that are more susceptible to agents that currently do not cause overt disease? There are still many questions that remain to be answered in the field of swine diseases and the impact of novel viruses.

It is hard to predict what will come next in the swine industry in terms of diseases, but the advent of PRRS by late 80s and beginning of 90s, PCV2-SD by late 90s, the pandemic influenza A/H1N1 by 2009 and PED in North America and Europe in 2013/14 implies to have new viral disease emergences every 7-8 years. Therefore, the risk of emerging and re-emerging diseases with significant economic losses and infections with unknown impact on production as well as on the human population is ensured, and deserves preparedness and proper basic and applied research. Pig production is a good example of a globalised industry, and swine veterinarians and researchers in conjunction with producers, consumers, and stakeholders should join efforts for more global, collaborative, and action-oriented approaches towards logical and practical solutions.



## KL04 - APPROACHES FOR NEW VIRUS DISCOVERY: SOLVING OR CREATING PROBLEMS?

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Historically virus discovery approaches involved filtration, isolation in tissue culture and electron microscopy. Culture techniques were the gold standard for the detection of viruses but vast majority of viruses are not easily cultivable. Recent developments in culture independent molecular analytical methods provided a breakthrough in virus discovery in human and veterinary medicine. These molecular methods can be sequence dependent or sequence independent.

The sequence dependent methods require prior knowledge of the nucleic acid sequence in order to amplify, sequence and analyse fragments of the genome. Novel species of bacteria have been discovered using DNA sequencing of conserved 16S rRNA gene. In contrast viruses do not have any common gene that could be targeted and serve as a phylogenetic marker. Generally missing sequence information can be obtained through the analysis of the conserved genome fragments of groups of known virus species (e.g. from one virus family) and the generation of consensus PCR primers allowing the amplification and DNA sequencing of novel viruses, even only distantly related to the ones that are already known. Phylogenetic analysis of the nucleotide sequences of the amplicons may eventually provide a hint of new virus species to exist. However, this approach is of no use for the discovery of novel viruses.

Metagenomics represents an alternative, sequences-independent approach, to detect novel viruses. In theory any virus present in the clinical sample, known or unknown, can be detected with this approach. In the recent years metagenomic technologies have developed rapidly and now are based on high throughput sequencing methods known as next generation sequencing (NGS). Metagenomic analysis consists of a sample preparation step, high throughput sequencing and bioinformatic analysis of massive amounts of DNA sequence data. Samples for metagenomic analysis has to be prepared in a way to enrich virus sequences present in minority in the sample hence by removing as much as possible of the non-virus related nucleic acids. The probability of detecting a pathogen depends on the proportion of the pathogen molecules in a given nucleic acid preparation. After nucleic acid extraction, DNA or cDNA has to be amplified randomly (sequence-independent) and the library of DNA fragments is prepared which are subjected to DNA sequencing. Nowadays there are several high-throughput sequencing platforms available. For example the Illumina platform is based on sequencing of the DNA fragments ligated to oligonucleotide adapters that act as sequencing primers. A sequencing run generates gigabytes of data consisting of millions of short (75-100 nucleotides) reads that have to be analysed to extract the genetic information. The reads are compared to previously documented virus sequences by similarity search tools, such as BLAST. In the next step selected reads are assembled bioinformatically to generate contigs (long sequences). However, this approach does not enable identification of completely new viruses, for which no "reference" sequences exist. To discover completely novel viruses *de novo* assembly has to be performed. This however may "generate" artefactual sequences. Thus to confirm that virus-like sequences obtained through *de novo* assembly really exist in nature it is essential that confirmatory PCR amplification and sequencing of the amplicons have to be performed.

Recently metagenomic analysis has allowed the discovery of a large number of novel viruses in all animal species including pigs. Some of these viruses were suspected to be involved in disease conditions however most of them were shown to be highly prevalent, also in apparently healthy animals. Historically, diseases caused by viruses were known before their etiologic agents identification. As the samples in which new viruses were detected were obtained from diseased individuals, a general view emerged that viruses are pathogenic and cause clinical diseases. Later we learned that some viruses (e.g. PCV2) can cause clinical signs and lesions only under specific circumstances (immune suppression, co-infections with other pathogens, etc.). Today we know that many viruses can be a part of a "normal" microbiome or virome in humans and animals, possibly with beneficial effects to the host. However other viruses considered today as harmless members of the "normal" virome may emerge into pathogenic entities due to the evolutionary processes.

Today viruses discovery is relatively easy however the identification of their true role in humans or animals is much more complex process, and the prediction of their future role is virtually impossible to be made. A simple discovery of a novel virus in an animal with clinical disease unlinked to any known pathogens is not a proof of the pathogenic role of this new virus. One of the Koch's postulates that requires the candidate etiological agent be isolated from diseased individual and grown in pure culture cannot be fulfilled for many viruses which are simply non-cultivable. One of the alternatives can be the comparison of genetic markers such as specific sequences (metagenomes) that can uniquely distinguish diseased from healthy individuals, or populations. Metagenomic surveillance of the taxonomic signatures representing the viruses present in a population can help in early identification of etiological factors of new epidemics in humans or animals.

In summary, modern high throughput DNA sequencing methods allow for fast, sensitive and relatively inexpensive new virus discovery. However, the advantages and limitations of metagenomic approaches in virus detection will have to be better understood by veterinary practitioners and laboratory diagnosticians.

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## KL05 - MICROBIOTA & DISEASES

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The intestinal microbiota has developed in co-evolution with domestic animals. The host genotype and external factors determine its composition and metabolic activity. Piglets are almost sterile at birth and first colonisation takes place in the birth canal. The development of a diverse microbiota is mandatory for the development of the digestive function, maturation of the gut-associated lymphoid tissue and the general immune function. The optimally balanced composition of the intestinal microbiota is still a matter of discussion, but it seems clear that an age related development is essential for animal health and welfare. The so-called „eubiosis“ of the gut microbiota allows optimal digestion, immune function and health. A „dysbiosis“, defined as undesirable composition of metabolic activity of the intestinal microbiota may be associated with diarrhea and other health problems. It has become obvious, that pre- and early- postnatal nutrition and environmental conditions affect the intestinal colonisation and function. Many European and national projects have addressed this issue, for instance the Cost Action „PiGutnet“ (<http://www.pigutnet.eu/>). The ban of in-feed antibiotics as growth promoters has stimulated more integrated approaches to the relationship between gut function, health and the intestinal microbiome. This does not only include research on defined pathogenic bacteria, for instance *Escherichia coli* (F4 and F18 fimbrial types) and the expression of their pathogenicity in the gut system, but is nowadays taking more and more interactive factors, mainly genotypes, feed composition and the environment of the pathogen („pathobiome“) into account.

The present state of science and technology in the field of the prevention of infectious diseases in farm animals, especially in piglets needs to consider the overall situation. In particular, basic data on the intestinal microbiota and its relationship to animal health as well as the use of antibiotics and innovative concepts for prophylaxis are often not yet mature enough for practical applications. In itself, the contribution of animal nutrition science to the reduction of diarrhea and the prevention of the useage of antibiotics is recognized as being important. However, in many farms the problem of intestinal diseases is still present despite considerable efforts of nutritionists to optimize diets and still belongs to the most common causes, why antibiotics are used therapeutically. Therefore nutritional physiological approaches are considered to be one important pillar of novel prophylaxis strategies, which are promising in combination with microbiome analysis and the optimization of other approaches, e.g. vaccinations. Important aspects of nutrition include in particular the optimal formulation of the feed by adapting the nutrient profiles as well as the selection of optimally digestible feed components, the avoidance of stress factors, for example impaired feed hygiene which is often adversely affected by mycotoxins and other hygiene deficits. The use of appropriate feed additives is of outstanding interest. Especially organic acids, probiotics, prebiotics, enzymes and phytogetic additives have been studied in detail regarding their impact on performance and health of pigs. From our own experience it is shown that often positive effects on animal health are achieved by means of changes in diet and recipe optimization. The current prophylaxis concepts, however, have numerous weaknesses, in particular it is difficult to assess the potential efficacy of different feed additives under practical conditions. Currently, the composition and metabolic activity of the intestinal microbiota is not yet regarded in depth when discussing the use of feed additives or corresponding dietary modifications. It is unquestionable that the composition and the activity of the intestinal microbiome is an important factor influencing the effectiveness or also the ineffectiveness of dietary measures. Methods for the characterization of the microbiome are methodically sophisticated and have hitherto been mainly subject to scientific investigations. Practical studies are urgently required to optimize the use of feed additives such as prebiotics and probiotics with a protective effect against intestinal diseases. It is important that future diagnostic procedures, at a reasonable cost and effort, will allow for a reliable assessment of the farm situation, regarding both, the occurrence of antibiotic resistant bacteria, pathogens and the diversity of the intestinal microbiome. The characterisation of the microbiome in the sense of a pathobiome, defined as the pathogen in the surrounding environment (Vayssier-Taussat et al., 2014), might offer new opportunities for innovative health strategies.

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## KL06 - WHAT CAN I DO IN CASE OF GASTROINTESTINAL DISORDERS?

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### Introduction

Optimal intestinal health is crucial to the psychological and physical well-being of animals and humans. Thus, intestinal health can be seen as a synonym for health and performance in modern livestock production.

The intestine comprises highly differentiated anatomical, histological, physiological and immunological structures and systems. In addition, the intestine is populated by about 100 trillion predominantly anaerobic bacteria, consisting of more than 10,000 different species.

These bacteria, the microbiota, have for a long time been of no great physiological importance in monogastric animals. Today the microbiota is even referred to as an "own organ system" with a metabolic activity greater than that of the liver.

Despite the superior importance of the bowel for health, there is still a lack of a clear definition of the requirements placed on this complex multifunctional organ system.

The most important tasks of the gastrointestinal tract are in the area of barrier function and immunomodulation, besides its digestive work for the supply of energy and nutrients for the maintenance of homeostasis in the organism. Its mucosal surfaces represent a boundary between the "outside world" (unsterile gut content) and the "inner world" (sterile submucosal tissue), the place of origin of the immunological responses, which we call mucosal immunity.

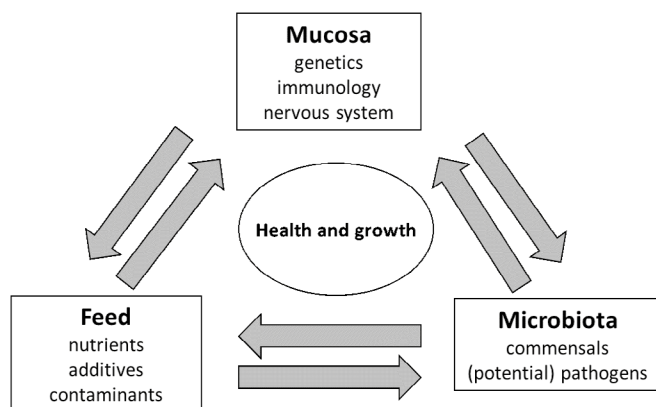
### Intestinal immune system

The immune system of the intestine is very active with more resident immune cells than elsewhere in the body. With approximately 10 weeks the lymphoid immune tissue is mature. In addition to the genetic factors and the environmental conditions (stress, heat, starvation) the immune responses of the pig are determined by the individual immunological experience of the animal.

In addition, the immune system is in a constant interrelationship with the resident microbiota, and with pathogens and other antigens (allergens from the feed) which are taken orally. A dense layer of epithelial cells covered by a thin mucus layer forms a physical barrier between the sterile mucosa and the non-sterile intestinal contents to prevent bacterial translocation and excessive immune activation. The gut immune system responds to the intestinal contents, which are the inducers of the immune system comprising of pathogenic or commensal bacteria, viruses, toxins, allergens, feed components and other substances. The reaction of the immune system can lead to the controlled defense of antigens, as well as to an overwhelming pathological immune reaction, such as an allergy or an autoimmune disease or to immune tolerance. In any case, it is the task of the immune system to ward off pathogens, to maintain intestinal integrity, to ensure an optimal nutrient retention, and all with minimum intestinal inflammation, since every inflammation in the body is an energy-consuming, performance-reducing process. The powerful enteric nervous system plays an important role in the process of attenuating the intestinal immune system and the prevention of excessive immune responses (Tracey, 2002). Afferent fibers of the vagus nerve, which also have receptors in the intestine, trigger an anti-inflammatory reflex in the CNS in the case of infections, injuries or cytokine releases via efferent cholinergic neurons, in order to protect the physiological functions of the organism in real time.

The intestinal bacterium *Bacteriodes fragilis* also has an anti-inflammatory effect with the membrane-bound sugar polysaccharide A (Mazmanian et al., 2008). The increased disappearance of this bacterial species from the intestine of humans could be responsible for the frequent occurrence of autoimmune diseases (e.g. multiple sclerosis, Crohn's disease) in the last decades.

The gastrointestinal tract fulfills its tasks through continuous interaction between its three most important components.



*Schematic representation of the three components of the intestinal ecology important in determining health and growth in production animals. Mutual interactions exist between the three components (feed, microbiota, and mucosa), and for each component the major factors of influence within the component are given. (Niewold, 2015)*



The intestinal **mucosa**, the **microbiota** and the **feed** are these 3 factors which influence each other and thus determine the functionality of the digestive system. In modern animal production, which aims at the greatest possible biological performance in all livestock, feed is certainly the most important factor in this trio.

### Feeding and intestinal health

The increased performance, both in the number of reared piglets and the output of meat or milk, require completely different feeding strategies than in the past to keep the animals healthy and productive. These performance targets can only be achieved by healthy animals in best physical and emotional well-being.

This fact implies the need for animal welfare, because only animals, which feel comfortable can permanently provide optimal performance. Nowadays pig feeding is as complicated as the nutrition of top athletes.

Even though pigs achieve higher biological performance through intensive breeding and selection, the morphological and functional properties of the intestine and the intestinal mucosa are apparently unchanged and an area of little attention.

Therefore it is very astonishing that this "stone age bowel" is able to fulfill its new tasks in today's high performance pig.

One of the few exceptions is the development of genotypes of pigs resistant to *E. coli*, where pig don't possess receptors for the fimbria antigens F18 and F4 (K88) on the intestinal mucosa.

Gut maturation starts immediately after birth and is mainly driven by the immediate feed intake and enteral nutrient presentation. Prolonged parturitions, hypoxia, delayed uptake of colostrum and lung infections short after birth delay the development of the gut and could be initial factors for neonatal diarrhea.

This problem is also present in human nutrition because within a few generations people have entered into a situation of food surplus after decades of food shortages and starvation. In humans, the intake of high energy food leads to civilization diseases such as metabolic syndrome, cancer, functional bowel disorders and postprandial inflammation (Margioris, 2009). It is argued that such postprandial inflammatory processes after the intake of high energy diets are also present in farm animals (Niewold, 2010).

It is a great challenge for future pig veterinarians to understand exactly the influence of feeding on animal health in order to control it.

### "A healthy bowel makes a healthy pig."

#### Calcium, phosphorus, zinc, polyphenols, yeasts

High levels of calcium and phosphorus reduce the gene expression of proinflammatory cytokines (Metzler-Zebeli et al., 2012). However, in the post-weaning period the acid binding capacity of the diet must not be increased too much.

Besides other physiological effects, zinc reduces the expression of K88 receptors and thus counteracts *E. coli* problems. By-products from food production, such as grape or apple remnants, have a high content of polyphenols. These polyphenols exert a protective effect against intestinal disorders in the post-weaning period. It was claimed that the yeast *Sacharomyces cerevisiae* boullardi reduced the bacterial translocation to the mesenteric lymph nodes after ETEC (enterotoxigenic *E. coli*) challenge.

#### Amino acids

Glutamine is an important amino acid to strengthen the intestinal barrier by expressing protective heat-shock proteins (Zhang et al., 2011). Furthermore it increases the villi/crypt-ratio as well as average daily gain and feed intake. Likewise, the amino acids tryptophan, threonine and arginine have an anti-inflammatory effect and protect the intestinal cells from oxidative stress. Porcine spray-dried plasma also has a protective effect in the intestine, on the one hand by a specific supply of antibodies and on the other by the nonspecific occupation of mucosal *E. coli* receptors. These dietary effects are mainly observed in the post-weaning period.

#### Mycotoxins

In animal studies, the chronic intake of mycotoxins caused anorexia, immune dysregulation, impaired weight gain and inhibits intestinal cell proliferation. There are indications that co-exposure to low concentrations of different trichothecenes synergistically harm the intestinal integrity. Thus, non-cytotoxic doses of deoxynivalenol (DON) may modify the composition of the microbiota and increases the translocation of bacteria across the intestinal epithelium.

#### Dietary fiber

The nutrient fraction, which has hitherto hardly been considered in animal nutrition, is the dietary fiber. It's the "food" for the gut bacteria and of huge importance for the morphological and functional development of the intestine.

Non-starch polysaccharides (NSP) and dietary fiber (DF) are often used as synonyms. Dietary fiber consists of carbohydrates that are resistant to digestion by digestive enzymes and absorption in small intestine. Dietary fiber includes non-starch polysaccharides (NPS), non-digestive oligosaccharides (NDO), resistant starch (RS), and lignin. Providing bulk to the gut lumen, it is the main substrate for bacterial fermentation, particularly in the large intestine. Chemically, dietary fiber consists of non-starch polysaccharides such as arabinoxylans, cellulose, and many other plant components such as resistant starch, inulin, lignin, chitins, pectins,  $\beta$ -glucans, and oligosaccharides. Dietary fibre is mainly found in protein crops, cereals and co-products from the agro and food industry, such as sugar beet pulp, pea husks or potato pulp.



Figure 1 - Dietary fibre concentration (g/kg dry matter) in different feedstuffs.

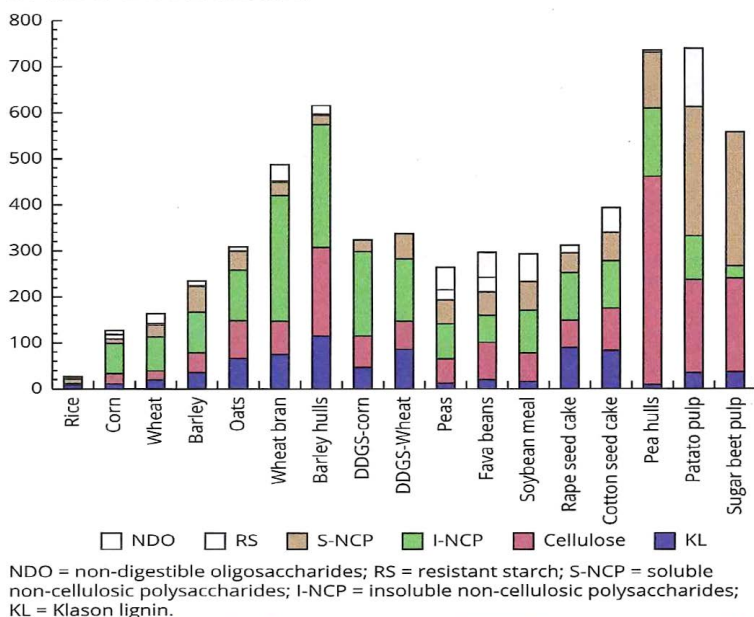


Figure 1 (Bach Knudsen 2016)

Dietary fibre concentration in different feedstuffs These carbohydrate compounds are chemically very inconsistent and differ in their chemical, physical and physiological properties. They are soluble or insoluble in the intestine, have the ability to swell and hold water in the cell wall matrix, thus they increase viscosity of the ingesta.

Thus, depending on their chemical structure, they influence the viscosity of the ingesta and its transit time through the intestine. For instance sugar beet pulp will primarily increase the water-binding-capacity of ingesta, whereas the viscosity elevating properties of sugar beet pulp are low. Contrary to that, barley, rich in  $\beta$ -glucan will to a larger extend solubilise from the cell wall matrix and raise luminal viscosity.

Soluble dietary fiber will increase luminal viscosity, thereby prolonging gastric emptying and hindering the contact between the ingesta and mucosal digestive enzymes. Furthermore it reduces the contact with the intestinal mucosa, which leads to a reduction in nutrient digestibility. In addition, a higher concentration of  $\beta$ -glucans from barley in the weaning diet increases the expression of E. coli K88 receptors and thus exacerbates post-weaning colibacillosis and diarrhoea (Ewaschuk et al., 2012).

Insoluble dietary fiber, such as wheat bran, oat husks or powder cellulose, promotes intestinal health by increasing villus length and villus / crypt ratio (Hedemann et al, 2006). These morphological changes improve intestinal integrity by thickening the protective mucin layer and thus prevent bacterial translocations from the intestinal lumen into the sterile submucosa. Thus, insoluble dietary fibers also show anti-inflammatory effects in the colon (Schedle, 2008).

Soluble and insoluble dietary fiber can be fermented at different degrees by the microbiota in cecum and colon, depending on their chemical, physical properties. The metabolites of fermenting dietary fiber, proceeding mainly in the cecum and in the anterior portions of the colon, are short chain fatty acids (SCFA), such as butyric acid, propionic acid and acetic acid. In pigs, these fermentation products lower the pH in the intestine and contribute up to 30% to the animals energy supply (Bergmann, 1990).

Wheat with its high content of fermentable fibers, the arabinoxylans boosts the fraction of butyrate-producing bacteria and thus increases the butyrate content in the colon. Butyrate is an important metabolite providing energy for mucosal cells. Without butyrates for energy, colon cells undergo self-digestion and die. This fatty acid also influences the gene expression and the morphological differentiation of the intestinal epithelium.

Interestingly, dietary fibers, rich in pectin (e.g. sugar beet pulp) have an impact, to stimulate the expression of heat shock proteins (HSP) (Liu H.Y. et al., 2012), regardless of the composition of the microbiota. Heat shock proteins play an essential role in the maintenance of the intestinal barrier and protects the intestinal epithelium from oxidative stress and chronic inflammation (Arnal M.E. et al., 2016).

Non-digestible oligosaccharides (NDO), which are found especially in legumes, improve the intestinal barrier and reduce post-weaning diarrhea. There are a large number of different dietary fibers, which influence the flow of the ingesta and non-starch-polysaccharides (NSP) into the hindgut at different rates.

This substrate supply for the mikrobiota of the large intestine stimulates all bacteria species at once, increasing SCFA production as well as the bulk mass. Large quantities of fermentable NSPs entering the large intestine can also result in diarrhea, induced by the osmotic effect of the metabolites from the bacterial fermentation, such as SCFA.

There are only few dietary fiber polysaccharides, such as NDOs, pectin, inulin, and resistant starch, which have the ability specifically to stimulate beneficial microbial groups.

Despite the fact that dietary fiber in diets is absolutely essential for gut health, it is not included in the nutrient requirement tables.



### **Dietary Protein**

Usually the most diets are high in protein in order to utilize the performance potential. It is very important to pay attention to the biological value of the protein sources to ensure a sufficient supply with essential amino acids. Dietary protein in excess provokes a redundant metabolic burden for animals and with negative impact on the environment due to odor emissions from barns.

Proteins that escape digestion in the small intestine are available for bacterial fermentation in the large intestine. Protein digestion results in a greater diversity of end products, including short chain fatty acids (SCFAs), indoles, thiols, amines, phenols and the gases CO<sub>2</sub>, H<sub>2</sub> and H<sub>2</sub>S, many of which have toxic properties.

### **Dietary influences on intestinal health**

There are several promising results from the various research areas of animal nutrition with an influence on intestinal health.

With the exception of some well-known additives (zinc, glutamine, acids, yeasts, etc.) the knowledge about the effect and also the interaction with other feed components, the environment and the herd-health status under field conditions is still limited.

Further controlled field trials are necessary to map out a final nutritional strategy to boost animal health in general by improving the gut health. We need such sophisticated nutritional strategies to keep the animals healthy and powerful and to reduce the use of antibiotics.

### **Gastrointestinal disorders**

In pigs the majority of clinical evident gastrointestinal disorders are infectious diseases. In addition to infectious diseases, gastric ulcers and the hemorrhagic bowel syndrome (intestinal torsion) play an important role.

### **How do I get to the diagnosis?**

In addition to the clinical examination, checking the environmental conditions (nutrition, housing, climate ) is essential. Always examine the environment in a house at pig level, and do this by walking around not by staying in the office.

### **Clinical examination**

The following tables give a very good overview of the clinical symptoms of some common gastrointestinal diseases in certain age groups (Zimmermann et al., 2012)



Differential diagnosis of some common gastrointestinal conditions of swine (mod. Thomson et al., 2012)

Cause	Age	Key Clinical Signs
Escherichia coli (ETEC, EPEC)	Neonatal: 1-4 days old Postweaning: 2-3 weeks after weaning	Watery, yellowish diarrhea; dehydration; sudden death Diarrhea, ill-thrift, deaths; neurological signs, edema, sudden deaths (edema disease)
Rotaviruses	1 day to 7 weeks; most frequent at 2-4 weeks of age	Watery to pasty diarrhea; may be subclinical; varying degrees of dehydration
Clostridium perfringens type C	1-14 days (rarely older)	Hemorrhagic/watery diarrhea; sudden death
Clostridium perfringens type A	2-10 days (rarely older)	Creamy, watery diarrhea (mild) decreased growth
Clostridium difficile	1-5 days (rarely older)	Creamy diarrhea and dehydration
Cryptosporidium spp.	3 days to 6 weeks	Mild yellowish diarrhea; varying degrees of dehydration
Isospora suis	5-21 days (sometimes older)	Watery/yellowish diarrhea; dehydration
Coronaviruses: TGEV and PEDV	All ages	Profuse watery diarrhea; rapid dehydration; deaths; vomiting often seen
Porcine circovirus type 2	6-16 weeks, occasionally older	Ill-thrift, depression, diarrhea; often respiratory or systemic signs
Gastric ulceration	Any age after weaning, mainly grow-finish pigs	Asymptomatic or if severe, melena, anemia, pallor, deaths
Lawsonia intracellularis	From approximately 5 weeks old to young adults	Usually sloppy diarrhea; PHE has watery hemorrhagic (port wine color) diarrhea, pale carcass, weakness, ataxia
Brachyspira hyodysenteriae	6 weeks old to adult	Pasty, sloppy diarrhea, mucus and blood in feces, lethargy
Brachyspira pilosicoli	4 weeks to 4 months old	Pasty, sloppy diarrhea
Salmonella spp.	All ages after weaning (rarely preweaning)	Variable, watery; feces with fibrin, necrotic or blood flecks; most infections are subclinical
Oesophagostomum dentatum	From weaning to adult	Mild, sloppy diarrhea
Trichuris suis	From weaning to adult	Pasty, sloppy occasionally mucohemorrhagic
Yersinia spp.	From approximately 6 weeks to 4 months old	Pasty, sloppy diarrhea

ETEC, enterotoxigenic E. coli; EPEC, enteropathogenic E. coli; PHE, proliferative hemorrhagic enteropathy; TGEV, transmissible gastroenteritis virus; PEDV, porcine epidemic diarrhea virus

ETEC, enterotoxigenic E. coli; EPEC, enteropathogenic E. coli; PHE, proliferative hemorrhagic enteropathy; TGEV, transmissible gastroenteritis virus; PEDV, porcine epidemic diarrhea virus

Approximate age at which certain causes of diarrhea in pigs are more common (Ramirez, 2012)





### Approximate age at which certain causes of diarrhea in pigs are more common (Ramirez, 2012)

1-2 days	3-4 days	5-6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults
<i>Clostridium difficile</i>												
<i>Clostridium perfringens</i> type A												
<i>Clostridium perfringens</i> type C												
<i>Escherichia coli</i>												
Rotavirus												
Porcine reproductive and respiratory syndrome virus												
Porcine epidemic diarrhea virus												
<i>Toxoplasma gondii</i>												
African swine fever virus												
Classical swine fever virus												
High fever (any condition causing a high fever)												
Sow feeding						Dietary conditions						
Hypoglycemia (agalactia)												
<i>Isospora suis</i>												
<i>Eimeria</i> spp.												
SBM (soybean meal hypersensitivity)												
<i>Salmonella</i> spp.												
<i>Oesophagostomum dentatum</i>												
Antibiotic-induced colitis												
<i>Brachyspira pilosicoli</i>												
Water quality												
Gastric ulcer												
Porcine circovirus type 2												
<i>Lawsonia intracellularis</i>												
<i>Trichuris suis</i>												
<i>Ascaris suum</i>												
<i>Brachyspira hyodysenteriae</i>												
Biotin deficiency												
Niacin deficiency												
Vitamin D toxicosis												
Vitamin E deficiency												
Tryptophan toxicity												
Salt toxicity												
T-2 toxin												
Vomitoxin												

### Sampling techniques for lab submission

The best specimens are acutely-ill live untreated pigs. Alternatively, necropsy or euthanized pigs with intestines collected in formalin within 10 minutes of death.

specimen	fresh chilled	formalin-fixed
Ileum	10 cm pieces	Two 2cm pieces
Jejunum	10 cm pieces	Two 2cm pieces
Colon	10 cm pieces	Two 2cm pieces
Brain ( <i>E. Coli</i> )	Swab (dd. <i>Strep. suis</i> )	
Lesions	10 cm	Several 2 cm pieces
Gut content ( <i>Brachyspira</i> )	2-5 ml (sterile container)	

The samples for histopathologic examination must be taken within 10 minutes of death and fixed in buffered 10% formalin solution. Take care that the mucosa is exposed to the fixative.



Fresh samples must be packaged per each pig and separately from other samples. Chill before mailing, but don't freeze. Swab samples from acutely diseased piglets can also be used additionally for microbiological examination. But an accurate diagnosis of diarrhea in suckling piglets requires submission of tissue. Whole dead piglets are not suitable for lab-submission, because intestines autolyze quickly.

The following table provides a very good overview of patho-anatomical findings on gastrointestinal disorders in different age groups (Thomson et al., 2012).

Pathology of some common gastrointestinal conditions of swine (mod. Thomson et al., 2012)			
Cause	Gross Lesions	Histological Lesions	Common Methods for Laboratory Confirmation
<i>Escherichia coli</i> (ETEC, EPEC, EC)	Fluid, watery gut content; stomach with milk in neonates; gut and eyelid edema	Mucosal congestion with bacteria attachment to intestinal epithelium; angiopathy; malacia in brain	Culture, serotype and/or genotype of isolates; PCR detection; tissue IHC
Rotavirus	Fluid ingesta, pale intestines; sparse stomach contents	Moderate villus atrophy	Virus detection: PCR, antigen-Capture ELISA (agELISA), EM, PAGE; antigen-tissue ICH, FAT
<i>Clostridium perfringens</i> type C	Hemorrhagic enteritis; mucosal necrosis; suppurative ulcerative enteritis	Mucosal necrosis, hemorrhage; gram-positive rods associated w lesions	Histopathology; culture with PCR confirmation of beta-toxin gene; beta-toxin ELISA
<i>Clostridium perfringens</i> type A	Watery-creamy intestinal content	Mild, loss of epithelium from villus tips, mild suppurative inflammation	Histopathology; culture with PCR confirmation of beta2-toxin gene; beta2-toxin ELISA
<i>Clostridium difficile</i>	Mesocolonic edema; creamy colon contents	Multifocal superficial ulcers and Suppuration in colon	Histopathology; toxin detection (ELISA); culture
<i>Cryptosporidium</i>	Fluid ingesta	None or mild villous atrophy; observe oocysts adjacent to surface epithelium	Histopathology-mucosal smear for oocysts; PCR
<i>Isospora suis</i>	Fluid ingesta, mild to moderate fibrinous exudates or necrosis of distal small intestine mucosa	Villous atrophy, fibrinonecrotic enteritis, intracellular coccidial forms	Histopathology; observe immature forms in lesions or mucosal impression smear
Coronaviruses TGE virus PED virus	Thin-walled pale intestine, sparse contents	Severe villous atrophy	Histopathology; detect in feces by PCR, ISH, or by ICH, FAT of tissue; serology
Porcine circovirus type 2 (PCV2)	Generalized lymph node enlargement, edema of mesocolon, pneumonia	Lymphoid depletion, histiocytic infiltration of lymphoid tissues; granulomatous enteritis	Histopathology; detection by ICH or ISH
Gastric ulceration	Ulceration and fibrosis of pars esophagea, possible hemorrhage into stomach and blood in intestines; melena	Mild: hyperkeratosis, epithelial loss severe: full depth necrosis, exposed lamina propria, hemorrhage, granulation, fibrosis	Gross pathology; close examination of the pars esophagea
<i>Lawsonia intracellularis</i>	Small or large intestine (ileum most common has thickened mucosa, sometimes necrotic or ulcerated; in PHE, blood clots in ileum, melena, carcass pale	Enterocyte hyperplasia with cells containing small curved rods (silver stain); in PHE, blood exudation into crypts through intact epithelium	Histopathology; detection by IFA, ICH, ISH on tissue; bacterial detection by PCR (feces or tissue); antibody Detection by serology
<i>Brachyspira hyodysenteriae</i>	Typhlocolitis with fibrinous exudates, diffuse erosions, and hemorrhage; mucus and blood in colon	Epithelial erosions, crypt mucus-goblet cell hyperplasia, fibrin exudation; large spirochetes present	Bacterial detection by culture or PCR of feces or tissue; histopathology with silver stains, IHC, ISH
<i>Brachyspira pilosicoli</i>	Mild to moderate colitis; lesions milder than <i>B. hyodysenteriae</i>	Similar to but milder than <i>B. hyodysenteriae</i> ; end-on attachment of organisms to surface epithelium seen in some cases	Bacterial detection by culture, PCR of feces or tissue; histopathology with silver stains; IHC, ISH
Salmonella spp.	Fibrino-hemorrhagic, local, or diffuse ulcers, lesions present in small and/or large intestine	Diffuse or focal ulcers; neutrophil infiltration, fibrinous thrombi; foci of inflammation in liver	Histopathology; bacteriaby culture; serotype, phage type; antibody by Mix-ELISA
<i>Oesophagostomum dentatum</i>	Erosions, edema, granulomas in cecum and proximal colon	Granulomatous typhlocolitis with nematode parasites	Fecal parasitology; histopathology
<i>Trichuris suis</i>	Typhlocolitis, erosions, sometimes mucus or blood	Erosion/ulceration and inflammation associated with migrating or adult phases of infestation	Gross (not visible for 3-4 weeks); histopathology; ova detected in feces 7 weeks prepatent
<i>Yersinia</i> spp.	Mild enteritis and/or colitis	Mild chronic, active enteritis and/colitis, granulomas, microabsces	Bacterial detection by culture

EDEC, edema disease *E. coli*, ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; PHE, proliferative hemorrhagic enteropathy; TGE, transmissible gastroenteritis; PED, porcine epidemic diarrhea; EM, electron microscopy; PCR, polymerase chain reaction; ICH, immunohistochemistry; FAT, immunofluorescence test; ISH, in situ hybridization; PAGE, polyacrylamide gel electrophoresis, IFA, indirect fluorescent antibody.



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### Nutrition check

In cases of **gastric ulcers, intestinal torsions, “bloody gut”** and **edema disease**, a check of the feed is obligatory. The composition of the diet, the quality of the feed components (DF, toxins, ..) and feeding hygiene have to be assessed properly

For all other intestinal infections, a feed check is highly recommended as a diet optimized is the prerequisite for successful therapy and also important in prophylaxis of enteral diseases.

Feed check parameters	
specifications	water, crude protein, fiber, sugar, ADF, NDF, energy, lysine, sodium, magnesium
ingredients	sources of energy, protein and dietary fiber (DF)
sensoric parameters	smell, taste, grip, dust, pH
technical parameters	particle size (sieve analysis), homogeneity, segregation of mixture
Storage conditions	hygiene, storage time, condensation in silos
contaminants	bacterial count, DON, ZON, yeasts, fungi, enterococci, clostrides, endotoxins (LPS), rodent faeces, insects,
feeding hygiene	equipment, feeders, water pipes, drinkers

### Climate - control

Temperature, humidity, smell, pigs lying behavior and air speed are the main parameters to be checked. Too high air speed is one of the greatest stressors in modern piggeries. For pigs, drafts are just as disturbing as for humans, so this problem is easy to diagnose. The maximum air speed on pig level must not exceed 0.2 m/sec if the incoming air is not preheated. Only if the temperature of the incoming air exceeds the thermo-neutral range of the animals, this limit may be raised, which is still a big challenge for technical ventilation systems. But pig health takes precedence over air quality.

Temperature recommendations for the critical periods are 24 °C room temperature in farrowing rooms in the first 48 hour after parturition and 40 °C on the heating plates for piglets. In the first week after weaning a room temperature above 23 °C and no drafts are recommended to avoid post-weaning problems.

### Gastric ulcers

Stomach ulcers in fattening pigs and occasionally also in sows around parturition cause the birth considerable problems. They can occur within 24 hours and rapidly lead to death. The main causes are an interruption of feed intake with irregular stomach filling, irregular feeding patterns and shortage of feeder space, low protein, low fiber and high energy diets, high levels of wheat in excess of 55% and fluctuating environmental temperatures. For an effective prophylaxis against gastric ulceration, the proportion of fine particles below 0.4 mm should not exceed the limit of 25% in the diet.

### Intestinal torsion, Hemorrhagic bowel syndrome (“bloody gut”)

The torsion of the intestine around the mesenteric root, with subsequent circulatory failure, is a frequent cause of death in fattening pigs. The torsion involves the small intestine or both the small and the large intestine. In most cases, the rotations of the intestines occur after food intake, particularly when the gut is filled with large volumes of fermentable liquid feed. Torsions are often associated with pigs making sudden movements. The hemorrhagic bowel syndrome (“bloody gut”) has a similar appearance as cases of torsion, but no dislocation of the intestines is detected at necropsy. This syndrome occurs under similar conditions as cases of torsion, when pigs receive highly fermentable rations.

An improvement of the diet and feeding hygiene, reduction of the protein content, an increase in non-fermentable dietary fiber (e.g. approx. 1% lignocellulose) combined with a brief intermittent use of an antibiotic (e.g. amoxicillin) to stop the excessive microbial activity, leads to a quick therapeutic success.

### Neonatal diarrhea in piglets

Caused by *E. coli* and / or *Clostridium perfringens* type C and type A (CpC7A) neonatal diarrhea is observed most commonly in pigs aged from 1 to 4 days. The cofactors are deficient colostrum immunity, delayed parturitions, low colostrum intake, too cold ambient temperatures (less than 25 ° C, less than 40°C on surface of the heating plates) or draft (above 0.2m / sec) in the area of the suckling piglets. Often neonatal diarrhea is related to problems in housing, hygiene and management. Neonatal diarrhea by *E. coli* and CpC/A can be prevented through lactogenic immunity induced by vaccination of late term pregnant gilts and sows. The vaccines are usually administered parenterally at about 4 weeks and 2 weeks prior to farrowing. In gilts it could be useful to give a third vaccination 6 weeks prior parturition, if a two time vaccination is not enough effective. In cases where vaccination with commercial vaccines is completely ineffective you can try to use an autogenous vaccine. Furthermore, an effective vaccination program against neonatal diarrhea usually reduces the losses of piglets, which are crushed by the dam. The immunity induced by these vaccines, however, disappears after weaning.

A rapid rehydration of the diarrheic and often hypoglycemic piglets by oral or intraperitoneal administration of an electrolyte is essential as an adjuvant therapy.





### Post-weaning diarrhea, edema disease

Weaning is the most critical period in the life of a piglet. It is well established that this process is multi-factorial and that post-weaning anorexia and undernutrition are major etiological factors. A variety of other cofactors, like housing, climate and management favor the occurrence of diarrhea by ETEC or EDEC.

*E.coli* infections occur worldwide and are endemic in most herds. Deficient preparation of the gut for starch digestion, and a too early switch from a highly-digestible milk diet to less-digestible solid feed, as well as cold accommodation after weaning, hygienic problems are the main on-farm risk factors to trigger these infections.

Good creep feeding practices, weaning ages not younger than 4 weeks, properly cleaning of the feeders at least once a day, supplying with pure drinking water and a strict mono-directional pig-flow reduce these problems considerably.

The temperature must be at least 30°C on piglets-level, since piglets do not gain full thermal body control until they are around 10 weeks-old.

The weaning diet must consist of highly digestible ingredients, similar to the milk of the sow. Lactose at an inclusion rate of 5% in the weaning diet shows positive effects.

The content of soybean extraction meal must not exceed 6% in the diet, as well as other highly digestible protein sources such as potato protein, egg protein, milk protein or spray-dried plasma can be beneficial. The energy content should be at least 15 MJ ME / kg. The pH value should be set between 3.5 - 4.5 and the acid binding capacity should not exceed 700 mEq / kg feed. Post-weaning colibacillosis is influenced by dietary fiber components, which generates high viscosity of the intestinal contents.

Dietary fiber components, like wheat bran, powder cellulose or small amounts of sugar beet pulp (2-4%) prove to be very advantageous since these do not increase the viscosity of the ingesta in the small intestine. The piglets must have unrestricted access to the feed throughout the day, any feed restriction is detrimental to intestinal health. The vaccination against EDEC infections with the shiga-toxin variant 2e (stx2e) shows a very good protective effect. Under optimal conditions, post-weaning diarrhea and edema disease can be controlled predominantly without any medication.

Own trials have shown that the occurrence of streptococcal infections (meningitis) in piglets aged 4 - 12 weeks, which are probably caused by intestinal translocation of the streptococci, can be prevented by appropriate dietary measures.

For this, however, good environmental and housing conditions, as well as a PRRSv stable herd situation are necessary. Other gut stabilizing products, such as certain polyphenols and selected dietary fibers (eg powdered cellulose), have a positive effect on intestinal health during the weaning period.

### Porcine intestinal spirochetosis

Porcine intestinal spirochetosis is a disease caused by *Brachyspira pilosicoli*.

The infection of healthy herds happens by introduction of infected carrier-animals, and horizontal transmission takes place on the oro-fecal route. The disease usually occurs after weaning, but can also affect all other age stages and is often associated with a change of feed. The pathogens penetrate the mucus layer in caecum and colon and attach to the enterocytes. This process is intensely influenced by diet composition.

An increase in viscosity of the intestinal content favors the colonization of the intestine. Feedstuffs, not increasing the viscosity and non-fermentable in the large intestine have positive effects for therapy and prophylaxis. Favorable are powder cellulose, oat chaff, as well as mealy instead of pelletized feedstuffs. High-protein diets also favor the occurrence of these diarrhea problems. The same dietary measures are also successful in therapy and prophylaxis of swine dysentery (*Brachyspira hyodysenteriae*) and salmonellosis (Thompson, 2009). Intestinal health is a prerequisite for the performance of livestock. In Europe, the most important diseases are bacterial infections caused by species of *E. coli*, *Clostridia*, *Brachyspira*, *Lawsonia* and *Salmonella*.

### Conclusion

In contrast to Asia, viral pathogens play a lesser role in Europe. The feed of the animals has a significant influence on the intestinal health. Veterinary dietetics are becoming more and more important, as the use of antibiotics and zinc oxide will be increasingly regulated in the future, at least in the EU. Due to its complexity, there is an urgent need for more veterinary practitioners, well trained in pig nutrition. Non-veterinarian feeding consultants, won't any longer meet the requirements of modern health-oriented nutrition. The veterinary educational institutions, when creating their curricula, also have to take into account that the veterinary practitioner of the future needs a thorough knowledge of pig nutrition for doing his work successfully in modern herd-health management.

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## KL07 - MEASURING AND INTERPRETING THE IMMUNE RESPONSE

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The immune system is a complex and interactive network of different organs, tissues and cells aimed at neutralizing and eliminating all “non self” agents, cells, and conditions. Immunity is continuously engaged in limiting the effects of microorganisms, chemical substances, “cells proliferation out of control” (pre-cancerous or cancerous), etc. by activating an immune response that is able to recognize, destroy, eliminate them and to create protection and memory for future exposure. The protection against these different pathological conditions depends on a complex system of overlapping and interlinked defence mechanisms that collectively destroy and control almost all invaders.

The interplay of the different components of the immune system (cells, chemical substances, proteins, receptors, etc.) is very complex and is based on interaction and signals that connect inflammatory, non specific and specific immune cells which determine the activation, proliferation and differentiation of these cells which are responsible for mounting an efficient defence mechanism.

### MEASURING THE IMMUNE RESPONSE

According to the Oxford Dictionary the verb “to measure” has two main definitions: 1) Ascertain the size, amount, or degree of (something) by using an instrument or device marked in standard units; 2) Assess the importance, effect, or value of (something). So, “to measure” expresses a concept of graduation and enumeration of the quality (importance, effect, value) and quantity of an item, of a phenomenon by an instrument (tools). The question is: “Are we able to efficiently measure immunity as a whole? Based on the complexity of the immune system we cannot directly measure the overall efficiency.

Therefore, the complexity of the mechanisms playing a role in this system makes it impossible to measure the efficiency of immunity as a whole. Consequently, there is no tests available that can estimate the overall function of the immune system. We know, for instance, that ageing compromises the efficiency of the immune system, as do several diseases that directly affect it. Toxic substances can impair the integrity and efficacy of the immune system. Some mycotoxins can play a detrimental role on the immune system based on experimental studies. Sometimes the reduced efficiency of the immune system is supposed to be the causative condition sustaining misdiagnosed diseases.

As mentioned above, each contact between the immune system and a “non self agent” generates a **signal**, a trace of the interaction (i.e. serological response) that can persist for a certain period of time, sometimes for the entire life. The immune response is commonly used for **diagnostic purposes (indirect diagnosis)**. Serology is not aimed at demonstrating the presence of the etiological agent, but at showing that a specific agent was in contact with the defences of the host. That is the most frequently used “measurement” of the immune response.

In human and veterinary medicine in general and in porcine health management in particular, the immune system is required to generate **protection following vaccination**. In fact, vaccination is an “artificial infection” by attenuated or inactivated infectious agents or otherwise assembled parts of immune agents. Vaccines are given primarily to protect against disease, though protection against infection and even infectiousness may also be an important and required effects. In porcine health the switch from **individual immunity** (single pig) to **herd immunity** (population immunity) is necessary.

**The most efficient way to measure the immune response to vaccination is the evaluation of clinical and immunological protection.** Protection is evident, and measured, in terms of the reduction in the risk of the clinical endpoint of interest. Thus, the (protective) **vaccine efficacy (VE)** is defined as the percent reduction in risk of (for example) disease amongst vaccinated individuals compared to equally exposed unvaccinated individuals, or  $VE = (R_{nv} - R_v) / R_{nv}$ , where  $R_{nv}$  and  $R_v$  represent incidence risk or rate of disease in non-vaccines and in vaccines respectively.

In the context of vaccines, **protection** implies an immunological mechanism (both humoral and cellular) to prevent or to reduce severity of infection or disease. Many aspects of these mechanisms are not yet understood. Protection is complex, not only in its intrinsic mechanism, but also in its manifestation. It may be **complete**, such that a protected individual suffers no ill consequences whatsoever if exposed to infection. It may be **incomplete**, implying that the severity of the consequences of the disease is reduced. (The term **partial protection** is sometimes used for this state, but is used in a different way for vaccines). Incomplete protection can be interpreted in two ways: a) the majority of the vaccinated population is protected but a few individuals are not protected at all (fully susceptible); b) the whole vaccinated population has an incomplete protection.

Sometimes, protection may be **situation-dependent**, related to the **infectious agent** (e.g. genetic plasticity, pathogenicity, exposure dose), to the **environment** or to **animal status** (e.g. age) and **concurrent infection**. These nuances complicate the evaluation of vaccines either directly, with disease reduction outcomes, or through **substitute immunological endpoints**, markers of the immune reaction correlated with protection. In figure 1, the induction of protective immunity by a vaccine and the **immune markers (IM)** also known as **correlates of protection** (parameters useful for **measuring the immune response**) are shown.

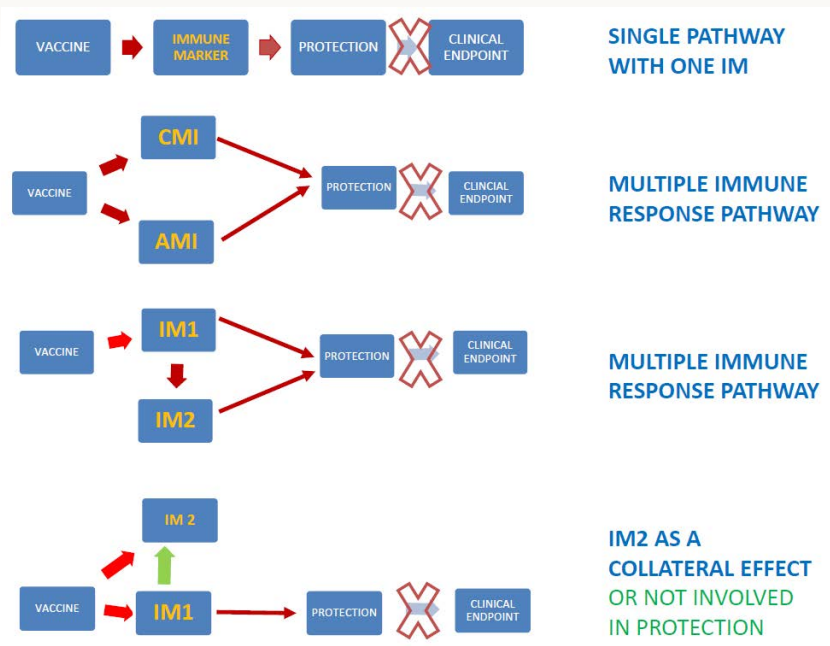


Figure 1. Different pathways of correlation between Immune Markers (IM or correlates of protection) and clinical protection.

#### THE IMMUNE SYSTEM AND IMMUNITY: GENERAL ASPECTS

The immune response to infectious agents is a very complex network of interactions among physical and humoral signals that interconnect inflammatory cells with those of innate and specific immunity, determining their activation, proliferation and differentiation with the ultimate goal of implementing an efficacious defence against pathogens.

Typically, immunity is divided into **innate or natural immunity** (non-adaptive immunity) and **acquired or specific immunity**. The latter is divided into the cell-mediated immunity and the humoral immunity. These two types of immune response are closely related through the close cooperation between their cellular components. Immunity is also coordinated with the neuroendocrine system.

#### The innate immunity: structural and functional features

The components of innate immunity are always ready to act as a “first, prompt line of defence against the pathogens” controlling and containing them at their entry site. Innate immunity is focused on the initial elimination of the pathogen and also on the differentiation and activation of the dendritic cell for efficient antigen presentation to specific immune cells such as lymphocytes.

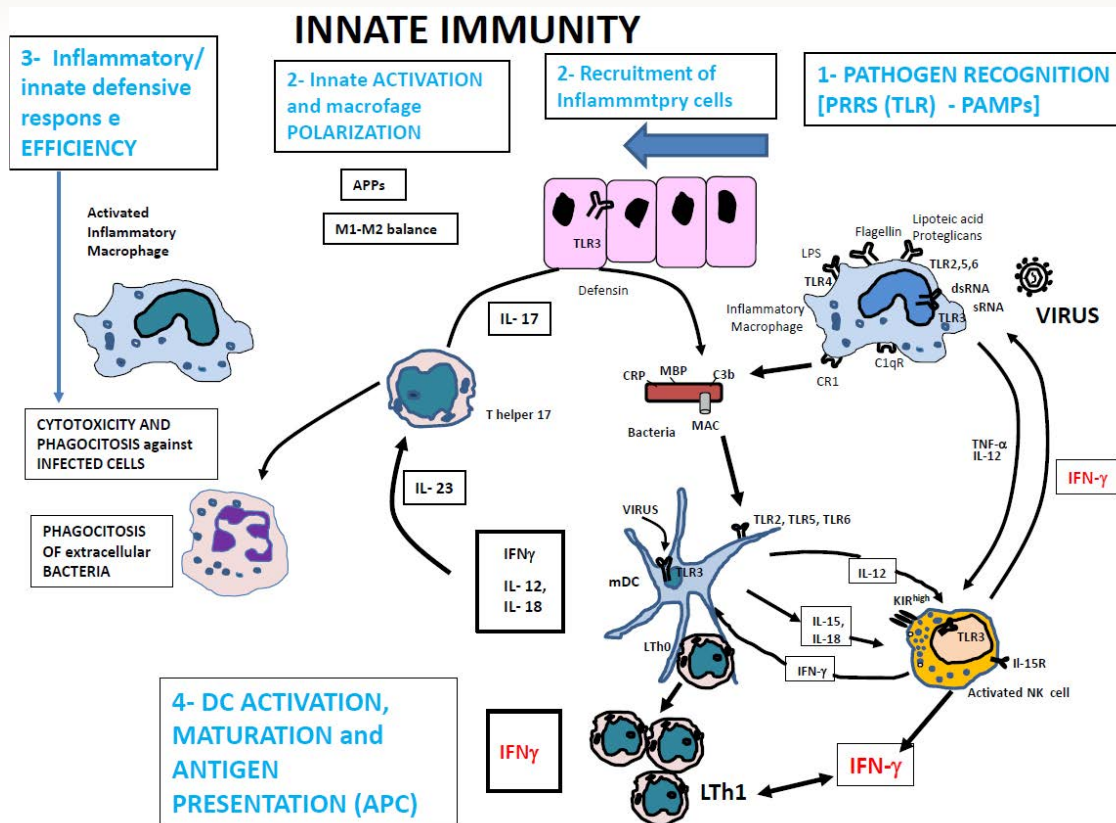


Figure 2. Cellular and molecular mechanism involved in the innate immune response.

**An efficient innate response is essential for an efficient adaptive immune response.** The natural recognition of the pathogen, mediated by natural receptors, with efficient production of inflammatory cytokines (IL-1, TNF $\alpha$ , IFN  $\alpha/\beta$ , chemokines) plays a major role in the connection between innate and acquired immunity; this allows the recruitment and activation of inflammatory and innate cells at the sites of infection and of lymphocytes and DC in lymphoid tissues and tributary lymph nodes. Moreover, cytokines from the innate cells induce the maturation of DCs for antigen presentation and for driving the differentiation of T helper Lymphocytes and establishing specific immunity.

#### Measurement of the innate immune efficiency upon viral infection.

The major markers for the measurement of the efficacy of the innate immune response are:

- 1) the expression of **TLR** by RT-qPCR from PBMC or in biological fluids (e.g. BAL fluid) or by IHC in the tissues;
- 2) the measurement of the main **proinflammatory** (type 1 INF, IL-1, TNF- $\alpha$ , IL-8, IL-6) and **proimmune cytokines** (IFN- $\gamma$ ) by gene expression (RT- qPCR) and/or as protein secretion by ELISA or by IHC in tissues;
- 3) the measurement of the **blood level of innate immune cells** (i.e. NK and gamma/delta T-lymphocytes by Flow cytometry);
- 4) the evaluation of the secretion of the above-mentioned cytokines associated with **cell phenotype** (e.g. macrophages, Gamma/delta lymphocytes, NK) by means of Intracellular Staining (IS) and Flow cytometry.

The best method could be the qualitative and quantitative evaluation of cytokines by RT-qPCR or ELISA comparing different conditions (e.g. vaccinated vs. non-vaccinated; infected vs. non-infected etc.).

In pigs, there are two major Acute Phase Proteins (APPs), pig-MAP (Major Acute Protein) and Aptoglobin (HP), that show a strong increase during acute inflammation, stress and acute bacterial or viral infections. Other cytokines have been studied for their anti-inflammatory role and field application such as SAA and adiponectin.

#### 2.2 Acquired (specific) immunity: structural and functional features

The acquired or specific immunity is more efficient both at systemic and mucosal level than innate immunity, and is able to definitively eliminate the pathogen from the tissues (clearance).

B and T immune cells have receptors for specific antigen determinants (epitopes) of the pathogen. The great capacity of B cells for antigen recognition is derived from genetic recombination processes that generate an enormous repertoire of immunoglobulins; the variability of antigenic recognition of receptors (TCR) of T-lymphocytes originates from similar mechanisms.

Specific immunity is triggered when **naïve B and T lymphocytes encounter their specific antigen in the secondary lymphoid tissues where the antigen arrives as it is or is presented by mature dendritic cells.**





The specific antigen recognition provides a survival stimulus for these cells, determining their activation and proliferation as specific B or T lymphocyte clones (**clonal proliferation**). The result is the onset of the effector immune response (**activation of the primary response**), with production of antibodies, cytotoxicity and phagocytosis of infected cells.

In parallel, a circulating pool of long-living immune cells (**T and B memory cells**) is established, which have memorized the first encounter (immunology memory) and that are prompt and more efficient to act upon a subsequent exposure to the same antigen.

The specific immunity against pathogens is regulated by **T helper lymphocytes (Th)**. Naive **helper T cells (CD4+)** recognize antigen-derived peptides of the pathogen that have been processed and associated with class II (MHC II) SLA molecules on the cell surface of **Antigen Presenting Cells (APC)**.

Once the pathogen is captured, the APC, derived from an immature DC, migrates into the lymph node and matures, thus increasing the expression of MCH II and costimulation adhesion molecules (CD80/86) and the production of cytokines and chemokines that act on T helper cells.

**T helper** cells modulate the response through cytokine secretion so different subsets have been defined by the different cytokine pattern of secretion.

**Th1** lymphocytes carry out their defensive role by reaching the sites of infection and secreting cytokines (IL-2, IFN-gamma), which mainly **regulate cell-mediated response** by activating specific **cytotoxic T cells** and innate immune cells, **NK cells and macrophages**, as effectors of cytotoxicity and phagocytosis. Thus, Th1 response plays a pivotal protective role in the defence against **intracellular microorganisms (viruses, intracellular bacteria and protozoa)**.

**Th2** lymphocytes act within the lymphoid tissue and promote the immune response by stimulating the proliferation and differentiation (isotype switching) of **B lymphocytes** involved in the **production of IgE and IgA (defensive immune response at mucosal level)**, via the main cytokines (IL-4, IL-5, IL-13, IL-10).

**Th17** lymphocytes are numerous at mucosal level and have a key role in the **innate defensive response against bacteria and fungi, by recruiting an early, acute neutrophilic inflammation**.

The effectors of acquired immunity are **cytotoxic T lymphocytes (CTL)**, in cooperation with NK cells and macrophages (**Cell Mediated Immunity**), and **antibodies [Immunoglobulins (Ig)]** produced by the activated B lymphocytes, that proliferate and differentiate in Antibody Secreting Cells (Plasma Cell) (Antibody Mediated Immunity). (**Figure 3**)

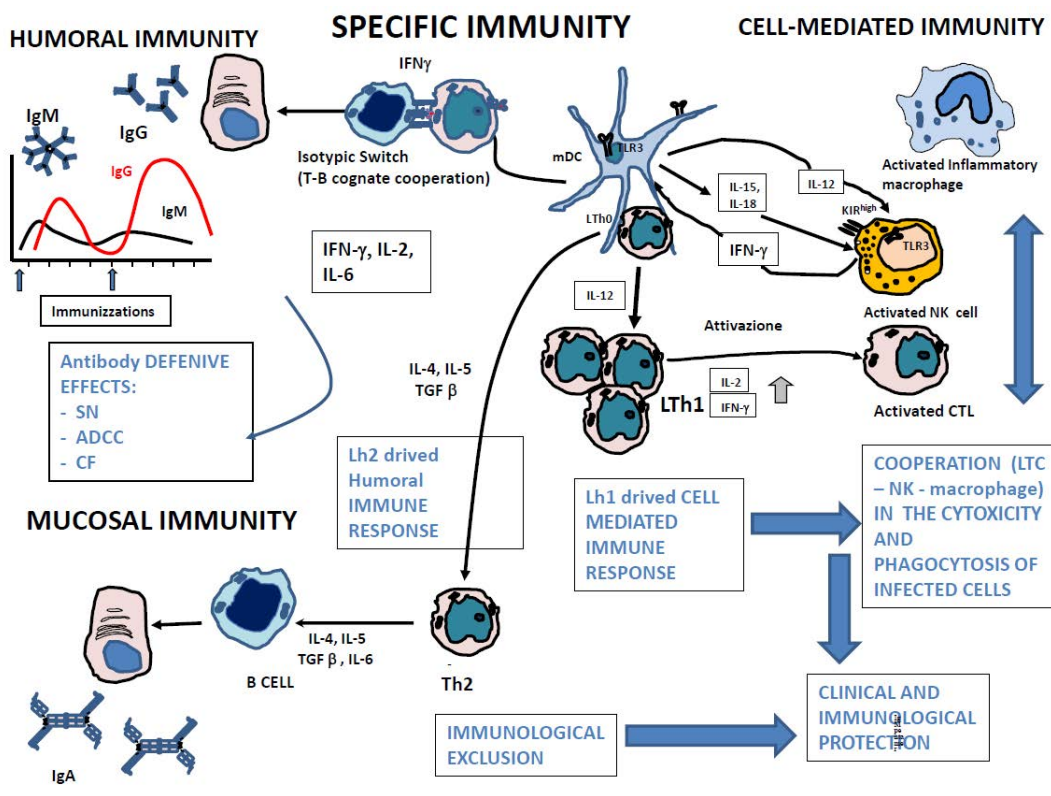


Figure 3. Cellular and molecular mechanism involved in the specific immune response to viruses at systemic and mucosal levels.

### 2.3 Control, "silencing" and immunological memory

**Regulatory T (Treg) cells, which secrete IL-10 and/or TGF-Beta**, play a fundamental role in limiting the clonal expansion and activation of effector T cells, which would otherwise lead to tissue damage. Regulatory T lymphocytes (Treg) are characterized by the **co-expression of CD4 and CD25 and transcription factor FoxP3** [the forkhead family factor (box P3)]. The protein encoded by the FoxP3 gene promotes the transcription of genes encoding for immuno-inhibitory cytokines (eg. IL-10, TGF-β).

A phase of "**silencing**" follows the clearance of the pathogen and it is characterized by the loss of effector T lymphocytes that undergo **energy and apoptosis**.



The passage from an acute effector phase to a state of immunological memory occurs. In fact, after the resolution of infection, the **immunological memory** provides the ability to respond more efficiently (secondary response) to a subsequent infection with a homologous antigen mediated by **B** (memory B cells and plasma cells) and **T memory lymphocytes**.

It is believed that in pigs, the **double positive T helper population (CD4 + CD8 + low)**, characterized by a reduced requirement of costimulation, high production of IFN-gamma and prompt ability to respond to antigenic recall, can play an important role in the secondary response to viral antigens, accelerating viral clearance. Memory CTLs also show a more efficient capacity of activating and performing effector response compared with the primary response.

**Together with neutralizing antibodies and amplification of the CD8+ response, the trend of cellular memory correlates with the degree of protection and is the immunological basis for vaccinology.**

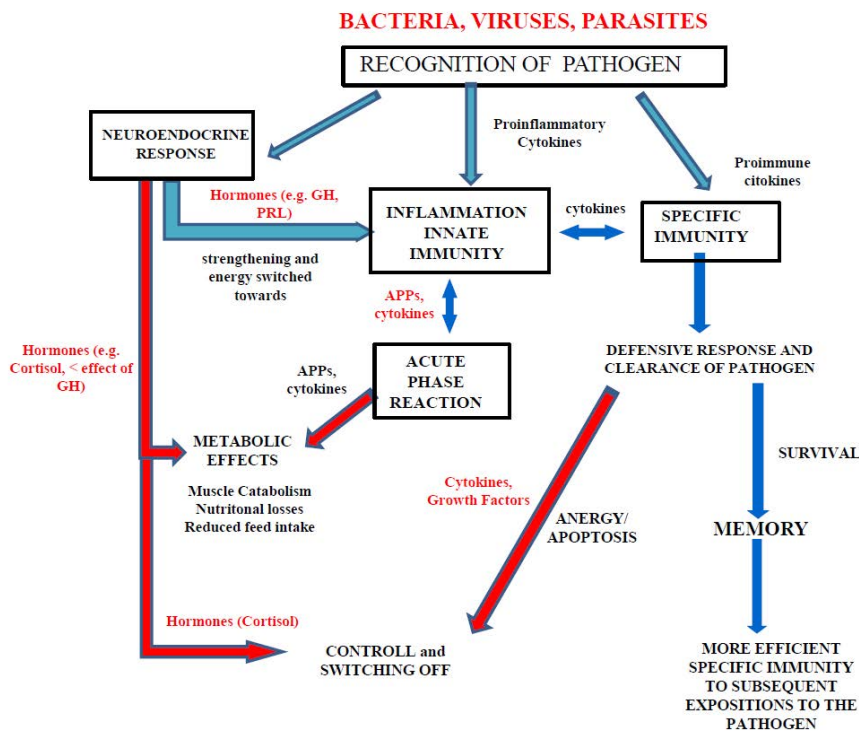


Figure 4. Summary of the drivers of the immune response (Innate immunity, inflammation, specific immunity and neuroendocrine response).

## MEASURING THE IMMUNE RESPONSE TO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV)

### 3.1 Effect of PRRSV infection on innate immunity

In order to measure the effect of PRRSV on innate immunity, it is important to recognize the early signals necessary to sustain the innate response following pathogen recognition. In fact, most data suggest that PRRSV induces a **reduced production of type 1 IFNs** (IFN- $\alpha$  and  $\beta$ ) through inhibition of the RIG-I pathway, associated with a **high production of IL-10**. Further studies have shown that the largest proteins in PRRSV play a role in the reduction of IL-1 $\beta$  and TNF- $\alpha$  and, in addition, negatively affect type1 IFN by NF- $\kappa$ B signalling pathway. The **IFN-gamma response to the virus is also poor**.

**CD163** is a member of the SSCR (Scavenger receptort cystein-rich) family which is strongly associated with the development of the innate response to infection. CD163 expression on macrophages increases after maturation and activation, and is also regulated by the TLR signal, resulting in IL-6 and IL-10 production (Weavewr et al. 2007). CD163 I is a receptor for PRRSV and a correlation between its expression and PRRSV infectivity has been reported. The role of CD163 in the pathogenesis of PRRSV infection is interesting in that its expression can be upregulated by IL-10 (Patton et al. 2009).

Lee et al. (2014) reported that different PRRSV isolates have differing capacity to induce IFN- $\alpha$  production. The **reduction of IFN- $\alpha$**  is correlated with significant **inhibition of NK cell activity**, independently of the number of these cells in the blood (Lunney et al., 2016). Early production of IL-4 could be involved in some aspects of PRRSV - mediated inhibition of the innate response.

It has been reported that diverse strains of PRRSV can **modulate differently the expression of TLR**, particularly TLR3. Furthermore, PRRSV infection of monocyte derived Dendritic Cells (moDC) enhance the production of IL-1 Receptor Antagonist (IL-1 RA), an intrinsic inhibitory mechanism to control inflammation (Nedumpoun et al. 2016).

**Higly Pathogenic (HP) PRRSV** isolates appear to stimulate a higher secretion of cytokines. Particularly, the infection of porcine alveolar macrophages (PAM) induces an overexpression of IFN- $\alpha$ , IL-8. Compared to "normal" strains, HP PRRSV are also stronger inducers of TLR3, 7, 8 expression (Zhang et al. 2013) and of several cytokines like IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IFN-gamma



**Reduction of early INF- $\alpha$  production is one of the key mechanism for viral persistence.** The reduced aptitude of a Modified Live Vaccine to compromise the early mediators of the innate immune response is a target for an efficacious vaccine. Furthermore, another important mechanism for **PRRSV persistence is the increased production of IL-10** due to the ability of the virus to interact with the dendritic cell, directly interfering with the induction of acquired immunity. This mechanism was recently correlated to the role of viral proteins N. The measurement of **TNF- $\alpha$  in association with IL-10 is theoretically a tool for the evaluation of the efficiency of the innate immune response in PRRSV infection** but these are not applicable nor useful in field conditions. Current research is aimed at evaluating other early mechanisms of evasion of innate immunity such as the modulation of early recognition signal (TLR) expression in PAM and dendritic cells or the evaluation of anti-inflammatory signals, such as IL-1 receptor antagonist or PD-L1 and FoxP3.

### 3.2 Effect of PRRSV infection on the acquired immunity

PRRSV induces suppression of inflammatory and antiviral cytokines (type 1 INF, IL-1, IL-8, IFN- $\gamma$ ) causing **dampened acquired immunity**.

In fact, even if an early humoral immune response can appear after 7–9 days post infection (dpi), these anti-N protein antibodies (measured by ELISA) are non-neutralizing and do not correlate with protection. So the prompt production of **ELISA antibodies** soon after infection is useful for diagnostic purposes but not for studying the immune response and consequent protection against infection. Moreover, these non-NA ELISA antibodies are thought to act as a “Trojan Horse”, enhancing the internalization of the virus in macrophages as a result of opsonisation. This phenomenon is called **antibody-dependent enhancement (ADE)**. (Lopez OJ, Osorio FA., 2004). Conversely, **serum neutralizing antibodies (NABs)** appear only after approximately 4 weeks and they are not associated with viral clearance (immunological protection) (Yoon et al, 1994; Loving et al, 2015). Furthermore, NABs are produced and detectable in association with viremia and viral persistence at the tissue level. However, high titres of NAb could transfer passive protection by both protecting passively iperimmunized sow to challenge infection and preventing transplacental PRRSV infection of the fetus, thus conferring sterilizing immunity to both sow and piglets *in utero* (Osorio et al, 202; Lopez et al., 2007).

Experiments on passive transfer in piglets have shown significant differences compared to the results obtained in gestating sows. High concentrations of passive antibodies (required end-point titer of 1:32) are needed to provide sterilizing immunity in piglets. Conversely, low titres of passive transferred NABs (required end-point titer of 1:8) can protect gestating sows. These results are likely the consequence of **differences in susceptibility** and overall capacity for PRRSV **replication and persistence in adult sows versus piglets**. It is thought that piglet target cells (macrophages) are much more permissive to PRRSV replication and consequently the viral load in tissues is higher in young pigs than in sows. So, in younger pigs, a vaccine may need to elicit higher titers of NAB to be effective (Loving et al., 2015). The potential mechanisms responsible for **delayed NABs** include (a) glycan shielding effects of N-linked glycosylation in GPs (Ansari et al., 2006); (b) presence of an immunodominant decoy epitope in GP5 upstream of the neutralizing epitope (Ostrowski M. et al., 2002); (c) antibody-dependent enhancement (ADE) of viral entry into target cells (Cancel-Tirado SM. Et al., 2004) (d) suppression of innate immune responses (Sang Y et al, 2011); and (e) prevention of normal B cell repertoire development (Butler et al., 2014).

Either natural exposure or vaccination provides only limited protection against secondary challenge. Protective levels of NABs usually requires multiple vaccinations or repeated infections and cross-protection (heterologous) can be limited (Vu HL et al., 2011; Zhou L. et al., 2012). Importantly, PRRSV viremia can be controlled even in the absence of detectable NABs.

Antigen-specific **lymphocyte proliferation** is first detected at four weeks post-infection (PI), peaks at 7 weeks PI, and declines after 11 weeks PI. Experiments that utilized blocking antibodies to specific porcine leukocyte antigens demonstrated that CD4+ T-cells were the main effector cells proliferating (Bautista E.M., Mollitor T.W., 1997). **Changes in PRRSV-specific T cell responses** following infection have been assessed by the **IFN- $\gamma$  ELISpot assay** which gives a measure of the number of NK cells, helper T cells and/or cytotoxic T cells producing IFN- $\gamma$ . Using IFN $\gamma$  ELISpot assays, Xiao et al. (2004) demonstrated that PRRSV-specific T cells in blood were observed as early as 2 weeks post-infection, with no significant difference in these T cells in lymphoid tissues, with no apparent correlation of tissue viral levels and PRRSV-specific T-cell frequencies and with wide variation over time and among animals (Xiao Z. et al., 2004)

When the IFN $\gamma$ -secreting CD8+T-cell (**Cytotoxic T-Lymphocytes - CTL**) response was evaluated, a late and low virus-specific response was observed (Ferrari L. et al., 2013)

There is limited data on the role of CD8+ cytotoxic T cells (CTLs) in controlling primary PRRSV infection, and anti-PRRSV-targeted CTLs have been detected only after clearance of the virus from blood (Costers S. et al., 2009). Temporary depletion of CD8+ T cells at the time of infection did not lead to an increase in infection, suggesting that cytotoxic T cells do not have a functional role in the control of acute infection (Lohse L., et al., 2004). Similarly, CTL activity was not detected against PRRSV-infected macrophages until after viremia was cleared. Memory CTL proliferation was observed at 14 days after infection but CTL activity was not detected until 49 days after infection (Costers S. et al., 2009).

The role of **memory T cells** in anti-PRRSV immunity has not been studied extensively. It has been reported that a recall response that is mainly dependent on CD4+ cells and SLA-II, is present from 4 weeks after infection and remains for more than 3 months (Lopez Fuertes et al., 1999).

The majority of studies evaluating T cell responses to PRRSV have investigated the response during the acute infection rather than following clearance of viremia. During infection, it is assumed that mainly T effector cells are detected as opposed to memory cells. The distinction between effector versus memory T cells is difficult in pigs due to the lack of phenotypic markers and functional characterization of individual T cell populations. Clearly, the evaluation of vaccine immunogenicity and efficacy warrants the measurement of PRRSV-specific memory T cells; however, memory T cells have not been clearly characterized in pigs.

PRRSV infection is reported to **increase the frequency of putative Foxp3+ T-regulatory cells (Tregs)** producing TGF- $\beta$  (Silva-Campa E. et al., 2012) and to **promote the secretion of IL-10**, inducing a strong **immunosuppressive response**, resulting in **delayed onset of a Th1 immune response** (Diaz I., et al., 2005; Johnsen CK et al., 2002, Dwivedi V., et al., 2011; Wongyanin P, et al., 2012)

As summarized by the above-mentioned studies, **dysregulated expression of immune molecules** following PRRSV infection results in weakened adaptive immunity.

The proportion of pig showing ELISA antibodies soon after vaccination is variable, ranging from 30 to 80%, very seldom 100%. Neutralizing antibodies are detectable after 3-4 weeks' post-vaccination with a high interindividual variability. After natural or experimental infections, non-NABs promptly increase and 100% of the animals are positive within a few days (week). The rise of the S/P value in ELISA is not correlated with clinical and immunological protection, so the information given by these serological data is limited to the diagnostic setting.

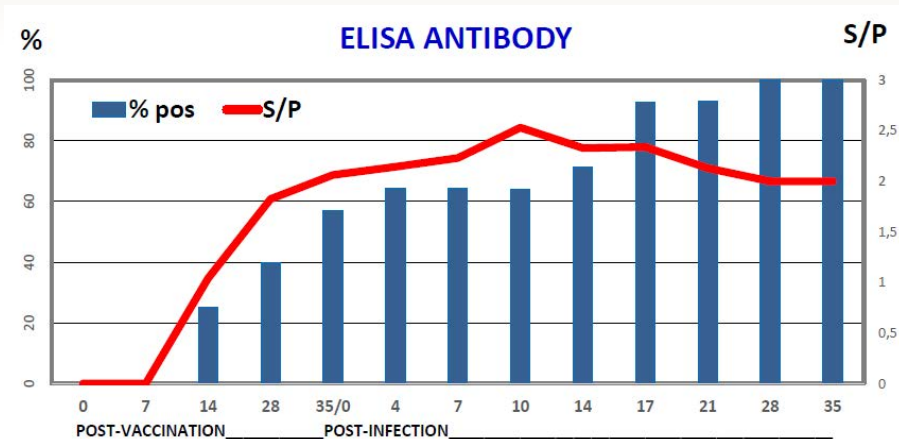


Figure 5. Time course of the ELISA antibodies following vaccination and natural exposure to PRRSV.

The measurement of NAb is not routinely performed and limited to research activity. To note that NAb titres depend on the cross reactivity between two isolates, the one infecting the animal and the one used in the test. Variability can occur and therefore these antibodies are not valuable for evaluating the immune response and the degree of protection and cross-protection. Measurement of cell mediated immunity is based on the ELISpot assay [enumeration of the INF-gamma Secreting Cells (SC) specific to PRRSV]. In naïve animals, both post vaccination and post-infection, the number of IFN-gamma SC starts to increase from 3-4 weeks onward. The course of the specific SC response is rather erratic with a tendency to increase over time.

As mentioned above for the ELISA antibodies, a boosting effect is detected after infection with a prompt and more intense increase of these cells. It is worth mentioning that both the antibodies and the cellular response feature an interindividual variability. Animals can be classified as “low” and “high” responders. These divergent responses to vaccination may be the result of the host’s genetic background (Ait-Ali T. et al., 2016).

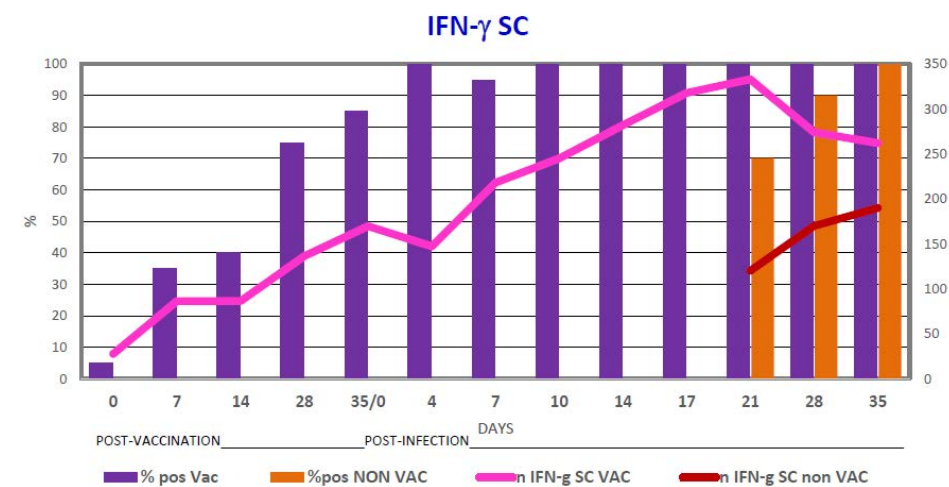


Figure 6. PRRSV-specific IFN-γ secreting cells (SC) following vaccination and post-exposure to natural infection.

## MEASURING THE IMMUNE RESPONSE TO PORCINE CIRCOVIRUS 2 (PCV2)

### Effect of PCV2 infection on the innate immunity

The internalization of PCV2 has been demonstrated for both conventional blood DCs and plasmacytoid DCs. The persistence of PCV2 in DCs, however, does not cause loss of viral infectivity or cell death, but actually helps the virus to evade the host immune system and facilitates the transmission of PCV2 by reducing the first signal of the innate immune response. In fact, PCV2 does not replicate in these cells, but accumulates within them. **PCV2-induced impairment of DC function** does not require viral replication and is mediated by the viral DNA, with a minimum concentration of double stranded (ds) DNA (replicative form) necessary to mediate such inhibition. Furthermore, the infection of pDC **reduces the production of IFN-alfa and TNF-alfa** and impairs the maturation of myeloid DCs. PCV2-mediated inhibition of natural IFN-producing cells appears to have a broad spectrum and can affect responses **induced by Toll-like receptors TLR7 and TLR9** agonists and by several other pig viruses. This would suggest that PCV2 modulation of the innate immune response may render the pigs more **susceptible to secondary or concurrent viral and bacterial infections**. PCV2 interaction with immune cells and with the lymphoid system appears to play an important role in the pathogenesis of PCV2 infection. The **increase of IL-10** and the **suppression of the innate cytokine** pattern play a major role in the pathogenesis of PCV2 and Porcine CircoVirus-Disease (PCVD). The whole PCV2 virus and some fragments of the genome are able to induce the suppression of cytokine production. Conversely, the Virus Like Particles (VLPs) do not show this suppressive effect.





#### 4.2 Effect of PCV2 infection on acquired immunity

The lymphoid tissues and immune cells are the primary target of PCV2 infection that alters the **cytokine responses** in infected pigs. Several studies have shown that anti-inflammatory and pro-inflammatory cytokines are differently regulated in PCV2 infection, depending on the lymphoid tissue considered. **IL10 plays a particularly important role in the immune dysregulation upon PCV2 infection.** (Ramamoorthy S, Meng XJ. 2009; Darwich L, Mateu E. 2012; Darwich L. et al., 2003) Pigs which are subclinically infected with PCV2 also develop a transient raise of IL-10 response during the peak of viremia (Darwich L. et al., 2008).

PCV2-infected pigs have a significantly **decreased number of CD8+ and CD4+CD8+ double-positive cell** subsets compared to PCV2-negative pigs, which further indicates that PCV2 infection impairs the host immune response. Nevertheless, both neutralizing antibodies and cell mediated immunity contribute to the protection against PCV2 infection (Darwich L, Mateu E., 2012). PCV2 infection induced virus-specific neutralizing antibodies at approximately 21 days postinoculation (10-28 days post inoculation) (Beach NM. Et al., 2010; Allan GM, Ellis JA. Lekcharoensuk P. et al., 2004). Low NAb titers have been related to increased virus replication and development of PCV-Systemic Disease. It is worth mentioning that some animals develop a **humoral response lacking NAb** or NAb develop later than non-NAb.

In gnotobiotic pigs experimentally infected with PCV2, neutralizing antibodies specific for PCV2 and IFN-gamma gene expression in PBMCs were detected, although the onset of adaptive immunity varied among the infected pigs. In fact, the absence of neutralizing antibodies is an important factor for increased PCV2 replication and protection against the development of PCVD (Meerts P. et al., 2005; Meerts P. et al. 200).

**IFN-gamma SC** develop specifically in response to PCV2 infection and may contribute to viral clearance in pigs (Fort M. et al., 2009; Martelli P. et al., 2011) and depletion of CD4+CD8+ T cells has been shown to weaken the virus-specific IFN-gamma responses (Steiner E et al., 2009). Therefore, IFN-γ appears to be an important immunological player. The contribution of cell-mediated responses in vaccine-induced protection is pivotal, because the effect of PCV2 antibodies is titre-dependent and the sole induction of a humoral response might not guarantee full protection against PCV2 infection (Blanchard et al., 2003; Opriessnig et al., 2009; Fort et al., 2009b).

After the onset of infection, non vaccinated pigs show a marked increase in IFN-gamma SC associated with a reduction of viremia. The IFN-gamma SC responses are also related to viral replication. Thus, in pigs in which PCV2 viral load is low or absent, as occurs more frequently in vaccinated animals, the number of these cells is rather low or is maintained at residual levels as a consequence of the primary activation by vaccination (ranging from 40 to 60 IFN-gamma SC on average).

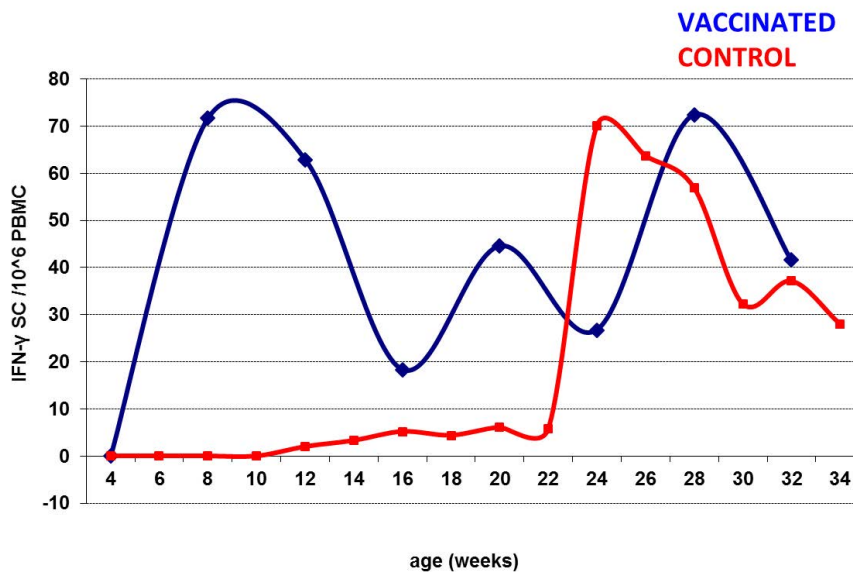


Figure 7: INF-gamma SC response after vaccination and natural infection.

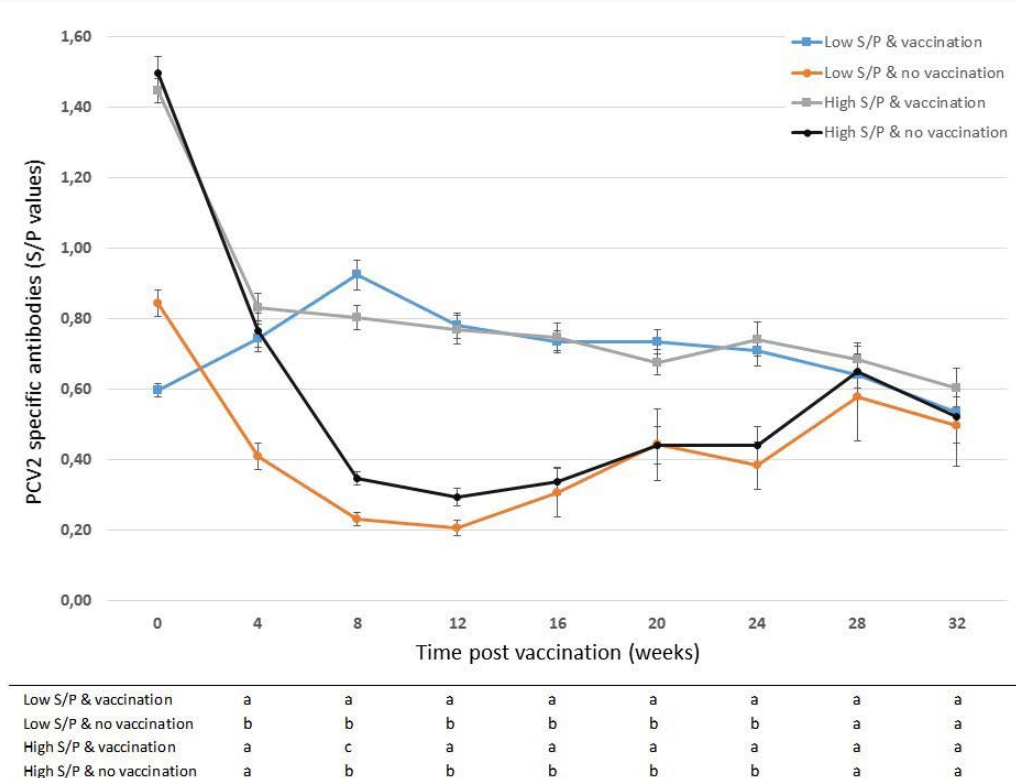


Figure 8. Course of the serologic response to PCV2 (Mean  $\pm$  STD anti-PCV2 ELISA antibodies) according to the levels of maternal derived antibodies (MDA) at vaccination and PCV2 infection (viremia) occurred approximately at 20 weeks of age. Overall, the animals were divided based on a low ( $\leq 0.9$ ) or high ( $\geq 1.2$ ) S/P value at 4 weeks of age when vaccinated animals were inoculated with the vaccine (“HIGH S/P vac” and “LOW S/P vac” groups) while unvaccinated animals were inoculated with the adjuvant alone (“HIGH S/P NV” and “LOW S/P NV” groups) (Martelli et al., 2016 -modified).

### MEASURING THE IMMUNE RESPONSE TO MYCOPLASMA HYPONEUMONIAE

Upon infection with *Mycoplasma hyopneumoniae* (Mh), immune response is both innate and adaptive. The innate response involves the ‘mucociliary system’, cytotoxicity by macrophages and neutrophils, increase of T-cell and NK cells, as well as the enhancement of the expression of cell receptors that trigger the complement cascade.

The adaptive immune response involves opsonization, antibody production (systemic and local) and cell-mediated immune stimulation. However, the immune response to *M. hyopneumoniae* acts as a “double edge sword” helping in protection and also playing a role in the exacerbation of lesions induced during mycoplasmal disease. Furthermore, TLR2 plays an important role in *M. hyopneumoniae*-activated inflammatory responses in porcine alveolar macrophages (Muneta et al., 2003). *Mycoplasma* lipopeptide-induced cytokine production is impaired in TLR2-deficient macrophages of mice (Takeuchi et al., 2000). Therefore, activation of *M. hyopneumoniae*-induced inflammation is thought to be mainly mediated by TLR2.

Alveolar macrophages and lymphocytes stimulated by *M. hyopneumoniae*, produce **pro-inflammatory cytokines** that are responsible for lung lesions and lymphoid hyperplasia (Rodriguez et al., 2004), suggesting the involvement of the immune response in the development of lesions. In general, mycoplasmas are capable of evading the host’s natural defenses. It is known that some pathogenic species use their genetic machinery to alter surface antigens, thus diverting the host immune response and allowing for chronic infection (Razin et al., 1998).

Substantial lymphoid cell infiltration occurs around the airways following infection with *M. hyopneumoniae*, thanks to the key role of macrophages. Indeed, macrophages are recruited as a result chemo-attraction induced by the release of immunogenic proteins and mediators during *M. hyopneumoniae* infection. Lymphoid hyperplasia of the bronchus-associated lymphoid tissue progressively leads to obliteration of the bronchiole’s lumen and atelectasis of the alveoli.

Helper T-cells are the most numerous subset in the lymphoid infiltration. Cytotoxic T-cells (CTL) are also detected in association with the specific lesions. Upon activation, Th1-cells are responsible for activating and increasing the phagocytic and cytotoxic activity of macrophages. T-cells and the overproduction of IL-1 and IFN-gamma worsen the host mediated tissue damage. Therefore, mycoplasmal pneumonia is not only dependent of the injury caused by ciliostasis and exfoliation of the epithelial cells, but also damage caused by the overexpression of some immune functions. The activation of humoral immunity leads to the production of antibodies with increases in IgG and IgA in the tracheobronchial secretions, lungs and serum of infected pigs. Predominantly, IgG participates in the opsonization of the microorganisms and in the phagocytosis by alveolar macrophages, while IgA provides local immunity interfering with the adherence of *M. hyopneumoniae*.

Despite the immune mechanisms of the host, the microorganisms persist in the respiratory tract of the pig (lack of immunological protection). In fact, phagocytosis by alveolar macrophages is severely impaired upon *M. hyopneumoniae* infection.



The serological response to *M. hyopneumoniae* after infection or vaccination is measured by ELISA tests. Commercial kits have low sensitivity. Serology is appropriate to determine the status of a population rather than of an individual pig or for vaccine compliance. Antibodies are first detected in serum of pigs by ELISA at 3-6 weeks post-exposure. Antibody levels following vaccination with a bacterin may vary depending on the vaccine, the infectious status of the pigs and the serological assay (Erladson et al, 2005). No correlation between the level of vaccine-induced antibodies and protection has been observed (Thacker et al., 1998).

In a vaccinated population, an increase of the ELISA S/P values as well as an increase of the proportion of seropositive pigs is commonly observed following challenge infection. In fact, active infection elicits a prompt secondary antibody response (booster effect).

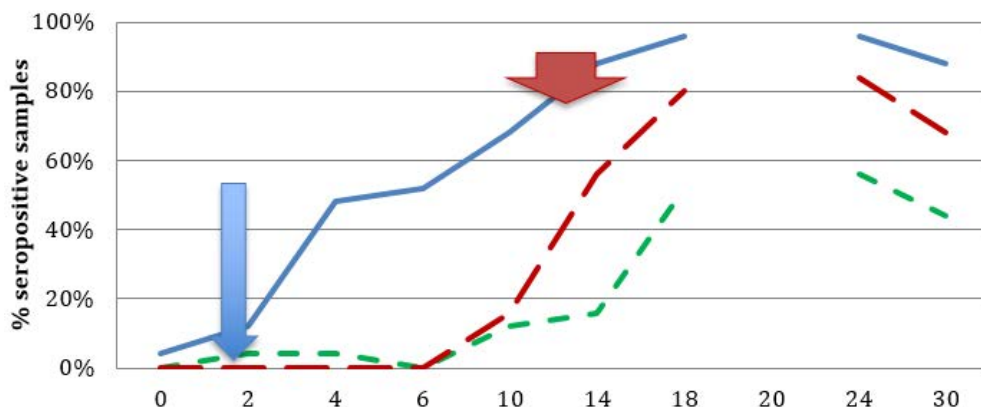


Figure 9: Time course of ELISA positivity following vaccination (blue arrow) and after the onset of infection (red arrow). Legend: blue line= vaccinated (vaccine A); red dotted line= vaccinated (vaccine B); green dotted line= negative control.

The ELISpot assay is used to measure the specific cell mediated immune response to the microorganism. The number of specific IFN-gamma Secreting Cells is usually low although marked differences between vaccines have been demonstrated.

Importantly, as mentioned above, the humoral immune protection to *M. hyopneumoniae* is due to local immune function, markedly of production of IgA. The quantification of IgA in the BAL fluid is a direct measure of local immunity. Laborious sampling and lack of repeatability (variability of quantity and location of the samples) make this technique impractical and rarely used in the field. BAL fluid sampling is commonly used for research purposes or as tracheal swabs for diagnostic purposes. Moreover, no commercial kits for the determination of the IgA are available, thus the measurement of this class of Immunoglobulins is not currently standardized.

## References

Available upon request



## KL08 - THE IMMUNE SYSTEM IN NEW BORN PIGLETS & PASSIVE IMMUNITY

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Development of the new-born immune system of different animal species differs mainly based on a length of pregnancy. Longer gestation is associated with the higher ability of the immune system either to respond to antigenic challenge including a vaccination or to cope with a real infection. The important stimulus for an activation of innate or adaptive immunity is represented by the fact that new-born leaves the sterile environment of the uterus and becomes to be exposed to influence of even non-pathogenic microflora colonising all surfaces incl. gut.

From protection point of view, crucial role of passive mother-derived immunity cannot be omitted. Based on a histological structure of placenta, new-borns are more or less dependent on the immunity transmitted through colostrum / milk.

### 1. PRENATAL DEVELOPMENT

Prenatal development of the immune system in piglets is very similar to the development in other higher mammals. Everything starts extraembryonally - in a yolk sac. Then the hemopoiesis moves into the fetal liver, spleen and finally into the bone marrow. First leukocytes were observed in the yolk sac and the fetal liver on the 17<sup>th</sup> day of gestation, the majority of them being of myeloid origin. The colonization of the thymus with leukocytes was observed 21 days later (Sinkora et al., 2002). First macrophages were identified by an electron microscopy in the fetal liver on the 25<sup>th</sup> day of gestation (Rehakova et al., 1998). It was also found that the major lymphocyte subpopulations started to appear at the beginning of the second third of the gestation period, with B cells being the earliest lymphocyte subpopulation to appear in the periphery (Sinkora et al., 1998). Finally, piglets are delivered around 115 days of the gestation. Their immune system is developed properly, but a redistribution of cells and an ability of the system intensifies with age probably due to influence of the gut being colonised by commensal microflora.

### 2. COLOSTRUM – CONTENT AND FUNCTION

Epitheliochorial type of the placenta in pigs does not allow a transplacental transmission of immunoglobulins (Stercl et al., 1966). Therefore, piglets are born agammaglobulinemic and depend on receiving of the antibodies from the sow's colostrum. At present, there is no doubt that the colostrum is the primary source of the antibodies for the new-born piglets (Kruse, 1983). IgG is the main isotype of these antibodies. Almost all IgG in the colostrum are transferred from the blood via so called neonatal Fc-receptor expressed in the mammary gland. The same receptor expressed on the intestinal epithelium of the new-born piglets allows the transport of the antibodies across its wall, mainly into the blood circulation. To prove that, we designed an experiment in which 4 pregnant sows were immunized with a model antigen. Two other sows were left as nonimmunized controls. Subsequently, blood samples were taken from the sows and their piglets. The samples were tested for a presence of antigen-specific antibodies by ELISA. The samples from piglets delivered by nonimmunized sows and the samples from piglets delivered by immunized sows but taken before first drinking of the colostrum were negative. After drinking of the colostrum, levels of the antigen-specific antibodies increased and stayed detectable until the end of experiment at day 28 (Nechvatalova et al., 2011; Figure 1).

Part of colostrum-derived antibodies - mainly IgA - can be redistributed also onto mucosal surfaces, incl. respiratory tract (Nechvatalova et al., 2011; Figure 2).

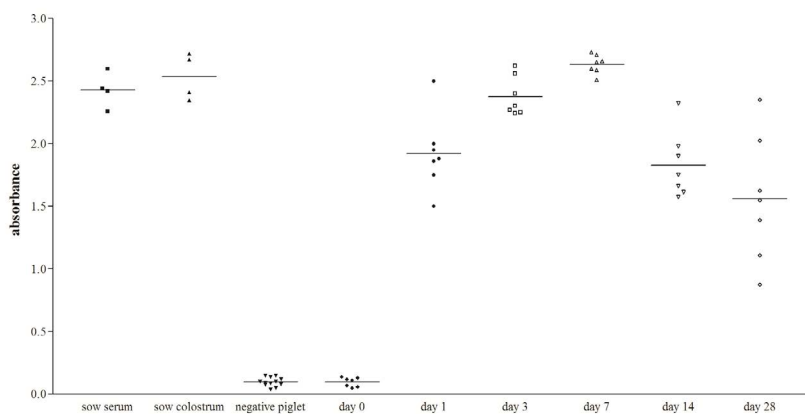


Figure 1.

Absorbances of IgG antigen-specific antibodies detected in colostrum and serum samples collected from sows and piglets at different intervals after birth and colostrum intake. Data are expressed as individual values and means of respective groups. Adapted from Nechvatalova et al., 2011.



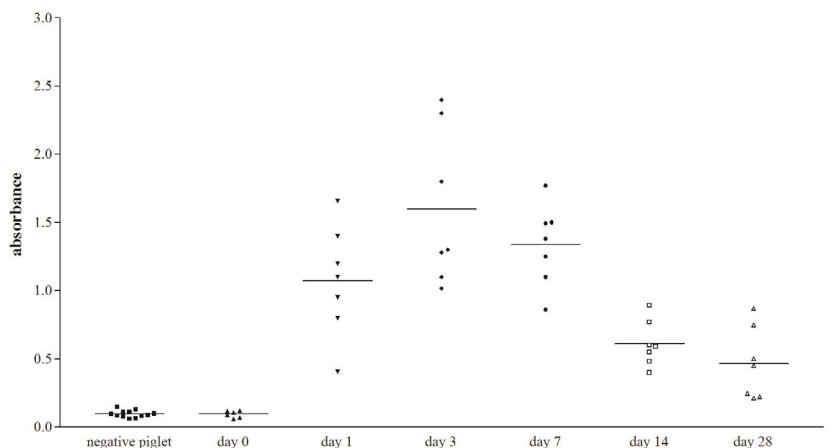


Figure 2.

Absorbances of IgA antigen-specific antibodies detected in brochoalveolar lavage fluid samples collected from piglets at different intervals after birth and intake of colostrum. Data are expressed as individual values and means of respective groups. Adapted from Nechvatalova et al., 2011.

Apart from the antibodies, the colostrum contains also large number of cells - epithelial cells, macrophages, neutrophil granulocytes but also huge number of lymphocytes (Evans et al., 1982; Le Jan, 1994). These lymphocytes can penetrate the intestinal wall and colonize blood and lymphatic and nonlymphatic tissues (Williams, 1993). Antigen-specific activity of these lymphocytes has been detected in the experiment in which such lymphocytes were found in the piglets obtaining colostrum from the sows immunized with a model antigen (Nechvatalova et al., 2011).

To understand the function of colostrum lymphocytes, it was important to identify the activation status of these cells and various cell surface markers were used to establish a more precise phenotype. Besides markers used for a detection and characterization of basic lymphocyte subsets - cytotoxic T lymphocytes, helper T lymphocytes, double positive cells, NK cells and  $\gamma\delta$  lymphocytes (i.e. CD2, CD3, CD4, CD8,  $\gamma\delta$  TCR), several other markers were used (Hlavova et al., 2014). CCR7 is a chemokine receptor, which regulates homing of lymphocytes to secondary lymphoid organs and is expressed on porcine naive CD4+ T cells and both naive and memory CD8+ T cells (Moreno et al., 2013). CD11b is a subunit of the adhesion molecule integrin  $\beta$ 2 that plays an important role in an adhesion, chemotaxis and diapedesis and which is expressed mainly on the activated lymphocytes (Mazzone and Ricevuti, 1995; Stepanova and Sinkora, 2012). CD25 is the interleukin (IL)-2 receptor  $\alpha$  chain, which is upregulated after an antigen stimulation of specific T cells and is generally considered to be a marker of activation (Bailey et al., 1992; Kaser et al., 2008). CD45RA isoform of the CD45 leukocyte common antigen, is considered to be a marker for naive cells, since its expression decreases upon an activation, with the replacement by other CD45 isoforms (Bullido et al., 1997). The expression of MHC class II molecules on the activated lymphocytes is somewhat species dependent. In pigs, it can be found on NK cells,  $\gamma\delta$ TCR and  $\alpha\beta$ TCR T lymphocytes as well as on resting T-cells (Bevan and Chisholm, 1986; Lunn et al., 1993; Saalmuller et al., 1991). Results of the study indicated that lymphocytes in the colostrum had been antigen-experienced cells and suggested these are central/effector memory cells (Hlavova et al., 2014).

Role of maternally-derived immunity in protection of the piglets has been tested on several infectious models, incl. *Mycoplasma hyosynoviae* (Leuritsen et al., 2017), *Mycoplasma hyopneumoniae* (Bandrick et al., 2008 and 2014) or *Actinobacillus pleuropneumoniae* (Faldyna et al., 2005; Krejci et al., 2005; Nechvatalova et al., 2005).

Although colostrum-derived immunity is important in very early protection of new-borns against pathogens, it can interfere with induction of active responses to a vaccine application. This is true for the antibody production (Figure 3) but not for ability of the antigen-specific lymphocytes to become stimulated upon *in vitro* stimulation with the specific antigen (Figure 4).

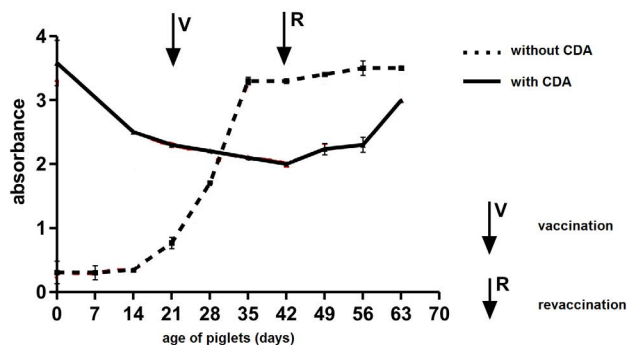


Figure 3.

Dynamic of IgG levels in piglets with or without colostrum-derived antigen-specific antibodies. Piglets were vaccinated and revaccinated at the age of 3 and 6 weeks of life, respectively. Piglets were delivered by vaccinated (full line) or nonvaccinated (dotted line) sows. Data show that presence of colostrum-derived antibodies can suppress active production of IgG upon vaccination.

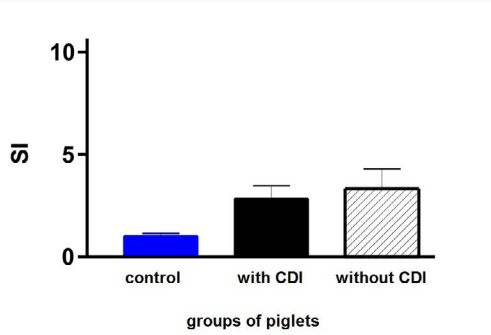


Figure 4.

Ability of antigen-specific lymphocytes to become activated. These lymphocytes were obtained from blood of piglets 3 weeks after vaccination at the age of 3 weeks. Piglets were delivered by vaccinated (full column) or nonvaccinated (dashed column) sows. Data show that presence of colostrum-derived antibodies does not suppress activation of antigen-specific lymphocytes upon vaccination. Piglets in control group were delivered by nonvaccinated sows and were not vaccinated.

### 3. POSTNATAL DEVELOPMENT

Studies in gnotobiotic piglets have revealed that the appearance of CD4CD8 double positive T cells and CD2-negative (i.e. activated) B cells is absolutely dependent on the contact of the immune system with live viruses and bacteria, respectively (Sinkora et al., 2002). Also percentages of gamma delta TCR CD8-positive cells and CD4CD8 double positive T cells increase with age (Stepanova et al., 2007). From functional point of view, although this ability intensifies with age (Pomorska-Mól et al., 2012), the immune system is developed properly to be able to recognise and process an antigen and respond to it by production of antibodies (Figure 5) or antigen-specific lymphocytes (Figure 6).

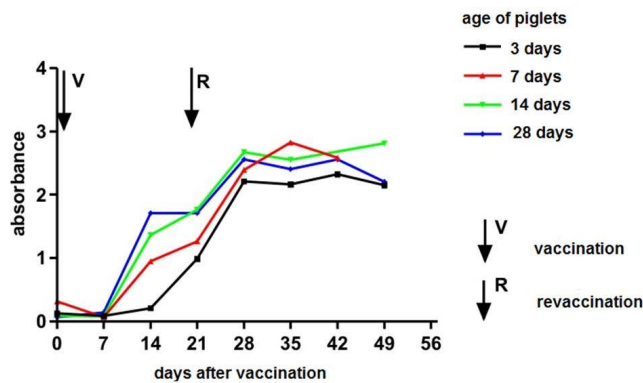


Figure 5.

Dynamics of antigen-specific antibodies in sera of piglets after vaccination (V) and revaccination (R) with a model antigen. Vaccination of respective groups started at different age (3 days, 7 days, 14 days and 28 days of life).

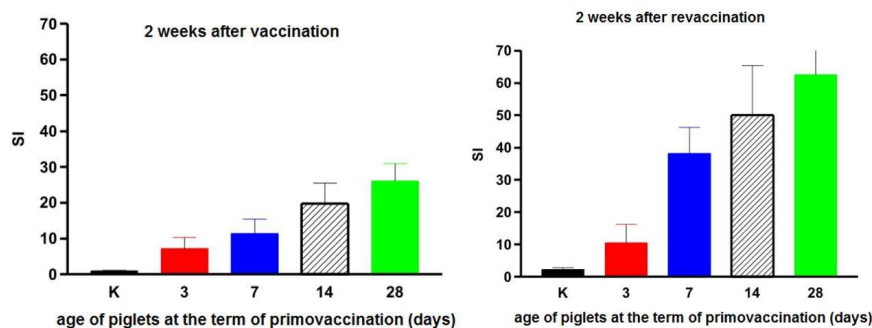


Figure 6.

Antigen-driven proliferative activity of lymphocytes 2 weeks after vaccination (left) and revaccination 3 weeks later (right). Vaccination of respective groups started at different age (3 days, 7 days, 14 days and 28 days of life).



#### 4. LACTOGENIC IMMUNITY

After the period of the colostral immunity, the lactogenic immunity starts. The lactogenic immunity is mediated by antibodies of IgA isotype. Almost all of the immunoglobulins in milk are produced locally in the mammary gland. Contrary to IgG from the colostrum, antibodies from milk are not absorbed; they are protected from enzymes by so called secretory component. IgA-producing B lymphocytes in the mammary gland are of dual origin: from gut and from upper respiratory tract (Bohl et al., 1972; Bohl and Saif, 1975; Lanza et al., 1995). Recruitment of these cells into the mammary gland is driven by the chemokine CCL28 produced in the mammary gland and the chemokine receptor CCR10 expressed on B-lymphocytes (Hieshima et al., 2003; Bourges et al., 2008; Lazarus et al., 2003). Molecule IgA is released into the cavity of the mammary gland as a dimer bound together by so called J-chain (Figure 7).

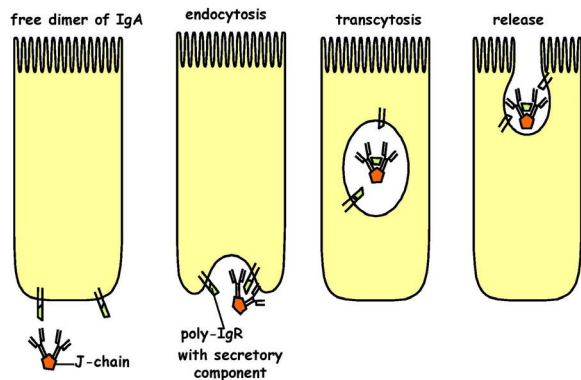


Figure 7.

Diagram of IgA release into the cavity of the mammary gland.

Role of IgA-mediated lactogenic immunity is to protect the gut from adhesion of pathogens. The most relevant pathogens in term of the lactogenic immunity include *Escherichia coli*, *Clostridium perfringens*, rotaviruses or coronaviruses.

#### Acknowledgements:

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References available upon request



# Oral Presentations





<b>VETERINARY PUBLIC HEALTH &amp; FOOD SAFETY</b>	<b>VPH-OP-01</b>	<b>VPH-OP-06</b>
<b>RESIDENT SESSION</b>	<b>RES-OP-01</b>	<b>RES-OP-06</b>
<b>VIRAL DISEASES</b>	<b>VVD-OP-01</b>	<b>VVD-OP-06</b>
<b>HERD HEALTH MANAGEMENT &amp; ECONOMY</b>	<b>HHM-OP-01</b>	<b>HHM-OP-06</b>
<b>BACTERIAL DISEASES I</b>	<b>BBD-OP-01</b>	<b>BBD-OP-03</b>
<b>WELFARE &amp; NUTRITION</b>	<b>AWN-OP-01</b>	<b>AWN-OP-03</b>
<b>BACTERIAL DISEASES II</b>	<b>BBD-OP-04</b>	<b>BBD-OP-07</b>
<b>MISCELLANEOUS</b>	<b>MIS-OP-01</b>	<b>MIS-OP-04</b>
<b>IMMUNOLOGY &amp; VACCINOLOGY</b>	<b>IMM-OP-01</b>	<b>IMM-OP-06</b>
<b>REPRODUCTION</b>	<b>REP-OP-01</b>	<b>REP-OP-06</b>

The pdf files of the oral presentations are available on [www.eaphm.org](http://www.eaphm.org)



## VETERINARY PUBLIC HEALTH & FOOD SAFETY

Wednesday, 3 May 2017, 15:30 – 17:30

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- VPH-OP-01 **LOW RISK FOR HUMANS ASSOCIATED WITH USE OF PLEUROMUTILINS IN PIGS**  
*Margit Andreasen (Denmark)*
- VPH-OP-02 **EFFECT OF TETRACYCLINE TREATMENT-DOSE AND TREATMENT-MODE ON SELECTION OF RESISTANT COLIFORM BACTERIA IN NURSERY PIGS**  
*John Elmerdahl Olsen (Denmark)*
- VPH-OP-03 **THE EFFECT OF AN ACID BASED FEED ADDITIVE ON THE INTESTINAL LEVEL OF ESBL-PRODUCING E. COLI IN FECES OF SWINE**  
*Nataliya Roth (Austria)*
- VPH-OP-04 **CHANGES IN HEMATOLOGICAL, CARCASS AND MEAT QUALITY PARAMETERS ASSOCIATED WITH LIVER MILK SPOTS IN SLAUGHTER PIGS**  
*Nikola Cobanović (Serbia)*
- VPH-OP-05 **TOXOPLASMA GONDII AND PARMA HAM: NO EVIDENCE OF TRANSMISSION**  
*Marco Genchi (Italy)*
- VPH-OP-06 **USING SEROLOGICAL MONITORING AT ABATTOIRS AND ON-FARM AUDITING FOR IDENTIFICATION OF PIG FARMS WITH A HIGH RISK FOR TOXOPLASMA GONDII**  
*Dorien Eppink (Netherlands)*



## VPH-OP-01 - LOW RISK FOR HUMANS ASSOCIATED WITH USE OF PLEUROMUTILINS IN PIGS

**L. Alban<sup>1</sup>, M. Andreasen<sup>2</sup>, J. Dahl<sup>3</sup>, E.O. Nielsen<sup>4</sup>, J. Ellis-Iversen<sup>5</sup>, U.W. Sønksen<sup>6</sup>**

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<sup>3</sup> Danish Agriculture & Food Council, Copenhagen, Denmark

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New treatment guidelines for antibiotic usage in animals are under development in Denmark. Their purpose is to assist veterinarians in choosing antimicrobials that combine best effect with lowest probability of developing resistance of importance for human health. These guidelines are made by the Danish Veterinary and Food Administration based on risk assessments.

Use of pleuromutilins in animals has been considered a very low risk for human health. However, recent international data contradict this. We reassessed the risk using the European Medicines Agency's risk assessment approach with Danish data.

Staphylococci, including livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) and enterococci were identified as relevant hazards.

For LA-MRSA, the release assessment showed that the probability of development of pleuromutilin resistance was high. The relevant exposure pathway is primarily occupational, which only exposes relatively few people. The consequences of exposure were assessed as high for vulnerable groups (severely ill patients at hospitals) and low for the general public, due to low transmission potential of LA-MRSA between people and current screening programmes in hospitals of patients with daily contact to pigs. For LA-MRSA, the overall risk was estimated as low (low uncertainty).

For enterococci, the probability of pleuromutilin resistance was low. Exposure pathways included both occupational and foodborne. The consequences were considered low to the general public due to low virulence in humans and high for vulnerable groups. There exist for the time being no national control measures, and enterococci have a wide resistance profile. The overall risk for enterococci was assessed as low but with high uncertainty and with potential for increasing consequences in the future due to emerging linezolid resistance.

If pleuromutilin consumption increases substantially, leading to increased prevalence of mobile, easily transmissible resistance mechanisms, the risk should be reassessed. Continuous monitoring of pleuromutilin resistance in selected human pathogens should therefore be considered.



## VPH-OP-02 - EFFECT OF TETRACYCLINE TREATMENT-DOSE AND TREATMENT-MODE ON SELECTION OF RESISTANT COLIFORM BACTERIA IN NURSERY PIGS

***J.E. Olsen<sup>1</sup>, K. Græsbøl<sup>2</sup>, J.P. Nielsen<sup>3</sup>, P. Damborg<sup>1</sup>, A. Herrero-Fresno<sup>1</sup>, I. Larsen<sup>3</sup>, A. Folkesson<sup>2</sup>***

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<sup>2</sup> *Danish Technical University, National Veterinary Institute, Frederiksberg, Denmark*

<sup>3</sup> *University of Copenhagen, Department of Large Animal Sciences, Frederiksberg C, Denmark*

### Background and objectives

Oral batch-treatment with antimicrobial is believed to contribute significantly to selection of resistant bacteria. However, a proper comparison of the selection power of batch-treatment versus pen-wise or individual treatments has not been performed. The aim of this study was to perform this comparison, and in addition to investigate whether lowered doses of oxytetracycline would result in less resistant bacteria.

### Materials and methods

A randomized clinical trial was performed including 75 batches of nursery pigs in five pig herds. Each batch was randomly allocated to one of five treatment groups: Oral batch treatment with high (20 mg/kg), medium (10 mg/kg) and low (5 mg/kg) dosage, oral pen-wise treatment with medium dose (10 mg/kg), or intramuscular injection treatment (10mg/kg). All groups were treated for five days. The number of tetracycline resistant coliforms was determined before treatment, two days after treatment, at the end of the nursery period, and for a smaller number of pigs, at slaughter.

### Results

In all groups, treatment was followed by a rise in the numbers and proportion of tetracycline resistant coliforms, followed by a significant drop at the end of the nursery period and a further drop at slaughter to below the starting concentration. Counts and proportion of tetracycline-resistant coliforms did not vary significantly between treatment groups, except immediately after treatment, where high dose resulted in the highest number of resistant coliforms. The changes in resistant coliforms were significantly different between batches of pigs undergoing the same treatment.

### Discussion and conclusion

Selection for tetracycline resistant coliform bacteria could not be reduced by lowering the dose of antimicrobial or by replacing oral-batch treatment with individual or pen-wise treatment. This shows that under practical field conditions, there will be no added value, in terms of lowering resistance development, by avoiding batch treatment of nursery pigs.





## VPH-OP-03 - THE EFFECT OF AN ACID BASED FEED ADDITIVE ON THE INTESTINAL LEVEL OF ESBL-PRODUCING *E. COLI* IN FECES OF SWINE

***N. Roth*<sup>1</sup>, *S. Mayrhofer*<sup>2</sup>, *B. Doupovec*<sup>1</sup>, *R. Berrios*<sup>1</sup>, *F. Waxenecker*<sup>1</sup>, *K. Domig*<sup>2</sup>**

<sup>1</sup> *Biomim Holding GmbH, Research and Development, Getzersdorf, Austria*

<sup>2</sup> *University of Natural Resources and Life Sciences, Institute of Food Science, Vienna, Austria*

### Background and objectives

Antimicrobial resistance is the serious threat to human and animal health. Evaluating the prevalence of extended spectrum  $\beta$ -lactamases (ESBLs) producing *E. coli* in swine and the effect of an antibiotic and acid based feed additives on the load of ESBLs was the aim of this study.

### Material and methods

Weaning pigs (120) were assigned to three groups with four replicates per group: negative control group containing no feed additives; group received amoxicillin in water from day 2 to 6; group received 2 kg/t of feed additive based on organic acids (Biotronic<sup>®</sup> Top3). Growth performance parameters were recorded. Individual fecal samples of three pigs per replication were collected on day 1, day 7 and day 56 of the experiment. ESBL-producing *E. coli* were isolated and enumerated within 24 hours on ChromID<sup>®</sup> ESBL medium.

### Results

The results showed that the prevalence of ESBL-producing *E. coli* in fecal samples of weaning pigs on day 1 of the experiment was  $1.0 \times 10^7$  which represents about 10% of total *E. coli*. On day 7 and day 56 of experiment, the level of ESBL-producing *E. coli* was lower ( $p < 0.05$ ) in OA group in comparison to the group that had received amoxicillin. The level of ESBL-producing *E. coli* in the amoxicillin group represented 15% of total *E. coli* counts on day 56 of the experiment, whereas these numbers were 0.5% in control group and 0.09% in the OA group. The growth performance results showed that at day 56 of the trial, daily weight gain was higher ( $p < 0.05$ ) in OA group compared with the two other groups.

### Discussion & Conclusion

According to results of the trial, a lower level of ESBL-producing *E. coli* in the feces of piglets was detected in the OA group. Follow-up studies in weaned piglets should be carried out in order to confirm the results.



## VPH-OP-04 - CHANGES IN HEMATOLOGICAL, CARCASS AND MEAT QUALITY PARAMETERS ASSOCIATED WITH LIVER MILK SPOTS IN SLAUGHTER PIGS

***N. Čobanović<sup>1</sup>, N. Karabasil<sup>1</sup>, D. Vasilev<sup>1</sup>, M. Dimitrijević<sup>1</sup>, V. Teodorović<sup>1</sup>***

<sup>1</sup> Faculty of Veterinary Medicine- University of Belgrade, Department of Food Hygiene and Technology, Belgrade, Serbia

### Background & Objectives

Liver milk spots cause significant financial losses to pig industry arising from reduced daily gain, feed conversion and growth, treatment costs, disposal of organs, lower carcass and pork quality. The aim of this study was to determine the influence of liver milk spots on hematological, carcass and meat quality parameters in slaughter pigs.

### Material & Methods

A total of 120 pigs with live weight of approximately 112 kg and six months old were examined. Any signs of liver milk spots were recorded as present or absent according to Welfare Quality<sup>®</sup> protocol (2009). A complete blood picture was investigated. The following carcass quality parameters were measured: live, hot and cold carcass weights, dressing percentage, backfat thickness and meatiness. pH and temperature measurements were performed 45 minutes postmortem. Pork quality classes (PSE, normal, DFD meat) were determined according to Adzitey and Nurul (2011) using pH<sub>45</sub> value.

### Results

Pigs with liver milk spots had significantly higher middle-sized cell count (monocytes, eosinophils, basophils) ( $0.27 \times 10^9/L$  vs.  $0.12 \times 10^9/L$ ), neutrophils count ( $7.77 \times 10^9/L$  vs.  $6.10 \times 10^9/L$ ), red blood cell count ( $7.22 \times 10^{12}/L$  vs.  $7.95 \times 10^{12}/L$ ), hemoglobin concentration (132.80 g/L vs. 146.40 g/L), hematocrit (39.37% vs. 40.76%) and MCV (49.28 fl vs. 51.10 fl) than unaffected pigs ( $P < 0.05$ ). The same group of pigs had significantly lower live weight (113.50 kg vs. 115.90 kg), hot carcass weight (92.55 kg vs. 95.23 kg), cold carcass weight (89.48 kg vs. 92.95 kg), dressing percentage (81.55% vs. 82.15%) and meatiness (36.58% vs. 44.10%) than unaffected pigs ( $P < 0.05$ ). Pigs showing liver milk spots had significantly higher pH<sub>45</sub> value (6.32 vs. 6.19), and incidence of DFD meat (26.09% vs. 7.84%) than unaffected pigs ( $P < 0.05$ ).

### Discussion & Conclusion

Assessment of liver milk spots at slaughter-line is a valuable tool for estimating pig welfare on farm of origin, carcass and pork quality.



## VPH-OP-05 - TOXOPLASMA GONDII AND PARMA HAM: NO EVIDENCE OF TRANSMISSION

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### Background & Objectives

*Toxoplasma gondii* is an Apicomplexan protozoa with a wide range of hosts. It has been estimated that it infects more than 2 million people every year in the European region. One of the most important routes of transmission is the consumption of undercooked or cured pork and pigs have been recognized as an important source of *T. gondii* because of the persistence of cysts in muscles. However, little information is available concerning the effect of curing and salting on *T. gondii* cysts in ham.

The aim of this study was to evaluate the survival and viability of tissue cysts of *T. gondii* in the most famous Italian cured ham, "Prosciutto di Parma", following the curing process as described by the legislative guidelines.

### Material & Methods

Twelve pigs were infected per os with 1000 sporulated *T. gondii* oocysts and slaughtered 4 months later. Twelve thighs were cured and 12 were immediately digested according to Dubey (Vet Parasitol. 15;74:75-7, 1998). To verify the infection, serology, meat juice serology, PCR from muscle tissue and bioassay in mice were carried out. After 12, 14 and 16 months of ageing, hams were digested and processed by bioassay in mice to evaluate the presence of viable parasites.

### Results

All pigs became seropositive after 21 days p.i. PCR was positive for parasite DNA either at slaughter and post curing. However, only bioassay in fresh thighs revealed the presence of viable cysts, because all mice inoculated with cured hams were negative.

### Discussion & Conclusion

The results of the present study, the first of its kind, shows that the production process for Parma ham is able to inactivate the parasite and that the product poses no risk to the health of consumers.



## VPH-OP-06 - USING SEROLOGICAL MONITORING AT ABATTOIRS AND ON-FARM AUDITING FOR IDENTIFICATION OF PIG FARMS WITH A HIGH RISK FOR TOXOPLASMA GONDII

**D. Oorburg<sup>1</sup>, D. Eppink<sup>1</sup>, M.J.A. Klein Koerkamp<sup>1</sup>, M. Bouwknegt<sup>1</sup>, H.A.P. Urlings<sup>1</sup>, J.W.P. Van der Giessen<sup>2,3</sup>, M.A.P.M. Van Asseldonk<sup>4</sup>, C.P.A. Van Wageningen<sup>4</sup>, M.F. Mul<sup>5</sup>, J.L. Gonzales<sup>3</sup>, M. Swanenburg<sup>3</sup>, H.J. Wisselink<sup>3</sup>**

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### Background & Objectives

*Toxoplasma gondii* is a relevant foodborne pathogen contributing to human disease burden. In the Netherlands, pork is estimated to contribute to 11% of the meatborne *T. gondii* infections. The European Food Safety Authority advised to perform serological testing of pigs and on farm audits on risk factors for *T. gondii* infection.

### Materials & methods

The within-herd seroprevalence of *T. gondii* was estimated for pig farms using longitudinal data (n = 135,584 samples collected from 2012-2014) from a private food safety monitoring programme at Dutch slaughter houses. A case-control study was performed in which case (n=13, >15% seroprevalence) and control (n=26, 0%) farms were audited for risk factors for *T. gondii*. For the audit the farms were visited and a HACCP based questionnaire was used to assess risk factors.

### Results

Farms with free-range facilities (organic farms) showed a higher seroprevalence than conventional farms. Risk factors such as 1) cats present on farms, 2) use of unheated feed products and 3) feeding liquid feed, were found in the case-control study.

### Discussion and conclusions

Serological screening of pig herds for *T. gondii* infections lead to the identification of pig herds in which typical risk factors for *T. gondii* infections are present (Kijlstra, 2004, Guo, 2015). These risk factors are controlled by Good Agricultural Practises and especially procedures controlling biosecurity. Biosecurity is relevant for veterinary care of the animals, which makes veterinarians important advisors for farmers to comply with and increase on-farm biosecurity. The use of health related (serological) data collected at the abattoir is valuable to guide and monitor the control of *T. gondii* in pork production. Changing farm management to reduce the exposure of pigs to *T. gondii* will likely reduce the human disease burden.

### References

Kijlstra et al, 2004. NJAS 52, 119-132

Guo et al, 2015. J Food Prot. 78(2):457-76.



## RESIDENT SESSION

Wednesday, 3 May 2017, 15:30 – 17:30

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- RES-OP-01 **DOES DENTAL HEALTH AFFECT YOUNG PIGS' PERFORMANCE?**  
*Lucia Dieste Perez (Netherlands)*
- RES-OP-02 **EFFECT OF DRINKING WATER ADDITIVES ON GROWTH PERFORMANCE AND INTESTINAL MICROBIOTA COMPOSITION IN WEANED PIGLETS.**  
*Juan Antonio Mesonero Escuredo (Belgium)*
- RES-OP-03 **INCREASED PREVALENCE OF HEMORRHAGIC DIATHESIS IN NEW BORN PIGLETS DUE TO THROMBOCYTE-SPECIFIC ALLOIMMUNE ANTIBODIES**  
*Rutger Jansen (Netherlands)*
- RES-OP-04 **DETERMINANTS FOR MYCOPLASMAL PNEUMONIA REPRODUCTION UNDER EXPERIMENTAL CONDITIONS: A SYSTEMATIC REVIEW AND RECURSIVE PARTITIONING ANALYSIS**  
*Beatriz Garcia Morante (Spain)*
- RES-OP-05 **EFFECT OF SOW VACCINATION AGAINST PORCINE CIRCOVIRUS TYPE 2 ON SEROLOGICAL, VIROLOGICAL AND REPRODUCTIVE PARAMETERS IN A SUBCLINICAL INFECTION SCENARIO**  
*Salvador Oliver-Ferrando (Spain)*
- RES-OP-06 **WASTING, COUGHING, ARTHRITIS AND CENTRAL NERVOUS SYMPTOMS IN 8 WEEKS OLD PIGLETS CAUSED BY MYCOPLASMA HYORHINIS IN COMBINATION WITH PRRSV**  
*Christine Unterweger (Austria)*





## RES-OP-01 - DOES DENTAL HEALTH AFFECT YOUNG PIGS' PERFORMANCE?

***L. Dieste-Pérez<sup>1</sup>, M. Sparreboom<sup>1</sup>, J. Allaart<sup>2</sup>, H. Van Hees<sup>2</sup>, H.E. Booji-Vrieling<sup>3</sup>, T.J. Tobias<sup>1</sup>***

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### Background & Objectives

Dental health affects general health in humans (Needleman et al., 2004) but in pigs this association is unclear. The aim was to assess dental health in young pigs and study its association with their performance.

### Material & Methods

180 male and female pigs from another experiment sub-clinically infected with *Salmonella* or *E.coli* and weaned at 24 days were used: Group 1 (G1), 60 pigs euthanized at weaning; G2, 72 pigs euthanized at 46 days; G3, 48 pigs euthanized at 53 days. After euthanasia, malocclusion and the presence (scoring 0/1) and severity (scoring 0 to 4) of black staining, plaque, caries, gingiva bleeding upon probing and condition of gingiva around the right and left lower premolar 3 were collected. Data on ADG (average daily gain), ADF (average daily feed intake), FE (feed efficiency) and BW (body weight) were also collected. Generalized linear mixed-effects models followed by an ANOVA test (R 3.2.2. Software) were used to study the association between dental and performance parameters.

### Results

The weaners showed high prevalence of black staining and plaque (>95%) and moderate prevalence of malocclusion patterns (>33%, G2; >23 %, G3) as well as red gingiva (>29%, G2; 44%, G3) and bleeding upon probing (>23%, G2; 31%, G3). Suckling piglets (G1) only showed high prevalence (>86%) of plaque. No effect of gender on dental problems was observed. None of the dental findings were related to ADG, ADF, FE or BW except for the bleeding of the gingiva that had a negative effect on ADG and a positive association with ADF in G3.

### Discussion & Conclusion

This study suggests that young pigs may have dental abnormalities but that only some of them, such as bleeding upon probing, are associated with performance.

### References

Needleman et al., J Clin Periodontol. 2004

Disclosure of Interest: None Declared



## RES-OP-02 - EFFECT OF DRINKING WATER ADDITIVES ON GROWTH PERFORMANCE AND INTESTINAL MICROBIOTA COMPOSITION IN WEANED PIGLETS

***J.A. Mesonero Escuredo<sup>1</sup>, D. Maes<sup>2</sup>, A.K. Oudshoorn<sup>3</sup>, M. Davids<sup>3</sup>, J. Carr<sup>4</sup>, P. Roubos-van den HiP***

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<sup>4</sup> Specialist Pig Practice, Melbourne, Australia

### Background & objectives

The drinking water additives (DWA) based on SCFA effectively reduce the pH in the water and stomach and support digestion. Additives can support microbiota by modifying bacterial populations (Zhu et al. 2014). The objective of this study was investigating the efficacy of DWA on performance and microbiota of piglets.

### Material and methods

220 weaned piglets 28 days old were divided over 2 treatments in the SRC (TN R&D), 3 animals per pen and 20 replicates per treatment. Piglets were fed with a standard 3 phase diet and were monitored 6 weeks. Piglets received standard water (Control) or DWA with 2.0 l/m<sup>3</sup> (Selko-pH). Body weight (BW), average daily gain (ADG), average feed intake (ADF), feed efficiency (FE) were measured weekly together with water intake (ADW) and diarrhea score daily. At the end 10 animals per treatment were sacrificed and stomach and intestinal content was collected to determine pH and microbiota. DNA was isolated from Ileum and Jejunum samples and sequenced using the Illumina HiSeq platform.

### Results

During the study, DWA showed BW higher (26,3 Kg,  $p < 0,001$ ) than control (24.4 kg). ADG of DWA (447 g/day,  $p = 0,006$ ) was higher than control (402 g/day). ADF was increased for DWA (645 g/day,  $p = 0,026$ ) compared to control (568 g/day). FE was not different between DWA (0,715,  $p = 0,695$ ) and control (0.707). DWA consumed more water (1.94 l/day pH 3,6;  $p = 0,003$ ) than control (1.63 l/day pH 7.6). No effects on mortality (4 deaths per group) and diarrhea (DWA scoring 59,83 vs. 60,06 control,  $p = 0,616$ ). A reduction of pH in the stomach was observed for DWA (pH 2.7 vs pH 3.4). DWA Microbiota showed a reduction of genus streptococcus in the Ileum (Jejunum pendent).

### Discussion & Conclusion

DWA increased the ADW and ADF of weaned piglets, and better ADG. DWA influenced microbiota in the intestinal tract showing reduced number of genus Streptococcus.



## RES-OP-03 - INCREASED PREVALENCE OF HEMORRHAGIC DIATHESIS IN NEW BORN PIGLETS DUE TO THROMBOCYTE-SPECIFIC ALLOIMMUNE ANTIBODIES

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### Introduction

During the period of over one year 172 piglets (125†, 43 litters) were affected by subcutaneous bleeding on a Dutch farm (875 sows; Topigs 50 sow x Tempo boar; liquid feed). Normally these symptoms are rarely observed on sow farms.

### Materials and methods

Blood samples of affected pigs and sows were analyzed with the direct Coombs test and by flow cytometry using FITC-conjugated rabbit anti-pig-IgG (Fab'2). Feed was analyzed by HPLC for the mycotoxins DON and ZEA and vitamin K3. Additionally Zinc was analyzed in the feed to exclude mixing errors in the feed mill or at the farm. Serum vit.K3 levels of 5 sows were analyzed by LC-MS/MS and were compared to 5 serum samples from sows originating from a control farm without similar problems.

### Results

All affected litters were born out of higher parity sows (3-9; mean 6.3). Necropsy of the affected piglets showed hemorrhagic skin, spleen and kidneys. The tonsils were negative for Classical Swine Fever virus. Blood samples of the affected piglets combined with blood samples of the sows were positive in the direct Coombs test. Flow cytometry proved the presence of maternally derived antibodies against the piglets' thrombocytes. Mycotoxins were below the detection limits (DON<100ppb and ZEA<5ppb). The amount of Zinc in the liquid feed was in line with the calculated premix dosage (97.9% recovered). Vit.K3 was below inclusion level (65% recovery) but in line with the documented press stability. Serum vit.K3 was higher in the involved farm (32.6nmol/L) compared to the control farm (2.3nmol/L).

### Conclusion

The hemorrhagic diathesis was the result of the proven presence of maternally derived alloimmune-antibodies against the thrombocytes of the neonatal piglets resulting in thrombocytopenia purpura. Advice to the farmer was to cull the involved sows since the problems were expected to occur (and did occur) in subsequent litters. The involved antigen couldn't be identified.



## RES-OP-04 - DETERMINANTS FOR MYCOPLASMAL PNEUMONIA REPRODUCTION UNDER EXPERIMENTAL CONDITIONS: A SYSTEMATIC REVIEW AND RECURSIVE PARTITIONING ANALYSIS

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### Background and Objectives

Variation in mycoplasmal pneumonia (MP) reproducibility and severity under experimental conditions is still a major concern. The present work aimed to identify those factors that may influence on the successful experimental reproduction of MP, determined by the macroscopic lung lesion score.

### Material and Methods

Firstly, we performed a systematic review approach to compile those *M. hyopneumoniae* (*Mhyo*) challenge experiments reporting MP prevalence and/or mean lung lesion score. Secondly, we followed a recursive partitioning approach to evaluate the influence of different experimental variables on the lung lesion outcome by means of regression trees. The variables included in such model were serological status against *Mhyo*, origin of the animals, age at inoculation, type of inoculum, strain, route, dose and times of inoculation, study duration and co-infection with other swine pathogens.

### Results

Eighty-five studies were included in the analyses. The final tree model explained 27% of the observed lung lesion score variability and included the following factors in decreasing order of importance: 1<sup>st</sup>: study duration (time elapsed between challenge and necropsies), 2<sup>nd</sup>: *Mhyo* strain, and 3<sup>rd</sup>: age at inoculation and/or co-infection with other swine pathogens. The highest likelihood to achieve severe MP in an experiment would require lung lesion assessment 4 weeks post inoculation, in SPF pigs above 3.5 weeks of age, intratracheally inoculated, better in 2 consecutive days, with a high dose of *Mhyo* pure culture and, preferably, in co-infection with other swine respiratory pathogens.

### Discussion and Conclusion

This study constitutes the first insight into the conditions that might contribute to reproduce MP under experimental settings and may serve as a basis for debate in the search for a universally accepted *Mhyo* challenge model.

*Acknowledgements:* This study was partly supported by Secretaria d'Universitats i Recerca del Dep. d'Economia i Coneixement de la Generalitat de Catalunya (DI20130039) and by Boehringer Ingelheim Veterinary Research Centre.



## RES-OP-05 - EFFECT OF SOW VACCINATION AGAINST PORCINE CIRCOVIRUS TYPE 2 ON SEROLOGICAL, VIROLOGICAL AND REPRODUCTIVE PARAMETERS IN A SUBCLINICAL INFECTION SCENARIO

**S. Oliver-Ferrando<sup>1</sup>, J. Segalés<sup>1,2</sup>, S. López-Soria<sup>1</sup>, A. Callén<sup>3</sup>, O. Merdy<sup>4</sup>, F. Joise<sup>5</sup>, M. Sibila<sup>1</sup>**

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### Background and Objectives

This study sought to evaluate the influence of sow vaccination against *Porcine circovirus* type 2 (PCV2) on serological, virological and reproductive parameters within two reproductive cycles.

### Material and Methods

The study was performed in a PCV2 subclinical infection scenario (PCV2 circulation but absence of major reproductive problems). Ninety-four sows (V) were primo-immunized with CIRCOVAC® (2 mL, intramuscular) and ninety-seven (NV) were injected with phosphate-buffered saline at 6 and 3 weeks before first farrowing, and then boosted at 2 weeks before the second one. Blood samples were taken throughout the study to assess PCV2 DNA load and antibodies. At farrowing, main reproductive parameters and piglet vitality index were registered. In those litters with more than three mummified or stillborn piglets, microscopic examination and PCV2 antigen detection in foetal myocardium was done.

### Results

V sows showed a significantly higher antibody levels compared to the NV counterparts. PCV2 DNA was only detected at farrowing in 2 (4.17%) NV sows. V sows had 1.3 more live-born piglets per litter at the second cycle ( $p < 0.05$ ) and 0.8 more weaned piglets per litter ( $p < 0.1$ ) than NV counterparts. Additionally, most reproductive parameters (return-to-oestrus (-0.6%), interval between expected and real farrowing date (-0.3 days) and number of mummified (-0.1) piglets per litter) were numerically favourable to V group in the second cycle. Piglets from V sows had significantly higher (+12.7%) vitality than the ones derived from NV sows. No PCV2 compatible lesions neither PCV2 antigen were detected in the tested foetal hearts.

### Discussion and Conclusion

Sow PCV2 vaccination in a subclinical infection scenario increased antibody levels, prolificacy, weaned pigs/litter and vitality of the offspring, but did not have a significant influence on other reproductive parameters.

*Acknowledgments: supported by Secretaria d'Universitats i Recerca del Dep. d'Economia i Coneixement de la Generalitat de Catalunya (2013 DI013)*





## RES-OP-06 - WASTING, COUGHING, ARTHRITIS AND CENTRAL NERVOUS SYMPTOMS IN 8 WEEKS OLD PIGLETS CAUSED BY MYCOPLASMA HYORHINIS IN COMBINATION WITH PRRSV

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### Background

Although considered a commensal in the upper respiratory tract, *Mycoplasma (M.) hyorhinis* has shown to be one of the causative agents of polyserositis (pleuritis, fibrinous pericarditis, peritonitis and arthritis) in piglets resulting in increased morbidity and mortality. So far, no reports on *M. hyorhinis* associated meningitis in piglets exist.

### Material and Methods

In an Austrian closed pig herd with 60 sows and places for 350 fattening pigs, 7 weeks old piglets developed symptoms such as wasting, coughing and head tilt resulting in 25% morbidity and 5% mortality. No all-in-all out system was implemented. The farm was positive for PRRSV and sows were routinely vaccinated against PRRSV with a modified live vaccine. Suckling piglets were vaccinated against PCV2 and *M. hyopneumoniae*. Two piglets (8 weeks old) were brought to the University Clinic for Swine, Vetmeduni Vienna for clinical examination. Necropsy was performed followed by bacteriological, virological and pathohistological examinations.

### Results

In both piglets interstitial peribronchial and intralobular pneumonia with fibrinous-purulent bronchopneumonia, as well as a fibrinous-purulent peri- and epicarditis were present. Furthermore, polyserositis, and leptomeningitis were found. PRRSV was detected in lung lymphnodes. *M. hyorhinis* was solely isolated from brain, serosal and synovial swabs from both piglets. *Haemophilus parasuis* or *Streptococcus suis* were not found. PCV-2 didn't play a role (qPCR:  $8 \times 10^1$  and  $8 \times 10^3$  copies/mg inguinal lymph node).

### Discussion

In this case report *M. hyorhinis* was solely isolated from brain, serosal and synovial swabs and thus was supposed to be the causative agent of meningitis, serositis and arthritis in combination with PRRSV. A PRRSV stabilization program and farm specific vaccination against *M. hyorhinis* was recommended as first step. Treatment with tulathromycin at weaning was implemented instead by the veterinarian and led to a decrease of losses, but didn't reduce respiratory symptoms. Finally the combination of treatment and PRRSV-vaccination of weaned piglets was successful.



## VIRAL DISEASES

Thursday, 4 May 2017, 10:30 – 12:30

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- VVD-OP-01 **COMPARISON OF PRRSV RNA DETECTION BY PCR IN DIFFERENT SAMPLE TYPES**  
*Tanja Opriessnig (United Kingdom)*
- VVD-OP-02 **EFFICACY OF AN ATTENUATED PRRSV-1 VACCINE IN PIGS UPON CHALLENGE WITH A HIGHLY PATHOGENIC EU PRRSV STRAIN**  
*Elena Canelli (Italy)*
- VVD-OP-03 **EVALUATION OF THE EFFECT OF MATERNAL IMMUNITY ON NEONATAL PIGLET VACCINATION PROTOCOLS FOR PRRS**  
*Thomas Wetzell (USA)*
- VVD-OP-04 **RECOMBINATION BETWEEN A FIELD AND A VACCINE STRAIN OF PRRSV DETECTED BY ROUTINE ORF5 SEQUENCING**  
*Adolf Steinrigl (Austria)*
- VVD-OP-05 **ATYPICAL PORCINE PESTIVIRUS ASSOCIATION TO CONGENITAL TREMOR IN THE LAST TWO DECADES IN SPAIN**  
*Mariano Domingo (Spain)*
- VVD-OP-06 **DETECTION OF HUMAN PANDEMIC INFLUENZA A VIRUSES H1N1/2009 IN SWINE HERDS IN THE NETHERLANDS IN 2016**  
*Karien Koenders - van Gog (Netherlands)*



## VVD-OP-01 - COMPARISON OF PRRSV RNA DETECTION BY PCR IN DIFFERENT SAMPLE TYPES

***T. Opriessnig<sup>1</sup>, F. Jinghui<sup>1</sup>, P. Gerber<sup>1</sup>, L. Eppink<sup>1</sup>, C. Wang<sup>2</sup>***

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Determining the PRRSV status on a herd and individual pig basis is essential to control the disease and reverse transcriptase (RT) PCR is commonly used. PRRSV detection is commonly done on serum samples, blood swabs, or FTA™ cards. Most research has focused on PRRSV-2. The objective of this study was to determine the accuracy of PRRSV RNA detection for sample type matrices typically used in the UK spiked with a PRRSV-1 isolate under experimental conditions. Dilutions of a PRRSV-1 strain were used to spike serum, dry or moist (immersed in saline) polyester or cotton swabs, and FTA™ cards. The different samples were stored for 24h, 48h or 7d at 4°C or 20°C and tested by a reverse transcriptase real-time PRRSV PCR assay. Under the study conditions, PRRSV was detected in all serum samples and under all storage conditions. A reduction in sensitivity occurred for swabs and FTA® cards. Despite similar detection rates for moist and dry swabs, dry swabs had lower number of PRRSV RNA copies indicating that moist swabs should be used whenever possible. Interestingly, PRRSV RNA detection in FTA™ cards was similar to dry swabs. Under the study conditions, the overall best sample type across all conditions was serum followed by moist swabs, followed by dry swabs and FTA® cards.



## VVD-OP-02 - EFFICACY OF AN ATTENUATED PRRSV-1 VACCINE IN PIGS UPON CHALLENGE WITH A HIGHLY PATHOGENIC EU PRRSV STRAIN

***E. Canelli<sup>1</sup>, A. Catella<sup>1</sup>, L. Ferrari<sup>1</sup>, E. De Angelis<sup>1</sup>, G. Ognò<sup>1</sup>, P. Bonilauri<sup>2</sup>, S. Guazzetti<sup>3</sup>, G. Sandri<sup>4</sup>, P. Martelli<sup>1</sup>***

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The efficacy of a commercial attenuated PRRSV-1 vaccine was assessed upon challenge with a highly pathogenic (HP) PRRSV strain (PR-402014) (IT171/2016). The challenge isolate is characterized by an 85.9% nucleotide identity of the ORF5 to the vaccine strain. The full genome sequence of the HP strain revealed a discontinuous deletion of 42nt and 366nt in the nsp2 region, and a 6nt deletion in the ORF4.

Seven pigs were vaccinated once with a commercially available modified-live PRRSV-1 vaccine (DV strain; Porcilis® PRRS) at 4 weeks of age. Four weeks after vaccination these animals and seven conventional PRRSV naïve pigs were inoculated intranasally with  $10^6$  TCID<sub>50</sub> PR-402014. A group of 5 negative controls was also considered. Clinical signs (respiratory disorders score, anorexia, level of consciousness, cyanosis) and rectal temperature were daily recorded daily from day 0 to 42 post-infection (DPI). Blood samples were collected at 0, 3, 7, 11, 14, 17, 21, 28, 35 and 42 DPI for virological and serological (ELISA, IPMA and VN antibodies).

The course of clinical signs in vaccinated/infected (V/I) pigs was significantly less severe and shorter compared with the infected animals (I). Overall, the clinical signs' occurrence in vaccinated animals was statistically less severe and shorter than in non-vaccinated. There was no mortality in V/I group. Rectal temperature in V/I was significantly reduced as well. In V/I pigs viremia was significantly less in terms of load (-3 log at the peak) and duration (-3 weeks). Interestingly, SN antibody in V/I animals were busted at 7 DPI and appeared at 21 DPI in HP-PRRSV infected animals.

It can be concluded that vaccination of pigs with an attenuated EU subtype 1 vaccine provides a partial but significant clinical and virological protection against a subsequent exposure to a HP PRRSV-1 subtype 1 strain.



## VVD-OP-03 - EVALUATION OF THE EFFECT OF MATERNAL IMMUNITY ON NEONATAL PIGLET VACCINATION PROTOCOLS FOR PRRS

**S. Dee<sup>1</sup>, J. Nerem<sup>1</sup>, D. Hanson<sup>1</sup>, R. Philips<sup>2</sup>, T. Wetzel<sup>2</sup>, E. Schmalting<sup>2</sup>, R. Edler<sup>2</sup>**

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The objective of this trial was to evaluate the effect of maternal immunity on Ingelvac PRRS<sup>®</sup> MLV vaccination at processing and protection against PRRSv challenge as measured by ADG performance. Trial pigs; n=1200, were PRRS virus negative, sourced from a PRRS stable sow farm. The sow herd received Ingelvac PRRS<sup>®</sup> MLV every 4 months and vaccination 45 days prior to the farrowing of trial pigs. PRRS ELISA testing in piglets confirmed high levels of maternal antibodies. Treatment groups included non-vaccinate controls, Ingelvac PRRS<sup>®</sup> MLV vaccination at processing and Ingelvac PRRS<sup>®</sup> MLV vaccination at weaning. The processing group received 2 ml I.M. of Ingelvac PRRS<sup>®</sup> MLV at 2-8 days of age. The weaning group received 2 ml I.M. of Ingelvac PRRS<sup>®</sup> MLV at 17-20 days of age. Vaccinated pigs were weaned to a wean to finish (WTF) site. Non-vaccinates were weaned to a separate nursery site until 7 weeks of age when they were moved to the WTF site and treatment groups were randomly assigned across all pens. Two pigs/treatment group/pen were challenged at 7 weeks of age with a PRRS field virus isolate I.M. Individual pig weights were taken at weaning, 7, 10, and 23 weeks of age. Pigs vaccinated at processing had significantly improved ADG from challenge to early finish (day 44 – day 79) period of the trial compared to the other treatment groups. Vaccination at weaning had an advantage in ADG compared to non-vaccinated controls during this critical post-challenge period. Both vaccinated treatment groups demonstrated higher ADG during the challenge to close (day 44 – day 161) period of the trial compared to non-vaccinated controls. There was no negative effect on ADG from wean to challenge following vaccination at processing. Early vaccination at processing or weaning, provided protection against field virus in the face of maternal antibodies.





## VVD-OP-04 - RECOMBINATION BETWEEN A FIELD AND A VACCINE STRAIN OF PRRSV DETECTED BY ROUTINE ORF5 SEQUENCING

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### Background & Objectives

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is widely distributed in Europe and is among the most economically important infectious diseases of swine. Administration of modified live vaccines (MLV) during PRRSV outbreaks is a common management strategy. While this practice theoretically creates a prerequisite for recombination between field and MLV-strains of PRRSV, this has not yet been observed in the field. Here, we report the detection of such recombinant PRRSV containing both field and vaccine virus sequences.

### Material & Methods

In spring 2015, an Austrian multisite pig operation became infected by a novel and highly virulent PRRSV strain that had not been recognized prior to 2015. To improve the health status of the farm, partial depopulation was applied as well as vaccination of replacement breeding pigs with a commercial MLV. However, in 2016, the farm experienced a relapse. Serum samples collected during the first and the recurrent phase of the outbreak were submitted for routine ORF5 sequencing. To validate the results, samples were also subjected to next generation sequencing (NGS) of the ORF2-ORF7 region.

### Results

ORF5 sequences obtained during the first phase of the outbreak confirmed the presence of a novel PRRSV-1 field strain. ORF5 sequences obtained from the same farm during the recurrent phase of the outbreak - after application of the MLV - resembled a recombinant between field and vaccine virus. NGS of the ORF2-ORF5 region indicated two recombination breakpoints, thus confirming the results obtained by conventional ORF5 sequencing.

### Discussion & Conclusion

Recombination is a well-known mechanism contributing to rapid evolution of many RNA viruses, such as PRRSV. For the first time we show that recombination may also happen between field and MLV strains of PRRSV, a fact which is likely influenced by common outbreak management strategies. Currently, we can only speculate about the fitness and potential virulence of such recombinant strains.



## VVD-OP-05 - ATYPICAL PORCINE PESTIVIRUS ASSOCIATION TO CONGENITAL TREMOR IN THE LAST TWO DECADES IN SPAIN

**A. Canturri<sup>1</sup>, M. Domingo<sup>2</sup>, S. Muñoz-González<sup>3</sup>, M. Pérez-Simó<sup>3</sup>, J.A. Bohórquez<sup>3</sup>, R. Rosell<sup>4</sup>, O. Cabezón<sup>3</sup>, J. Segalés<sup>2</sup>, L. Ganges<sup>3</sup>**

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<sup>4</sup> Centre de Recerca en Sanitat Animal CReSA- IRTA-UAB and Dept. Agricultura- Ramaderia i Pesca- Generalitat de Catalunya, Bellaterra, Spain

### Background and objectives

The newly described Atypical porcine pestivirus (APPV), thus far reported in USA, The Netherlands and Germany, has been associated to congenital tremor (CT). The aim of this study was to detect APPV in serum samples of pigs submitted for necropsy to *Universitat Autònoma de Barcelona* Veterinary Pathology Diagnostic Service and to search for possible associations of APPV with clinical-pathological conditions found in necropsied pigs.

### Material and Methods

Blood was collected from pigs submitted alive for necropsy. Samples were centrifuged, and the serum was aliquoted and stored frozen at -20°C. A total of 642 samples from the period 1997 to 2016 were investigated with quantitative TaqMan RT-PCR for the APPV NS2-3 genome region. The final diagnosis of necropsied pigs was established based on clinical signs, gross, microscopic lesions and eventual detection of infectious agents. Age of animals was grouped in weeks, according to the clinical history and standard production system in the country.

### Results

Globally, 89 out of 642 samples (13.9%) were positive for APPV RT-PCR. This percentage was higher (54/161, 33.5%) for piglets in the first week of age. Viraemic pigs at older ages ( $\geq 10$  weeks of age) were also detected (11/116, 9.5%). Forty-five out of 78 (57.7%) CT cases were viraemic for APPV. No other clinical-pathological condition was consistently associated with APPV infection.

### Discussion and conclusion

Regular presence of APPV since 1997 to date was found in Spain. Infection was regularly associated to CT, but not to other clinical-pathological conditions. APPV-positive animals in the absence of specific clinical signs may explain APPV spread in Spanish farms in the last twenty years.

*Acknowledgements.* This research was supported by grant AGL2015-66907 from Spanish government. S.M-G. has a predoctoral fellowship FI-DGR 2014 from AGAUR, Generalitat de Catalunya. Additionally supported by CERCA.



## VVD-OP-06 - DETECTION OF HUMAN PANDEMIC INFLUENZA A VIRUSES H1N1/2009 IN SWINE HERDS IN THE NETHERLANDS IN 2016

**K. Koenders - van Gog<sup>1</sup>, D. Henritz<sup>2</sup>, P. van der Wolf<sup>3</sup>, H. Veldhuis<sup>3</sup>, J. Biermann<sup>4</sup>, L. Kateman<sup>5</sup>, D. Struik<sup>6</sup>, S. Wacheck<sup>7</sup>, T. Harder<sup>2</sup>**

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The influenza-A-virus (IAV) subtypes H1N1, H1N2 and H3N2 circulate in domestic pigs at variable rates in The Netherlands (NL). Following the emergence of the human pandemic H1N1\_2009\_IAV (H1N1pdm), variants of this virus occurred in global swine populations. Here we describe the detection of H1N1pdm and H1pdmN2 in swine farms in NL.

Nasal swab or oral fluid samples from clinical cases of porcine flu were sent to the FLI for detection (RT-qPCR), typing and sequencing of IAV.

In four farms H1pdm IAV were found.

In case #1 coughing, fever and increased mortality were seen in weaned piglets and gilts in a 1600 sow herd, vaccinated against IAV at the end of gestation. In nasal swabs from 1 out of 7 gilts and 7 out of 19 piglets reassortant H1pdmN2 was found.

In case #2, in a 3000 sow herd, IAV vaccinated at the end of gestation, hard non-productive coughing occurred in suckling piglets. Also, some gestating sows showed fever and inappetance. In 1 out of 19 nasal swabs from suckling piglets H1N1pdm was detected.

In case #3 coughing was heard in weaned piglets. Sows were vaccinated against IAV at the end of gestation. In oral fluids from piglets (3 out of 3 positive) H1N1pdm was detected.

In case #4, in a 500 sow herd, nasal swabs from weaned piglets were collected, after typical clinical signs of flu occurred. 18 out of 20 swabs were positive for H1N1pdm.

Pigs appear to be highly susceptible to infection with human pandemic IAV strains. These are the first findings of H1N1pdm in NL in pigs. H1pdmN2 has previously been found in NL. Both the H1N1pdm and H1pdmN2 strains closely resemble strains found in Germany.

Current European flu-vaccines do not confer sufficient cross protection against these pandemic strains of IAV. Development of vaccines based on such pandemic strains is warranted.



## HERD HEALTH MANAGEMENT & ECONOMY

Thursday, 4 May 2017, 10:30 – 12:30

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- HHM-OP-01 **LEVERAGING DATA ACROSS A LARGE GEOGRAPHIC AREA TO INCREASE CONTEXT AND UNDERSTANDING OF PRRSV TRANSMISSION**  
*James Lowe (USA)*
- HHM-OP-02 **UNDERSTANDING THE CULTURE OF ANTIMICROBIAL USE BEHAVIOURS IN AGRICULTURE: A MIXED METHODS STUDY OF UK PIG FARMERS**  
*Lucy Coyne (United Kingdom)*
- HHM-OP-03 **THE EFFECTS OF AMOXICILLIN USE IN NEWBORN PIGLETS ON THEIR UMBILICAL PROBLEMS, OTHER TREATMENTS AND RESISTANCE OF INTESTINAL COLIFORMS**  
*Mari Heinonen (Finland)*
- HHM-OP-04 **STABILITY OF ANTIMICROBIALS IN CONTACT WITH DISINFECTANTS IN DRINKING WATER FOR PIGS**  
*Anne Hemonin (France)*
- HHM-OP-05 **SOW VACCINATION AGAINST PORCINE CIRCOVIRUS TYPE 2 AT DIFFERENT PHYSIOLOGICAL STAGES: EFFECTS ON REPRODUCTIVE PARAMETERS AND PASSIVE IMMUNITY IN PIGLETS**  
*Sergio Lopez-Soria (Spain)*
- HHM-OP-06 **SOW MORTALITY DUE TO LIVER LOBE TORSIONS (FOUR CASES).**  
*Theo M.J. Geudeke (Netherlands)*



## HHM-OP-01 - LEVERAGING DATA ACROSS A LARGE GEOGRAPHIC AREA TO INCREASE CONTEXT AND UNDERSTANDING OF PRRSV TRANSMISSION

**J. Lowe<sup>1</sup>, E. Lowe<sup>2</sup>**

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### Background

Area Regional Control (ARC) projects have been successful in increasing the understanding of local risk for PRRSV introduction into herds and have helped coordinate locally vaccination, pig movement and herd elimination strategies. One of the weakness of ARC projects is that while they provide granularity at the local level they do not provide a broad context for current and potential sources of new viruses to that region.

### Materials and Methods

To address that information gap we established a network, the Illinois Swine Health Network (ISHN), with the goal of developing the data collection methods, infrastructure and collaborations to facilitate dynamic, real time estimates of the risk of disease introduction. ISHN employs four key tactics: 1) building collaborations 2) aggregating diverse data streams 3) robust data analysis, and 4) synthesizing information resulting in the following outputs: describing what disease is where, understanding how often disease occurs, understand what disease moves where and understand how farms are connected. The Disease Bioportal Tool (U. California -Davis) is employed for analytics and visualization of data. Each participant maintains its own database to allow for local analysis of each data stream, data is aggregated via the cloud based Disease Bioportal tool to provide global analytics. Standard, routine reporting is provided to participating veterinarians.

### Results

572 individual sites have been enrolled across 6 Midwestern states. Each site has a robust set of metadata including Geo-location, production type, size, and Prem ID. Current focus is on collecting all PRRSV and Influenza Virus A (IAV) sequences including metadata (Location, Date) for each isolate. As of July 2016 ISHN contained 1978 unique PRRS ORF5 sequences and 508 unique IAV HA sequences.

### Discussion

Future work includes capturing animal movement data to provide better context for disease movement patterns and integrating poultry sites to understand cross species IAV movement.





## HHM-OP-02 - UNDERSTANDING THE CULTURE OF ANTIMICROBIAL USE BEHAVIOURS IN AGRICULTURE: A MIXED METHODS STUDY OF UK PIG FARMERS

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The prudent use of antimicrobials is essential to reduce selection pressures and to preserve efficacy and availability for use by human and veterinary medicine. This study investigated the drivers and attitudes around antimicrobial use behaviours in the UK pig industry. Farmers have a unique position in antimicrobial use decisions as whilst the veterinary surgeon may prescribe, the role of administering and overseeing treatment usually falls to the pig farmer.

Initially, qualitative interviews were conducted with farmers (n=22) selected using a purposive sampling approach; which aimed to encompass the spectrum of farming systems and individuals working within the UK pig industry. The key themes and ideas from these were explored further in a quantitative cross-sectional questionnaire study on a representative sample of pig farmers (n=261) across England, Wales and Scotland.

There was an overall perception that farmers identified that both themselves and their respective veterinary surgeons were responsible in their antimicrobial use, however, there were diverse opinions over the definition of responsible use and what practices were identified as prudent. The farmer and veterinary surgeon relationship was considered to be a key factor with farmers relying on their veterinary surgeon for trusted information on antimicrobial use as well as antimicrobial prescriptions. One major concern identified by farmers was that low profitability and unstable retailer contracts resulted in farms with limited scope to reinvest in buildings and management systems that would allow reduced antimicrobial use.

Gaining in-depth insight and understanding into the influences behind prescribing decisions can identify behaviours associated with over or inappropriate use. Such studies have been used in human medicine to identify potential interventions and assess their efficacy on promoting prudent use. It is hoped that by better understanding antimicrobial use practices in livestock, similar interventions may be developed to promote the judicious use of antimicrobials.



## HHM-OP-03 - THE EFFECTS OF AMOXICILLIN USE IN NEWBORN PIGLETS ON THEIR UMBILICAL PROBLEMS, OTHER TREATMENTS AND RESISTANCE OF INTESTINAL COLIFORMS

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Antimicrobials have been used in newborn piglets to reduce umbilical infections. We investigated the effects of a single amoxicillin treatment of newborn piglets on the prevalence of umbilical problems until the age of nine weeks and the associations of the treatment with mortality and the need for treating other diseases until weaning. The prevalence of ampicillin (AMP)-resistant coliforms and antimicrobial resistance patterns of intestinal *E. coli* was also studied. All piglets born every other week (n=3661) were treated with intramuscular amoxicillin (75 mg) on the first day of life (AMOKS) and every other week all piglets (n=3495) were left untreated (CONTR). The umbilicus was palpated at nine weeks. Mortality and disease treatments were recorded until weaning. Faecal samples were collected after weaning and the proportions of AMP resistant coliforms were determined and the susceptibilities of *E. coli* isolates were tested against 14 antimicrobials.

Altogether 2.3% of piglets in CONTR and 0.7% in AMOKS groups ( $P < 0.05$ ) had an umbilical abscess and/or hernia. The treatment did not affect the need to treat skin problems, diarrhoea or the total amount of piglets treated, but the piglets in AMOKS group needed fewer treatments for leg problems (1.9%) than the CONTR pigs (3.4%,  $p < 0.01$ ) and their mortality was lower (6.9% versus 10.5%,  $p < 0.05$ ). There were no differences between the groups in the proportions of AMP resistant coliforms. However, resistance in *E. coli* was more common than on average in indicator *E. coli* in Finland.

In conclusion, single amoxicillin treatment decreased umbilical defects and mortality, but to minor extent and a large proportion of resistant *E. coli* was detected. These results do not support preventive use of antibiotics in newborn piglets, especially considering the possible influence on the development of the normal intestinal microbiota and the selection of antimicrobial resistance at herd level.



## HHM-OP-04 - STABILITY OF ANTIMICROBIALS IN CONTACT WITH DISINFECTANTS IN DRINKING WATER FOR PIGS

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### Background & Objectives

In farms, when antimicrobials are administered through drinking water, they may be in contact with a disinfectant used to improve the bacteriological quality. The objective of this study is to evaluate the stability of antimicrobials in contact with a disinfectant.

### Material & Methods

Six antimicrobials (amoxicillin, colistin, doxycycline, tylosin, trimethoprim and sulfadiazine) and three disinfectants (sodium hypochlorite, hydrogen peroxide and electrolyzed water) were tested. Each antimicrobial was tested as an active ingredient and as a veterinary medicinal product to assess the potential effect of the formulation. In a first step, antimicrobial assay was performed by high performance liquid chromatography in a stock solution with and without disinfectant, at T0, T12 and T24 hours, which is the usual storage period in farms. In a second step, the same assay was performed after neutralization of the disinfectant by sodium thiosulfate or sodium bisulfite. In a third step, the stock solution was incorporated at 5 % into disinfected water, to simulate a treatment administered by a dosing pump.

### Results

All antimicrobials were stable with sodium hypochlorite. With hydrogen peroxide, amoxicillin was deteriorated by 13 % in step 1, but not in step 2 with sodium bisulfite. This disinfectant also impacted tylosin in step 3 (- 11 %). With electrolyzed water, colistin and sulfadiazine were rapidly deteriorated in step 1 (respectively -20 % and - 11 %), but not in step 2 with sodium thiosulfate. However, with the exception of trimethoprim, no antimicrobial was stable in step 3. This implies to connect the dosing pump to a circuit without electrolyzed water.

### Discussion & Conclusion

This study contributes to know the stability of antimicrobials in contact with disinfectants in the drinking water, which is a very usual situation. Precautions should be taken with hydrogen peroxide and above all electrolyzed water that can deteriorate some antimicrobials.



## HHM-OP-05 - SOW VACCINATION AGAINST PORCINE CIRCOVIRUS TYPE 2 AT DIFFERENT PHYSIOLOGICAL STAGES: EFFECTS ON REPRODUCTIVE PARAMETERS AND PASSIVE IMMUNITY IN PIGLETS

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### Background and Objectives

Porcine circovirus type 2 (PCV2) infection has been linked to reproductive disorders. The prevalence and economic importance of this condition is still under debate. The present study aimed to elucidate the benefits of sow vaccination at different physiological stages to prevent these reproductive problems. In addition, maternal antibodies transfer to piglets was assessed.

### Material and Methods

The study was conducted in a conventional sow farm where PCV2 infection was detected in sows and placental umbilical cords and without historical breeding stock vaccination. A total of 246 sows were included in the study: 63 vaccinated (V, PCV2 Ingelvac Circoflex<sup>®</sup>; 1 ml i.m.) at 10 days pre-mating, 61 at mid gestation and 62 at late gestation, plus 60 non-vaccinated (NV saline solution; 1 ml i.m.). Reproductive parameters were recorded at farrowing. PCV2 antibodies were measured from 5 piglets/litter at weaning (3 weeks of age).

### Results

Average piglet birth weight for sows V at mid ( $1.668 \pm 0.382$  Kg) or at late gestation ( $1.646 \pm 0.387$  Kg) were significantly higher than from NV sows. Proportion of mummies tended ( $p=0.1$ ) to be lower in sows V pre-mating (1.1%) compared to NV sows (2.2%). Proportion of stillbirths tended ( $p=0.1$ ) to be lower in sows V pre-mating or mid gestation (4.3%) compared to those V in late gestation or NV (5.5%). Average number of live born piglets was higher in V sows compared to NV, although not statistically significant. Finally, antibody levels of piglets at weaning were higher ( $p<0.05$ ) in the three V groups compared to NV.

### Discussion and Conclusion

A single PCV2 sow vaccination improved reproductive parameters as well as increased maternally derived antibody levels in their offspring compared to non-vaccinated sows. The present study explores the effects of blanket vaccination in a sow herd.

*Acknowledgments: Supported by the 2013 Boehringer Ingelheim European PCV2 award.*



## HHM-OP-06 - SOW MORTALITY DUE TO LIVER LOBE TORSIONS (FOUR CASES)

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### Introduction

GD Animal Health in Deventer (GD) receives about 2500 submissions for post mortem investigation yearly of which 7.5% are sows. The most frequently made diagnoses in deceased sows are acute heart failure (18%) and torsions of abdominal organs (stomach, spleen, intestines; 18%). During the summer and early autumn of 2016, submitted sows from four herds with a relatively high sow mortality rate showed clear symptoms of liver lobe torsions.

### Material / method

Suddenly deceased sows submitted to the pathology lab of GD were thoroughly examined. Anamnestic information from the sow herds was collected by telephone or during farm visits.

### Results

In four submissions of suddenly dead sows, clear signs of liver lobe torsions were observed. Livers were swollen, congested, brittle and ruptured. In the abdomen large amounts of (bloody) fluid were seen. Related pathological findings were (acute) peritonitis (n=3) and spleen and stomach torsions (n=2).

Anamnestic information revealed that problems with high mortality (> 10%) were seen mainly during summer and early autumn and that these problems persisted for at least two months. In three farms tympanic sows were observed, in three farms sows were fed with liquid feed containing several easy fermentable byproducts. In three farms gestating sows were fed once daily.

### Conclusion

The most feasible explanation for the described problems are serial dilatations of the stomach. These dilatations can be caused by accumulation of gas related to feed composition (fermentation). Moreover, feeding sows once a day, especially if they get large volumes of liquid feed, results in stomach dilatations as well. Sequential stomach dilatations can damage liver ligaments resulting in a higher risk for torsions of liver lobes. These torsions can lead to infarction, ruptures and fatal abdominal bleedings. So in case of liver lobe torsions in sows, evaluation of feed and feeding system is strongly recommended.





## BACTERIAL DISEASES I

Thursday, 4 May 2017, 15:20 – 16:20

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- BBD-OP-01**    **ENTEROXINS OF F4+ ESCHERICHIA COLI INDUCE IL17 IN THE PIG SMALL INTESTINE AND ENHANCE COLONIZATION WITH F18AC+ VEROTOXIGENIC E. COLI**  
*Eric Cox (Belgium)*
- BBD-OP-02**    **ASSOCIATION BETWEEN ISOLATION OF ENTEROTOXIGENIC E. COLI AND NUMBERS OF E. COLI F18 GENES IN FAECES IN NURSERY PIGS**  
*Nicolai Weber (Denmark)*
- BBD-OP-03**    **DETECTION OF SALMONELLA ANTIBODIES IN THE SALIVA OF PIGS FROM SALMONELLA TYPHIMURIUM-VACCINATED AND UNVACCINATED HERDS**  
*Alessia De Lucia (Italy)*



## BBD-OP-01 - ENTEROXINS OF F4+ ESCHERICHIA COLI INDUCE IL17 IN THE PIG SMALL INTESTINE AND ENHANCE COLONIZATION WITH F18AC+ VEROTOXIGENIC E. COLI

***E. Cox<sup>1</sup>, Y. Luo<sup>2</sup>, M. Atef Yekta<sup>3</sup>, M. Loos<sup>4</sup>, A. Coddens<sup>5</sup>, S. Arnouts<sup>6</sup>, V.D.B. Wim<sup>7</sup>, U. Lundberg<sup>8</sup>, B. Devriendt<sup>9</sup>***

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Enterotoxigenic *E. coli* (ETEC) play a major role in post-weaning diarrhoea in pigs. Upon weaning, F4+ETEC rapidly colonize the small intestine and produce LT, STa and/or STb enterotoxins which induce diarrhoea. Colonisation with F18+verotoxigenic *E. coli* (VTEC) is more variable. We examined if the LT enterotoxin might change the small intestinal barrier in such a way that it might enhance colonization with F18+VTEC. Furthermore, we studied the effect of enterotoxins on IL-17A, a cytokine that has been implicated in protection of the host against bacteria.

In first experiments we analysed the effect of different concentrations of LT on fluid secretion and mucus coverage in 20 cm long small intestinal segments (3 pigs/group). A dose of 10 µg LT induced fluid secretion and decreased the mucus coverage, comparable to this of an infection with 10<sup>8</sup> CFU F4+ETEC. Since the length of the small intestine of newly-weaned pigs is approximately 8 meter, 40 times higher LT doses were administered in vivo (n=11), whereafter pigs were inoculated with F18ac+VTEC. LT administration significantly increased colonization with F18+VTEC in comparison with pigs receiving PBS (n=7) indicating that infection with an F4+ETEC strain can increase susceptibility to infection with F18+VTEC.

In a second series of experiments, the effect of different enterotoxins was analysed on IL-17A using among others mutant strains expressing one or more enterotoxins. A strong IL-17A expression was induced by STb. Surprisingly IL-17A was first seen in enterocytes, followed by goblet cells. Blocking experiments showed that this STb-mediated epithelial IL-17A controls the expression of genes involved in maintaining the epithelial barrier, such as β-defensin-2, mucins and plgR. On top of this epithelial response, ETEC infection induced an influx of CD3<sup>+</sup>IL17A<sup>+</sup> cells in the intestinal lamina propria. Our findings unravel a role of IL-17A in innate immunity against ETEC infection and show this might be a therapeutic target.



## BBD-OP-02 - ASSOCIATION BETWEEN ISOLATION OF ENTEROTOXIGENIC *E. COLI* AND NUMBERS OF *E. COLI* F18 GENES IN FAECES IN NURSERY PIGS

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### Background & Objectives

The study was designed to investigate the association between bacteriological findings of enterotoxigenic *E. coli* (ETEC) in nursery pigs and the quantitative detection of *E. coli* F18+ in faeces.

### Material & Methods

A cross-sectional study was conducted in 20 pig herds. When a diarrheic outbreak occurred in a nursery room, 80 randomly selected pigs were clinically examined. From each pig a faecal sample was collected and diarrheic status was assessed by visual inspection. From each herd, 8 pigs with and 8 pigs without diarrhoea was euthanized and necropsied. Bacterial culture for *E. coli* was performed from jejunum and faecal samples. Pigs with demonstration of haemolytic *E. coli* in pure/dominant culture from faeces or jejunum content were classified as ETEC positive pigs (Coli+) and were used as golden standard. Excretion levels of *E. coli* F18+ in pig faecal samples were assessed by qPCR analyses. ROC analysis on excretion level of *E. coli* F18+ was performed to determine the optimal cut-off value to detect Coli+ pigs. The optimal cut-off was determined as the point with the largest combined value of sensitivity + specificity.

### Results

A total of 313 pigs (156 diarrheic, 157 non-diarrheic) was used for analysis. 63 (20.1%) were classified as Coli+ after bacterial culture. The ROC analysis provided an optimal cut-off value of 5.2 log<sub>10</sub> *E. coli* F18+ CFU/g faeces. This cut-off provided a diagnostic sensitivity of 60.3 and diagnostic specificity of 90.4. Using the cut-off value of 5.2 log<sub>10</sub> in pigs with diarrhoea provided a diagnostic sensitivity and specificity of 76.6 and 90.4 respectively.

### Discussion & Conclusion

The results of the study showed that pigs with an excretion levels of >5.2 log<sub>10</sub> *E. coli* F18+ CFU/g faeces suffered from ETEC infection. The diagnostic value of qPCR testing was highest in samples from diarrheic pigs.



## BBD-OP-03 - DETECTION OF SALMONELLA ANTIBODIES IN THE SALIVA OF PIGS FROM SALMONELLA TYPHIMURIUM-VACCINATED AND UNVACCINATED HERDS

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This study aimed to investigate the oral fluid saliva (OF) collected passively via chewed sampling ropes as a potential sample type for assessing anti-*Salmonella* antibodies in 2 *Salmonella*-vaccinated (V) and 2 non- vaccinated (NV) pig farms, comparing the results with direct bacteriological diagnostic methods.

Gilts and sows in the V farms were vaccinated prior to farrowing with a live attenuated vaccine (Salmoporc STM - IDT Biologika). For each age class (sows - S, weaners - W and grower pigs – G) pooled of faecal and OF samples were collected. *Salmonella* was isolated according to a modification of ISO6579 Annex D. In parallel, IgG antibody titres were assessed in OF samples using an ELISA assay (IDEXX Swine *Salmonella* Ab Test - IDEXX Laboratories).

*Salmonella* was detected in 90.6% of faecal samples in NV farms, and in 36.1% of V farms ( $p < 0.001$ ). A lower prevalence (47.8%) was observed in W+G of V farms compared to 97.1% in NV farms. In V farms, the IgG levels in OF were higher in S and declined in W+G. In NV farms, W+G had higher IgG levels than S.

The lower *Salmonella* prevalence in S of V farms probably correlates with the higher antibody titer, which was lower in weaners and growers. Antibodies measured in V farms are likely to be partially related to vaccination. Conversely, in NV farms a high IgG antibody titre was observed in W+G and correlated with high level of shedding, suggesting that the antibody response was related to infection with field *Salmonella* strains. The use of OF to assess antibodies in pigs represents a valid alternative to serum. However, to assess vaccination efficacy in stimulating antibody response, further studies are necessary to discriminate between IgG produced in response to vaccination and that resulting from infection with field *Salmonella* strains.



## WELFARE & NUTRITION

Thursday, 4 May 2017, 15:20 – 16:20

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- AWN-OP-01 ACCESS TO CHEWABLE MATERIALS INCREASES PIGLET ACTIVITY DURING LACTATION PERIOD**  
*Kirsi-Marja Swan (Finland)*
- AWN-OP-02 MEAT INSPECTION DATA HIGHLIGHT HEALTH PROBLEMS IN FREE-RANGE PRODUCTION SYSTEMS**  
*Hanne Kongsted (Denmark)*
- AWN-OP-03 BEHAVIORAL ANALYSIS OF PIGLETS TREATED WITH METACAM® THIRTY MINUTES BEFORE AND AT TIME OF CASTRATION**  
*Jessica Law (Canada)*





## AWN-OP-01 - ACCESS TO CHEWABLE MATERIALS INCREASES PIGLET ACTIVITY DURING LACTATION PERIOD

***K.M. Swan<sup>1</sup>, H. Telkänranta<sup>1</sup>, C. Munsterhjelm<sup>1</sup>, O. Peltoniemi<sup>1</sup>, A. Valros<sup>1</sup>***

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Several studies have shown the benefits of enrichment materials after weaning. Still, there is little data on the influence of chewable materials on piglet behavior during early weeks of life.

We divided 58 sows farrowing in crates into two groups. Half of their litters, the rope-paper group (RP) had access from the first day of life to 10 sisal ropes, one plastic ball, newspaper and sawdust (n=29). The other half, the control group (C), had access to sawdust and plastic ball (n=29).

Behavior of the piglets was recorded for a four-hour period during the first 7-18 days of life. From the recordings piglet-sow interactions, udder manipulation, manipulation of other body parts of the sow and the number of successful nursings were analyzed.

The statistical analysis was done for two age groups separately: younger than 14 days (RP n=21, C=18) and 14 days or older (RP n=8, C n=11). Treatments were compared with nonparametric Mann-Whitney U-test.

In age group 1 RP piglets manipulated the udder more often ( $p=0.00$ , Med 98 times, Min 43 Max 163) than the C group (Med 64 times, Min 43 Max 151).

In age group 2 RP piglets made body contacts with the sow more often ( $p=0.02$ , Med 124 times, Min 41 Max 178), had more successful nursing ( $p=0.03$  Med 5 Min 4 Max 6) and manipulated the udder more ( $p=0.01$  Med 73 Min 41 Max 119) than the C group (Med 52, Min 12 Max 116; Med 4, Min 3 Max 5 and Med 49 min, Min 27 Max 63).

We conclude that access to enrichment materials during early weeks of life increase the general activity of the piglets which may affect the sow-piglet and piglet-piglet interaction and lactation performance.



## AWN-OP-02 - MEAT INSPECTION DATA HIGHLIGHT HEALTH PROBLEMS IN FREE-RANGE PRODUCTION SYSTEMS

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### Background & Objectives

Free-range production systems provide better possibilities for natural behaviour than conventional indoor systems. However, these systems may challenge the prevention and handling of diseases. Our objective was to evaluate the prevalence of lesions at meat inspection in conventional indoor-, conventional free-range and organic free-range production systems taking seasonal-, yearly- and herd effects into account.

### Materials & Methods

We used routine meat inspection data from 1.096.756 pigs slaughtered at one Danish abattoir during 2013-2015. Abattoir-codes were aggregated into overall disease complexes related to specific organ systems. Odds for these disease complexes within the three different systems were evaluated in generalized linear mixed models.

### Results

Free-range pigs had increased odds for white liver spots, tail lesions, arthritis, skin lesions, bone fractures, septicaemia and abscesses at slaughter compared to conventionally raised pigs. We saw no significant difference between the effects of conventional and organic free-range systems. Approximately 3% of free-range pigs compared to 1% of conventional pigs had tail lesions at slaughter. Within all three systems, approximately 20% of the pigs had airway-related lesions. This was by far the most prevalent disease complex.

Three disease complexes; swellings/ scars, herniae and hoof abscesses had lower odds in free-range pigs than in conventionally raised pigs.

### Discussion & Conclusion

The study found some lesions associated with conventional indoor and several disease complexes associated with free-range production systems. A substantial herd variation appeared within all three systems, indicating good possibilities for herd-specific management interventions. Parasitic liver spots, tail lesions, arthritis and bone fractures were pointed out as points of concern in relation to animal welfare under free-range conditions. The concern of under-treatment of sick animals in organic production systems with larger restrictions on antibiotic use was not supported, since we saw no significant difference between the effects of the two free-range systems.



## AWN-OP-03 - BEHAVIORAL ANALYSIS OF PIGLETS TREATED WITH METACAM® THIRTY MINUTES BEFORE AND AT TIME OF CASTRATION

**J. Law<sup>1</sup>, G. Cunningham<sup>2</sup>**

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<sup>2</sup> *Boehringer Ingelheim Canada Ltd., Kelsey, Canada*

### Introduction

This study was done to expand the understanding of objective behavioral observation tools and test the hypothesis that Metacam® 5mg/ml would be effective for pain control given at castration or 30 minutes prior.

### Materials and Methods

Ninety-five piglets 6-8 days of age from a research barn were randomly placed into six treatment groups: Uncastrated groups: A (saline at sham castration), B (Metacam® at sham castration) and E (Metacam® 30 minutes prior to sham castration), Castrated groups: C (Saline at castration), D (Metacam® at castration) and F (Metacam® 30 minutes prior to castration). Piglets were trained prior to the trial in the chute/hurdle course, and 4 baseline runs were timed to establish individual baselines. Minimum 1 hour after baselines were completed, piglets were collected, groups E and F were treated with Metacam®. Piglets were timed through the chute course 15 minutes prior to castration. At castration/ sham-castration the remaining groups were treated with either 0.08ml/kg of saline or Metacam® (5mg/ml). Castration was performed according to North-American standards. Immediately after castration (T0) the piglets were run through the chute, timed and repeated at 15, 30, 60, 120 and 1440 minutes. Statistics completed in IBM® SPSS® Statistics version 22, 2013.

### Results

Group C (castrated, untreated) was significantly ( $p < 0.05$ ) slower indicating increased pain compared to baseline values when compared to all other groups both treated/castrated and uncastrated groups at T=15, 30 and 120 minutes. There was no significant difference,  $p > 0.10$  between the Metacam® treated groups or between the Metacam® groups and the uncastrated groups.

### Conclusion

Metacam® treatment of at the time of castration or 30 minutes prior provided increased piglet comfort according to this model.

### Disclosure of Interest

Third party study funded partially by the product manufacturer and supported by Alberta Agriculture and Forestry.



## BACTERIAL DISEASES II

Thursday, 4 May 2017, 16:40 – 18:00

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- BBD-OP-04**    **A LAWSONIA INTRACELLULARIS FECAL QUANTITATIVE POLYMERASE CHAIN REACTION ASSAY AND THE CORRELATION TO PIG PERFORMANCE IN LAWSONIA INTRACELLULARIS CHALLENGED PIGS.**  
*Jessica Seate (USA)*
- BBD-OP-05**    **EFFECT OF PRE-FARROWING SOW VACCINATION AGAINST MYCOPLASMA HYOPNEUMONIAE ON OFFSPRING COLONIZATION AND LUNG LESIONS**  
*Ioannis Arsenakis (Belgium)*
- BBD-OP-06**    **USE OF MOLECULAR CHARACTERIZATION TOOLS TO INVESTIGATE M. HYOPNEUMONIAE OUTBREAKS**  
*Eduardo Fano (USA)*
- BBD-OP-07**    **PREVALENCE AND SEROTYPE DISTRIBUTION OF STREPTOCOCCUS SUIS IN CLINICALLY ILL PIGS AND IN HEALTHY-CARRIER PIGS AT DIFFERENT STAGE OF PRODUCTION**  
*Vahab Farzan (Canada)*



## BBD-OP-04 - A LAWSONIA INTRACELLULARIS FECAL QUANTITATIVE POLYMERASE CHAIN REACTION ASSAY AND THE CORRELATION TO PIG PERFORMANCE IN LAWSONIA INTRACELLULARIS CHALLENGED PIGS

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Sub-clinical manifestations and performance losses due to *Lawsonia intracellularis* (Li) infections are difficult to diagnose. Fecal qPCR testing has been suggested as diagnostic tool to evaluate the impact when obvious clinical signs are lacking. The objective of this study was to investigate if fecal qPCR levels correlate with artificial challenge doses and performance losses.

In total, twenty-eight 5-week old pigs were stratified by weight and gender, and assigned to four challenge groups ( $10^9$ ,  $10^7$ ,  $10^5$ , or  $10^3$  CFU/mL per pig) or left as strict controls (SC). Pigs were weighed at day of challenge (d0) and 20 days later. The Li challenge treatments were orally challenged with 25 mL of gut homogenate. Fecal samples were collected on d0, d7, d14, and d20 and tested by qPCR at HMC (Ames, Iowa). Three weeks post challenge pigs were euthanized, necropsied and lesions grossly scored. Immunohistochemistry and Hematoxylin & Eosin staining of ileum and cecum were analyzed.

Total weight gain by treatment was: SC = 106.7,  $10^3$  = 93.4,  $10^5$  = 93.4,  $10^7$  = 53.3, and  $10^9$  = 26.7 kg. The qPCR results by treatment day were (d0, d7, d14, d20): SC= negative at all-time points;  $10^3$  = 0%, 0%, 25%, 50% positive;  $10^5$  = 0%, 71%, 93%, 86% positive;  $10^7$  = 0%, 96%, 89%, 89% positive;  $10^9$  = 0%, 100%, 96%, 93% positive.

Daily and total weight gain was inversely related to the dose level of challenge. The qPCR results were positively correlated to the dose level of challenge. Macroscopic and microscopic lesions in the ileum, cecum, and colon were positively correlated to the higher dose level of Li challenge. Based on the results of this study qPCR might be considered a useful tool to evaluate subclinical cases of Li infection. It should be kept in mind that these data were collected from non-vaccinated animals.





## BBD-OP-05 - EFFECT OF PRE-FARROWING SOW VACCINATION AGAINST MYCOPLASMA HYOPNEUMONIAE ON OFFSPRING COLONIZATION AND LUNG LESIONS

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This study investigated *Mycoplasma hyopneumoniae* (*Mhyo*) colonization and lung lesions at slaughter in pigs from vaccinated and non-vaccinated sows, in two herds. In each herd, two sow groups received *Mhyo* vaccination with Ingelvac MycoFLEX® at six and three weeks before farrowing and two sow groups remained non-vaccinated. From each sow group, per herd, the litters of five primiparous sows were sampled with laryngeal swabs at weaning, and with laryngeal swabs and oral fluids at seven days post-weaning. All samples were tested for *Mhyo* by nested PCR. In total, 488 piglets were sampled. Upon slaughter, the severity of *Mycoplasma*-like lung lesions (LLS) was assessed. In herd A, 14.17% and 20.00% of the piglets from the vaccinated and non-vaccinated sows, respectively, were laryngeal swab-positive at weaning ( $P=0.225$ ). At seven days post-weaning those values were 0.81% and 6.08%, respectively ( $P=0.031$ ). In herd B, 0.87% and 0.79% of the piglets from the vaccinated and non-vaccinated sows, respectively, were laryngeal swab-positive at weaning ( $P=0.948$ ). At seven days post-weaning, those values were 1.81% and 2.46%, respectively ( $P=0.738$ ). In herd A at seven days post-weaning, 0% of the pens with piglets from the vaccinated sows were oral fluid-positive, while 25.00% of the pens with piglets from the non-vaccinated sows were positive ( $P=0.473$ ). In herd B, those values were 5.88% and 10.52%, respectively ( $P=0.619$ ). The average LLS in herd A was 15.54 for the piglets of the vaccinated sows and 26.40 for the piglets of the non-vaccinated sows ( $P=0.021$ ). In herd B, those values were 9.70 and 8.51, respectively ( $P=0.541$ ). In conclusion, in herd A, with a higher level of piglet colonization at weaning, offspring from vaccinated sows had a significantly lower colonization rate seven days post-weaning and a significantly lower LLS at slaughter, when compared to the offspring of the non-vaccinated sows.



## BBD-OP-06 - USE OF MOLECULAR CHARACTERIZATION TOOLS TO INVESTIGATE *M. HYOPNEUMONIAE* OUTBREAKS

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### Background & Objectives

Clinical disease and *Mhp* detection can be evidenced post-elimination, questioning the success of the program or potential lateral transmission. Therefore, this investigation assessed *Mhp* outbreaks employing molecular characterization tools after elimination efforts.

### Materials and Methods

Six 2,500 farrow-to-wean farms completed and succeeded a *Mhp* elimination program. Fourteen to sixteen months post-completion of the elimination program, a *Mhp* outbreak was confirmed at all six sites by detecting the pathogen by PCR. For all sites, P146 sequencing was performed in DNA from positive swabs to characterize *Mhp* variants and a phylogenetic tree was created to analyze the sequences obtained from the six sites as well as the original variant(s) identified prior to the implementation of sow farm elimination strategies.

### Results

Sequences obtained post-elimination clustered together but differently and independently from the original variants identified prior to elimination, indicating that the *Mhp* outbreaks were produced by a new *Mhp* variant; therefore the elimination program failure was ruled out. By investigating a GDU shipment records, it was identified that sourced all six farms approximately 2.5 months prior to the detection of *M.hp* post-elimination. It is important to note that all gilts tested (n=30) negative (serology) prior to entry into the farms. Despite this outcome, the possibility of the GDU becoming positive through lateral transmission and the lack of detection due to insufficient sample size, timing for seroconversion, and diagnostic method cannot be ruled out.

### Conclusion and recommendations

To aid veterinarians in the investigation of *Mhp* activity post-elimination efforts, transmission, and variant origin, the molecular characterization of variants should be an essential and vital component of an elimination program. Through the use of molecular characterization tools, insights regarding new *Mhp* outbreaks can be provided in multiple different scenarios, such as assessing a potential failed elimination attempt or lateral transmission between pig sites.



## BBD-OP-07 - PREVALENCE AND SEROTYPE DISTRIBUTION OF STREPTOCOCCUS SUIIS IN CLINICALLY ILL PIGS AND IN HEALTHY-CARRIER PIGS AT DIFFERENT STAGE OF PRODUCTION

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<sup>2</sup> University of Guelph, Pathobiology, Guelph, Canada

### Background and Objectives

*Streptococcus suis* serotypes are present as commensal bacteria or opportunistic pathogens in the nasal cavity and tonsils of the majority of pigs but only some serotypes can cause clinical disease. The objective of this study was to investigate the prevalence and serotype distribution of *S. suis* in healthy and diseased pigs at different stage of production.

### Materials and Methods

Nasal and tonsillar swabs from healthy young pigs (suckling, weanling, and grower), nasal and vaginal swabs from sows, as well as tonsillar and meningeal swabs and tissue from tonsil and lymph nodes from clinically-ill pigs were obtained. Samples were cultured, and the *S. suis* isolates were identified by MALDI-TOF or PCR. A multiplex PCR was used to serotype the isolates. A mixed-effect multilevel logistic regression modeling method was used to compare the presence and serotype distribution of *S. suis* in diseased and healthy pigs and among pigs at different stages of production.

### Results

*Streptococcus suis* was recovered from 219 (68%) and 74 (59%) of samples collected from healthy and diseased pigs, respectively ( $P < 0.001$ ). *S. suis* was more likely to be recovered from suckling and nursery piglets than from sows and finishers ( $P < 0.001$ ). Twenty-two different serotypes were identified; serotype 31 was the most common serotype in healthy pigs while serotypes 4, 8, and 9 were the most common serotypes in sick pigs. However, 205 (57%) of isolates were untypable; the isolates from healthy pigs were more likely to be untypable than those isolated from sick pigs ( $P < 0.001$ ).

### Conclusion

These findings indicate which serotypes are most problematic in causing *S. suis* disease as well as which serotypes may be only found in healthy pigs. This can be used to develop a more effective vaccination program to prevent *S. suis* infection outbreaks in pigs and to design appropriate management changes.



## MISCELLANEOUS

Thursday, 4 May 2017, 16:40 – 18:00

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- MIS-OP-01 **BUTORPHANOL INDUCES ANXIETY-LIKE BEHAVIOUR AND DISTRESS IN PIGLETS**  
*Veronika Cap (Switzerland)*
- MIS-OP-02 **SHORT- AND LONG-TERM EFFECTS OF EARLY METAPHYLACTIC USE OF CEFTIOFUR ON THE FAECAL MICROBIOTA IN SUCKLING AND GROWING PIGS**  
*Ursula Ruczizka (Austria)*
- MIS-OP-03 **UROLITHIASIS IN FINISHING PIGS**  
*Joris Vrielinck (Belgium)*
- MIS-OP-04 **CLOSING THE INNOVATION GAP - HOW TO TUNE RESEARCH TO FARMERS NEEDS**  
*Sarah De Smet (Belgium)*



## MIS-OP-01 - BUTORPHANOL INDUCES ANXIETY-LIKE BEHAVIOUR AND DISTRESS IN PIGLETS

**V.H. Cap<sup>1</sup>, M. Abass Mossa<sup>2</sup>, P.J. Hug<sup>1</sup>, D. Kümmerlen<sup>3</sup>, C. Hug<sup>1</sup>, R. Bettschart-Wolfensberger<sup>1</sup>**

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To provide analgesia, pig anaesthesia protocols often include butorphanol. When we used butorphanol for castration under inhalation- or injectable anaesthesia, severe restlessness and cyanosis were recorded in piglets receiving butorphanol, even if azaperone was given. As such effects in pigs have not been reported before, the aim of this study was to investigate the effects of butorphanol in piglets. Ten 27 days old piglets weighing  $8.5 \pm 1.3$  kg were randomly allocated (double-blinded) to a group administered  $0.2 \text{ mg kg}^{-1}$  butorphanol (B) and to a control group receiving saline 0.9% (C) intramuscularly. The animals were video recorded for 60 min and behaviour was retrospectively assessed by two independent observers. Room temperature was  $28 \text{ }^{\circ}\text{C}$ .

Within 2 to 15 min after application, piglets in group B showed restlessness and distress and started to vocalize excessively ( $p < 0.05$ ). Locomotor activity was increased, the piglets laid considerably less frequently and for shorter time periods during the first 35 min compared to group C ( $p < 0.1$  and  $p < 0.01$ ). Animals in group C were calm and slept during the most time of the experiment ( $45.1 \pm 2.9$  min in group C vs  $12.7 \pm 2.9$  min in group B,  $p < 0.01$ ). Butorphanol treated pigs showed anxiety and signs of panic, trying to escape by jumping repeatedly against the wall (average 1.2 times per min during the first 30 min vs 0 times per min in group C,  $p < 0.05$ ). One animal of group B depicted lead-press. Severe panting and gasping were observed in four animals of this group. Moreover 30 min after butorphanol application piglets became hyperthermic ( $41.1 \pm 0.7 \text{ }^{\circ}\text{C}$  in group B vs  $39.7 \pm 0.3 \text{ }^{\circ}\text{C}$  in group C,  $p < 0.01$ ).

The results of this study show that butorphanol induces severe side effects in piglets that are similar to those reported in other species.



## MIS-OP-02 - SHORT- AND LONG-TERM EFFECTS OF EARLY METAPHYLACTIC USE OF CEFTIOFUR ON THE FAECAL MICROBIOTA IN SUCKLING AND GROWING PIGS

**U. Ruczizka<sup>1</sup>, C. Unterweger<sup>1</sup>, B.U. Metzler-Zebeli<sup>1</sup>, E. Mann<sup>2</sup>, L. Schwarz<sup>1</sup>, C. Knecht<sup>1</sup>, I. Krauss<sup>1</sup>, S. Mayerhofer<sup>1</sup>, I. Hennig-Pauka<sup>1</sup>**

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### Background & Objectives

The metaphylactic use of ceftiofur during the first days of life is a common preventive strategy against different bacterial diseases in swine production. As a broad-spectrum antibiotic, ceftiofur inhibits the targeted pathogens but may also impair the commensal microbiota composition important for gastrointestinal health. This study aimed to evaluate short- and long-term effects of early metaphylactic use of ceftiofur on the faecal microbiota in suckling and growing pigs.

### Material and Methods

In total, 64 male and female piglets from 8 litters (n=4/litter/sex) were assigned to the antibiotic group (AB; n=32) or control group (control; n=32). At 12h postpartum AB piglets received an intramuscular injection of ceftiofur (5.0 mg/kg BW), whereas control piglets received a placebo (0.2 ml/kg BW; phosphate-buffered saline). DNA was extracted from faecal samples that were collected from piglets 12h postpartum before treatment and at days 12, 28 and 97 postpartum. 16S rRNA gene sequencing using Illumina MiSeq was performed. Bioinformatics were performed using the QIIME<sup>®</sup> pipeline and data were analysed in SAS<sup>®</sup>.

### Results

At day 97, AB pigs tended to weigh less compared to control pigs. AB-related differences in the faecal microbiota were visible at all taxonomic levels at all sampling time points. For instance, AB pigs comprised more *Enterococcaceae* at day 12, and *Campylobacteraceae* at day 97 than control pigs (P<0.05). Moreover,  $\alpha$ -diversity indices indicated reduced (P<0.05) species richness and evenness in AB pigs compared to control pigs at days 12, 28 and 97. Alpha-diversity indices positively correlated with body weight (P<0.001).

### Discussion & Conclusion

The present results showed that ceftiofur injection on the first day of life markedly affected the successional changes in the faecal microbiota composition in male and female pigs with long-term consequences for the microbial community and host phenotype.





## MIS-OP-03 - UROLITHIASIS IN FINISHING PIGS

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<sup>2</sup> Provet, Veterinary Practice, Torhout, Belgium

### Background and objectives

Urinary calculi have already been described in fattening pigs in the past (1). Nevertheless many aspects of the epidemiology and pathogenesis have not been solved at the moment. The present study aims at bringing insight in the prevalence and composition of urinary tract calculi.

### Material and methods

Fifty farms, with fattening pigs, were selected. A slaughterhouse examination was performed. Urinary bladders of approximately 50 pigs per farm were sampled. After excision, the bladders were opened, a urine sample was taken for microscopic examination. The bladders were checked for abnormal content (stone and grit) and for inflammatory epithelium lesions. The composition of stones was examined by infraredspectrophotometry.

### Results

In 8.18% of the bladders, macroscopic abnormalities were observed: stones 4.6%, grit 2.7%, amorphous sediment 1.7%. Microscopical crystalluria was detected in 57.1% of the urine samples: struvite crystals (29.5%), calciumoxalatedihydrate (COD) (22.2%), calciumcarbonate (5.4%), amorphous crystals (8.4%), calciumoxalatemonohydrate (COM) (0.2%). Stones of mixed composition occurred frequently.

### Discussion and conclusion

Struvite, which is considered as an infection crystal (2), was the most common crystal component (29.52%). In a previous study, calciumcarbonate was most frequently found (1). It is unlikely that all the struvite is caused by infection, but rather is influenced by feed and water composition. Infraredspectrophotometry of stones also showed carbonate-apatite as a component, especially where amorphous sediment was detected.

Uroliths are present in a large proportion of the examined male fattening pigs (8% at macroscopic and 57% at microscopic level) with risk for urethra obstruction and bladder rupture. Further analysis will focus on risk factors related to management, housing, feed, drinking water quality for urolithiasis and ways to prevent the condition

1. DGD Maes, J Vrielinck, S Millet, GPJ Janssens, P Deprez, Urolithiasis in finishing pigs, Veterinary Journal 168(3): 317-22, dec 2004

2. R Miano, S Germani, G Vespasiani, Stones and urinary tract infections, Urol Int 2007, 79(suppl 1): 32-36



## MIS-OP-04 - CLOSING THE INNOVATION GAP - HOW TO TUNE RESEARCH TO FARMERS NEEDS

**S. De Smet<sup>1</sup>, S. Van Gansbeke<sup>2</sup>, S. Millet<sup>3</sup>**

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Scientific research results are often insufficiently translated and exploited in practice. In the other direction specific knowledge gaps, research questions and innovative ideas of farmers are not captured in a structured manner and do not always reach researchers. To stimulate knowledge exchange, the Flemish government funded the Pig Information Counter in 2012.

The mission of the Pig Information Counter is to enhance sustainable pig production by providing farmers with up-to-date vulgarized research results and practical information. As the access point of the Pig Research Network, the core business is answering practical questions of farmers in an understandable objective way. To date, 357 written advices have been given based on (inter)national applied and fundamental research experience and practical experience within the Pig Research Network. All areas of pig farming are covered: farm management (27%), feed (13%) and drinking water (3%) strategy, housing (13%), manure/environment (11%), hobby pig husbandry (10%), animal health (5%), biosecurity (4%), reproduction (4%), indoor climate (3%), organic pig husbandry (3%), slaughter quality (2%) and animal welfare (2%). Research results, best practices, calculation tools and advices are communicated through [www.varkensloket.be](http://www.varkensloket.be), newsletters, press articles, symposia and workshops. To stimulate knowledge exchange on practical farming approaches, more focus will be put on peer to peer learning through farmers' network groups and visual material.

To capture needs and innovative signals of farmers, these need to be translated to appropriate research questions. For this aim, a digital questionnaire was sent to approximately 3500 Flemish pig farmers to inventory and prioritize their research topics and needs. Major concerns from Flemish pig farmers towards the research community will be presented on the symposium.



## IMMUNOLOGY & VACCINOLOGY

Friday, 5 May 2017, 10:30 – 12:30

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- IMM-OP-01 CONSERVED HA-PEPTIDES AS MULTIVALENT VACCINE AGAINST INFLUENZA VIRUSES**  
*Marta Sisteré (Spain)*
- IMM-OP-02 STUDY OF THE EFFICACY OF AN AUTOGENOUS VACCINE DIRECTED AGAINST ARTHRITIS DUE TO TRUEPERELLA PYOGENES**  
*Dominique Marchand (France)*
- IMM-OP-03 DIFFERENTIATION OF PORCINE POST-INFECTION AND POST-VACCINATION ANTIBODIES AFTER SALMONELLA TYPHIMURIUM INFECTION AND VACCINATION WITH SALMONELLA TYPHIMURIUM-BASED INACTIVATED VACCINE**  
*Jan Gebauer (Czech Republic)*
- IMM-OP-04 STRATEGIC PIGLET VACCINATION AS A TOOL TO ELIMINATE PRRS VIRUS RECIRCULATION IN A COMMERCIAL NURSERY**  
*Marta Jiménez (Spain)*
- IMM-OP-05 VACCINATION AGAINST POST-WEANING DIARRHOEA WITH COLIPROTEC® F4: PRODUCTION RESULTS FROM A LARGE SCALE FARM IN SPAIN.**  
*Pedro Sánchez Uribe (Spain)*
- IMM-OP-06 GOOD VACCINATION PRACTICES: IT ALL STARTS WITH GOOD REFRIGERATOR TEMPERATURE**  
*Frédéric Vangroenweghe (Belgium)*



## IMM-OP-01 - CONSERVED HA-PEPTIDES AS MULTIVALENT VACCINE AGAINST INFLUENZA VIRUSES

**M. Sisteré-Oró<sup>1</sup>, J. Vergara-Alert<sup>1</sup>, J. G. Zabala<sup>1</sup>, S. Pina-Pedrero<sup>1</sup>, L. Córdoba<sup>1</sup>, S. Martínez-Pulgarín<sup>2</sup>, J. M. Escribano<sup>2</sup>, A. Darji<sup>1</sup>**

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<sup>2</sup> Alternative Gene Expression S.L. ALGENEX, Centro empresarial – Parque Científico y Tecnológico Universidad Politécnica de Madrid Campus de Montegancedo, Pozuelo de Alarcón, Spain

### Background and Objectives

Influenza A viruses, the causal agents of swine influenza disease, are not only transcendental to control for the economic losses in the swine industry, but also are zoonotic or even pandemic pathogens. Vaccination is one of the most important and effective strategies to control and prevent Influenza infection. Current human vaccines against Influenza induce strain-specific immunity and must be updated annually. Our group has previously reported the use of conserved hemagglutinin peptides (HA-peptides) of Influenza virus as potential multivalent vaccine candidates. Immunization with these peptides elicited antibodies that recognize and neutralize heterologous Influenza viruses' *in vitro* and demonstrated strong hemagglutination-inhibiting activity.

### Material and Methods

Conserved immunogenic HA-peptides were selected and reverse-translated to DNA and combined with two different adjuvants that were either interfering with innate (flagellin) or adaptive immune response (CTLA-4). One HA-peptide (ng34) was cloned into the pCMV-IgG2a-ct14a plasmid. Another HA-peptide with flagellin was cloned into a pcDNA3.1(+) plasmid. For immunization and challenge studies, animals were divided into three groups (one infection control group immunized with saline solution and the other two with the DNA formulations). A needle-free IDAL (Intra Dermal Liquid Application) vaccination procedure was applied twice with a 3-week interval and was followed by an intranasal challenge with two swine Influenza virus subtypes (H1N1 and H3N2). Nasal swabs, sera and peripheral blood mononuclear cells were collected for analysis at defined time points.

### Results

A complete elimination or significant reduction of the viral shedding was observed within the first week after the challenge for both viruses. Serology and analysis of cellular immune response elicited by vaccination will further be discussed.

### Discussion and Conclusion

We assume these HA-peptides could potentially be used as multivalent vaccine against Influenza viruses.

*Acknowledgements: This study was funded by a doctoral fellowship from the Ministry of Economy, Industry and Competitiveness (AGL2013-48-923-C2-2-R).*



## IMM-OP-02 - STUDY OF THE EFFICACY OF AN AUTOGENOUS VACCINE DIRECTED AGAINST ARTHRITIS DUE TO TRUEPERELLA PYOGENES

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<sup>4</sup> Ecole Nationale Vétérinaire d'Alfort, 94, Maisons-Alfort Cedex, France

### Objectives

*Trueperella pyogenes* (Tp) is an ubiquitous pathogen causing purulent infections in pigs. Arthritis due to Tp is an increasing epidemiological and economical problem in some pig farms in France. The clinical signs are characterized by peri and intra-articular abscesses, which can be observed concomitantly in several joints and at different physiological stages. Antimicrobial treatments are often ineffective. Administering an autogenous vaccine in sows in order to reduce contamination to their offspring and to protect them with the maternally-derived antibodies is an option developed in this study.

### Materials & Methods

The study was conducted during the second semester of 2015 in a 500-sow farrow-to-finish herd with a four week management and where arthritis in piglets from which Tp had been isolated several times was observed during the entire weaning period. The incidence of arthritis before the beginning of the vaccination was up to 6.83% despite an individual injectable antibiotic treatment at 3 days of age and an oral 5 day collective treatment at weaning. An autogenous vaccine including the strain isolated in the lesions was administered to 4 batches of sows, which represented 440 sows and 5764 piglets. The sows were vaccinated intramuscularly 6 and 3 weeks before farrowing at a dose of 5 mL. Clinical signs were recorded and each case of arthritis described in detail: localization, treatments.

### Results

After vaccination, the farmer stopped metaphylactic[MY1] treatments and administered injectable antimicrobials only in case of finding swelling joints. The prevalence of arthritis progressively decreased from 5.67% in the first vaccinated batch to 3.33% in the last vaccinated batch.

### Conclusion

This study shows that in the case of arthritis due to Tp, autogenous vaccines could be a good option to reduce antimicrobial consumption. Other factors may concomitantly contribute to the persistence of arthritis in the farm.



## IMM-OP-03 - DIFFERENTIATION OF PORCINE POST-INFECTION AND POST-VACCINATION ANTIBODIES AFTER SALMONELLA TYPHIMURIUM INFECTION AND VACCINATION WITH SALMONELLA TYPHIMURIUM-BASED INACTIVATED VACCINE

**J. Gebauer<sup>1</sup>, H. Kudlackova<sup>1</sup>, R. Tesarik<sup>1</sup>, M. Faldyna<sup>1</sup>, J. Matiasovic<sup>1</sup>**

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### Background & Objectives

Anti-pathogen antibodies detected in animal's blood make this animal considered as recently or currently infected with this pathogen. Vaccination is one of the tools to decrease the burden of animals by many pathogens. However, the vaccine should also enable us to differentiate between vaccinated and naturally infected animals - so-called DIVA vaccine. We developed an inactivated *Salmonella* Typhimurium-based vaccine that is efficient for suckling piglets when the sows had been vaccinated. Together with the vaccine, we developed a procedure for finding *Salmonella* antigens that would be useful for serological differentiation of vaccinated from infected pigs.

### Material & Methods

We took advantage of that *Salmonella* express slightly different proteins during infection of host and when it is cultivated in a growth medium for a vaccine batch. Antibodies produced after infection or vaccination were analysed using immunoaffinity chromatography and IgG-bound *Salmonella* proteins were identified by LC-MS/MS. Candidate proteins were prepared as recombinant proteins and then used in western blot and ELISA assays to test the specificity of post-infection and post-vaccination antibodies.

### Results

Proteins BamB, OppA and FliC showed the higher intensity of reaction with serum from vaccinated animals. On the other hand, proteins SipB, SipD and SseB were confirmed as post-infection antigens as they were found to bind post-infection antibodies only. Subsequently, we proved specificity and measured the level of antibody response against SipB and FliC proteins using ELISA with individual porcine sera.

### Discussion & Conclusion

We proved that *Salmonella* proteins differentially expressed during infection and cultivation can be used for differentiating infected and vaccinated animals when used as antigens in western blot and ELISA. This DIVA approach could facilitate the use of a vaccine that is prepared without any intervention into the genome of a vaccine strain.

This work was supported by funding programs QJ1210115 and LO1218.





## IMM-OP-04 - STRATEGIC PIGLET VACCINATION AS A TOOL TO ELIMINATE PRRS VIRUS RECIRCULATION IN A COMMERCIAL NURSERY

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<sup>4</sup> MSD Animal Health, Swine Technical Service, Madrid, Spain

### Background & Objectives

The efficacy of MLV PRRS vaccines in controlling clinical signs related to PRRS has been widely demonstrated. Nevertheless PRRS vaccination also has epidemiological properties, like reduction of shedding and viral transmission. The objective of this trial was to evaluate the efficacy of Strategic Piglet Vaccination to control PRRS circulation in a commercial nursery.

### Materials & Methods

The trial was done in a multisite farrow (400 sows) to finish farm (site 1+2) that works in a 4 week batch system. The farm was PRRS positive and classified as stable. From January to March 2016, a progressive increase in mortality and growth retardation was detected in the nursery. PRRS infection was confirmed by positive PCR and serology of diseased animals of 6 weeks of age. PCR of 40 pre-weaning piglets was done to confirm absence of PRRS circulation in sows. As depopulation of the nursery was not feasible, Strategic Piglet Vaccination was implemented by vaccinating 4 consecutive batches of 14 day old piglets with Porcilis® PRRS IDAL.

### Results

After Strategic Vaccination period, non-vaccinated 8 week old piglets were serologically and PCR negative for PRRSv, and this situation was maintained at least the following 5 batches that were tested, confirming that PRRSv was not circulating in the nursery. Mortality rate was reduced from 4.4% in the pre-vaccination batches to 2.8% in the vaccinated and 1.8% in the post-vaccination batches ( $p < 0,001$ ). Considering only the reduction in mortality, the strategic piglet vaccination investment paid off after the improved mortality results in the first 4.5 batches.

### Discussion & Conclusion

Strategic piglet vaccination is an effective and profitable tool to control viral circulation in the nursery and is therefore a valid and simple alternative to a sanitary depopulation of the facilities.



## IMM-OP-05 - VACCINATION AGAINST POST-WEANING DIARRHOEA WITH COLIPROTEC® F4: PRODUCTION RESULTS FROM A LARGE SCALE FARM IN SPAIN

**P. Sánchez Uribe<sup>1</sup>, Á. Lamrani<sup>1</sup>, P. Núñez<sup>1</sup>, Á. Hidalgo<sup>2</sup>, J. Martínez<sup>3</sup>, J.E. Oliva<sup>3</sup>, G. Márquez<sup>3</sup>, É. Nadeau<sup>4</sup>**

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Post-weaning diarrhoea (PWD) is mainly caused by enterotoxigenic *Escherichia coli* (ETEC), with F4-ETEC and F18-ETEC being the most prevalent in Europe (Luppi et al., 2016). Coliprotec® F4 is a live non-pathogenic *E. coli* vaccine for active immunization of pigs against PWD caused by F4-ETEC. This study investigates changes in production parameters after vaccination with Coliprotec® F4 in a large commercial pig farm in Spain.

A nursery farm (weaning to 25 kg) with a recent history of F4-ETEC PWD was selected. Before the introduction of Coliprotec® F4 on the farm, PWD control involved colistin and zinc oxide (ZnO, 3100 ppm/ 14 d) in feed. A total of 15384 piglets from 3 consecutive batches were vaccinated with Coliprotec® F4 in drinking water 1 day after entering the nursery (CPF4 group). ZnO (1500 ppm/ 14 d) was used in the CPF4 group but no specific antibiotic medication against *E. coli* was used. Mortality, average daily weight gain (ADWG), medication costs (MC), Kg of feed/piglet and feed conversion ratio (FCR) were recorded for the CPF4 group and results were compared with the 3 previous batches of pigs (Control group, n=15534).

Mortality was lower in the CPF4 group (1.9 %) than in the control group (2.2%). ADWG was similar in both groups (CPF4=0.354; Control= 0.350). However, less feed was needed in the CPF4 than in the control group (28.94 and 30.05 kg feed /piglet, respectively), resulting in an improved FCR for the CPF4 group (CPF4= 1.531; Control= 1.590). Finally, vaccination reduced MC by 1.06 € (CPF4= 0.52 €; Control= 1.58 €).

Under the conditions of this study, Coliprotec® F4 controlled F4-ETEC PWD in a cost effective way. Pigs vaccinated with Coliprotec® F4 presented an improved FCR and mortality rate, with lower medication costs, when compared to a previous control strategy based on medication and high inclusion of ZnO.



## IMM-OP-06 - GOOD VACCINATION PRACTICES: IT ALL STARTS WITH GOOD REFRIGERATOR TEMPERATURE

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### Background & objectives

Due to the forced reduction in antibiotic use, a major increase in the use of vaccinations against most currently present swine pathogens, such as *M. hyopneumoniae*, PRRSv, SIV, *H. parasuis*, *A. pleuropneumoniae* and PCV-2, has been observed. Based on a survey held in 2014 and practical on-farm data collection, many farmers and farm workers still do not realize the strong impact of wrong refrigerator temperatures on the quality and efficacy of vaccines. The aim of the current study was to obtain data on the real in-refrigerator temperatures by direct measurement of in-refrigerator temperatures.

### Materials & Methods

Data of in-refrigerator temperatures of over 100 farms have been collected using a digital thermometer. The refrigerator sampled was the main refrigerator designated by the farmer to stock the main supply of vaccines on the farm. Data were collected after at least 45 min of temperature measurement using the temperature probe of the digital thermometer.

### Results

Analysis shows only 58% of all refrigerators monitored have a well equilibrated temperature range (+2°C to +8°C) suitable for guaranteed preservation of stored vaccines. No significant differences were observed in refrigerator temperature between Belgium and The Netherlands. Most extreme situation has been observed with a refrigerator freezing the vaccine vials down to -4°C, resulting in frozen content and subsequent loss of efficacy of the stored vaccine.

### Discussion & Conclusion

It is clear that the awareness of refrigerator temperature for vaccine storage is low to non-existing. The need for regular training on Good Vaccination Practice seems crucial in order to improve the effectiveness of vaccination protocols. In conclusion, basic knowledge on GVP, and more specifically the continuous monitoring of refrigerator temperatures for vaccine storage has still room for major improvement in order to maximize efficacy of applied vaccines under Belgian and Dutch conditions.



## REPRODUCTION

Friday, 5 May 2017, 10:30 – 12:30

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- REP-OP-01 **PROLONGED FARROWING MAY INCREASE WEIGHT LOSS DURING LACTATION**  
*Olli Peltoniemi (Finland)*
- REP-OP-02 **RELATIONSHIP BETWEEN AGE AT FIRST FARROWING AND LONGEVITY IN SWEDISH SOW HERDS**  
*Lena Eliasson-Selling (Sweden)*
- REP-OP-03 **INFRARED THERMOGRAPHY AND ULTRASOUND AS POSSIBLE TOOLS FOR INVESTIGATING UDDER HEALTH IN SOWS**  
*Alexandra Von Altröck (Germany)*
- REP-OP-04 **USE OF AN ANTI-GNRF VACCINE TO SUPPRESS ESTRUS IN IBERIAN GILTS REARED IN COMMERCIAL EXTENSIVE CONDITIONS IN SPAIN**  
*Antoni Dalmau (Spain)*
- REP-OP-05 **REDUCED FERTILITY IN A BOAR DUE TO MULTICYSTIC DEGENERATION OF THE BULBOURETHRAL GLANDS**  
*Alexander Grahofer (Switzerland)*
- REP-OP-06 **OPTIMIZATION OF CASTRATION UNDER INHALATION ANAESTHESIA WITH ISOFLURANE USING PARENTERAL BUTORPHANOL, MELOXICAM OR INTRATESTICULAR LIDOCAINE IN 7-14 DAYS OLD PIGLETS**  
*Petra Julia Hug (Switzerland)*



## REP-OP-01 - PROLONGED FARROWING MAY INCREASE WEIGHT LOSS DURING LACTATION

**S. Björkman<sup>1</sup>, C. Oliviero<sup>1</sup>, O. Peltoniemi<sup>1</sup>**

<sup>1</sup> University of Helsinki, Department of Production Animal Medicine, Saarentaus, Finland

### Background

Prolonged farrowing has been found to be associated with reduced post-weaning fertility and reduced fertility after lactation is usually associated with high weight loss during lactation. Recently it has been shown that sows with prolonged farrowing show a prolonged reduction of appetite after farrowing and post-farrowing appetite is known to be associated with sow feed intake during the remainder of lactation. Therefore, the hypothesis is that prolonged farrowing duration affects negatively body weight loss during lactation.

### Methods

At day 90 of gestation, the weight of 107 sows was determined with a scale and half of the estimated piglet birth weight and placenta weight per piglet subtracted according to the litter size. At parturition, the farrowing duration (time interval between expulsion of first and last piglet) was determined in 8 batches. At weaning, the weight of the sows was determined with a scale. The effect of parity, weight of the sow at parturition, farrowing duration, and lactation length were analyzed through a general linear model and sow and batch were included as random factors (PASW Statistics v.18.0.0) and weight loss (%; = weight at weaning – weight at farrowing) as outcome.

### Results

The sows had a parity of  $3.8 \pm 1.0$  and farrowed for  $401 \pm 257$  min. The lactation length was  $30 \pm 4$  days. Sows lost  $3.3 \pm 6.9$  % of their body weight between farrowing ( $275 \pm 29$  kg) and weaning ( $265 \pm 30$  kg). The farrowing duration ( $P = 0.051$ ) and lactation length ( $P = 0.058$ ) had positive correlations with weight loss.

### Discussion

Besides lactation length, farrowing duration seems to negatively affect subsequent weight loss and that, in turn, could explain the reduced subsequent fertility. Prolonged farrowing may deteriorate the catabolic state and/or feed intake of the sow and therefore increase weight loss during lactation.



## REP-OP-02 - RELATIONSHIP BETWEEN AGE AT FIRST FARROWING AND LONGEVITY IN SWEDISH SOW HERDS

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<sup>1</sup> *Farm&Animal Health, Uppsala, Sweden*

<sup>2</sup> *Swedish University of Agricultural Sciences, Uppsala, Sweden*

### Introduction

Sow longevity is a key component for profitable pig farming. However annual sow culling is on average 50%. Poor sow longevity has detrimental influence on economic efficiency, animal welfare and infectious diseases.

One central question is: how replacement gilt should be raised, which characteristics can be used as predictors for a productive life. The aims of this study were to analyze the influence of age at first farrowing on stayability and reason for culling.

### Material and Methods

Longevity was studied in 8 Swedish sow herds (Landrace\*Yorkshire crosses). Data from the herd monitoring program (PigWin) was used, and the analyses were restricted to sows having had their first litter in 2013 – 2014 (study 1), or that were culled 2013-2015 (study 2).

The sows were for the analyses grouped according to age at first farrowing into four 'almost equal sized' groups: (A: 300-345 days; B: 346-369 days; C: 370-399 days; D: 401-500 days).

### Results

Study 1 comprised information on 12171 and study 2 on 17768 sows.

The proportion of gilts that farrowed a 3<sup>rd</sup> litter in study 1, were for the four groups: A: 74%; B: 70%; C: 68%; D: 62%.

The proportion of gilts farrowing 7<sup>th</sup> litters before culling in study 2, were for the four groups A: 20%; B: 14%; C: 15%; D: 16%. No significant differences were found for culling reason inferior reproduction and leg problems. However young sows had less udder problem (A: 21%) than elder sows (B: 26%, C: 6%, D: 24%).

### Conclusions

This study shows that age at first farrowing to some degree can be used as a predictor for the productive life of the gilt. Sows that get pregnant at a younger age seem to stay longer in the herd. Of course, this requires that the gilts are in a good body condition to be able to show their reproductive capacity.





## REP-OP-03 - INFRARED THERMOGRAPHY AND ULTRASOUND AS POSSIBLE TOOLS FOR INVESTIGATING UDDER HEALTH IN SOWS

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<sup>2</sup> University of Veterinary Medicine Hannover, Institute for General Radiology and Medical Physics, Hannover, Germany

### Background & Objectives

Udder diseases in sows usually result in reduced milk production, and are directly related to survival and growth of the piglets. In view of animal welfare and economical aspects early diagnosis of udder changes is most important. Ultrasound and infrared thermography are potential noninvasive tools to monitor the udder health status of sows. Both methods were used to assess udder health in sows at time of weaning.

### Material & Methods

Ultrasound and thermographic examinations were performed in 107 sows on three different farms after clinical investigation including visual and palpatory examination of the udder. Thermal images were taken from both sides of the sows using a high resolution inspect infrared thermographic camera (VarioCAM®, Infratec GmbH, Germany), positioned 150 cm off the lateral skin surface at the level of the mammary complexes picturing all mammary glands. Ultrasound scans were done with a Fazone CB (Fujifilm, Physis GmbH, Germany) equipped with a 5 to 10 MHz linear transducer, applied cranial, caudal and at both sides of the teats of each mammary complex.

### Results

Nine sows showed nodular structures in the gland parenchyma by inspection and palpation. These chronic alterations were also detected by ultrasound. Additionally, two more sows were recognized with further nodes only by ultrasonography. Using thermography, these alterations could be illustrated by measuring a decrease in the average surface temperature of this area of  $1.24 \text{ }^{\circ}\text{C} \pm 0.69$  compared to the total skin area of the altered complex.

### Discussion & Conclusion

Although ultrasound examination of the mammary glands of sows is time consuming, it is well suited to diagnose chronic alterations. Those alterations can also be illustrated by thermal images. But using infrared thermography as diagnostic tool, factors influencing the surface temperature of the skin like the environmental temperature, moisture, dirt, scratches or scabs must be considered.



## REP-OP-04 - USE OF AN ANTI-GNRF VACCINE TO SUPPRESS ESTRUS IN IBERIAN GILTS REARED IN COMMERCIAL EXTENSIVE CONDITIONS IN SPAIN

**A. Pall Dalmáu<sup>1</sup>, J. Pallisera<sup>2</sup>, V. Rodriguez<sup>3</sup>, J. Hernandez<sup>3</sup>, A. Romero<sup>3</sup>, M.A. Sierra<sup>3</sup>, A. Velarde<sup>2</sup>, Isera**

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<sup>2</sup> Irtá, Monells, Spain

<sup>3</sup> Zoetis, Madrid, Spain

### Introduction

The aim of the study was to test the use of an anti-GnRF (gonadotropin-releasing factor) vaccine (Vacsincel<sup>®</sup>, Zoetis) in commercial conditions, as a tool to prevent oestrus in Iberian gilts reared in extensive conditions, avoiding attracting wild boars and mating in vast and difficult to control farms.

### Materials and Methods

Thirty Iberian gilts maintained in extensive conditions were enrolled in the study and randomly allocated to two treatment groups. At 18, 22, 34 and 46 weeks of age, 15 gilts were vaccinated subcutaneously with 2 mL of Vacsincel, and the remaining 15 gilts received 2 mL of saline solution and remained as control group. Efficacy was assessed by measuring anti-GnRF antibody titres, progesterone levels and estradiol levels at regular intervals throughout the study, and also by comparing the size of reproductive organs between groups at the end of the study, after slaughtering all animals them at 60 weeks of age.

### Results

Negative control gilts reached sexual maturity and vaccinated gilts failed to mature due to GnRF inhibition induced by the vaccine. Examination of reproductive organs at the end of the study showed that uterine weight and length were significantly greater in control females than in vaccinated gilts, 712 g vs 69 g, respectively ( $p < 0.0001$ ) and 2889 mm vs 615 mm, respectively ( $p < 0.0001$ ). Similar results were found in ovarian weight and activity. Additionally, two gilts in the control group were pregnant at slaughter, due to uncontrolled mating of wild boar.

### Discussion and Conclusions

In conclusion, the results of this field study confirm that vaccination with Vacsincel is effective in preventing oestrus in Iberian gilts reared in big extensive areas, avoiding the threat posed by surrounding wild boars being attracted for mating sexually active and cycling gilts. This vaccine is a great management tool to prevent the associated risks.



## REP-OP-05 - REDUCED FERTILITY IN A BOAR DUE TO MULTICYSTIC DEGENERATION OF THE BULBOURETHRAL GLANDS

**A. Grahofer<sup>1</sup>, H. Nathues<sup>1</sup>, C. Gurtner<sup>2</sup>**

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<sup>2</sup> Vetsuisse Faculty- University of Bern- Switzerland, Institute of Animal Pathology- Department of Infectious Diseases and Pathobiology, Bern, Switzerland

### Introduction

Reduced fertility in boars can be attributed to many different factors, such as environmental impacts, loss of libido and anatomical problems of the reproductive tract. Therefore, a thorough history taking and a detailed clinical examination are necessary to identify the cause of any reduced fertility.

A spectrum of congenital and acquired anomalies of the bulbourethral gland has been reported in farm animals and often cause reduced fertility.

### Case Description

The present report describes a case of multicystic degeneration of the bulbourethral gland in a 1.3 year old purebred intact Large White boar with reduced fertility. General physical examination, followed by an andrological investigation and ultrasound was performed. The physical examination revealed no abnormalities apart from findings in the genitals, where several pathological conditions were found. The tissue of the testis was softer than normal. The size of the paired bulbourethral gland was slightly increased, the texture was rough, and the manual palpation was painful for the pig. A transrectal ultrasonography was conducted and multiple cysts in the bulbourethral gland filled with fluid were seen. For further diagnostics, a pathological examination including histopathology of the gland was performed. On gross examination there were multiple cysts filled with mucous within the glandular tissue. In the histopathological examination the epithelium of the cysts of the endpieces and excretory ducts of the glands were flattened and the cysts were filled with a large amount of basophilic mucous compressing the surrounding tissue.

### Conclusion

The case indicates that cystic degeneration of the bulbourethral gland should be contemplated in the differential diagnoses of andrological disorders even though it has not been described in pigs so far. While selecting breeding boars, a morphological check of the bulbourethral gland could be performed, since degeneration of the gland potentially would have an impact on future fertility.



## REP-OP-06 - OPTIMIZATION OF CASTRATION UNDER INHALATION ANAESTHESIA WITH ISOFLURANE USING PARENTERAL BUTORPHANOL, MELOXICAM OR INTRATESTICULAR LIDOCAINE IN 7-14 DAYS OLD PIGLETS

**P.J. Hug<sup>1</sup>, V.H. Čáp<sup>1</sup>, J. Honegger<sup>2</sup>, A. Schwarz<sup>1</sup>, G. Schüpbach-Regula<sup>3</sup>, R. Bettschart-Wolfensberger<sup>1</sup>**

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This blinded prospective study investigated the analgesic effects of parenteral butorphanol, meloxicam or intratesticular (IT) lidocaine for the castration of 7-14 days old piglets under isoflurane anaesthesia. 66 piglets were randomly allocated in 5 groups: meloxicam (0.4mg kg<sup>-1</sup> intramuscular (IM); group M), butorphanol (0.2mg kg<sup>-1</sup> IM; group B), or both (group BM) 20 minutes prior to castration, or lidocaine (4mg kg<sup>-1</sup> or 8mg kg<sup>-1</sup>; group ML4 or ML8) IT together with meloxicam (0.4mg kg<sup>-1</sup> IM) immediately before castration. Castration was performed under inhalation anaesthesia, with end-tidal Isoflurane 1.8%. Heart rate (HR), respiratory rate (RR), blood pressure (MAP), end-tidal carbon dioxide (EtCO<sub>2</sub>), intensity and number of movements, amount of bleeding after castration and time until recovery were recorded.

Piglets receiving butorphanol exhibited dyspnoea and excitation. Therefore, butorphanol use was stopped. Group M showed a significantly higher incidence of movements (11/17) than group ML4 (3/18) and ML8 (4/17). Additionally, the number and the intensity of movements in group M were significantly higher than in groups ML4 and ML8. There was no significant difference between the groups regarding RR, HR or MAP during castration. RR increased significantly over time in groups M and ML4 in all the different steps of castration. In group ML8, a significant increase in RR could only be detected between baseline and cut as well as between baseline and exteriorization of the testicle. HR decreased significantly over time in all groups. No significant difference was found between the groups regarding time until recovery or amount of postoperative bleeding. No adverse or toxic effects were observed in both groups treated with lidocaine.

For castration in 7-14 days old piglets under inhalation anaesthesia with isoflurane, IT lidocaine 4mg kg<sup>-1</sup> or 8mg kg<sup>-1</sup> provides side effect free additional analgesia and improves animal welfare compared to meloxicam alone.



# Poster Presentations



<b>Bacteriology and Bacterial Diseases</b>	BBD-001	BBD-083
<b>Herd Health Management and Economy</b>	HME-001	HME-055
<b>Miscellaneous</b>	MIS-001	MIS-017
<b>Parasitology and Parasitic Diseases</b>	PPD-001	
<b>Reproduction</b>	REP-001	REP-017
<b>Residents' ECPHM Session</b>	RES-001	RES-005
<b>Vaccinology &amp; Immunology</b>	VAC-001	VAC-057
<b>Veterinary Public Health &amp; Food Safety</b>	VPH-001	VPH-010
<b>Virology and Viral Diseases</b>	VVD-001	VVD-060
<b>Welfare &amp; Nutrition</b>	WEL-001	WEL-042

The pdf files of the poster presentations are available on [www.eaphm.org](http://www.eaphm.org)





## Welfare & Nutrition

### WEL-001 - OVERVIEW OF A BIBLIOGRAPHIC AWARENESS SERVICE ON SCIENTIFIC PUBLICATIONS ON ALTERNATIVES TO ANTIBIOTICS IN SWINE PRODUCTION OVER 1.5 YEAR

**V. Dedeř<sup>1</sup>**

<sup>1</sup> *Auzalide Santé Animale, Auzalide Santé Animale, Paimpont, France*

#### **Introduction/Aim**

Since the implementation of national programmes aiming at reducing antimicrobial usage in animal production, a large number of products have been presented as partial or complete alternatives to the preventative usage of antibiotics – mostly towards enteric conditions. Some of these also claim an oxidative modulation effect, other an immune modulation effect... Our aim was to identify the different categories of products whose trials undergo peer-reviewed publication.

#### **Methods**

Scientific publications reporting on trials using such products have emerged, and reached a substantial number. A bibliographic awareness service has been implemented in July 2015. It is performed through the weekly consultation of over 400 websites of scientific journals in relation with biology, animal production and/or veterinary medicine.

#### **Results and Conclusion**

this work allowed to distinguish over 20 categories of products for which trial results have been published. The frequency of such publications is sustained, with more than 2 articles per week, on average. There is a dominance of publications on probiotics, organic acids and plant extracts. Antimicrobial peptides have a lower but significant importance. In about 20% of the publications, the tested product combines compounds belonging to the same or different categories of alternatives. There is, however, a minority of articles that compare their product to an antibiotic as a positive control. Beyond the categories corresponding to products that have already reached the market, new categories/ strategies appear, such as phage therapy or the use of nanoparticles. Hence, it seems that peer-reviewing the results of field/experimental trials is not enough to discriminate between products with alleged ability to be an alternative to antibiotics in swine production.



## WEL-002 - THE POSITIVE EFFECT OF A BUTYRATE BASED SUPPLEMENTATION OF PIGLET FEED ON INTESTINAL INFLAMMATION

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### Introduction

Butyrate has a biological role in the gut, as it is naturally produced by the beneficial intestinal microbiota. The anti-inflammatory effect of butyrate through IL-12 and subsequently Th1 inhibition in the gut is recently well documented in literature.

### Aim

The objective of this trial was to evaluate the effect of a synergistic butyrate based product on intestinal inflammation in weaned piglets through evaluation of a biomarker, the % of TNF $\alpha$  colored cells in intestinal samples.

### Materials and methods

A trial was carried out at PUCPR located in Brazil. 48 weaned piglets (average body weight of 7 kg), were housed in nursery pens during 35 days and divided into 2 groups with 8 replicates: 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 C (standard feed without inclusion of additives) and B (standard feed supplemented with a synergistic butyrate based product at 0,15% in prestarter and 0,075% in starter phase). At 14 and 35 days of evaluation, 8 animals of each group were euthanized to collect samples of duodenum and jejunum for immunohistochemistry by TNF $\alpha$  coloring, a biomarker for intestinal inflammation.

### Results

Animals of group B showed a significant lower amount of TNF $\alpha$  positive cells comparing to group C animals in duodenum at all time points, as well as at day 35 in jejunum. Group B animals had a numerically lower amount of TNF $\alpha$  positive colored cells comparing to group C animals in jejunum at day 14.

### Conclusion

This trial indicates that butyrate based additives could have an anti-inflammatory effect in the gut.



## WEL-003 - EVALUATION OF THE NUTRITIONAL SUPPLEMENT VIUSID VET POWDER ON THE QUALITY OF SEMEN OF BOARS

***J.C. Rodriguez-Fernandez<sup>1</sup>, V. Mendez-Garcia<sup>1</sup>, I. Calero-Herrera<sup>1</sup>, K. Peña-Calzada<sup>2</sup>***

<sup>1</sup> *Universidad de Sancti Spiritus, Medicina Veterinaria, Sancti Spiritus, Cuba*

<sup>2</sup> *Universidad de Sancti Spiritus, Agronomia, Sancti Spiritus, Cuba*

### Background & Objectives

During the semen collection regime, the boar has different nutritional demands than growing and fattening pigs. Maintaining spermatogenesis requires an input of energy and protein on different levels, as well as macro and micro elements that may be limiting. The objective of this research was to evaluate the effect of administering VIUSID vet powder on the quality of semen of boars.

### Material & Methods

A totally randomized group comparison design was used for the experiment. Two groups were formed, each with 12 boars of similar age, genotype and spermatic quality. The clinical trial lasted 98 days. The treatment consisted in administering 10 grams of VIUSID vet powder with feed to each animal, once a day for 6 weeks (week 1 to 6); from week 7 onwards the supplement was suspended. The other group was used as control. Evaluations were carried out at three points: at the beginning of the experiment (week 1), 8 weeks after the start of the experiment (week 9) and in the last week of the experiment (week 14).

### Results

Only the variable Spermatic motility differed statistically ( $p = 0.002$ ), which was higher in treated boars by 4.5%, showing similar values in the samples from treated animals, evidenced by the coefficient of variation obtained (4.44%). The experimental error was low in all the variables, which confirms high accuracy in measurements and observations made. Normal sperm (%), Pathological sperm (%) and Colour and odour of semen did not differ either, and were within the range considered normal in Cuba

### Discussion & Conclusion

VIUSID vet powder, used as a nutritional supplement in boars in production for 6 weeks has improved spermatic motility and does not affect the other variables representative of the quality of the semen.



## WEL-004 - EVALUATION OF THE NUTRITIONAL SUPPLEMENT VIUSID VET POWDER ON THE PRODUCTIVE BEHAVIOUR OF SOWS

***J.C. Rodriguez-Fernandez<sup>1</sup>, V. Mendez-Garcia<sup>1</sup>, I. Calero-Herrera<sup>1</sup>, K. Peña-Calzada<sup>2</sup>***

<sup>1</sup> *Universidad de Sancti Spiritus, Medicina Veterinaria, Sancti Spiritus, Cuba*

<sup>2</sup> *Universidad de Sancti Spiritus, Agronomía, Sancti Spiritus, Cuba*

### Background & Objectives

The sows' feed has to be supplemented in the last third of gestation, especially the protein and vitamin-mineral content, which positively influences the survival rate of the piglets in the first days of life due to their higher farrowing weight. The objective of this research was to evaluate the effect of administering VIUSID vet powder on the productive behaviour of sows

### Material & Methods

A totally randomized group comparison design was used for the experiment. Two homogeneous groups of gestated sows (between second and fifth farrowing) were formed, with 35 sows in the control group and 37 in the treated group. Each sow daily received 10 grams of VIUSID vet powder, mixed with food, from 21 days before the probable date of farrowing and until weaning (33-day lactation period).

### Results

The group in which the sow received VIUSID from 21 days before the probable date of farrowing, showed a significant reduction ( $p < 0.05$ ) of diarrhoea in piglets (7.58% less) and the treatment had a significant influence ( $p < 0.05$ ) on the variables: size of the litter upon weaning, weight of the litter at farrowing, weight of the litter at weaning and increase in weight. The piglets born alive through parturition in the treated group differed significantly from the control group (10.62 vs. 9.91). The weaning-fecundation interval and body condition was not affected with regards to the control, despite the treated sows having more numerous and heavier litters.

### Discussion & Conclusion

VIUSID vet powder, used as a nutritional supplement in sows from 21 days before the probable date of farrowing until weaning, significantly improved the principal post-partum productivity indicators and significantly reduced diarrhoea.



## WEL-005 - IN VITRO STUDY OF SYNERGETIC EFFECTS BETWEEN TETRACYCLIN AND AN ESSENTIAL OIL BLEND AGAINST A PASTEURELLA MULTOCIDA RESISTANT STRAIN

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Antibioresistance is a key challenge. Antibiotics and phytochemicals have synergistic effects against several bacteria, but there are few *in vitro* tests against resistant strains. The aim of this study is to evaluate the synergistic effect of tetracycline and an essential oil mix (EO mix), against a resistant strain of *Pasteurella multocida*. Firstly, this work compares the minimal inhibition concentrations (MIC) of tetracycline and the growth inhibition concentration (GIC) of the EO mix. Both are determined against the resistant strain or the sensitive strain of *P. multocida* using CLSI 2009 guideline recommendations. MIC is defined as the lowest concentration of the antibiotic where there is no bacterial growth visible to the eye at 37°C during 18h. EO mix GIC is determined under the same conditions. Secondly, three two-fold serial dilutions of antibiotic are performed with a constant EO mix concentration (GIC, GIC/2, and GIC/4). Tetracycline concentration varies from MIC calculated against the resistant strain ("resistant" MIC) to MIC calculated against the sensitive strain ("sensitive" MIC). The aim is to measure GIC of the "tetracycline and EO mix" association against the resistant strain of *P. multocida*. A synergistic effect is observed when "tetracycline and EO mix" GIC is lower than tetracycline "resistant" MIC. This study shows that EO mix GIC is equal for sensitive or resistant strains, suggesting that the EO mix mechanism of action is different from the antibiotic one. A synergistic effect is observed with EO mix at GIC/2 or GIC/4. In both cases, "tetracycline and EO mix" GIC is 2.82 mg/mL when tetracycline "resistant" MIC is 22.6 mg/mL. This *in vitro* study proves that the sensitivity of resistant *P. multocida* strain against tetracycline can be reduced by adding EO mix sub-GIC. Further works could investigate exact EO mix mode of action against resistant *P. multocida*.



## WEL-006 - EVALUATION OF DIFFERENT DOSE RATE COMBINATIONS OF KETAMINE, ROMIFIDINE AND AZAPERONE FOR CASTRATION OF 3-4 AND 5-6 WEEKS OLD PIGS

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The aim of this study was to establish a satisfactory intramuscularly injectable anaesthesia protocol for castration of 3-4 (G3-4) and 5-6 weeks (G5-6) old pigs.

Based on previous studies and the known effects of ketamine, azaperone and romifidine a dose rate decision tree was elaborated that determined for each undesirable anaesthesia stage a dose optimization protocol including maximal dose rates. According to it, different dose rate combinations of ketamine, romifidine and azaperone were applied intramuscularly and tested. The aim was to determine of each tested drug the optimal dose to provide excitement-free anaesthesia induction, reaction-free anaesthesia for castration as well as smooth and complete recovery within two hours. When a combination failed to meet those criteria in two pigs the next dose was investigated according to the dose rate decision tree. The trial was started with the combination of 10 mg/kg ketamine, 0.15 mg/kg romifidine and 3 mg/kg azaperone in both groups. In G3-4 four combinations were tested in 14 piglets. Induction was smooth in all pigs, but either anaesthesia quality was insufficient (eleven pigs) or recovery was stormy (four pigs) or prolonged (two pigs). In G5-6 five combinations were tested in 37 pigs with dose rates of 3-4 mg/kg azaperone, 10-20 mg/kg ketamine, 0.15-0.20 mg/kg romifidine. Induction was smooth in all pigs except of two. Quality of anaesthesia was insufficient in 16 pigs and recovery was stormy in one and prolonged in six animals.

In the present study with 3-4 and 5-6 week old pigs acceptable quality of anaesthesia and recovery could not be achieved despite using very high drug dose rates.





## WEL-007 - EFFECT OF FENBENDAZOLE IN DRINKING WATER ON ASCARIS SUUM IN FINISHING PIGS UNDER FIELD CONDITIONS

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*Ascaris suum* is a globally distributed helminth commonly infecting swine. During its life cycle *Ascaris* migrates from the intestine and back via liver and lungs. In the liver the migration process causes typical lesions, hepatic white spots, which are reason for liver condemnations at slaughter carcass inspection. We investigated if a treatment with 2.5 mg fenbendazole per kg weight of the pig, given in the drinking water supply, one and six weeks after pigs arrived to fattening units would reduce the presence of *Ascaris suum*-infections, improve the average daily weight gain, reduce the liver condemnations with 50% and increase lean meat percentage with 1%. The study included 427 pigs for a group that was given placebo and 420 pigs for a group given fenbendazole from four different farms with a history of liver condemnations  $\geq 15\%$ . The treatment was given for two consecutive days, one and six weeks after the pigs arrived to the fattening unit. Individual faecal samples were collected week one, six and twelve from all pigs and examined for *Ascaris suum* eggs. Pigs were individually weighted on week one and twelve to calculate average daily gain during treatment. Information on liver rejections and lean meat percentage was collected at the slaughterhouse. Fenbendazole prevented new *Ascaris suum* infections ( $P < 0.01$ ) and reduced the liver condemnations with 69.8% ( $P < 0.001$ ). The treatment could not demonstrate an effect on the average daily weight gain or lean meat percentage. However, pigs with *Ascaris suum* eggs in faeces at week six had a lower average daily weight gain of 61.8 grams/day ( $P < 0.05$ ). In conclusion, the proposed treatment may be applied in farms with persisting *Ascaris suum* problems improving the weight gain of the animals shedding eggs in faeces and reducing liver condemnations at slaughter.



## WEL-008 - ANTI-OXIDATIVE EFFECTS OF LIQUORICE EXTRACT, GLYCYRRHIZIC ACID, AND GLYCYRRHETINIC ACID IN AN INTESTINAL PORCINE EPITHELIAL CELL LINE (IPEC-J2)

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Disruption of the equilibrium between the cellular anti-oxidant defense system and the production of reactive oxygen species (ROS) can lead to oxidative stress, responsible for damage of cellular structures, immune system activation, and dysfunction of the gastrointestinal system.

In piglets, oxidative stress, which can be triggered by different factors including social, pathological, or physical events, is considered to reduce overall health state and impair growth performance of the animals. To overcome accompanying problems like substantial economic losses in farm industry, screening for phytochemical substances, which are able to reduce oxidative stress is of particular importance.

Therefore, this *in vitro* study aimed determination of anti-oxidative properties of an extract of the known medicinal plant liquorice (*Glycyrrhiza* sp.) [15 – 60 µg/ml], its ingredient glycyrrhizic acid [2.5 – 10 µg/ml], and the resulting metabolite after hydrolysis, glycyrrhetic acid [2.5 – 10 µg/ml], using an intestinal porcine epithelial cell line (IPEC-J2). The cell-based 2', 7'- dichlorodihydrofluorescein diacetate (DCFH-DA) assay was used to assess cellular ROS and ROS-scavenging potential of the test substances. 24 hours after cell seeding, cells were incubated with DCFH-DA. Subsequently, cells were pre-incubated with ethanolic extracts of the test substances, before oxidative stress was induced by H<sub>2</sub>O<sub>2</sub>. Fluorescence, which is directly proportional to cellular ROS, was measured following a 1 hour incubation period with H<sub>2</sub>O<sub>2</sub>.

Results demonstrated a dose-dependent anti-oxidative effect of liquorice extract (maximum ROS reduction of 40% [at 60 µg/ml] compared to H<sub>2</sub>O<sub>2</sub> stimulated cells). Interestingly, glycyrrhizic acid did not reduce ROS, whereas glycyrrhetic acid even enhanced ROS values (up to 183% compared to H<sub>2</sub>O<sub>2</sub> stimulated cells).

Summarized, results indicated that the anti-oxidative effect of liquorice extract on IPEC-J2 cannot be explained by the commonly known active compounds glycyrrhizic acid or glycyrrhetic acid. Thus, a more detailed evaluation of the active compounds seems mandatory.



## WEL-009 - SUBSTITUTION OF ZINC OXIDE WITH A CLOSTRIDIUM BUTYRICUM PROBIOTIC (MIYA-GOLD® S)

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### Introduction

The field experience substituting zinc oxide in post weaning feed with *Clostridium butyricum* (Miya-Gold® S) in a Danish farm is reported.

### Material and Methods

The case farm produces pigs from 7 to 30 kg sourced from its own closed sow herd in an all-in all-out batch system during a fixed period of 55 days. One batch consists of approximately 1600 pigs and every batch is treated for post weaning diarrhea caused by *E. coli*. Four batches were tested. In the two control batches 2500 ppm zinc oxides was used during the first 14 days post weaning. In the case batches zinc oxide was substituted with 0.8 kg Miya-Gold® S per ton of feed. Consecutive feeds were identically between batches with the exception of one case batch were also benzoic acid 0,5 kg/MT was excluded to challenge the pigs.

### Results

No statistical significant difference was obtained for average daily gain, feed conversion and mortality. The preventive use of zinc oxide in the diet was associated with lower antibiotic use.

### Conclusion

Piglets not receiving preventive zinc oxide in an environment challenged with enteropathogenic *E. coli* need therapeutic antibiotic treatment at a later age. This leads to a numerical difference in antimicrobial use. However substitution of therapeutic zinc oxide at 2500 ppm with Miya-Gold® S is proven to be a cost effective alternative with conservation of feed conversion and daily gain.



## WEL-010 - REDUCTION OF ZINC OXIDE IN POST WEANING DIET BY SUPPLEMENTING CLOSTRIDIUM BUTYRICUM (MIYA-GOLD® S)

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### Introduction

The field experience excluding zinc oxide but supplying the probiotic *Clostridium butyricum* (Miya-Gold® S) in a Danish farm with home mixed feed is reported.

### Material and Methods

The case farm was bacteriological positive for *Salmonella* and used Miya-Gold® S as a control measure. All diets were homemade with wheat and barley as main carbohydrate source. The main protein sources consisted of milk powder, HP300 and soybean. In the first period (3189 piglets) a post weaning diet with 3000 ppm zinc oxide was used for 14 days. In the second period (3400 piglets) zinc oxide was excluded from the feed. Miya-Gold® S was included at 2 kg/mT during both periods. Consecutive feeds were identically between periods.

### Results

A total of 23,6 kg zinc oxide was used during the first period. The average daily gain in the first period was 493 g/day and 505 g/day in the second period. Feed conversion measured in energy decreased from 14,24 kJ/kg gain to 13,5 kJ/kg gain between both periods. Mortality was 2,2 and 3,0% respectively.

### Conclusion

Withdrawal of therapeutic zinc oxide at 3000 ppm during the first 14 days after weaning was applied without significant impact on intestinal health, feed conversion and daily gain. Mortality was slightly elevated caused by polyserositis and respiratory infections. The farm continued production without zinc oxide at 3000 ppm, but including Miya-Gold® S without reduction in digestible protein in the feed or loss of pig performance.



## WEL-011 - VIRTUAL TRAINING FACILITIES FOR ANIMAL WELFARE ASSESSMENT IN DANISH PIGGERIES

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### **Background & Objectives**

Herd visits during education in agriculture are important for training of practical skills. With increasing student uptake, such visits become more time consuming and expensive, hence, more limited. This is unfortunate since assessment of welfare is subjective and difficult to perform in a consistent and uniform fashion in complex environments. Several different professions assess animal welfare in Danish pig herds - farm personnel, pig advisors, veterinarians and veterinary officers - and so far it has proven very difficult to reach consensus. A possible solution could be to provide a safe, virtual environment to practice such skills in an easy-accessible, interactive setting. Hence, the objective of this research project is to develop, document and test a game-based learning concept where agricultural and veterinary students can visit virtual pig farms, interact with farmers and veterinarians, examine the animals, identify and diagnose welfare problems and suggest appropriate action. The project will increase the general awareness of animal welfare and improve communication and consensus when different professions assess welfare issues.

### **Material & Methods**

Researchers joined control visits along with veterinary officers from the Danish Veterinary and Food Administration (DVFA). Veterinary officers from the DVFA and researchers developed representative cases that are in conflict with legislation and/or welfare.

### **Results**

In the virtual pig farm, the control visit is conducted together with the responsible manager from the farm. Player must examine sows and piglets on the farm and decide, whether legislation is complied with or not. The attitude and behaviour of the manager can vary between game sessions. After game session the level of compliance from other players are presented.

### **Discussion & Conclusion**

The game provides a virtual farm, where players must examine sows and piglets, communicate with farm personal and make decisions based on obtained information whether legislation is complied with or not.



## WEL-012 - FEEDING COFFEE MILK OR LEMONADE TO PREVENT STARVATION SHORTLY AFTER BIRTH DOES NOT INFLUENCE IGG UPTAKE IN NEONATAL PIGS

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### Background & objectives

During the past years a huge increase in litter size has been established that was accompanied by high pre-weaning piglet mortality up to 15%. Farmers try to diminish piglet mortality by supplying liquid products e.g. milk replacer, coffee milk or lemonade to prevent the animals from starvation immediately after birth. It is hypothesized that foreign (non-porcine) proteins could accelerate gut closure and thus inhibit macromolecular transport in the neonatal pig. The objective of this study was to determine if early life oral administration of sugars or a fat/protein mixture is impairing maternal antibody uptake.

### Material & methods

Within 12 hours after birth, piglets were force-fed 5 ml of one of the following liquids: coffee milk (energy/protein; CM), lemonade (energy; L) or water (control; C). Coffee milk and lemonade were given isocaloric. Of each litter (n=20) 12 piglets were selected and assigned to treatment (4 CM; 4L; 4C) equally distributed amongst weight differences. Piglets had ad lib access to the udder. Piglets were bled within 24 to 48 h after birth. Serum IgG was measured using the immunocrit method.

### Results

No differences were found in IgG levels in serum (CM:  $39,8 \pm 12,3$  vs. L:  $38,3 \pm 14,01$  vs. C:  $40,0 \pm 12,9$  mg/ml;  $P > 0,10$ ) or in weaning weight of the piglets (CM:  $7,2 \pm 1,8$  vs. L:  $6,8 \pm 1,6$  vs. C:  $6,7 \pm 1,4$  kg;  $P > 0,10$ ). There was no correlation between treatment and growth until weaning.

### Discussion & conclusion

Feeding neonatal pigs lemonade or coffee milk did not interact with uptake of immunoglobulins from sow's colostrum and will thus not intervene with achievement of sufficient passive immunity. It might decrease the risk to die from starvation by supplying energy to the newborn and thus contribute to the international goal to decrease piglet mortality before weaning.





## WEL-013 - EFFECTS OF LIANOL® FERTI AROUND FARROWING ON BODY CONDITION AND LITTER PERFORMANCE IN LACTATION SOWS

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### Background & Objectives

Previous research demonstrated a positive effect of Lianol®, a complementary feed based on fermented potato protein, on lactation and growth. Objective of this research was to evaluate the effect of feed stuff on sows' body condition and lactation performance.

### Material and Methods

Total 50 crossbred sows were equally divided in a control and treatment group and followed over two consecutive parities. The Lianol group received 10 g Lianol® from 5 days before farrowing to 2 days after. The sows were fed a common die. The amount of feed was gradually increased from ± 3.6 kg to ± 8.4 kg and individual recorded (kg/day).

Back fat thickness was measured via ultrasonic scan to at farrowing (d1) and day before weaning (d24). Litter traits like # born and # born alive, body weight (kg) at birth and weaning, litter gain (kg/litter), and ADG (g/piglets) were recorded.

### Results

The back fat change (in mm) during lactation was significantly improved for the Lianol group over control group with respectively 3.41 vs 3.91 for 1<sup>st</sup> lactation ( $p = 0.069$ ) and 2.38 vs 2.95 for 2<sup>nd</sup> lactation ( $p < 0.05$ ). The average feed intake per sow was numerically affected for the Lianol group respectively 3.6 % and 6.7 % for 1<sup>st</sup> and 2<sup>nd</sup> lactation. On body weight loss, no difference was noticed.

Lianol had no significant effect on the # piglets born, born alive or weaned. In 2<sup>nd</sup> period, birth weight was significantly increased in the Lianol group with 130 g / piglet ( $p=0.024$ ) in both periods. Litter weaning weight was improved in Lianol group with 3.4 kg and 5 kg ( $p<0.001$ ) for respectively 1<sup>st</sup> and 2<sup>nd</sup> period. Piglets average daily gain was also increased significantly.

### Conclusion

Giving Lianol® around farrowing increased sow feed intake what resulted in a better condition, improved litter weight gain and heavier weaned piglets.



## WEL-014 - ASSESSING CALCIUM PHOSPHORUS METABOLISM, USING BONE BIOMARKERS FOR DIAGNOSTIC PURPOSES IN GROWING PIGS

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### Introduction

Evaluating bone metabolism in pigs by determining Calcium (Ca) and Phosphorus (P) in serum is not a very sensitive method. However, measuring bone markers like osteocalcin (OC) and C-telopeptide (CTx) might provide useful information about Ca- P metabolism of bone.

OC is the most abundant non-collagenous protein of the bone matrix. It is synthesized by osteoblasts and partially built in the bone matrix. A proportion is released into the circulation. This circulating OC is a marker of osteoblast activity and indicates the rate of bone formation. Over 90% of the bone matrix consists of type I collagen. During bone regeneration, type I collagen is degraded into small peptide fragments like CTx which are excreted into blood and urine. So CTx is an indicator of bone resorption.

### Method

The laboratory of GD Animal Health uses the N-Mid Osteocalcin ELISA for the quantitative measurement of OC and an adaptation of the Serum CrossLaps ELISA for the quantification of CTx. Serum samples from healthy pigs are used to establish reference values. Besides, samples from well-defined cases of lameness are evaluated.

### Results, conclusion

An inventory in serum samples of pigs at different ages revealed divergent reference values for OC and CTx. In healthy young, growing pigs OC is high (>30 µg/L) and CTx is low (< 0.2 µg/L). In adult pigs OC is much lower (<15 µg/L) and CTx higher (> 0.4 µg/L), indicating the steady state of bone metabolism. In certain cases of lameness in young finisher pigs remarkably low OC values (<20 µg/L) were measured, suggesting poor bone formation (afterwards confirmed with post mortem investigation). After intervention at the farm, improvement of locomotive health was seen and subsequently higher OC levels were found. In conclusion, the use of serum tests for bone markers in pigs can provide useful diagnostic information about Ca-P metabolism and bones.



## WEL-015 - INFLUENCE OF VARIOUS LEVELS OF MILK BY-PRODUCTS ON GROWTH PERFORMANCE, BLOOD PROFILES, INTESTINAL MORPHOLOGY, MUSCLE FIBER IN WEANING PIGS

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This experiment was conducted to evaluate various levels of milk by-products in weaning pig diet on growth performance, blood profiles, intestinal morphology, muscle fiber diameter and incidence of diarrhea on weaning pigs. A total of 160 weaning pigs ([Yorkshire × Landrace] × Duroc), average  $5.97 \pm 1.53$  kg body weight (BW), were allotted to one of four treatments in 5 replications with 8 pigs per pen by BW and sex in a randomized complete block (RCB) design. Pigs were fed each treatment diet with various levels of milk by-product (phase1: 30, 20, 10 or 5%; phase2: 15, 10, 5 or 0%, respectively) for 5 weeks (phase1: 0-2 week, phase2: 3-5 week). Growth performance was lowered linearly as milk by-product level decreased during phase 2 and whole experimental period (linear response,  $P < 0.05$ ). However, pigs fed milk by-product 10%-5% diet showed similar growth performance compared to pigs fed milk by-product 30%-15%. In blood profiles, there was no significant difference in BUN, IGF-1, IgA and IgG among treatments. The villus height and crypt depth of small intestine (proximal, mid and distal small intestine) had no significant difference among dietary treatments. Muscle fiber diameter was also not affected by dietary milk by-product levels. The incidence of diarrhea had no significant difference by dietary treatments. Consequently, inclusion of milk by-product 10%-5% had no negative influence on growth performance and other measurements compared with higher inclusion of milk by-product 30%-15%.



## WEL-016 - EUROPEAN CEREAL AND CORN HARVEST 2016

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### Background

The annual BIOMIN Mycotoxin Survey provides good insights in the threats posed to livestock by mycotoxins worldwide. Especially pigs are very sensitive to mycotoxins, therefore it is of high importance to know where and at what level mycotoxins occur.

### Materials and Methods

813 grain and 305 corn samples from European harvest season 2016 were analysed with ELISA and LC-MS/MS method (Spectrum 380®).

### Results

Wet conditions around the flowering time of cereals in many parts of Europe in 2016 favored the development of *Fusarium* head blight disease. This has contributed to higher prevalence and concentrations for deoxynivalenol (DON) and zearalenone (ZEN) compared to 2015. All European grain samples were analyzed for DON and the majority were also analyzed for ZEN. For the whole of Europe, DON was found in 84% of samples in 2016, compared to 72% reported in 2015. 45% of the samples were contaminated with ZEN compared to 20% in 2015. Cool and wet conditions around silking time in corn can contribute to the development of *Fusarium* species ear rot diseases resulting in production of the mycotoxins DON, ZEN and fumonisins (FUM). All European corn samples were analyzed for DON and most were also analyzed for ZEN and FUM. For the whole of Europe, DON was detected in 73% of samples, FUM in 54% and ZEN in 50% of samples. The presence of DON may increase the risk from both FUM and ZEN through synergistic interaction.

### Discussion and Conclusion

The types, concentration levels and combinations of mycotoxins in the feed, along with animal age and health status, all influence the actual mycotoxin impact. As the latest survey results show, multiple mycotoxins often contaminate swine feed and some of them have known synergistic effects – so even low levels can reduce pig performance and affect pig health.



## WEL-017 - INFLUENCE OF ALFALFA FED TO PIGS (25-45 KG) ON GROWTH PERFORMANCE AND THE COEFFICIENTS OF APPARENT ABSORPTION OF THE FIBRE

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The use of fibres in pig feeding is of real interest because the degradation of the microbial fibre in the cecum and colon generate volatile fatty acids that can supply 5-28% of the energy requirement for the growing pigs. We conducted a 4-week balance experiment on 9 male, castrated, half-brothers, TOPIGS pigs (25 kg live weight), assigned to 3 groups (C, E1 and E2). All three groups received the same basal diet (corn, soybean meal, sunflower meal) with 3200 kcal/kg feed and 17.5% CP/kg feed. The 3 compound feeds differed through the proportion of dietary alfalfa: 0% (C), 5% (E1) and 10 % (E2). Consequently, the dietary fibre ranged from 4.5% (C) to 6% (E1) and to 8% (E2). With the view to facilitate nutrient digestibility and to promote a healthy digestive tract, the experimental formulations have been supplemented with 15g pre/probiotic/kg feed and 10 g organic acids/kg feed. The experimental results show that there were no significant differences between the groups in terms of the weight gain, as follows: 19.75±3.182 kg gain/period (C); 19.833±1.607 kg gain/period (E1) and 20.00±2.00 kg gain/period (E2). The average daily feed intake (kg feed/pig) decreased significantly ( $P\leq 0.05$ ) in the experimental groups: 2199.24±365.34 g feed/pig/day (E1) and 2060.46±431.05 g feed/pig/day (E2) compared to 2422.39±247.47 g feed/pig/day (C). No significant differences were noticed in the apparent absorption of the fibre, even though numerically, the data for the experimental groups 59.90±7.58 % (E1) and 58.54±11.05 % (E2) were higher than the data for the control group (52.06±11.77 %). In conclusion, the use of 5% and 10% alfalfa in pig feeding (25-45 kg), accompanied by the use of symbiotics, didn't affect the growth performance and the digestive tract health of the experimental animals.



## WEL-018 - EFFECTS OF GESTATING SOWS HOUSED IN GROUP WITH ELECTRONIC SOW FEEDING (ESF) SYSTEM OVER THREE CONSECUTIVE PARITIES

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This study was conducted to investigate the effects of gestating sows housed in groups with electronic sow feeding (ESF) system over three consecutive parities. A total of 83 pregnant gilts [Yorkshire × Landrace] were housed into 1) ST (N=41): gilts housed in conventional stall, 2) ESF (N=42): gilts housed in groups with ESF system on the basis of body weight (BW) and backfat thickness (BFT) in a completely randomized design. In gestation, ESF tended to increase BW gain in second parity (P=0.08), and consistently showed the significance during third parity (P<0.01), resulting in higher BW at d 110 (P=0.10, P<0.03 in parity 2 and 3, respectively). Similarly, BFT gain tended to be higher in ESF than ST (P=0.08, P= 0.10 in parity 1 and 2, respectively). Estimated body fat contents changes was higher in ESF regardless of parities (P<0.01, P<0.02, P=0.10 in parities 1, 2, and 3, respectively). However, there were no significant differences on sow BW and BFT changes during lactation. In endocrinal analysis, high serum cortisol level of ST treatment was observed at d 110 of gestation (P<0.01 in parity 1), whereas no detectable difference was observed in serum oxytocin level. Higher incidence of body scratch were recorded in ESF in early gestation in all parities (P<0.01), resulting in higher locomotor disorders in middle and late gestating period (P=0.07). In conclusion, our result suggested that ESF system showed higher growth performance as well as survival rate of piglets. However, more incidences of body scratch and higher locomotion disorder scores in ESF due to in combination with persistent fighting around ESF machines and inadequate bedding materials. Consequently, it is necessary to consider the proper bedding materials as well as adequate space divider or barrier for gestating sows to avoid escaping aggression in ESF system.





## WEL-019 - EFFECTS OF FEEDING FREQUENCY ON THEIR REPRODUCTIVE PERFORMANCE AND STRESS RESPONSE IN GESTATING SOWS

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A total of 20 F1 multiparous sows (Yorkshire×Landrace) were used to investigate the effects of feeding frequency on their reproductive performance and stress response in gestating sows. The sows were allotted to one of two treatments into 1) once daily feeding (OF) and 2) twice daily feeding (TF) in a completely randomized design (CRD) based on their parity, body weight (BW), and backfat thickness (BFT). Gestating diet was provided at 2.2 and 2.4 kg/d for 2<sup>nd</sup> and 3<sup>rd</sup> parity respectively, while lactating diet was provided *ad libitum* during lactation regardless of treatment. In gestation, BW and BFT were not affected by feeding frequency, however body weight gain is significant higher in day 35 to 90 and day 35 to 110 of gestation were observed in OF treatment ( $P<0.05$  and  $P<0.10$ , respectively). In lactation, although litter and piglet performance were not affected by feeding frequency during gestation, litter weight at birth was significantly higher in OF treatment ( $P<0.05$ ). Sows in OF treatment showed significantly or numerically lower average daily water consumption from day 35 to 70 of gestation ( $P<0.05$ ), day 70 to 105 of gestation ( $P<0.10$ ) and overall period of gestation ( $P<0.05$ ). While there were no significant differences in stereotypic behaviors and salivary cortisol levels during gestation between treatments. OF treatment showed lower activities at day 70 and day 105 of gestation ( $P<0.10$  and  $P<0.05$ , respectively). In conclusion, once daily feeding(OF) treatment have no negative influence on sow reproduction and reduced the stress-related responses.



## WEL-020 - DIETARY NUCLEOTIDE SUPPLEMENTATION IN SOWS DURING LACTATION: EFFECTS ON PIGLETS PERFORMANCE AND MORTALITY

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### Background & Objectives

Weaning is a challenging event for piglets. Providing an adequate nutritional support could help piglets overcome this stressful period. Dietary nucleotide supplementation has been shown to improve immunity, promote blood flow and enhance intestinal cell proliferation. Therefore, piglets could benefit from nucleotide supplementation in sows. The aim of this study was to evaluate the effects of supplementing sows with nucleotides during lactation on performance and mortality rate of piglets during weaning and transition.

### Material & Methods

Seven days before the birth of piglets and during lactation period, sows received a diet supplemented with 500 ppm of a nucleotide formulation specifically designed for pigs. At weaning and during the transition period, several parameters were evaluated in 11,711 piglets (11 batches of piglets from sows that had received nucleotides and 9 batches from sows that were fed a commercial diet without nucleotides, used as Control group). The Statistical Analysis System (SAS) was used for the statistical analyses and alpha significance level was set at 0.05.

### Results

When groups were compared, piglets from sows that had received nucleotides showed significant increases ( $p < 0.05$ ) in average weight at weaning (6.02 vs 5.61 kg), in average daily gain during transition (0.459 vs 0.412 kg/day) and in average weight at the end of transition (25.2 vs 21.5 kg); and a significant reduction ( $p < 0.05$ ) of mortality rate during transition (1.13 vs 1.43 %).

### Discussion & Conclusion

These findings suggest that dietary nucleotide supplementation in sows during lactation results in transmission of nucleotides to their piglets, allowing an improvement in several performance parameters as well as a reduction in the mortality rate.



## WEL-021 - THE USE OF AN ALGAE-BASED COMPLEMENTARY FEED HELPS LIMITING PEDV DAMAGE ON SUCKLING PIGLETS

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The porcine epidemic diarrhea virus (PEDv) is an enteric disease of swine. Once ingested, the virus rapidly causes enterocyte lysis, leading to decreased nutrient absorption capacity resulting in watery diarrhea followed by a rapid dehydration, anorexia, and, in some cases, mortality. Managing symptoms and initiating a plan for barn inoculation is the best way to control a localized infection. In support to these measures, the use of a complementary feed capable of protecting the intestinal mucosa may be beneficial. The aim of this study was to test the efficacy of such complementary feed (Ecopiglet), based on algae extracts and montmorillonite clay, on piglet performance in a context of PEDv infection.

The trial was performed in a farrow-to-finish farm of 450 gestating sows located in the center of Mexico. Twenty-two litters from the same batch and placed in the same room were involved in the trial. Eleven litters (127 piglets) constituted the control group, which did not receive any complementary feed, and eleven litters (120 piglets) constituted the test group which received the algae-based complementary feed from day one to weaning (21 days) at the dose of 50g/litter/day. The farm was diagnosed positive for PEDv at day eleven of the trial.

Results showed that despite a lower birth weight (-160g,  $P<0.05$ ), piglets from the test group had a higher growth rate than control (respectively 187 and 150 g/day,  $P<0.05$ ). In addition, the use of the complementary feed reduced the mortality rate from 26.8% to 14.2%.

In summary, the use of an algae-based complementary feed in this farm, which was positive for PEDv, helped to lower mortality rate and improve growth rate. During the six months following the trial, the continuous use of this complementary feed in this farm helped to minimize mortality rate until PEDv outbreak was completely controlled.



## WEL-022 - REPLACEMENT OF SIMPLE ACID BASED PRODUCTS BY ENHANCED ACID BASED PRODUCT IN WEANED PIGLET DIETS

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A combination of organic acids, cinnamaldehyde and a permeabilizing complex (Biotronic® Top3, BTR) can partially or fully replace conventional dietary acid supplementation in weaned piglet diets at lower inclusion levels while delivering similar or better growth performance of the animals.

Three trials were conducted to determine whether BTR in inclusion level between 1.5-2.0 kg/t of feed, can partially or even fully replace benzoic acid based product (BA) at 5.0 kg/t of feed, formic acid based product (FA) at 6.0 kg/t of feed and potassium diformate based product (PD) at 6.0 kg/t of feed. In the trial with BA also a combination group (BAC) was tested, where BTR in inclusion level of 1.0 kg/t of feed, replaced 3.0 kg/t of BA from the diet.

Average final weight gain of the piglets was higher in the BTR group than in the simple acid based product groups. In the trial with BA group (30.53 kg) the average body weight was 1.7% lower compared to BTR group (31.04 kg) and 4.9% lower compared to BAC group (31.78 kg), the average weight gain in BA group (20.08 kg) was 2.4% lower compared to BTR group (20.57 kg) and 6.1% lower compared to BAC group (21.31 kg). In the trial with FA group (34.90 kg) the average body weight was 2.2% lower compared to BTR group (35.30 kg), in FA group (25.50 kg) the average weight gain was 2.6% lower compared to BTR group (25.90 kg). In the trial with PD group (10.90 kg) the average body weight was 5.6% lower compared to BTR group (11.51 kg) and the average weight gain in the PD group (4.85 kg) was 8.3% lower compared to BTR group (5.25 kg).

The supplementation of BTR improved the performance of pigs compared to the simple acid based products, demonstrating that successful replacement is possible.



## WEL-023 - EFFECT OF EMULSIFIER(SOLMAX®) SUPPLEMENTATION ON LACTATING SOWS AND SUCKLING PIGLETS

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A total of 40 mixed-parity sows (Yorkshire × Landrace, average parity 6) were used in this experiment to determine the effect of emulsifier (SOLMAX®) supplementation on lactating sows and their progeny. Sows with an initial body weight (BW) of 248.6±19.71kg were assigned in a 2×2 factorial arrangement with 10 sows per treatment after 24hrs postpartum based on BW, BF thickness, and litter weight in a completely randomized design. The first factor was two dietary energy level (3,200 or 3,265 kcal of ME/kg), and the second factor was supplementation of emulsifier (0 or 0.05%). Lactating sows were fed experimental diet *ad libitum* after 5 days postpartum. Body weight, backfat thickness, lactation feed intake, weaning to estrus interval of sows were not affected by dietary energy and emulsifier effect. There were no significant differences in litter weight and piglet weight at 24hrs postpartum and 21d of lactation. However, interaction of dietary energy and emulsifier was shown in piglet weight gain during 0-21d lactation (E×E, p=0.10). Although high energy diet (3,265 kcal of ME/kg) with emulsifier decreased piglet weight gain, low energy diet (3,200 kcal of ME/kg) with emulsifier showed higher piglet weight gain. In blood profiles, dietary energy level and emulsifier supplementation had no influence on glucose, total protein, creatinine in lactating sows and piglets. Sows fed high energy diet (3,265 kcal of ME/kg) had higher serum triglyceride, and VLDL-cholesterol at 21d of lactation. Also, blood urea nitrogen and albumin were affected by interaction of energy and emulsifier (E×E, p=0.06, p=0.02). There was no significant differ in milk composition of sows by energy and emulsifier effects. Consequently, supplementation of emulsifier showed positive effect on growth performance of piglet when gestating sows fed low energy diet.



## WEL-024 - THE EFFECT OF SUPPLYING LIANOL® SOLAPRO IN THE LACTATION FEED ON SOW LACTATION PERFORMANCE AND BODY CONDITION

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### Introduction

Previous research demonstrated a positive effect of Lianol®, a complementary feed based on fermented potato protein, on lactation and growth. The objective of this controlled field study was to evaluate effect of Lianol® Solapro supplementation for sows during lactation.

### Material and Methods

On a Danish farm, 4 production groups were alternately allocated to respectively a treatment and a control group. The treatment group, received 20 gram/ Lianol® Solapro/sow/day from entry of the farrowing room (day 1) till 1 day after farrowing and 10 gram Lianol Solapro/sow/day from the 2<sup>nd</sup> day after farrowing till weaning (day 29).

Feeding, management and housing was according to the normal procedure on the farm for all sows. Movement of piglets between treatment and control groups was not allowed. Foster-sows were excluded from the trial. At day 29 total litter weight and # weaned piglets were recorded. Back-fat measurement was performed at day 1 and 29, while daily feed intake was recorded for each sow throughout the study period.

### Results

There was a tendency for higher feed intake during lactation for control vers treatment group, respectively 149.9 kg/ sow to 151.6 kg/sow. The loss of back-fat during lactation was not significantly different between both groups. The # weaned piglets/sow was higher for the treatment group with resp. 12.5 vrs 12.9 piglets/sow. The weaning litter weight was increased in the treatment vrs control group with respectively 70.1 kg/litter vrs 65.7 kg/litter (p=0.11). This resulted in a 3 % increase in piglet weaning weight in the treatment group.

### Discussion

In conclusion supplementation of Lianol® Solapro for sows during lactation had no significant effect on loss of back-fat, total feed intake and number and weight of weaned piglets. However, there was a tendency for higher feed-intake, number and weight of weaned piglets in sows supplemented with Lianol® Solapro.





## WEL-025 - EFFECT OF LYSINE CELL MASS AS ALTERNATIVE TO L-LYSINE • HCL ON GROWTH PERFORMANCE, BLOOD PROFILES, AND DIARRHEA INCIDENCE IN WEANING PIGS

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This experiment was conducted to evaluate the effect of lysine cell mass (LCM) levels as an alternative to L-lysine-HCl on growth performance, blood profiles, immune response and diarrhea incidence in weaning pigs. A total of 200 crossbred pigs ([Yorkshire × Landrace] × Duroc), average  $6.89 \pm 1.043$  kg body weight (BW) and weaned at  $28 \pm 3$  days of age, were allotted to one of 5 treatments in 4 replicates of 10 pigs per pen in a randomized complete block (RCB) design. The dietary treatments were composed by dietary supplementation level of LCM as an alternative to L-lysine-HCl (0, 0.25, 0.5, 0.75 or 1.0%) in weaning pig diets. There was no significant difference in growth performance when pigs were fed various level of LCM. However, serum cortisol concentration of 5<sup>th</sup> week was significantly increased as dietary LCM level increased (linear,  $P < 0.01$ ). Also, serum insulin concentration of 5<sup>th</sup> week was linearly increased as dietary LCM level was increased (linear,  $P < 0.05$ ). In the case of immune response, IgG concentration of 5<sup>th</sup> week was linearly decreased (linear,  $P < 0.05$ ). However, the changes in blood profile and immune response could not alter growth performance and the incidence of diarrhea was not affected by dietary LCM levels. Consequently, LCM can be supplemented in weaning pig's diets as an alternative lysine source.



## WEL-026 - IN-FEED ANTIBIOTICS OR SODIUM SALT OF COCONUT FATTY ACID DISTILLATE ON PERFORMANCE IN POST-WEANED PIGLETS

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### Objective

The objective of the present study was to evaluate the effect of DICOSAN (sodium salt of coconut fatty acid distillate) on performance parameters in post-weaned piglets.

### Material & Methods

A total of 240 piglets, with 28 d of age ( $7.7 \pm 0.80$  kg) were randomly distributed according to their initial body weight (BW) into 24 pens with 10 piglets per pen. There were three treatments: CON-, control diet with no additives nor antibiotics; CON+, control diet plus 120mg/kg of colistin and 3000ppm of ZnO; DIC, control diet plus DICOSAN at 3kg/t of feed in the pre-starter diet and 1 kg/tn of feed in the starter diet. The trial lasted 42 days, the pre-starter diets were offered *ad libitum* from 28 d to 42 d and the starter diets from 43 d to 70 d of age. At the end of each period, animals were weighted and data from feed intake and mortality were recorded. Results were analyzed by one way ANOVA using GLM procedure of SSPS v. 19.0, with the initial BW as a covariable.

### Results

There were no differences in the productive parameters at 42 d. At 70d, piglets fed CON+ diet were the heaviest (22.53, 24.42 and 22.63 kg, for CON-, CON+ and DIC,  $P=0.006$ ). Moreover, the feed conversion ratio (FCR) of piglets fed DIC diets were the same than those fed CON+ (1.62, 1.54 and 1.54, for CON-, CON+ and DIC,  $P=0.108$ ). The mortality of DIC group was the lowest (5.0, 2.5 and 0.0 for CON-, CON+ and DIC,  $P=0.073$ ).

### Discussion & Conclusion

It can be concluded that the better results achieved by medicated diets (CON+) can be also achieved by DICOSAN as showed by the results in the FCR and in the low mortality. These results reinforce DICOSAN as a good alternative to antibiotics in pig diets.



## WEL-027 - VITAMIN B<sub>12</sub> STATUS IN PIGS WITH AND WITHOUT EXPERIMENTALLY-INDUCED EXOCRINE PANCREATIC INSUFFICIENCY

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### Background & Objectives

Cobalamin (vitamin B<sub>12</sub>) absorption, which occurs mainly in the ileum, depends on binding proteins, including intrinsic factor (IF). The IF-cobalamin complex enables absorption via receptor-mediated endocytosis in the ileal mucosa. In humans and dogs, IF is synthesized by gastric parietal cells and pancreatic acinar cells (PAC), respectively, whereas in pigs no information is available. Experimentally-induced exocrine pancreatic insufficiency (EPI) could mimic the absence of IF from PAC. Vitamin B<sub>12</sub> status can be quantified in serum by measuring cobalamin and methylmalonic acid, which reflects the availability of cobalamin on the cellular level. Our aim was to evaluate the effect of experimentally-induced EPI on the vitamin B<sub>12</sub> status in pigs.

### Material & Methods

Fourteen age-matched pigs were randomly allocated to a control-group (n=4), induced EPI-group-I (n=5; 7 weeks old) and induced EPI-group-II (n=5; 16 weeks old). The control-group received a sham laparotomy, whereas the other two groups underwent surgical ligation of the pancreatic duct. Serum samples were obtained from all pigs at 9, 15, 21, and 26 weeks of age. Cobalamin and methylmalonic acid concentrations were measured using an electro-chemiluminescence-immunoassay and high-performance-liquid-chromatography, respectively. The effect of experimentally-induced EPI was evaluated by using a MANOVA model.

### Results

Both variables (cobalamin [ $p=0.0057$ ] and methylmalonic acid [ $p=0.0037$ ]) were significantly different among the groups. Cobalamin and methylmalonic acid concentrations in the induced EPI-group-I differed significantly from the control-group ( $p=0.0068$  and  $p=0.0155$ , respectively) and induced EPI-group-II ( $p=0.0038$  and  $p=0.0135$ , respectively). In contrast, no differences were observed between the control-group and induced EPI-group-II for both variables ( $p>0.05$ ).

### Discussion & Conclusion

Experimentally-induced EPI in 7 weeks old pigs did affect the vitamin B<sub>12</sub> status which may suggest that IF is mainly synthesized by PAC in pigs. The difference in the response to the experimentally-induced EPI at 7 and 16 weeks of age warrants further investigation.



## WEL-028 - EFFECT OF DIFFERENT VACCINES ON THE BEHAVIOR OF SUCKLING PIGLETS

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### Background & Objectives

The aim of this study was to examine the effect of two combined porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (M. hyo) vaccines on the activity of piglets regarding suckling and resting behavior as well as average daily gain (ADG).

### Material and Methods

Overall 44 piglets out of 4 litters were divided in two groups and vaccinated with either (A) FLEXcombo<sup>®</sup> (mixture of Ingelvac CircoFLEX<sup>®</sup> and Ingelvac MycoFLEX<sup>®</sup>, water based adjuvant, n=21) or (B) Porcilis<sup>®</sup> PCV M hyo (mineral oil based adjuvant, n=23). All animals were individually weighed three days before vaccination and at weaning (28 days). Three animals of each litter (one lightweight, one middleweight, one heavy piglet) were marked and chosen as focus animals. Their suckling- and resting behavior was video monitored in 24 hour intervals the day before vaccination, on day of vaccination and two days afterwards.

### Results, Discussion & Conclusion

The animals from group A (FLEXcombo<sup>®</sup>, water based adjuvant) showed only minimal changes in the total resting time. Compared to the day before vaccination, they only showed an increase of 6 % in resting time.

The suckling time was 25% less during the same period.

In the observation period the ADG in this group was 295 gram.

Piglets from group B (Porcilis<sup>®</sup> PCV M hyo, mineral oil based adjuvant) showed an increase in resting time of 28%, accompanied with a drop in suckling time of 40%. The ADG was 277 g.

### Discussion

This study indicates that vaccines and their adjuvants can impact the behavior of piglets. Beside vaccine efficacy, tolerability and impact on suckling behavior should be considered when it comes to vaccine choices.



## WEL-029 - PREVALENCE OF TAIL LESIONS IN FATTENING PIGS SLAUGHTERED IN SWITZERLAND

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The pig's tail is frequently hit by lesions that can affect the skin only or can include deeper structures of this organ. From weaning to slaughter, cannibalism, i.e. tail biting, is considered as one of the most important reasons for tail lesions and loss of the tail's tip, if this has not been docked. Concurrently, swine inflammation and necrosis syndrome (SINS) can contribute especially to lesions at the tail's tip. In order to prevent both aetiologies, the pig's tail is routinely docked in many countries and raising pigs with undocked tails is assumed to be impossible without significant cannibalism. The aim of the present study was to estimate the prevalence of tail lesions in fattening pigs in Switzerland, where tail docking has been banned years ago.

In two different slaughter plants in Switzerland, all fattening pigs slaughtered on a particular day have been examined by the first author. Pigs were studied during bleeding and tails were scored according to the extent of lesions (0=no lesion; 1=1-25% missing, 2=26-50% missing, etc.). Moreover, lesions were scored as either 'acute' or 'chronic'.

Overall, 3066 pigs from 81 batches have been examined. The median batch size was 24 pigs (range 2-190). The overall and intra-batch prevalence of tail lesions was 39.7% (SD 23.9%). The majority of these pigs (72.0%) showed 'chronic' lesion of score '1'. In total, 5.7% of all slaughtered pigs showed 'acute' tail lesions (score 1-4) characterized by fresh bleeding. Male pigs were significantly more often affected compared to female pigs ( $p < 0.001$ ).

Tail lesions were observed more frequently than expected. However, only a small proportion of pigs with tail lesion showed evidence for cannibalism. The majority of pigs with tail lesions was affected by slight chronic lesions only and it is hypothesized that several of these cases must be attributed to SINS.



## WEL-030 - GILTS VS. BARROWS: THE EFFECT OF GENDER ON SKIN WOUNDS IN FINISHERS REARED OVER 160 KG

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Injuries due to fighting and sexual behaviour are a major welfare issue in pigs, with health and economic impact. In Italy, according to the cured meat production, pigs are slaughtered later (over 9 months of age, 27 weeks of fattening and 160 kg live-weight) than in the other EU systems. The prolonged fattening period after reaching sexual maturity might be a risk factor for females (males are castrated).

Hence, the aim of this epidemiological survey was to evaluate the effect of sexual behaviour on skin wounds of heavy pigs reared in single-sex pens. Barrows were assessed as a control group. 6486 gilts and 6967 barrows from 27 farms were scored for skin wounds on 3 body regions (ears, front, back) at 4 weeks of fattening and again before slaughtering (24 weeks of fattening), according to Welfare Quality® Protocol for finishing pigs (score 0-1-2). Scores 1 and 2 were grouped together and considered as score 1. In further 7 farms, wounds were scored at the abattoir, from the carcasses of 724 gilts and 781 barrows.

At 4 weeks of fattening, 81% of animals of either gender had an overall score 0 (i.e. less than five lesions in each body region). In particular, 96% was scored 0 at ears and in hind-quarters, while 92% was scored 0 in front. At 24 weeks, 86% of animals of either gender had an overall score 0. Ears and hind-quarters were assessed as 0 in 97% of animals. As regards front region, 95% of pigs were scored 0.

Unexpectedly, this study found a small percentage of skin wounds in finishers reared over 160 kg, with no gender effect. No gender variations were also detected at the abattoir, even though the majority of animals were scored 1, probably due to transportation, lairage and slaughtering operations. RC IZSVE 10/13.



## WEL-031 - GROWTH PERFORMANCE OF PIGLETS AS INFLUENCED BY DIETARY PLANT ALKALOIDS OR MEDIUM-CHAIN FATTY ACID SUPPLEMENTATION

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### Background & Objectives

In piglets changes associated with the post-weaning phase often result in reduced feed intake, causing energy deficiency, modifications in the intestinal morphology, reduced absorptive and immune functions and shifts in the composition of the intestinal microbiota. Isoquinoline alkaloids (IQ) and glycerol monolaurate (GML) have shown anti-inflammatory or anti-microbial effects, respectively, hence having potential to help maintain performance in the post-weaning phase. Thus, this study was designed to determine the effects of IQ, GML alone and in combination on growth performance of post-weaning piglets from 25 to 66 days of age.

### Material & Methods

160 piglets (Danbred x Piétrain) with an initial age and body weight of 25 days and 6.7 kg, respectively, were assigned to 4 dietary treatments, using 8 replicates/treatment and 5 piglets/replicate. Treatments were: (T1) Control (no supplementation); (T2) IQ (Sangrovit® Extra, 120 mg/kg); (T3) GML (2 g/kg); (T4) IQ (120 mg/kg) + GML (2 g/kg). Piglets were subjected to a starter (25 – 38 days of age) and grower diet (39 – 66 days of age). Mash feed based on corn, wheat and soybean meal and drinking water were provided *ad libitum*.

### Results & Discussion

Supplementation of the diet with IQ, GML or IQ+GML improved overall daily gain (T1: 0.48, T2: 0.52, T3: 0.54, T4: 0.56 kg/d;  $p < 0.001$ ). GML increased feed intake in the starter period ( $p < 0.01$ ), whereas overall feed intake did not differ between treatments ( $p > 0.05$ ). Overall FCR was improved by supplementation with GML or IQ+GML (T1: 1.46, T2: 1.40, T3: 1.35, T4: 1.30;  $p < 0.001$ ). Faecal scores were improved in pigs fed IQ, GML or IQ+GML ( $p < 0.001$ ) indicating a positive influence on intestinal health.

### Conclusion

Dietary supplementation with IQ, GML or their combination significantly improved weight gain and/or feed efficiency, representing a tool for supporting growth performance in pigs.





## WEL-032 - KEEPING PIGS WITH UNDOCKED TAILS IN CONVENTIONAL GERMAN PIG FARMS WITH AN ENRICHED ENVIRONMENT

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### Background & Objectives

Tail biting is one of the largest animal welfare problems in modern pig production. As the most commonly regarded preventive strategy suckling pigs have their tails docked in most countries. In Europe amputations carried out for other than therapeutic purposes are prohibited by the Council Directive 2008/120/EC but more than 95% of the pigs in Germany have their tails docked. In Germany conventional housing and management conditions are characterised by suckling periods  $\leq 4$  weeks, restricted pig to feeding place relations, barren environments and fully slatted floors. Therefore this study was focused on the effects of improved enrichment in conventional pig herds facilitating the prevention of tail biting.

### Material & Methods

A total of 799 pigs with non-docked tails from two conventional piglet producers and three fattening herds were included in the present study. Due to technical constraints (slurry, hygiene) the organic enrichment materials (CCM, straw) were provided in racks or feeders and not simultaneously available for all pigs in a group. The examination of the pigs (general health status, tail lesions) was performed on a weekly basis (principle investigator). Tail lesions were documented using a validated score.

### Results

During the final week of the rearing period 46.2% of the piglets had an intact tail (no injury, original length) but only 19.8% were slaughtered with an intact tail. Tail biting was especially observed just after weaning, at the end of rearing and at the beginning of the finishing period.

### Discussion & Conclusion

Under the housing and management conditions given in the study herds, the enrichment strategy was not effective in preventing tail biting. Tail biting was closely correlated with climatic hazards, problems during feeding process and respiratory tract infections. Enrichment with organic materials, not simultaneously available, proved insufficient to compensate for technical failure and challenges due to infectious diseases.



## WEL-033 - BENTONITE AND VITAMIN BINDING

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### Background

Adsorbing specific mycotoxins to limit their negative effects in livestock is a well-established method for mycotoxin deactivation. One of the most important features of binder products containing clay minerals such as bentonites is specificity meaning that only the targeted material (aflatoxins) is adsorbed. Not specific materials would bind all sorts of other compounds, such as vitamins and nutrients, reducing feed quality.

### Materials and Methods

Weaning piglets were divided into two experimental group (n=8) and kept in floor pens for seven days. The animals received a standard diet for weaning piglets. One group received feed supplied with bentonite at a concentration of 2 mg/kg. Blood samples (Vena cava cranialis) were taken on day seven of the trial to investigate the status of vitamins A, K, B1, B2, B6 and B12 in serum of the pigs.

### Results

No significant differences in serum vitamin levels were observed between the groups. Vitamin A levels of 0.33 mg/L and 0.28 mg/L were detected in serum of the control group and the bentonite group, respectively. Vitamin K levels of 259 ng/L and 244 ng/L were detected in serum of the control group and of the bentonite group, respectively. For the analyzed group of B-vitamins the following results were obtained: 236 µg/L (control) and 221 µg/L (bentonite) for vitamin B1, 517 µg/L (control) and 474 µg/L (bentonite) for vitamin B2, 66 µg/L (control) and 79 µg/L (bentonite) for vitamin B6 as well as 272 µg/L (control) and 306 µg/L (bentonite) for vitamin B12.

### Discussion and Conclusion

Some commercially available clay binders for aflatoxins used in feed are known to interfere with the feed quality by binding essential feed components. Results of this study revealed that there was no significant change in the vitamin levels in serum of weaning piglets when a bentonite based binder was applied.



## WEL-034 - OXIDATIVE STRESS AS INDICATOR OF PIGLET HEALTH AT WEANING

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Weaning is a source of social, nutritional and environmental changes for piglets, that challenge its health. This study aims to assess the opportunity to use plasma hydroperoxide (dROM) concentration and antioxidant capacity (BAP), two indicators of oxidative status, as indicator of piglet health in the context of weaning. In order to dissociate effects of age and management conditions on the evolution of oxidative status around weaning, a study was carried out, where piglets were followed from 12 to 147 days of age, and weaned at 21 or 28 days of age in optimal (OC) or deteriorated (DC) conditions (n=16 per group). Oxidative products (dROM), antioxidant capacity (BAP), vitamin E and A were measured in plasma. DC piglets had lower ADG ( $P<0.001$ ) and more diarrhea ( $P<0.01$ ) between 0 and 19 days after weaning compared to OC piglets. Oxidative stress index (OSI, dROM/BAP) increased for DC but not OC piglets after weaning ( $P<0.01$ ) due to increased dROM ( $P=0.05$ ) and decreased BAP ( $P<0.05$ ). Piglets which exhibited diarrhea between 0 and 19 days after weaning had higher OSI ( $P<0.001$ ) and dROM ( $P<0.01$ ) 12 days after weaning. The concentrations of vitamin E and A dropped after weaning ( $P<0.001$ ). Present data indicate that oxidative stress index can be affected by sub-optimal weaning conditions such as deteriorated management conditions and exhibition of diarrhea. Therefore, oxidative stress index seems to be a relevant candidate as an indicator of piglet health in the context of weaning.



## WEL-035 - EFFECT OF A LATE PREGNANCY DIET SUPPLEMENTED WITH HYDROLYZED YEAST ON SOW COLOSTRUM YIELD AND ITS COMPOSITION

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The aim of this study was to examine whether yeast derivative (YD) based on brewery yeast hydrolysate added to a late pregnancy diet affected colostrum composition and yield (CY) in sows. 37 sows were randomly allocated to two groups as follows: a negative control diet (n= 19) and the same diet supplemented with 3.5 g YD/kg (n=18) during the last 3 weeks of pregnancy. The YD used was Progut® (Hankkija Oy/Suomen Rehu, Hyvinkää, Finland). Within the first 2 hours from the beginning of farrowing, a 10 ml colostrum sample was obtained to check for nutritional composition (protein, fat, lactose, dry matter, with Fourier transform infrared spectroscopy - FTIR), and immunoglobulin content (IgA, IgM and IgG with ELISA analysis). All piglets were individually weighted at birth and 24 hours later in order to calculate CY. Colostrum content of protein, lactose and dry matter did not significantly differ between the two groups, while YD fed sows had higher level of fat in colostrum (5.1% vs 4.2%;  $p < 0.05$ ). IgA, IgM and IgG levels in colostrum did not significantly differ between the two groups. CY was 3701 g in the control group and 4580 g in the YD fed group ( $p < 0.05$ ). In conclusion, adding YD to late pregnancy diet in sows did not affect immunoglobulin level, protein and lactose content in colostrum, but contributed to higher fat content and increased the CY. Therefore YD added to sow diet may increase colostrum availability and also its energy content through fat increment for neonate pig.



## WEL-036 - ON THE EFFECTIVENESS OF A MICROBIAL FEED ADDITIVE IN PREVENTING DEOXYNIVALENOL-RELATED TOXICITY IN GROWING PIGS

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The trichothecene deoxynivalenol may cause significant economic losses in pig farming due to reduced feed intake and growth performance. Biological detoxification of mycotoxins has been in focus over the last decade. Besides adsorption, one of the most studied biological methods is microbial detoxification of trichothecenes. There are bacterial strains described in literature, which have been shown to detoxify deoxynivalenol (DON) *in vitro*. However, *in vivo* studies on their ability to detoxify DON in the digestive system of animals are still sparse. The main aim of our study was to evaluate the effect of a non-commercially available formulation of a bacterial strain on DON uptake and toxicity in growing pigs. In a 43-day experiment, 48 crossbred (Landrace-Yorkshire/Duroc-Duroc) weaning pigs were fed pelleted feed made from naturally-contaminated oat, with DON at four concentration levels: (1) control diet (DON < 0.20 mg/kg), (2) low-contaminated diet (DON = 0.86 mg/kg), (3) medium-contaminated diet (DON = 2.25 mg/kg), (4) highly-contaminated diet (DON = 4.11 mg/kg); with and without the feed additive incorporated. Growth performance, clinical signs, hematological and biochemical parameters, as well as DON, deoxy-DON and DON-glucuronide serum concentrations were measured at the beginning, middle and end of the feeding study. In the first week of the exposure period, the pigs in the highest dose group showed 5-8% reduction in feed intake and 7-10% reduction in weight gain compared to pigs in the control and low-dose groups. The addition of the additive had no influence on the observed clinical effects of DON, nor on the serum concentrations of DON and its metabolites. The reason for the lack of effect of the strain in this study is not known but may in part be related to negative effects on the viability of the bacteria by the stringent pelleting process.



## WEL-037 - COMPARISON OF PERFORMANCE IN WEANED PIG FED EITHER HIGH MOISTURE MAIZE GRAIN SILAGE OR A STANDARD STARTER DIET

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### Background & Objectives

An experiment was performed to evaluate high moisture maize grain silage (MG) inclusion in a weaned pig diet.

### Material & Methods

The trial was performed using a concentrate mixed 1:1 with MG compared to a standard starter diet (SD). Concentrates were produced to obtain diets with equal nutrient levels on a dry matter basis; the dry matter content of MG was 61%. 144 piglets weaned at 28 days were allotted in 12 pens of 12 piglets. During 28 days, after a transition period, 6 groups of pigs were fed with MG and 6 groups of pigs were fed with SD. Pigs were individually weighed at the beginning and at the end of the trial as well as every week. Feed consumption was measured every week at the pen level.

### Results

The average weight of piglets at weaning was  $7.6 \pm 1.0$  kg. At the end of the transition period, one piglet died. During the trial, 3 more pigs fed with the standard starter diet died. The average daily weight gain of piglets fed MG was significantly higher:  $0.341 \pm 0.031$  kg vs.  $0.300 \pm 0.019$  kg ( $P=0.021$ ). The mean feed intake (corrected for 87.5% dry matter content) was not significantly different ( $0.594$  kg/piglet.day). The difference between the mean feed conversion ratio was highly significant, *i.e.*  $1.71 \pm 0.07$  with MG vs.  $2.02 \pm 0.14$  with the standard starter diet ( $P=0.001$ ). Feed cost was  $0.65$  vs.  $0.77\text{€}$ /kg gain respectively with MG and SD.

### Conclusion

Significantly better performance was obtained in piglets fed with high moisture maize grain silage.



## WEL-038 - EFFECT OF A SEAWEED-CLAY COMBINATION ON THE NEONATAL DIARRHEA IN PIGLETS

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Neonatal diarrhea is one of the most frequent problem in piglets. It can result in high mortality and morbidity if it's not properly managed. Moreover, neonatal diarrhea leads to an over use of antibiotics. In this context, Olmix developed a new product based on seaweed extract and clays in order to improve gut health and help in case of neonatal diarrhea. This product contains specific seaweed extract called MSP Mucin, which has the property to stimulate mucin production (a glycoprotein composing the intestinal mucus layer) and micronized montmorillonite obtained via a soft milling process preserving its layered structure and increasing contact surface and coating properties. The objective of the present study was to evaluate the capacity of this MSP Mucin & clay combination to improve health status of newborn piglets in case of neonatal diarrhea. Several trials were set up in different countries (France, Italia, Ireland) in more than 15 farms with different managements. So far 90 litters from mainly Large-White/Landrace genetic were taken into account for the analysis of the results. The MSP Mucin & clay combination was administrated to litter with diarrhea before 5 days after farrowing at 2 ml/piglet/day during one to two days depending of the piglet's status. After two days, if diarrhea persisted, the farmers were allowed to use antibiotic. The age for piglet diarrhea occurrence, number of MSP Mucin & clay combination administration/piglet, ability of the combination to control the diarrhea, time to recover from diarrhea were recorded. The litter distribution was 36 % from gilts and 64 % from sows (average lactation rank: 3.1). This multisite trial, taking into account diverse farming situations, has shown the efficacy of the MSP Mucin & clay combination on stopping diarrhea in 84% of the cases. In 70% of successful cases, only a single application was needed.





## WEL-039 - CAN SODIUM SALICYLATE AND HYDROLYSABLE TANNINS AFFECT POST-WEANING DIARRHOEA IN ARTIFICIALLY INFECTED PIGLETS?

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Despite a multifactorial aetiology, post-weaning diarrhoea (PWD) are often related to pathogen infections, such as enterotoxigenic *E. coli* F4 (ETEC). The large use and misuse of antibiotics has led to increased occurrence of resistances, which strongly incites the development of alternative solutions. Nutritional solutions would represent a good alternative. Hydrolysable tannins are known to have antimicrobial properties and salicylate have been proposed to decrease the severity of diarrhoea.

The present experiment aimed to study whether a standard diet (C; CP: 18.5%; DE: 14 MJ/kg) supplemented with hydrolysable tannins from chestnut (T; Silvafeed Nutri P/ENC for Swine, Silvateam, Italy) combined (SA) or not with sodium salicylate (NSA) could reduce the incidence of PWD in piglet artificially infected with ETEC. At  $26 \pm 1$  d of age, 72 piglets were weaned and allocated in a 2 x 2 factorial design balanced for weaned body weight and litter. Piglets were orally infected with a suspension at  $10^8$  CFU/ml of ETEC (F4ac, LT+, STb+) 4 d after weaning. Each piglet had free access to an electrolytes solution containing or not SA. Growth performances were recorded weekly and faecal score was monitored daily for 14 d using a 4-level scale (1=dry to 4=watery diarrhoea).

The addition of SA had no effect ( $P > 0.05$ ) on growth performances nor on faecal score. At the opposite, during the first week post-infection, T supplementation increased ( $P = 0.03$ ) average daily gain by 58% and decreased ( $P < 0.001$ ) faecal score compared with the C group (1.46 vs 2.07 respectively). In addition, the number of days in diarrhoea during the whole experiment was reduced ( $P < 0.001$ ) with the T diet compared with the C diet (2.8 vs 6 d respectively).

Unlike SA, hydrolysable tannins could be a promising product to improve growth performance and reduce PWD directly after weaning.



## WEL-040 - THE INFLUENCE OF SELENIUM INTAKE ON SOME ERYTHROCYTE-RELATED BLOOD PARAMETERS IN HIGH-YIELDING NORWEGIAN SOWS

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Breeding sows are exposed to different kinds of stress, e.g. weaning stress and oxidative stress. Selenium (Se) is an essential trace element, exerting its functions as part of selenoproteins whereof several are antioxidants. Se- deficiency seem to re-emerge in high-yielding, Norwegian pigs independent of the production phase. We describe the influence of Se supplementation on some red blood cell parameters in breeding sows.

Landrace\*Yorkshire sows and gilts were included one month prior to farrowing and allocated to four groups (A-D, n=8). Experimental diets added sodium selenite contained A:  $0.40 \pm 0.03$  and B:  $0.61 \pm 0.05$  mg Se/kg feed. Diets enriched with L-selenomethionine contained C:  $0.26 \pm 0.04$  and D:  $0.40 \pm 0.08$  mg Se/kg. Hematological analysis was performed on heparinized blood samples using the ADVIA 120 analyzer.

Changes in MCH, MCHC, RDW, and hemoglobin were evaluated [(a) from initiation to farrowing, (b) farrowing to weaning, and (c) initiation to weaning], compared and correlated by employing the R software. The significance level was  $p < 0.05$ .

MCV showed a similar curve in A-D (a-c). Period (a): MCH tended to increase more in A compared to D ( $p = 0.06$ ). MCHC tended to increase stronger in A and C ( $p = 0.05$ ) compared to B and D. Period (b): RDW decreased more in C compared to A and D ( $p < 0.05$ ). RDW showed less modulation in B ( $p < 0.05$ ) compared to A. Overall RDW and hemoglobin concentration changes correlated significantly only in A ( $-0.49$ ,  $p < 0.05$ ).

A review of the literature highlights the lack of reference intervals on RDW and other blood parameters in sows in conjunction with Se supplementation in feed. Here, erythrocyte related parameters in group A seemed to be stronger influenced than in groups B to D. In hens, cattle and elderly persons, low serum Se has been associated with anemia. It is possible that the red blood cell indices of group A reflect a suboptimal Se status.



## WEL-041 - EFFECTS OF SOME NUTRITIONAL STRATEGIES ON PERFORMANCE AND VIRAL-LOAD IN GROWING GILTS CHALLENGED WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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The objective of this study was to assess the effect of different nutritional strategies on gilt performance and infection dynamics under porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) infection. One hundred gilts (46.5±3.5 kg) were blocked as small (S) and large (L) gilts, and distributed into five groups: control (C-), high dose of vitamin A (vitA), increasing ratios of some essential amino acids to lysine (AA), and increasing omega 3 (Ω3) groups; that were infected through intra muscular injection (IM). Twenty gilts were the positive control (C+) in a separate facility. Gilt body weight (BW) and feed intake (by pen) were recorded weekly. The trial lasted 89 days (d) although for logistic issues C+ was only followed up to d 40. Viral load (VL) was quantified as the area under the curve for 0–40 d post infection (PI) of the log of PCR-based serum viremia. Infection was at d 11 and by d 15 all IM injected gilts showed viremia. At d 20, C+ was 7.8 kg above infected groups ( $P < 0.0001$ ). An interaction showed that this difference was 12.6 kg at d 40 while S and L were equal in BW for C+, however S were smaller than L for the other groups ( $P = 0.032$ ). Only comparing infected groups, gilts from AA group did not lose BW after 9 d PI while other groups lost at least 1 kg ( $P = 0.085$ ). In fact, AA group showed a higher average daily feed intake for the whole period ( $P = 0.037$ ) and a higher average daily gain than group Ω3 ( $P = 0.081$ ) until d 70. The VL of the infected gilts did not show differences among groups. Therefore, amino acids requirements may be higher under a PRRSV infection and show a potential to modulate infection dynamics and this can have an effect on performance.



## WEL-042 - ALTERNATIVE TREATMENTS IN CONJUNCTION WITH CASRATION: EFFECT ON PAIN BEHAVIOUR IN PIGLETS

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We examined the effect of different castration protocols on behavioural observation related to pain expression and social cohesion of 5-day-old piglets. A total of 145 male piglets (Y×L, 5 piglets per litter) were allocated to five separate castration protocols: 1) NC: non-medicated castration, 2) PK: castration with meloxicam injection 0.4 mg/kg i.m., 3) LA: local anaesthesia with lidocaine (2%), 0.5ml into each testicle and meloxicam injection 0.4 mg/kg i.m., 4) GA: inhalation anaesthesia with isoflurane (1.5%) and meloxicam injection 0.4 mg/kg i.m., 5) IM: immunocastration with Improvac at 10 and 20 weeks of age. Behavioural observations related to pain expression (i.e., prostration, head shaking, leg crossing or shaking, tail wagging, rump rubbing, and abnormal walking), and social cohesion (i.e., isolation and desynchronization) were monitored for a 10 min period at 0, 1, and 2 hours immediately after castration. Repeated measures were used for data analysis. Duration of prostration in the NC piglets was higher than in the PK or IM piglets ( $P < 0.05$ ,  $P < 0.01$ , respectively), but it did not differ with the other treatments. The IM piglets showed less frequency of leg crossing or shaking than the PK, LA and GA piglets ( $P < 0.05$  for all). Frequency of tail wagging of the GA piglets was not different from that of the PK or LA piglets, but higher than the NC and IM piglets ( $P < 0.05$ ,  $P < 0.01$ , respectively). The PK piglets showed higher frequency of rump rubbing than the IM piglets ( $P < 0.01$ ), but no difference compared to the other treatments. There was no difference between treatments in head shaking, abnormal walking and sociality. In conclusion, pain behaviour such as tail wagging or rump rubbing was more present in piglets that were physically castrated regardless of method of pain medication or anaesthesia.



## Bacteriology and Bacterial Diseases

### BBD-001 - COMPARISON OF FIVE VACCINES AGAINST ACTINOBACILLUS PLEUROPNEUMONIAE LICENSED IN EUROPE

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#### Introduction

Porcine pleuropneumonia still represents an important problem among respiratory diseases in pigs in Europe. The infection results in substantial production losses including high mortality and reduced growth rate in growing and fattening pigs. Vaccination can provide efficient protection by decreasing the prevalence and extension of pneumonia and pleuritis. The aim of this study was to compare the efficacy of Coglapix® (Ceva Sante Animale), a toxoid+bacterin vaccine, with four different A.p. vaccines licensed in Europe.

#### Materials and methods

Six weeks old pigs were vaccinated in a prime-boost regime three weeks apart either with Coglapix® or with one of four competitor vaccines. Two were bacterin (A, B), 1 toxoid (C) and 1 toxoid+OMP (D). Pigs were challenged with A.p. serotype 2 strain via aerosol with a dose approx. 10<sup>6</sup>CFU/pig. After one week observation lung lesions and parietal pleura alterations were scored (LLS). By analyzing the weighted LLS and mortality rate, the results obtained for the vaccinated, un-vaccinated challenged, and un-vaccinated non-challenged groups were compared.

#### Results

The mortality was 0% in the Coglapix® group, 18%; 18%; 9%; 18% and 36% for vaccines A,B,C, D and control pigs, respectively. The weighted lung scores were 0.6; 1.67; 1.69; 0.9; 1.34 and 2.3 for Coglapix®, vaccines A,B,C,D and the control, respectively. The difference versus the positive control was statistically significant only for Coglapix® and vaccine C. The calculated vaccine efficiency was 75% for Coglapix® in comparison with 29%; 28%; 62% and 43% for vaccines A, B, C and D.

#### Conclusions

A.p. serotype 2 belongs to the most prevalent serotypes in Europe. Coglapix®, as demonstrated in this controlled experimental challenge study, had the highest efficacy in preventing the mortality and development of lung lesions due to the infections with A.p. 2.



## BBD-002 - EDEMA DISEASE IN FRENCH PIG PRODUCTION: ASSESSMENT OF THE KNOWLEDGE ACQUIRED THANKS TO THE GENOTYPING OF ESCHERICHIA COLI SINCE 2014

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### Background and Objectives

Edema disease (ED) is a disease of weaned pigs that is the cause of high economic losses, due to the high mortality rate and general bad technical performances of affected pigs. ED is caused by shigatoxin producing *E. coli* (STEC; coded by Stx2e gene). To complete the information given by clinical signs and necropsies, laboratory analyses are needed to have a definitive diagnosis. The aim of this study is to summarize the laboratory analyses carried out since 2014 on samples from farms suspected to have ED.

### Material and Methods

345 fecal samples or intestinal contents were considered in this study. In the two investigating diagnostic laboratories (Labocea and Labofarm), the genotyping by multiplex PCR was implemented on isolated *E. coli*. DNA from these *E. coli* was tested for the presence of two adhesins (F18 and F4) and several toxins (Stx2e, LT, Sta and Stb) genes.

### Results

The Stx2e gene was found in 224 samples (65%). The average age of the sampled piglets was 7 weeks. ED in older pigs (10 weeks and above) was common (17% of the Stx2e positive results). ED was found in different regions of France, in areas with various pig densities. Indeed, the highest number of submitted samples and of Stx2e positive results came from Brittany (high pig density area) and Reunion Island (low pig density area). The STEC belonged to 3 serotypes: O139:K82 (61%), O141:K85 (27%) and O138:K81 (5%). 7% of the STEC were non-serotypable.

### Conclusion

This study shows that ED in France can occur in any region (low and high density) and in any age group (mainly 7 weeks but older is possible). The results of the genotyping show that STEC can not only belong to different serotypes, but can also be non-serotypable.



## BBD-003 - ERADICATION OF ACTINOBACILLUS PLEUROPNEUMONIAE FROM CHRONICALLY INFECTED FARROW-TO-FINISH HERDS USING A PARTIAL DEPOPULATION PROCEDURE TOGETHER WITH A FLUOROQUINOLONE

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*Actinobacillus pleuropneumoniae* (APP) can cause severe pleuropneumonia with high mortality. In Denmark the use of fluoroquinolones together with a partial depopulation procedure seem to be promising as a control measure. The objective was to test this elimination protocol in many herds to obtain evidence of efficacy.

### Materials and Methods

Nine infected sow herds (6,867 sows in total) and associated finisher production were included. The herds were culture positive of APP serotypes 2, 5 or 6, had a high mortality and level of pleurisy and more than 500 m to nearest other pig farm.

All breeding stock was vaccinated against APP prior to the medication period. Four weeks prior to medication no farrowings took place and simultaneously all weaners and finishers were sold (partial depopulation). At the time of medication only boars, sows and gilts more than 10 months of age were present on the farm. All breeding stock was injected twice with a four day interval in the farrowing-free period (enrofloxacin: 5 mg/kg). Not earlier than 90 days after medication new breeding stock was allowed into the herd.

### Results

Seven out of nine sow herds were declared free of at least one APP serotype. The success of the elimination of APP was substantiated by clinical control on days 14, 30, 90, 150 and 365 and serologically by an ELISA on day 365 on 20 blood samples from offspring born after the medication.

In spite of farm to farm variations, occurrence of pleurisy, mortality as well as antibiotic usage in the offspring was greatly reduced, when comparing data 6 months prior to medication to data 6 months after medication.

### Conclusion

This project has shown that there is a fairly high success rate of eliminating APP from chronically infected sow herds by medication with a fluoroquinolone together with temporary changes in the pig flow.

### Disclosure of interest

None Declared.





## BBD-004 - LARYNGEAL SAMPLING TO ASSESS MYCOPLASMA HYOPNEUMONIAE INFECTION DYNAMIC ACCORDING TO THE ACCLIMATION PROCESS

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### Introduction

A proper acclimation program of the gilts to *M. hyo* before entering the breeding herd need to be implemented to control respiratory disease. This study documents the use of laryngeal swabs to assess the pathogen dynamic in a gilt flow with or without acclimation program.

### Materials and Methods

This case study was in a *M. hyo* positive system in Spain. A single *M. hyo* positive herd sends 12 weeks of age gilts to two continuous flow gilt development units (GDU).

In GDU A, ten seeders animals are placed with the incoming gilts two weeks after arrival in a 1 to 10 ratio. The seeders are moved among pens every 3 weeks to increase the probability of mycoplasma infection.

In GDU B, no physical contact between different age animals is actively promoted. In each GDU, 30 laryngeal swabs were collected at different ages as a cross-sectional profile. To determine the presence of *M.hyo* real-time PCR in pools of 3 samples was used.

### Results

In GDUA no positive animal was detected at 14 weeks, but 6 weeks after placement of seeders 100% of PCR positive pools were found. At 30 weeks of age, 40% of the gilts could be detected shedding. In GDU B first positive gilts were detected at 20 weeks of age in 70% of the pools. At 30 weeks of age 100% were positive.

### Conclusions

A proper gilt acclimation program is an integral part of systematic *M. hyo* control particularly in herds receiving naïve gilts. Laryngeal swabs are a good tool to evaluate acclimation systems. Properly acclimated gilts in GDU A achieved early exposure and had reduced the shedding before entering the sow unit. In contrast, the gilts from GDU B, late naturally exposed are shedding around first farrow. Avoiding shedding of gilts during the first pregnancy is the goal of the acclimation concept.



## BBD-005 - INACTIVATION OF *E. COLI* K99 INOCULATED IN PORCINE PLASMA AND SUBMITTED TO DIFFERENT SPRAY-DRYING PROCESSING CONDITIONS

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### Introduction

Spray dried porcine plasma (SDPP) is a common ingredient in post-weaning pig diets due to its well-recognized health benefits and improved growth and survival. Manufacturing of SDPP involves several safety features, including spray-drying at high temperatures. *E. coli* is a common pathogen of concern in the feed industry. The aim of this study was to check the effectiveness of different spray-drying conditions on *E. coli* K99 (EC-K99) inactivation in previously inoculated liquid plasma.

### Material & Methods

EC-K99 was grown during 24h in LB at 37°C. After centrifugation, precipitate was suspended in PBS buffer and inoculated into 50L of fresh (8.4±0.09% solids) or 25L of concentrated (23.0±0.47 % solids) porcine plasma. Inoculated plasma was then spray dried in a pilot plant spray drier (Anhydro Compact) at different inlet temperatures (170, 200 and 250 °C). At each inlet temperature, three different outlet temperatures were tested (70, 80 and 90°C). Dried samples were analyzed for presence of EC-K99 after incubation at different dilutions in TBX medium agar. The study was conducted in triplicate for each condition combination.

### Results and Discussion

After inoculation EC-K99 was detected at 7.66±0.27 logs/g dry matter (DM) in fresh plasma and 7.15±0.15 logs/g DM in concentrated plasma. Following spray drying, EC-K99 was not found in any of the dried samples obtained at all combinations of the conditions tested (limit of detection <10 cfu/g DM). These results indicate the effectiveness of the spray-drying process on *E. coli* inactivation. Typical processing conditions for commercial blood products involve drying at a minimum of 80°C outlet temperature and the results from this study indicate that even the lowest processing conditions used were enough to inactivate more than 7 logs of *E. coli* K99.



## BBD-006 - SURVEY OF THE SPANISH SWINE INDUSTRY ON MYCOPLASMA HYOPNEUMONIAE ACCLIMATION OF GILTS

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### Introduction

The introduction and management of replacement gilts has to be considered as a risk factor of the perpetuation of *Mycoplasma hyopneumoniae* in the farm. Improper acclimation may result in sub-populations and then in increased *M. hyo* colonization of the suckling piglets.

The objective of the survey was to learn more about the *M. hyo* gilt acclimation protocols in the Spanish swine industry.

### Materials and Methods

A survey composed by 14 questions was designed to identify which gilt acclimation methods for *M. hyo* are being used in Spain. The survey was completed by 45 veterinarians and producers.

### Results

The most important findings were:

- In 71% of farms, gilts from *M. hyo* positive multipliers are introduced.
- 37% of the producers consider that it is not necessary to acclimate sows for *M. hyo*
- 34% of the producers start the acclimation process late.
- 62.5% use vaccines against *M. hyo* during the acclimation.
- 92.5% do not perform diagnostics to verify an adequate acclimation
- In 67% of the farms the assessment of the stability in the sow herd is based on clinical signs.
- 17.5% of the responders feel that their acclimation protocol keeps their sow herd stable
- 50% of the veterinarians or farmers think that their acclimation process is not ideal.
- 87.5% of the responders believe that a proper gilt acclimation plays a major role in commercial *M. hyo* control of pigs

### Conclusions and Discussion

87.5% of the producers or veterinarians (87.5%) are convinced that a proper gilt acclimation program plays a major role in the *M. hyo* stability of their farms. Although 50% of the respondents believe that their acclimation process is perfect, many of them do not have a clear definition of the necessary time for a proper acclimation and start the process too late. Besides, the 92.5 of the farms do not verify the acclimation process of the gilts.



## BBD-007 - EVALUATION OF EFFICACY OF AIVLOSIN® 62.5 MG/G WSG (TYLVALOSIN) FOR THE TREATMENT OF ILEITIS AND ENZOOTIC PNEUMONIA IN FINISHING PIGS

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### Background and Objectives:

This study was designed to evaluate the effectiveness of tylvalosin (Aivosin®) compared to tiamulin (Denagard™) for the treatment of ileitis in a pig herd infected with *Lawsonia intracellularis*. *Mycoplasma hyopneumoniae* was also present and the effects on enzootic pneumonia were measured.

### Material and Methods

422 pigs were split randomly into two treatment groups on entry to fattening units:

- Tylvalosin (Aivosin® 62.5 mg/g Water Soluble Granules). 5 mg/kg/day in drinking water for 5 days.
- Tiamulin (Denagard™ 12.5% Liquid Concentrate). 8.8 mg/kg/day in drinking water for 5 days.

Treatment began when 5% of pigs showed clinical signs.

### Results

Pigs treated with Aivosin® exhibited a statistically significant better faecal score at Day 4 and significantly reduced coughing score at Week 1 after treatment, compared to the pigs treated with tiamulin.

Pigs treated with Aivosin® were sent to slaughter earlier on average (NS).

At slaughter, the Aivosin® treated group showed a lower pneumonia rate (68.36% vs 74.86%) and a lower mean pneumonia lung lesion score (4.63 vs 4.99) (NS).

### Discussion

Both products were effective for the treatment of ileitis; however, the Aivosin® treated pigs had better diarrhoea scores at Day 4 and coughing scores at Week 1.

Pigs from the Aivosin® group were sent to slaughter earlier and had better pneumonia scores, although the differences were not significant. An additional study with more animals could further confirm the benefits observed in the Aivosin® group.

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## BBD-009 - APPLICATION OF HIGH ENERGY DIETS IN COMBINATION WITH THE COLIPROTEC® F4 VACCINATION AGAINST POST-WEANING DIARRHEA

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### Background & Objectives

Post-weaning *Escherichia coli* diarrhea (PWD) is due to enterotoxigenic *Escherichia coli* (ETEC). Therapy to control PWD typically consists of antibiotic treatment, addition of zinc oxide (ZnO) and changes in feed composition. The objective was to compare vaccination with Coliprotec® F4 vaccine in piglets fed with a high energy (HE) diet (2530 kcal/kg) with/without acid addition with two standard therapeutic approaches, namely ZnO and a safe feed formulation (30% barley) with addition of acid in a farm with F4-ETEC PWD.

### Materials and methods

Piglets from a 600-sow farm with F4-ETEC PWD were vaccinated at 18 days of age with Coliprotec® F4. At weaning, piglets (n=640) were randomly distributed into 5 groups: (A) ZnO; (B) safe formulation with acid; (C) 1<sup>st</sup> safe formulation followed by HE diet + vaccination; (D) HE diet + vaccination; (E) HE diet + acid + vaccination. During the 7-week post-weaning period several technical production parameters were recorded.

### Results

No significant differences were observed among vaccinated groups. In the first 3 weeks post-weaning, vaccination with Coliprotec® F4 (C-D-E) resulted in statistically better fecal score ( $AUC_{3wk}$  56) higher average daily gain (150 g/d) and lower antibiotic treatment incidence ( $TI_{100}$  15) as compared to the piglets fed with the safe formulation with acids ( $AUC_{3wk}$  116, 124 g/d,  $TI_{100}$  52). Vaccination with Coliprotec® F4 (C-D-E) resulted in statistically lower antibiotic treatment incidence ( $TI_{100}$  15) as compared to the piglets on ZnO ( $TI_{100}$  25).

### Discussion & Conclusion

This comparative study shows vaccination with Coliprotec® F4 has advantages on technical performance parameters. The use of HE diets in combination with Coliprotec® F4 resulted in better growth with lower antibiotic use. In conclusion, control of PWD through oral vaccination is a good option to prevent piglets from the negative clinical effects of F4-ETEC infection in the post-weaning period.



## BBD-010 - SURVEY OF PULMONARY LESIONS AND PLEURITIS IN SLAUGHTERED PIGS IN SPAIN

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### Objectives

Lung scoring at slaughterhouse is a valuable tool for the assessment of the respiratory health status of pig farms. The aim of this study was to investigate the prevalence and extension of lungs lesions observed at slaughter in pigs from Spain.

### Material and Methods

Between January 2016 and November 2016 a total of 423 batches and 58.377 lungs from different farms in Spain were scored at the slaughterhouse for Enzootic pneumonia (Ep) -like lesions, and dorsocaudal pleurisy (*Actinobacillus pleuropneumoniae*, A.p.-like lesions) using Ceva Lung Program scoring methodology.

For the EP-like lesions each lobe was quantified according to the following: 0 (no lesions), 1 (1-25% of the surface of the lobe affected), 2 (26-50%), 3 (51-75%), 4 (76-100). Since each lobe does not represent an equal proportion of the lung, the results were assigned according to percentage of total lung capacity represented by each lobe (Christensen 1999).

A.p. like lesions included the presence, extension and position of pleurisy on both lungs.

For each batch was calculated:

- Percent of affected lungs with Ep-like lesions.
- Average percent of affected surface out of all lungs.
- Average percent of affected surface of pneumonic lungs.
- Percent of scarring lungs
- Percent of lungs with cranial pleurisy (SPES =1)
- Percent of lungs with dorsocaudal pleurisy (SPES>1)
- APPI index

### Results

52.8% of the lungs showed Ep-like lesions, with a 4.3% of affected surface out of all lungs, and 7% of affected surface of pneumonic lungs.

17% of the lungs showed scarring, and 14% cranial pleurisy.

12% of the lungs showed dorsocaudal pleurisy with an Appi index of 0.35

### Conclusions

There is a high frequency of Ep-like and pleural lesions in slaughter pigs in Spain.

This results suggest the need of implementing more effective programs against *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*.



## BBD-011 - VOLUNTARY ANTIMICROBIAL USE BENCHMARKING PROJECT ON 36 CANADIAN PIG FARMS

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### Introduction

Antimicrobial use (AMU) in farming is under scrutiny in all countries including Canada. Available Canadian farm AMU information such as the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) is informative but incomplete. A source of high quality data available is from the sale of antimicrobials to the farm. Voluntary benchmarking between farmers is a tool to help quantify and rank current status for production and disease information, and may therefore also provide needed incentive for change in AMU at the farm level.

### Aim

Our aims were: to measure AMU, using sales data as a proxy for usage, on a subset of Ontario farms; to understand key factors driving antimicrobial use and choice; to test a benchmarking strategy as a tool for AMU reduction.

### Methods

Volunteer farms were solicited based on convenience. Data for sale of antibiotics to the farm were collected from the veterinary practice and feed supplier. Boehringer-Ingelheim's "Compass" calculator was used to calculate animal doses based on Canadian medication labels. A short questionnaire on health status, and antimicrobial use decisions was administered. AMU data and survey results were summarized and presented to the volunteer farmers.

### Results

36 farms were enrolled, ranging from 100 to 4200 sows. The total number of sows was approximately 35 000, over 10% of the Ontario pig industry. Thirteen feed suppliers and four veterinary practices supplied antimicrobial sales information. AMU was calculated in mg/kg pork produced and animal daily doses per 100 animals days. Survey answers yielded valuable information about who the key influencers are on antimicrobial use choices, and why antimicrobials are used. The top three bacterial pathogens driving AMU were *S. suis*, *L. intracellularis*, and *M. hyopneumoniae*.

### Conclusion

This project highlights the value of AMU benchmarking as a tool to allow farm-to-farm comparisons and therefore stimulate positive changes in behaviour around AMU.





## BBD-012 - EFFICACY OF STELLAMUNE® MONO USING A NEEDLE FREE INJECTION DEVICE UNDER FIELD CONDITIONS IN FRANCE

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### Introduction

*Mycoplasma hyopneumoniae* (Mhyo) causes enzootic pneumonia (EP), a chronic respiratory disease of pigs resulting in major economic losses. Stellamune® Mono is a Mhyo vaccine administered as a single intramuscular (i.m) dose. Needle free injection devices (NFID) present some advantages over conventional needle application and can be an alternative for i.m. administration of Stellamune® Mono (Mouzin et al., 2014). This study aims to evaluate the efficacy of Stellamune® Mono using a NFID under field conditions.

### Material / Methods

A 450-sow farrow to finish farm operating a 4-batch farrowing system located in Brittany, France, was selected for this study. Initially Mhyo and PRRS-free, the farm broke out with EP and PRRS during summer 2015 (EP-like lung lesion score 4.81, 75% of affected lungs). Subsequently, Mhyo vaccination with Stellamune® Mono was implemented at weaning (21 days of age) and its efficacy evaluated when administered with a NFID (MS Pulse 250). For that, a batch of 1300 piglets was randomly allocated in 2 groups and vaccinated with Stellamune® Mono using syringe and needle (Group 1) or a NFID (Group 2). Average daily weight gain (ADG), carcass quality measured as % of lean meat (TMP) and EP-like lung lesion scores were recorded at the abattoir.

### Results

Average EP-like lung lesion scores were similar between vaccinated groups (Group 1= 0.99; Group 2= 1.03) and 57% of the lungs were free of EP-like lesions in both groups. Group 1 showed an ADG of 747 g/d and Group 2 of 745 g/d. Carcass quality was similar in the two vaccinated groups (Group 1 TMP= 61.5; Group 2 TMP = 61.6).

### Discussion / Conclusion

Our study shows that under field conditions Stellamune® Mono controlled Mhyo effectively after an outbreak of EP in a recently infected farm and that NFID can be an alternative for the i.m. administration of this vaccine.



## BBD-013 - ASSESSING THE USE OF INDIVIDUAL ORAL FLUID SAMPLES FOR IDENTIFICATION OF MYCOPLASMA HYOPNEUMONIAE USING A QUANTITATIVE REAL-TIME PCR ASSAY

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### Background & objectives

There is a need for an easy, quick, non-invasive and non-stressful samples collection ante mortem for the diagnosis of *Mycoplasma hyopneumoniae* (*Mhyo*). Therefore, the objective of this study was to evaluate the diagnostic sensitivity of individual oral fluid (OF) samples compared with trachéo-bronchic (TB) swabs for direct identification of *Mhyo* by quantitative Real-Time PCR assay.

### Materials & Methods

One farrow-to-finish farm located in Brittany, chronically affected by respiratory disorders attributed to *Mhyo* was selected. Gilts from a negative herd enter the farm at 24 weeks of age and enter the sow herd at 30 weeks of age. 30 gilts in gestation, 30 sucklers at time of weaning (28 days old) and 30 weaners (7 weeks old) were sampled. Individual OF were collected, on the one hand for gilts using a chewable piece of nonwoven polymer (Sodibox, Névez, France) inserted in the mouth for 2 min, and on the other hand for each piglet using a cotton swab inserted deeply in the mouth. Direct identification of *Mhyo* was then performed using a quantitative Real-Time PCR assay.

### Results

29 gilts among the 30 sampled were positive for *Mhyo* by TBS with bacterial loads ranging from  $4.5 \times 10^2$  CFU/ml to  $3.8 \times 10^7$  CFU/ml. 15 gilts were positive by individual oral fluid samples. 38 % (8/21) of gilts without clinical signs and 78 % (7/9) of gilts coughing at time of sampling were positive by salivas. Bacterial loads in OF ranged from  $4.0 \times 10^2$  CFU/ml to  $1.4 \times 10^5$  CFU/ml. No statistical correlation was demonstrated between bacterial loads using TBS or OF.

At 28 days old, only 2 TBS were positive, and 1 OF. At 7 weeks, 4 TBS were positive and 1 OF.

### Conclusion

Individual OF samples on gilts might provide a good diagnostic samples for diagnosis of *Mycoplasma hyopneumoniae* in a high prevalence sow herd.



## BBD-014 - SUCCESSFUL ELIMINATION OF MYCOPLASMA HYOPNEUMONIAE IN FIVE OUTDOOR PIG UNITS IN THE SOUTH OF ENGLAND USING AIVLOSIN®

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This paper describes the implementation of a *Mycoplasma hyopneumoniae* (M. hyo) elimination protocol in five outdoor, 3-week batch herds, with a total inventory of 4,000 sows.

### Material and Methods:

On-site nurseries were depopulated. Gilt and boar replacement was stopped to ensure that no breeding animals younger than 9 months of age remained in the herd when treatment started.

Dry and lactating sows were medicated for six weeks via the feed with Aivosin® 4.25% premix at an inclusion rate of 212 ppm. Start of treatment was carefully timed to coincide with the week before farrowing, and end of treatment with week after weaning. Any inappetent sows were injected with a macrolide (Draxxin®, Zoetis, Inc.) once.

After completion of the elimination protocol, gilts from a known EP negative source were brought in and used as sentinels, being tested every quarter until a year after completion of the elimination protocol. The sample size aimed to ensure a 90% certainty of detection of a M. hyo prevalence of 10% or higher. The IDEXX® ELISA M. hyo test was used; positives were retested using the OXOID Ltd ELISA test to rule out false positives.

### Results and Conclusion

Following twelve months with negative screening, the elimination was deemed successful in all five herds.

Successful M. hyo elimination allowed health to be maintained across the whole pyramid with significantly lowered antimicrobial use and major performance improvements in the system.

We believe this represents a novel technique for health improvement in 3-week batch units.

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## BBD-015 - EFFECTIVENESS OF SHORT AND MEDIUM CHAIN FATTY ACIDS, PLANT EXTRACTS AND ESSENTIAL OILS (LUMANCE®) ON POST-WEANING DIARRHOEA AND WEIGH GAIN

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### Introduction

Post-weaning diarrhea (PWD) cause mortality and morbidity in pig production and is a reason for regular antibiotic treatment. Two concerns are related with PWD:

1. Lost profit due to mortality, lower average daily weight gain (ADWG), additional medication costs.
2. Recommendation of European Medicines Agency for reducing the use of Colistin in animal feed related to the risk of antimicrobial resistance.

Gut integrity, balanced microbiota, anti-inflammatory, anti-oxidant and antibacterial activity are crucial for the strategy of reducing antibiotics and increasing performance. This study investigated the effectiveness of in feed combination of short and medium chain fatty acids (butyric, propionic, sorbic, lauric, capric, caprylic), plant extracts and essential oils (Lumance®, Innovad, Belgium) on PWD and ADWG in a commercial farm in Bulgaria.

### Materials and Methods

In two trials (T1 and T2), a treatment of 400 piglet each was compared with a control group of 400 piglets. The treatments in T1 and T2 included Lumance® at respectively 1,5 kg/T and 1 kg/T in the feed from the day of weaning (30 days) for 20 days and ZnO for 20 days 3000 ppm and 2000 ppm respectively starting from weaning. The control group for both trials included Colistin (12% ) in the feed at 2 kg/T for 10 days from weaning and ZnO, 3000 ppm for 20 days from weaning. PWD and ADWG were monitored from D30 till D94.

### Results

No symptoms of PWD were observed in none of the groups during both trials. ADWG in control groups were 610 g and 620 g. ADWGs obtained in Lumance® groups were 675 g in T1 and 709 g in T2.

### Conclusion

Lumance® is an effective concept to replace Colistin and reduce ZnO for preventing PWD, while having a positive effect on ADWG of the treated animals.



## BBD-016 - IMPROVED ENZOOTIC PNEUMONIA CONTROL WITH STELLAMUNE® MYCOPLASMA IN FRANCE

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### Background - Objectives

*Mycoplasma hyopneumoniae* (*Mhyo*) is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs, and is involved as well in the porcine respiratory disease complex. Commercial vaccines are commonly used to control *Mhyo* infection (Maes et al., 2008). Nevertheless, several factors can influence the efficacy of the vaccination against *Mhyo* and optimization of the vaccination strategy can be required. This study reports the use of Stellamune® Mycoplasma for an improved *Mhyo* control compared to previous vaccination strategies in French farms with chronic respiratory disease in fattening pigs.

### Materials - Methods

Twelve farrow-to-finish farms with chronic respiratory disease in fattening pigs located in Brittany were included in this study during 2015 and 2016. Those farms were included because *Mhyo* vaccination program didn't give satisfaction; program consisting of single-dose *Mhyo* vaccine from other manufacturers around weaning. Stellamune® Mycoplasma was implemented in those 12 farms with a first injection one week after birth and a second injection at weaning. Lung lesions were scored in the abattoir (Madec et al., 1982) before and after the implementation of Stellamune® Mycoplasma.

### Results

A total of 1677 lungs were scored before implementation of Stellamune® Mycoplasma, with an average EP-like lesion score of 4.26 and 62% of lungs affected. A score of >7 was shown in 18% of the lungs. After implementation of Stellamune® Mycoplasma, the farmers reported an improvement of the respiratory status of their fatteners and 1768 lungs were investigated with an average EP-like lesion score of 1.90 and 38% of lungs affected. Only 6% of lungs had a score >7.

### Discussion - Conclusion

In this study, the optimization of *Mhyo* vaccination strategy with Stellamune® Mycoplasma improved respiratory symptoms in the finishing units and reduced EP-like lesion scores at slaughter when compared to previous vaccination strategies in French farms with chronic respiratory disease in fattening pigs.



## BBD-017 - EVALUATION OF THE ABILITY OF AIVLOSIN® ADMINISTERED TO SOWS PRE-FARROWING TO REDUCE COLONIZATION OF MYCOPLASMA HYOPNEUMONIAE IN WEANED PIGS

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*Mycoplasma hyopneumoniae* (MHP) prevalence at weaning in piglets is considered to be the main predictor of the severity of mycoplasmal pneumonia in pigs (1).

The study was conducted in a system with a history of PRDC in late finishers, believed to be the result of introducing MHP-naïve gilts into a positive sow herd.

The goal of this trial was to evaluate whether Aivlosin® administered to sows pre-farrowing could reduce MHP shedding by the sow and produce a MHP-stable weaned pig population. MHP stability is defined as less than 10% of weaned pigs testing positive for MHP.

### Trial Design

Forty-five pre-weaned pigs from Parity 1 litters were tested for MHP via PCR of laryngeal swabs. Samples were submitted to Iowa State University and tested in pools of five. Positive pools were tested individually to determine the incidence rate.

In a subsequent group, sows were medicated with 62.5% Aivlosin® WSG (5 mgs/kg/day) as a top dressing for the first 5 days of placement in the farrowing crates. Forty-five weaned pigs from these sows were tested for MHP following the protocol above.

### Results

In litters from untreated sows, MHP prevalence was 13.3%; in litters from Aivlosin® treated sows, MHP prevalence was 2.2%. These results are the first evidence that an oral antibiotic given to sows pre-farrowing can reduce vertical transmission of MHP to piglets.

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## BBD-018 - PREVALENCE OF VIRULENCE FACTORS OF ESCHERICHIA COLI ISOLATED FROM PIGLETS WITH POST-WEANING DIARRHOEA IN BELGIUM AND THE NETHERLANDS

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### Background & Objectives

Post-weaning *Escherichia coli* diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by entero-toxigenic *Escherichia coli* (ETEC). The most common adhesins found in ETEC from pigs with PWD are fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are LT, STa and STb. The objective of the present study was to determine the prevalence ETEC subtypes causing PWD in Belgium and The Netherlands.

### Materials & Methods

A total of 504 pig herds distributed in the Benelux showing clinical signs of PWD were sampled between January 2014 and December 2016. Rectal swab samples (n=5) from diarrheic pigs were collected and submitted to IZSLER (Brescia, Italy) to analyze the presence of virulence factors - adhesins (F4, F5, F6, F18 and F41) and toxins (LT, STa, STb, Stx2e).

### Results

In total, 526 non-hemolytic and 784 hemolytic *E. coli* strains were isolated and subsequently tested by PCR. The prevalence of the different ETEC subtypes was as follows: F4-ETEC (24.4%) and F18-ETEC (19.2%). On a herd level, the prevalence of the different ETEC subtypes was as follows: F4-ETEC (45.8%) and F18-ETEC (37.5%). Besides ETEC, 22 isolates (1.7%) were classified as Shiga toxin-producing *E. coli* (STEC). The most prevalent virotypes in the necropsy study were F18STaSTb (10.8%); F4STaSTbLT (8.7%); F4STaSTb (6.1%); F4STbLT (4.9%).

### Discussion & Conclusions

This study confirms that fimbriae type F4 was slightly more prevalent than F18 among *E. coli* isolates from PWD cases in Belgium and The Netherlands. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of *E. coli* in PWD outbreaks and initiate appropriate preventive and control measures such as live oral vaccination.





## BBD-019 - THE ETIOLOGICAL SIGNIFICANCE OF STREPTOCOCCUS SUIIS CPS TYPES FOR HERD PROBLEMS WITH ARTHRITIS AND MENINGITIS IN PIGS IN NORTHWESTERN GERMANY

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*Streptococcus suis* (*S. suis*) infections in pigs are the cause of invasive diseases including meningitis and arthritis and lead worldwide to massive problems. Though numerous studies have investigated *S. suis* strain collections, very few data have been published on the role of the different serotypes of this pathogen in herd problems with arthritis and meningitis.

In the period from 05/01/2016 to 10/31/2016 90 pigs from 45 herds were necropsied, whose preliminary report recorded increased herd problems with arthritis or central nervous system disorders. From all pigs a swab of an inflamed joint and/or of the meninx were taken and were investigated by bacterial culture and subsequently bacterial specific PCRs. *S. suis* strains were characterized by multiplex PCR targeting capsular genes to differentiate serotypes (*cps* type) 1, 2, 7, and 9. When the bacteriological examination was negative, a multiplex PCR for *S. suis*, *Haemophilus parasuis* (*Hps*), *Mycoplasma hyorhinis*, and *Mycoplasma hyosynoviae*, was performed.

In 21 of the 45 herds (40 of the 90 animals) *S. suis* was detected from joint or meningeal swabs. Twenty-six of 59 joint swabs (44%) were *S. suis*-positive. The percentages of the isolates (n=23) for *cps* type 1, 2, 7, 9 and untypable strains were 4%, 39%, 4%, 44% and 9%, respectively. The following other pathogens were detected: *Mycoplasma hyorhinis* (n=4), *Mycoplasma hyosynoviae* (n=4), *Trueperella pyogenes* (n=3), *Staphylococcus aureus* (n=2), *Streptococcus dysgalactiae* (n=2), *Hps* (n=1), *Escherichia coli* (n=1) and *Salmonella* spec. (n=1). Thirty of 51 meningeal swabs (59%) were positive for *S. suis*; 86% (n=28) of these strains were *cps* types 2 (n=16) and 9 (n=8).

In pig herds affected by arthritis and meningitis, *S. suis* was the most frequently detected causative agent. Most of the *S. suis* strains isolated were *cps* types 2 and 9. Where *S. suis* was not the responsible pathogen, the etiology was very diverse.



## BBD-020 - COMPARISON OF PRODUCTIVE PARAMETERS IMPACT OF TWO VACCINES ONESHOT (HYOGEN® AND VACCINE A) AGAINST MYCOPLASMA HYOPNEUMONIAE IN A FIELD TRIAL

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### Introduction

Enzootic pneumonia (Ep) is one of the main causes of economic losses in swine production due to its negative impact on swine productive parameters. Vaccination against *Mycoplasma hyopneumoniae*, has become an effective tool to control and reduce economic losses associated. The aim of the field trial was to compare the effect on the productive parameters between two commercial vaccines oneshot against *M. hyopneumoniae*, Hyogen® and Vaccine A.

### Material and methods

Field trial was performed between January and November of 2016. Productive parameters and lung lesions scores from 43 batches from a 2.500 sows farm were collected, 21 pig batches vaccinated with Vaccine A and 23 pig batches vaccinated with Hyogen®. All parameters were compared. The economic impact of both vaccination protocols were calculated with Respinomics®.

### Results

Pigs vaccinated with Vaccine A, spent 3.3 days less at the fattening unit than the vaccinated with Hyogen®, however, Hyogen® vaccinated group consumed less feed (14.01 Kg/animal) to gain the same weight ( $86.2 \pm 0.1$  Kg). Thus, Feed Conversion Ratio (FCR) was 0.16 points better for Hyogen® group (2.33 vs 2.49). While, the Average Daily Gain was similar in both groups (0.75 and 0.73 Kg/animal/day). In Hyogen® batch the average percent of lungs with Ep-like lesions was 40% better, and the affected surface average was 3.55% better ( $p < 0.05$  and  $p < 0.001$  respectively). Antibiotic treatment costs were 682 € lower in Hyogen® group than in Vaccine A group. The economic analysis of the two groups showed a profit of 3.34 €/animal and a return of investment of 32 €/animal.

### Conclusion

Vaccination with Hyogen® improves the productive parameters compared to Vaccine A. FCR improvement and reduction on antibiotic treatments are the main factors to improve the profit after using Hyogen®. Thus, vaccinate with Hyogen® could contribute to reduce economic impact of enzootic pneumonia in swine farms.



## BBD-021 - EFFICACY OF HYOGEN® IN COMPARISON TO OTHER MYCOPLASMA HYOPNEUMONIAE INACTIVATED ONESHOT VACCINE UNDER FIELD CONDITIONS

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### Introduction

Vaccination against *Mycoplasma hyopneumoniae* (Mh) is a common tool used for prevention and control of Enzootic pneumonia (Ep). Vaccines efficacy has been demonstrated by the reduction on average of percent of affected lungs with Ep-like lesions and its severity at slaughterhouse. The aim of this field trial is to compare the efficacy of two oneshot Mh vaccines, Hyogen® and Vaccine B.

### Material and methods

The study was performed from May 2015 until June 2016, in a 1.200 sows farm. 19 pig batches and 1.847 lungs were scored at the slaughterhouse for Ep-like lesions and dorsocaudal pleurisy (A.p.-like lesions) using Ceva Lung Program scoring methodology. The parameters measured were:

Percent of affected lungs with Ep-like lesions, Average percent of affected surface out of all lungs, Average percent of scarring lungs, Percent of cranial pleurisy, Percent of affected lungs with dorsocaudal pleurisy, APPI index

### Results

Pigs vaccinated with Hyogen® showed an improvement on percent of affected lungs with Ep-like lesions (38%) over Vaccine B group (58%) ( $p < 0.001$ ). Average percent of affected surface out of all lungs were lower in Hyogen® group (1.31%) than in Vaccine B group (6.40%), ( $p < 0.001$ ). Scarring lungs percent was lower in Hyogen® batches (10%) than in Vaccine B batches (25%,  $p < 0.001$ ). Percent of dorsocaudal pleurisy results showed an improvement in Hyogen® group (7%) over Vaccine B group (11%) ( $p < 0.05$ ). APPI index was better in Hyogen® batch (0.17) than in Vaccine A batch (0.26)

### Conclusions

All parameters scored showed better results in Hyogen® batches. Also, parameters related to *Actinobacillus pleuropneumoniae* (A.p.) have improved, thus control over Mh could reduce impact of A.p. in swine. Related to this field trial, Hyogen® vaccination presents more efficacy in control of EP than Vaccine B.



## BBD-022 - ANTIBODY RESPONSE IN SERUM AND COLOSTRUM AFTER PRE-FARROWING VACCINATION WITH A MULTIVALENT ETEC E. COLI VACCINE ON A MULTIPLYING FARM

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### Objectives

This study aimed to evaluate the antibody profiles in serum and colostrum in gilts vaccinated with NEOCOLIPOR<sup>®</sup>, an hexavalent *E. coli* vaccine registered for the passive immunization of piglets against neonatal enterotoxigenesis.

### Material and Methods

The study was carried out on a multiplying farm where no noticeable mortality or clinical signs due to enteric disease in neonates had been reported. The routine vaccination program in force on the farm included the immunization of breeders with another *E. coli* vaccine. Eighty-one healthy gilts from 9 consecutive breeding batches were randomly allocated into two experimental groups: vaccinated (n=41) and unvaccinated (n=40). Within each farrowing batch, the same number of gilts were allocated to each experimental group. Vaccinated gilts were primo-immunized in their first gestation 5 and 2 weeks before farrowing and boosted 2 weeks before second farrowing. Blood samples and colostrum samples were taken throughout the study and assayed for antibody levels by specific agglutination antibody tests against K88-F4 (F4ab, F4ac, F4ad); K99-F5; 987P-F6 and F41.

### Results

Fifty-three gilts were monitored until the completion of their second gestation. Culling was mainly due to selection and was balanced between groups. At the beginning of the study, the gilts showed low anti-K88 antibody titers contrary to the other valences. Sera: Vaccinated gilts showed for each valence a antibody increase as early as after the first injection ( $p < 0.01$ ) and remained higher than unvaccinated animals at first farrowing ( $p < 0.01$ ). Following booster injection, K88-, K99- and 987P- antibody levels were higher ( $p < 0.01$ ) and a numerical difference was observed in F41 antibody response ( $p > 0.05$ ) at second farrowing. A definite increase of colostrum antibody levels was observed in vaccinated breeders at both farrowings for all tested valences ( $p < 0.05$ ).

### Conclusion

The evidence of NEOCOLIPOR potency for a significant colostrum immunity increase was demonstrated towards various components of the vaccine.



## BBD-023 - OUTBREAK OF MYCOPLASMA HYOSYNOVIAE: CLINICAL PRESENTATION AND DIAGNOSIS

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### Background and Objectives

In a farm placed in northern Italy a serious problem of lameness was observed. 30-40% of animals between 80 and 100 days-old showed clear lameness of front or back legs, not always associated to swelling of the affected part. Stiffness, decreased appetite or fever were never observed. Laboratory testing allowed the identification of *Mycoplasma hyosynoviae* as the cause of the disease.

### Materials and Methods

Three swine carcasses were sent to the Diagnostic Service of the IZSve, placed in Pordenone, for post mortem examination. Gross pathology lesions were evaluated and different specimens were collected to perform bacteriology test (Blood Agar, Eosin Methylene Blue Agar, and Chocolate Agar), specific PCR for *Haemophilus parasuis*, *Mycoplasma spp.* cultivation and histopathology. The M.I.C. (Minimum Inhibitory Concentration) was assessed for two *M. hyosynoviae* isolates to evaluate the performance of different drugs.

### Results

The gross pathology lesions were fibrinous synovitis of the metacarpal and metatarsal region, swollen appearance of the hock, the elbow and the carpal region, hemorrhage of the coxo-femoral joints associated to hyperemia of the ligament of the head of the femur and chronic synovitis between the superficial and deep flexor tendon. The histopathology results confirmed the presence of a fibrinous purulent inflammation of the tissues. All the bacteriology exams resulted negative, as well as the *Haemophilus parasuis* PCR. *Mycoplasma spp.* cultivation was performed from 9 samples and 7 resulted positive. The 16s-rDNA-PCR-DGGE allowed the detection of a single *Mycoplasma* species, which was identified as *Mycoplasma hyosynoviae*. The M.I.C. results showed that the isolates were susceptible to most of the tested drugs.

### Discussion & Conclusion

Basing on the reported results, the clinical case can be related to *Mycoplasma hyosynoviae* infection. As this infection showed a relapsing behavior, despite the antibiotic treatment, thus the application of an inactivated autologous vaccine is under evaluation.



## BBD-024 - EVALUATION OF THE EFFICACY OF A VACCINE AGAINST ACTINOBACILLUS PLEUROPNEUMONIAE. CLINICAL OUTCOME, MORTALITY AND PLEURITIS LESIONS AT SLAUGHTERHOUSE

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Vaccination is one of the major tools for prevention and reduction of the economic losses due to pleuropneumoniae. This study aims at assessing the effects of vaccination against *Actinobacillus pleuropneumoniae* (APP) on mortality and pleuritis at slaughterhouse in comparison with non-vaccinated (controls).

A randomised, controlled and blinded study was performed in a farrow to finish 1200-sow herd with a history of Pleuropneumonia based on the occurrence of acute disease associated with isolation of the microorganism, high incidence of dorso-caudal pleural lesions in slaughter pigs and no previous use of specific vaccination. Five-hundreds piglets were randomly assigned to the two groups, vaccinated and control. The animals of both groups were housed and managed in the same barn. The piglets in the test group were vaccinated with COGLAPIX® (Ceva) twice at 7 and 10 weeks of age. Mortality, body weight at admission, at 24 weeks of age and prior to slaughter were recorded. Lung lesions (pleuritis and pneumonia) were recorded at slaughterhouse by SPES and Madec's grids, respectively. Moreover, blood samples were collected from 20 individually identified ear-tagged piglets/group at first vaccination (7 weeks of age), 4 weeks after the second vaccination (14 weeks of age) and before slaughter for serology to APP, PRRS, SIV and *M. hyopneumoniae*.

The diagnosis of Pleuropneumoniae was confirmed by the isolation and serotyping of App (serotype 1 and 9). In vaccinated group, mortality associated with App isolation was reduced by 66% (7 vs 21 pigs; vaccinated vs control). Lung slaughter-checks revealed a statistically significant reduction of the dorso-caudal pleural lesions in vaccinated animals ( $p < 0.001$ ). Serological investigations also revealed that App infection occurred concurrently with PRRSV and *M. hyopneumoniae* infections.

This study confirms that two different serotypes of App can coexist in the same affected animals and that vaccination can significantly reduce mortality and specific lung lesions at slaughterhouse.



## BBD-025 - IS ENTEROCOCCUS HIRAE A PART OF THE NORMAL INTESTINAL FLORA IN NEWBORN PIGLETS?

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### Background & Objectives

Enteroadherent *Enterococcus* (*E.*) *hirae* has recently been put forward as a potentially emerging cause of neonatal porcine diarrhoea (NPD). Although *E. hirae* is known to be a part of the normal intestinal flora in older piglets, less is known about its presence in piglets <1 week of age. The aim of this study was to investigate whether *E. hirae* could be cultured from healthy newborn piglets and to obtain *E. hirae* isolates from healthy animals.

### Material & Methods

Rectal swabs were collected from ten healthy piglets <1 week of age and ten healthy piglets at nine weeks of age, as *E. hirae* previously has been detected in the latter age group. The pigs originated from a SPF herd unaffected by NPD. Samples were cultured for enterococci on Slanetz Bartley agar. Up to ten pink colonies from each sample were subcultured on horse-blood agar and analysed by MALDI-TOF-MS (Bruker Daltonics) for species identification.

### Results

In total, 170 presumptive enterococci isolates were analysed by MALDI-TOF. Identity on species level (score  $\leq 2$ ) was established for 125 isolates and 115 were identified as enterococci. *E. faecalis* was the most abundant species in both age groups (n=58).

*E. hirae* was found in six of the newborn piglets but was not the dominating species in any of the samples. Among the nine-week-old piglets, *E. hirae* was only detected in one pig. In total, 13 isolates of *E. hirae* were obtained from the healthy newborn piglets and six from the nine-week-old pig.

### Discussion & Conclusion

These results show that *E. hirae* can be detected in the faecal flora of healthy newborn piglets. Isolates from healthy piglets and enteroadherent *E. hirae* previously isolated from diarrhoeic piglets will now be subjected to comparative genetic analyses to investigate the possibility that only certain strains of *E. hirae* are associated with NPD.





## BBD-026 - ASSESSMENT OF LUNG LESIONS IN SLAUGHTERED PIGS FROM FARMS VACCINATED WITH DIFFERENT MYCOPLASMA HYOPNEUMONIAE VACCINES

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### Background

Vaccination of piglets against *Mycoplasma hyopneumoniae* (*Mh*) has become an effective way to reduce lungs lesions induced by *Mh* infection. Lung scoring at slaughterhouse is a valuable tool for the assessment of the respiratory health status. The aim of this study was to investigate the prevalence and extension of lungs lesions observed at slaughter in pigs vaccinated with either Hyogen<sup>®</sup> or other 6 commercial *Mh* vaccines or unvaccinated pigs.

### Material and Methods

Between January 2016 and September 2016 a total of 182 batches within 23.636 lungs from different farms in Spain were scored at the slaughterhouse for Enzootic pneumonia (EP)-like lesions and dorsocaudal pleurisy (A.p. -like lesions) using Ceva Lung Program scoring methodology.

### Results

Lungs from vaccinated pigs showed statistically lower lung lesions than lungs from unvaccinated pigs. Percentage of lungs with EP like lesions was 81% in non vaccinated and 51% in vaccinated pigs ( $p < 0.001$ ). The affected surface out of all lungs was 11.64% in unvaccinated pigs and 3.7% in vaccinated pigs ( $p < 0.001$ ). In unvaccinated pigs Appi index was 0.54 vs 0.33 and lungs with dorsocaudal pleurisy was 19% versus 12% ( $p < 0.001$ )

Lungs from farms vaccinated with Hyogen<sup>®</sup> showed statistically lower EP lesions (42.4% of bronchoneumonic lungs and 2% of affected surface out of all lungs) than average of other groups (60% and 5.3% respectively,  $p < 0.001$ ) and a decrease in the percentage of dorsocaudal pleurisy (*Actinobacillus pleuropneumoniae*-like lesion) 11% vs 13% and APPI index 0.30 vs 0.36.

### Conclusions

Vaccination of piglets against *Mh* reduced the severity of Ep-like lesions, pleurisy and APPI index in slaughtered pigs.

Lungs from farms vaccinated with Hyogen<sup>®</sup> showed less EP-like lesions, pleurisy and APPI index than the average from farms vaccinated with other vaccine.

This results suggests that control of *Mh* can help in the control of problems associated with *Actinobacillus pleuropneumoniae*.



## BBD-027 - IMPORTANCE OF SINGLE ANIMAL VACCINATION WITH A TOXOID VACCINE AGAINST EDEMA DISEASE

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### Background and objectives

A sow farm raises its piglets at different locations. One of them receives 450 piglets every three weeks. In September 2015 edema disease (ED) occurred at this site in six weeks old piglets. After the diagnosis, the farm began to vaccinate three week old piglets destined for this site with Ecoporc SHIGA. Subsequent, vaccinated groups were not affected by ED. In September 2016 however 32 piglets died of ED, as was confirmed via necropsy and microbiological analyses. The herd veterinarian suspected that the site may have received unvaccinated piglets. The event was reported as a pharmacovigilance case.

### Material and Methods

For the use in pharmacovigilance cases only, a serum neutralization test has been developed which can detect antibodies produced after vaccination against shigatoxin 2e. The interpretation of the test is at herd level. After correct vaccination with Ecoporc SHIGA it is expected that in about 80% of the pigs antibodies are detectable (experience of own field trials). For this case 11 sera of the dead piglets and 12 sera of healthy piglets were analyzed.

### Results

No antibodies could be detected in the sera of the dead piglets, while 11 of the 12 (92%) healthy piglets had antibodies against shigatoxin.

### Discussion

Based on these results, the question was raised, if the vaccination management in the farm was performed correctly. The most likely reason for the death of piglets is that they had not been vaccinated with Ecoporc SHIGA.

### Conclusion

This case study shows that Ecoporc SHIGA is a recombinant toxoid vaccine that has no influence on the dynamics of shigatoxin producing *E. coli* in a farm. In order to be protected against the effects of ED, it has to be ensured, that each individual piglet is vaccinated correctly.



## BBD-028 - INFLUENCE OF IN-WATER ADMINISTRATION OF TILMOVET® 250 MG/ ML ON THE DAILY WATER INTAKE OF PIGS

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Next to the stability and solubility, the palatability of a watersoluble veterinary product is of importance. A change in water consumption will influence the zootechnical parameters, but more important also the intake of the medication and consequently the clinical outcome. In this study, Tilmovet® 250 mg tilmicosin/ ml oral solution was administered in the drinking water of nursery pigs to control the influence on the daily water consumption.

928 Pigs, weaned at 21 days old, were housed in 2 completely identical nurseries at an initial temperature of 29° C, with a weekly decline of 1°C. The water was supplied by nipple drinkers, separated from the troughs and no remarkable wastage of water was observed. The first nursery was the non-treated control group (478 pigs). The second nursery (450 pigs) was treated with Tilmovet® at a daily dose of 16 mg/ kg bodyweight for 5 consecutive days from day 25 until day 29 after weaning. The mean bodyweight of the pigs was determined on days 1, 3 and 5 of the treatment period in order to ensure correct dosing. The daily dose was determined using the Dose Calculator and administered continuously over 24 hours by a proportioner. In both groups, each day the mean daily water consumption per pig was digitally registered.

The daily water intake of the Tilmovet® group showed exactly the same evolution as the non-treated group during the 5 treatment days. Following mean daily water intake (day -1 to 5) was respectively registered in the control and treatment group: 1.76, 1.78, 1.96, 2.12, 2.39, 2.32 l/day and 1.89, 1.88, 2.03, 2.17, 2.36, 2.22 l/ day.

Tilmovet® 250 mg/ ml administered via the drinking water of pigs at 16 mg/ kg bodyweight over 24 hours does not affect the daily water consumption, avoiding under or over dosing.



## BBD-029 - EFFICACY OF RHINIFFA T® FOR THE PASSIVE IMMUNIZATION OF PIGLETS USING AN ATROPHIC RHINITIS CHALLENGE MODEL

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### Introduction

Vaccination of sows is among the most effective strategies used for the prevention of progressive atrophic rhinitis (PAR) in piglets. The efficacy of RHINIFFA T® was tested in laboratory conditions.

### Material and Methods

Five SPF sows were included in the study from which two were primo-immunized in 2 injections with RHINIFFA T 7-8 and 2-3 weeks before the expected farrowing date. The remaining sows were left unvaccinated. Piglets born from two unvaccinated sows (NV-C, n=18) and piglets born from the vaccinated sows (V-C, n=17) were intranasally challenged with  $9.0 \log_{10}$  CFU of *Bordetella bronchiseptica* (Bb) strain at 2 days of age and with  $9.0 \log_{10}$  CFU of a toxigenic *Pasteurella multocida* type D (Pm) strain 3 days later. The remaining piglets were administered culture medium (NV-NC, n=11). A clinical monitoring was conducted until 6 weeks of age. Growth of piglets was monitored between 4 and 6 weeks of age. Necropsy was performed at 6 weeks of age for lesions evaluation and bacterial isolation.

### Results

Sneezing was observed in NV-C piglets few days following Bb challenge which increased following Pm challenge concomitantly to the appearance of cough. Clinical signs in these groups remained high. V-C piglets definitely show lower clinical signs which tended to disappear. Growth for NV-NC and V-C piglets was comparable (340g/day) and definitely higher than NV-C piglets (281g/day). At necropsy, pneumonia and severe to total destruction of nasal turbinates was observed in 44% and 88% of NV-C piglets respectively and in 6% and 0% of V-C piglets. In lungs, only Bb was reisolated from lungs of NV-C piglets (44%). In nasal cavities, Pm and Bb were reisolated in NV-C piglets in respectively 94% and 77% cases as well as in 23% and 47% in V-C piglets.

### Conclusion

This study showed the efficacy of RHINIFFA T for the prevention of PAR caused by Bb and toxigenic Pm.



## BBD-030 - INFLUENCE OF IN-WATER ADMINISTRATION OF VETMULIN® 450 MG/G ON THE DAILY WATER INTAKE OF PIGS

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Next to the stability and solubility, the palatability of a watersoluble veterinary product is of importance. A change in water consumption will influence the zootechnical parameters, but more important also the intake of the medication and consequently the clinical outcome. In this study, Vetmulin® 450 mg tiamulin hydrogen fumarate/ g was administered in the drinking water of nursery pigs to control the influence on the daily water consumption.

704 Pigs, weaned at 21 days old, were housed in 2 completely identical nurseries at an initial temperature of 29° C, with a weekly decline of 1°C. The water was supplied by nipple drinkers, separated from the troughs and no remarkable wastage of water was observed. The first nursery was the non-treated control group (478 pigs). The second nursery (226 pigs) was treated with Vetmulin® at a daily dose of 8.8 mg/ kg bodyweight for 5 consecutive days from day 25 until day 29 after weaning. The mean bodyweight of the pigs was determined on days 1, 3 and 5 of the treatment period in order to ensure correct dosing. The daily dose was determined using the Dose Calculator and administered continuously over 24 hours by a proportioner. In both groups, each day the mean daily water consumption per pig was digitally registered.

The daily water intake of the Vetmulin® group showed exactly the same evolution as the non- treated group during the 5 treatment days. Following mean daily water intake (day -1 to 5) was respectively registered in the control and treatment group: 1.76, 1.78, 1.96, 2.12, 2.39, 2.32 l/day and 1.86, 1.95, 2.15, 1.99, 2.13, 2.50 l/ day.

Vetmulin® 450 mg/ g administered via the drinking water of pigs at 8.8 mg/ kg bodyweight over 24 hours does not affect the daily water consumption, avoiding under or over dosing.



## BBD-031 - TOWARDS A BETTER UNDERSTANDING OF LEPTOSPIROSIS IN SWINE HERDS

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### Background and Objectives

Leptospirosis is a worldwide re-emerging infectious disease that can produce considerable reproduction losses in swine herds. However, diagnosis is difficult because of the limitations of diagnostic tests and absence of pathognomonic signs. The study objective was to determine a diagnostic algorithm at herd level with 4 different laboratory tests (MAT, PCR, ELISA, histology) and to investigate usefulness of kidney sampling at the abattoir.

### Material and Methods

Six French breeding-fattening units were purposively selected based on a strong leptospirosis suspicion. Sample size per herd was fixed to detect disease assuming a herd sensitivity of 95%, a prevalence of 10% and a high sensitivity diagnostic test. Differential diagnosis was performed for Parvovirus, Flu A, PRRS, PCV2. Data from 150 pigs and 112 aborted fetuses was analyzed with Excel.

### Results

Proportion of positive adults per herd varied between 26% and 65% for MAT $\geq$ 100 and between 4% and 35% for MAT $\geq$ 200. Bratislava was the predominant serovar. All herds were positive by PCR on kidneys except one. Kappa statistics showed a very low level of agreement ( $\kappa=0$ ) between PCR on kidneys and MAT. Four sera were ELISA positive. Histology revealed mild lesions of chronic nephritis and multifocal tubular necrosis on PCR positive kidneys without macroscopic lesions. Out of 11 litters following sow abortion, 8 were PCR positive with a proportion of positive fetuses per litter ranging from 11.7% to 31%. Differential diagnosis revealed positivity for Flu A and PCV2. Thus, *Leptospira* were circulating in the herds in which leptospirosis was strongly suspected.

### Discussion & Conclusion

When suspecting leptospirosis as abortion cause, as many fetuses as possible should be sampled per litter. Field test performances should now be investigated using latent class models. A larger study should also enable to better investigate possible interactions of *Leptospira* with other pathogens commonly identified in herds with reproductive disorders.



## BBD-032 - IN VITRO SUSCEPTIBILITY OF BRACHYSPIRA HYODYSENTERIAE AGAINST A COMBINATION OF VETMULIN® AND PAROFOR®

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Tiamulin is frequently used for treatment of swine dysentery, but sometimes the veterinarian is confronted with a decreased susceptibility. The aim of this study was to examine *in vitro*, the susceptibility of *Brachyspira hyodysenteriae* isolates for a combination of tiamulin with paromomycin, the active ingredients of respectively Vetmulin® and Parofor®.

Minimal Inhibitory Concentrations (MIC) were determined for 23 *Brachyspira hyodysenteriae* isolates using the agar dilution technique. The isolates were tested for two-fold dilutions of paromomycin (P) (0.5-128 µg/ml), tiamulin (T) (0.06-16 µg/ml) and for tiamulin (0.06-16 µg/ml) with a fixed amount of paromomycin (4 (TP4), 8 (TP8), 16(TP16) and 32(TP32) µg/ml respectively).

The range of MIC's was the same in the 5 tiamulin groups: <0.06→16 µg/ml. MIC<sub>50</sub> in the tiamulin and paromomycin group was respectively 4 and 64 µg/ml. In all tiamulin-paromomycin groups MIC<sub>50</sub> was lower, with a MIC<sub>50</sub> of 1 µg/ml for TP4, TP8, TP16 and 0.25µg/ml for TP32. When looking in detail, 12 isolates (52%) in the TP4 and 14 isolates (61%) in the TP8 group had a MIC which was more than two dilutions lower than with tiamulin alone. 15 isolates in TP16 (65%) and 17 isolates in TP32 (74%) had an MIC >2 dilutions lower compared to the tiamulin group. In the tiamulin group 9 isolates (39%) could be classified *in vitro* as susceptible (MIC ≤1 µg/ml), while in the TP4 group and the TP8,16 and 32 group respectively 15 isolates (65%) and 16 isolates (70%) were *in vitro* susceptible.

An increase of 66% of strains classified as susceptible to tiamulin could be recorded when adding 4 µg/ml of paromomycin. Since a paromomycin concentration was chosen lower than the MIC<sub>50</sub> for paromomycin (MIC<sub>50</sub> 64µg/ml, determined on 23 isolates), the activity of paromomycin itself cannot explain completely these results, suggesting a beneficial effect of combining both molecules.





## BBD-033 - TILDIPROSIN SUSCEPTIBILITY OF PASTEURILLA MULTOCIDA TYPE A, TYPE B AND ERYSIPELOTHRIX RHUSIOPATHIAE ASSOCIATED WITH ACUTE MORTALITY IN SWINE

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### Background & Objectives

*Pasteurella multocida* is a commensal microorganism of the upper respiratory tract of both domestic and wild animal species, and is the causative agent of numerous, important economic losses in swine. Haemorrhagic Septicaemia (HS) is an acute systemic infection caused by serotypes B and E strains of *P. multocida* that occasionally affects pigs. Outbreaks of HS have been reported in pigs in geographic areas as Asia, Australia and Europe (Ujvari et al., 2015). *Erysipelothrix rhusiopathiae* causes erysipelas in swine, other animals and humans. This bacterium has been isolated from sick and healthy animals and even from the environment they lived. In Spain, *E. rhusiopathiae* causes outbreaks of acute diseases in swine. Since swine erysipelas reappeared as a clinical problem in pig populations in Japan and the United States, it has been considered as a reemerging disease that contributes substantially to economic losses in the swine industry (Zou Y et al., 2015). The aim of this study was to determine in vitro tildipirosin susceptibility of *Pasteurella multocida* type A, type B and *Erysipelothrix rhusiopathiae* associated with acute mortality in swine.

### Materials & Methods

Twenty five strains of *P. multocida* type B, fifteen of *P. multocida* type A and twenty-one of *E. rhusiopathiae* previously isolated of clinical cases in southwest Spain were selected. All of the strains were tested for their antimicrobial susceptibility by the disk diffusion method according to the performance standards VET01–A4 of the CLSI against tildipirosin (Mastdisc, 60 µg).

### Results

Tildipirosin inhibited the growth of more than 80% of *P. multocida* type B strains and was effective in inhibiting the growth of 100% of the *P. multocida* type A and *E. rhusiopathiae* strains.

### Conclusion

tildipirosin is an effective alternative in the treatment of the major diseases causing acute mortality in pigs.



## BBD-034 - TILMOVET®: OPTIMAL ABSORPTION TO AVOID SUBOPTIMAL DOSING

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The absorption and distribution rate of a product in the body has a direct and critical impact on the clinical outcome of the treatment. Often veterinary products containing the same amount of active substance are considered as equivalent. This study was performed to compare the pharmacokinetic behaviour (total absorption and speed of absorption) of Tilmovet® 20% with another 20 % tilmicosin containing veterinary premix.

Twenty two pigs were orally administered 16 mg tilmicosin / kg bodyweight as a single dose in a cross over design. Plasma concentrations of tilmicosin were measured in each animal at 12 time points for 48 hours starting from time of administration. Quantification of tilmicosin in the plasma was performed with Liquid Chromatography tandem mass spectrometry (LC-MS/MS), compliant with principles of GLP and validated. Following parameters were calculated: area under the curve (AUCt), maximum reached plasma concentration (C max) and time required to reach maximum plasma concentration (Tmax). The results were statically analysed using ANOVA.

The following results were obtained for respectively Tilmovet® 20% premix versus the other premix: AUCt: 249,197 versus 222,579 µg min/L, Cmax 756.7 versus 629.6 µg/L and Tmax: 169.4 versus 213.4 min. Based upon these results, it can be stated Tilmovet® was supra-equivalent and reached values of 20.2% higher, in a time (Tmax) of 17% faster in comparison with the other premix.

In conclusion, this study showed Tilmovet® 20 % premix is constantly better and faster absorbed from the gastro-intestinal tract. Previous studies have shown the direct correlation between plasma and lung concentrations, the major target tissue for tilmicosin. This 20 % difference between both products is important to ensure sufficient tissue concentrations especially in diseased animals with a decreased feedintake. This explains the differences in clinical outcome between Tilmovet® 20% premix and the competitive premix often observed in the field.



## BBD-035 - IMPACT OF PARENTERAL ANTIMICROBIAL ADMINISTRATION ON THE STRUCTURE AND DIVERSITY OF PORCINE FECAL MICROBIOTA

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### Background and objectives

Antibiotic administration in swine production systems are used for the prevention, control and treatment of bacterial diseases. More recently concerns about the emergence of antibiotic-resistant bacteria (AMR) and the role of agricultural antibiotics in human AMR have been raised. The purpose of this study was to characterize the impact of antibiotics administration on the composition and diversity of the resident fecal microbiotas in pigs.

### Materials and Methods

Five antimicrobial treatment groups each consisting of four, eight week old piglets were treated with one of the antimicrobials (Tulathromycin, Ceftiofur Crystalline free acid, Ceftiofur hydrochloride, Oxytetracycline, and Procaine Penicillin G) at label dose and route. Individual fecal swabs were collected before antibiotics administration (day 0) and again on days 1, 3, 7, and 14. Genomic DNA was extracted, and the V1-V3 region of 16S rRNA gene was amplified and sequenced using Illumina- based sequencing.

### Results

The core fecal microbiome was dominated by *Firmcuties* and *Bacteroidetes*. Discriminant analysis showed pronounced microbial shift in the fecal microbiota after different antibiotics administration. The changes in the relative abundance of bacterial taxa at different sampling day in different antibiotic groups were temporal in nature and the interval needed for the fecal microbiota to be normalized after different antibiotics treatment varied according to different microbial species.

### Discussion

Based on our results, exposure to various antibiotics administration has distinct effects on the composition of the porcine fecal microbiotas with no significant effect on bacterial diversity. Understanding these effects is a critical step in designing comprehensive health management programs that optimize local immunity to minimize the disease and the need for antibiotics.



## BBD-036 - EVALUATION OF MYCOPLASMA SPP PREVALENCE IN A MULTISITE PRODUCTION FLOW IN ITALY

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### Background and Objectives

The aim of the present study was to improve the knowledge on *Mycoplasma spp.* distribution in different stages of an Italian 3 sites production system, with particular attention to *Mycoplasma hyopneumoniae* (MHYOP) and *Mycoplasma hyorhinis* (MHYOR). Pigs of this particular flow were not vaccinated for MHYOP.

### Materials and Methods

Samples were collected during a 15 months long period from pigs of different ages in different sites/stages of production. 35 suckling piglets and 28 weaned piglets were subjected to *post-mortem* evaluation and conjunctival, nasal and lung swabs were collected. 9 to 10 months-old finishers were scored at the abattoir and samples were collected from lungs showing lesions referable to *Mycoplasma spp.* infection. MHYOP PCR and *Mycoplasma spp.* cultivation from all collected samples were performed.

### Results

Macroscopic lung lesions were found in 20/35 suckling piglets and the most common ones were bronchopneumonia and pleuritis. 25/28 weaned piglets showed signs of bronchopneumonia and 27/271 of the lungs of finishers showed enzootic pneumonia lesions. Samples from the suckling piglets were negative for MYHOP PCR, but MHYOR was successfully cultivated from 23/103 samples. During the nursery period, only 1/28 sample tested positive for MHYOP PCR, but only MHYOR was isolated from 9/21 lungs. 19/27 samples from finishers were positive in MYHOP PCR and MHYOP was also isolated from 13 samples.

### Discussion & Conclusion

MHYOR was isolated in most of the samples collected during the suckling and nursery period. Its presence was not always connected with lung lesions, even if isolation rate was higher in lungs with pathological lesions. Despite the wide distribution in the first stages of production, no evidence of MHYOR was found from samples collected from finisher pigs. On the contrary MHYOP was detected *via* PCR and *via* cultivation from lungs with enzootic pneumonia lesions collected at the abattoir.



## BBD-037 - EFFICACY OF COLIPROTEC® F4 IN PIGLETS WITH POST-WEANING DIARRHEA DUE TO F4-EPEC IN AN ITALIAN FARM

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Post-weaning diarrhea (PWD), mainly caused by enterotoxigenic *Escherichia coli* (EPEC), remains a major cause of economic losses for the pig industry. Coliprotec® F4 is a live oral vaccine for active immunization of pigs against PWD caused by F4-EPEC.

This study investigate the efficacy of Coliprotec® F4 administered to piglets before weaning in a commercial pig farm in Italy.

This study was conducted in a 1600-sow farm with a history of PWD located in Northern Italy. The farm is a multisite operation with separated nursery and a 1-week batch farrowing system. Before the start of the study, PWD due to F4-EPEC was confirmed based on laboratory diagnostics. Full litters of pigs from one batch were randomly distributed into 2 groups at 18 days of age (0 dpv, days post-vaccination). *Group-A* (200 piglets) was vaccinated with Coliprotec® F4 at 0 dpv and *Group-B* (200 piglets) remained unvaccinated. Pigs were weaned a week after vaccination. Average daily weigh gain (ADWG), mortality, diarrhea and PWD-treatments were investigated by group during the 71-day nursery period.

Following vaccination with Coliprotec® F4, clinical signs of PWD decreased and PWD-specific antibiotic treatment was not needed in the *Group-A*. Colistin treatment was required in *Group-B* due to a PWD outbreak. Mortality was reduced in the vaccinated group (*Group-A*: 3.5%; *Group-B*: 8%). An improvement of 23 g/d in ADWG was shown during the first 3 weeks post-weaning for the vaccinated group, with an overall improvement of 53 g/d for entire nursery-period (*Group-A*: 364 g/d; *Group-B*: 311 g/d). Coliprotec® F4 vaccinated pigs were 3.6 kg heavier at the end of the nursery.

This study shows that Coliprotec® F4 is efficacious in reducing clinical signs and mortality due to PWD caused by F4-EPEC. In addition, pigs vaccinated with Coliprotec® F4 had better performance parameters as shown by an increased ADWG during the nursery.



## BBD-038 - INFLUENCE OF MATERNAL MICROBIAL COMMUNITIES ON THE MUCOSAL MICROBIOME OF NEONATAL PIGS

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### Background

Colostrum is vital to the newborn pig. Hence, cross-fostering is employed to equalize the number of piglet between litters ensuring colostrum intake for their survival and growth. However, little is known about its impact on the intestinal microbiome of the neonatal pig.

### Materials and Methods

Twenty-four piglets were enrolled in the study to determine the influence of maternal microbial communities on the mucosal microbiome of the young pig. Piglets were randomly assigned to 1 of 3 treatments according to colostrum source and postcolostral milk feeding for 21 days, as follow: treatment 1 (n = 8), received colostrum and post-colostral milk feeding from their own dam; treatment 2 (n = 8), received colostrum from foster dam and returned to their own dam for post-colostral milk feeding; and treatment 3 (n = 8), received colostrum and post-colostral milk feeding from foster dam. DNA was extracted from nasal, fecal, and gastrointestinal (GI) tract of the piglets and from colostrum, vaginal, and fecal samples of the sows.

### Results

Discriminant analysis revealed that bacterial communities varied with biogeographical location in the GI tract, with colon being the most diverse section. *Firmicutes* and *Bacteroidetes* were the dominant phyla in the GI tract of the young pig. Bacterial communities in both maternal colostrum and vaginal samples were significantly associated with those present in the GI tract, feces, and nasal passage of piglets. Treatment did not affect bacterial communities present in the piglet GI tract, however, the bacterial communities present in piglet fecal and nasal samples changed over time

### Discussion

Although cross-fostering did not impact microbial communities in the piglet, this study suggests an impact of colostrum and maternal influence on the development of the microbiome of the piglet.



## BBD-039 - CHARACTERIZATION OF COLISTIN RESISTANCE IN SALMONELLA TYPHIMURIUM 1,4 [5],12:i- ISOLATED FROM DIFFERENT SOURCES

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Monophasic *Salmonella* Typhimurium 1,4 [5],12:i- (mSTM), has been increasingly responsible for Salmonella outbreaks in Europe in the last fifteen years, with pig being considered as the main reservoir of the infection. The aim of the present work is to detect the presence of the plasmid-mediated colistin resistance genes, *mcr-1* and *mcr-2*, in isolates of mSTM of different origin, in order to detect the spread of plasmid-borne resistance determinants to humans via a zoonotic infection.

Two hundred and seven mSTM Italian isolates collected from 2013 to 2016 from pigs (n= 56), foods and environment (n= 43), humans (n= 108) were included in this study. The isolates were tested for the colistin susceptibility using the broth microdilution method (MIC), according to the European Committee on Antimicrobial Susceptibility Testing recommendations. Isolates classified as resistant were tested for the presence of *mcr-1* gene by PCR (Liu *et al.*, 2015). Isolates tested negative for *mcr-1* were further investigated for the presence of the *mcr-2* gene using PCR (Xavier *et al.*, 2016).

Forty-five isolates (45/207, 21.7%) were phenotypically resistant to colistin. Twenty two of them (22/56, 39,3%) were from pigs, 14 out of 108 (13%) from humans and nine out of 43 (20.9%) from food and the environment. The distribution of MICs did not show a bimodal pattern. Among the forty-four resistant isolates, ten, nine from swine and one of human origin, tested positive for *mcr-1* (10/45, 22.2%). No *mcr2+* isolates were detected.

Our data confirm the presence of *mcr-1* in colistin-resistant isolates of pig origin. Conversely, only one isolate from humans showed the presence of the *mcr-1* gene. In most cases, the phenotypic resistance was not linked to the presence of a plasmid-borne determinant. These results reinforce the idea that the selective pressure determined by the use of colistin in pig must be reduced..





## BBD-040 - ANTIMICROBIAL ACTIVITY OF A CITRUS EXTRACT AGAINST BRACHYSPIRA HYODYSENTERIAE

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### Background & Objectives

*Brachyspira hyodysenteriae*, an anaerobic spirochaete, is the main etiological agent of swine dysentery (SD). Nowadays, treatment and control of SD is increasingly difficult due to the emergence of antimicrobial resistance in *B. hyodysenteriae* together with the restrictions on the use of antibiotics in veterinary practice. So, there is an urgent need of new alternative products that can be used in the control of this disease. The aim of this study was to evaluate the antimicrobial activity of BIOCITRO<sup>®</sup>, a citrus extract commercialized as feed additive in the EU, against *B. hyodysenteriae* and its main mechanism of action.

### Material & Methods

Ten isolates of *B. hyodysenteriae* were used to assess the minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) of BIOCITRO<sup>®</sup> by broth microdilution method. Moreover, stationary phase cultures of two *B. hyodysenteriae* isolates were subjected for 90 min to four different concentrations of BIOCITRO<sup>®</sup> and compared with the untreated controls by flow cytometry (FC), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

### Results

MIC and MBC values ranged from 32 to 128 ppm. Membrane integrity was assessed by measuring the intake of propidium iodide by FC, showing a minimum of 35% and 76% damaged cells when exposed to 128 and 256 ppm of BIOCITRO<sup>®</sup>, respectively. Moreover, alterations of the bacterial shape were observed by SEM and changes on the cellular structure and composition were demonstrated in the medium infrared spectra by FTIR.

### Discussion & Conclusion

BIOCITRO<sup>®</sup> has a relevant bacteriostatic and bactericidal effect against *B. hyodysenteriae*. It induces damage in the membrane as well as relevant changes in the composition and structure of the bacterial cells. According to this results, BIOCITRO<sup>®</sup> seems to be an alternative to the use of antibiotics in the control of SD although further studies to investigate its efficacy in field conditions are need.



## BBD-041 - RESISTANCE TO COLISTIN IN ENTEROTOXIGENIC E.COLI ISOLATED FROM PIGS AFFECTED BY PWD AND ITS ASSOCIATION WITH PLASMID-MEDIATED RESISTANCE GENES

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Colistin is now considered as an ultimate line of refuge against multi-resistant bacterial pathogens. Last year, the report of a plasmid-mediated colistin resistance gene, *mcr-1*, in Enterobacteriaceae from humans, pigs, and retail meat in China raised concerns about the possible horizontal spread of this resistance. More recently, a novel plasmid-mediated colistin resistance determinant, *mcr-2*, was identified in Belgium. The aim of this study was to analyse the presence of plasmid-mediated colistin-resistance genes in *E. coli* isolated from pigs affected by Post Weaning Diarrhea (PWD) in 2016.

One hundred and twenty-five enterotoxigenic *E. coli* (ETEC) isolates collected from pigs affected by PWD in 2016 from Italy (n= 34), Belgium (n= 48) and Spain (n= 43) were included in this study. The isolates were tested for the colistin susceptibility using the broth microdilution method (MIC), according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. Isolates classified as resistant were tested for the presence of *mcr-1* gene by PCR (Liu *et al.*, 2015). Isolates tested negative for *mcr-1* were further investigated for the presence of the *mcr-2* gene using PCR (Xavier *et al.*, 2016).

Fifty-one out of 125 isolates (40.8%) were phenotypically resistant to colistin. The distribution of MICs showed a clear bimodal pattern. Thirty-two colistin-resistant isolates out of 51 (62.7%) tested positive for *mcr-1*, while only three, originated from two herds in Belgium, were *mcr2+*.

The presence of a discrete jump in the distribution of MICs is consistent with an acquisition of resistance through a genetic modification. We confirmed the presence of *mcr-1* in colistin resistant porcine isolates belonging to different European countries, while the presence of *mcr-2* was limited to Belgium. Taken together, these data suggest that pathogenic isolates, which are extensively exposed to antibiotics, can be used as early warning indicators of antibiotic resistance in pigs.



## BBD-042 - VACCINATION AGAINST ACTINOBACILLUS PLEUROPNEUMONIAE (APP); THE NEED TO CONSIDER MATERNALLY DERIVED ANTIBODIES

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### Background & Objectives

Virulent strains of *Actinobacillus pleuropneumoniae* are an important bacterial swine pulmonary pathogen. Although various vaccines provide high levels of protection, non-satisfactory results may occur due to maternally derived antibodies (mAbs) interference.

### Material & Methods

Due to an APP-history, all pigs in a farrow-to-finisher farm were vaccinated at 7 and 11 weeks of age with a commercially available APP subunit toxin-based vaccine (Porcilis® APP). Weaned pigs originated from a single source and were vaccinated against *M. hyopneumoniae* and PCV2. Mild coughing occurred consistently from 80kg Body Weight onwards. In September 2016, an acute APP outbreak affected two fattening units. For diagnostics, pigs were submitted for necropsy and nasal swabs were taken for SIV-PCR. In addition, 30 blood samples from 4 age groups (4-weeks (A), 7-weeks (B), 11-weeks (C) and 14-weeks (D)) were evaluated for APP-Abs by APX-IV ELISA (IDEXX®, IVD GmbH, Seelze-Letter).

### Results

Nasal swabs were negative for SIV. Macroscopically, pericardial effusion, firm non-collapsed lungs displaying no necrotizing lesions and pleurae covered with mild white-yellowish fibrin were present. An APP serotype 9/11 was isolated from lung tissue while PCRs for SIV, HPS and PRRS (EU/NA/HP) turned out negative. All sera were positive for APP-Abs (APX-IV ELISA, IDEXX®). Group A displayed highest S/P% values ranging from 58 to 180 (mean: 148.2), followed by group B (S/P%: 64 to 174; 141.6 (mean)), C (S/P%: 56 to 117; 85.4 (mean)) and D (S/P%: 52 to 117; 98.6 (mean)).

### Discussion & Conclusion

APP Serotypes 9/11 tend to be highly virulent. Laboratory testing confirmed high levels of mAbs (group A and B) that apparently interfered with the efficacy of the vaccine, which was administered correctly. In this case, herd mortality stopped following antibiotic treatment. No relapse occurred nor did any subsequent respiratory disorder occur after vaccination was pushed back to 10- and 14-weeks of age.



## BBD-043 - ANALYSIS OF SOCK, SWAB AND FECAL SAMPLES FOR PRESENCE OF SALMONELLA FROM SOW FARMS IN THE NETHERLANDS

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Salmonella on farm can be detected by different types of samples: sock, swab and fecal. Here we describe the results of 281 samples collected at 15 different sow farms.

From corridors only a sock and dust sample were taken. In a compartment, a pooled fecal sample (mixed feces from the floor of each pen, max 50 ml total) was taken additionally. For each set of samples, new gloves and overshoes were used to prevent cross-contamination. For socks we used non-sterile gauze tissue and for the dust samples we used Swiffer® pads. All samples were analyzed via a standard bacteriological technique for Salmonella and suspicious colonies were identified with respect to serotype.

In total, 42% of the samples were positive for *Salmonella*, of which 58% was *S. Typhimurium* (ST). Out of 117 socks 54 were *Salmonella* positive (30 were ST); of 89 dust samples 43 were positive (25 were ST) and of 66 fecal samples 17 were positive (10 were ST). Relative number of ST out of *Salmonella* positive samples was comparable for all sample types. Dust tended to be more often positive than socks in corridors (54% vs. 43%). Socks tended to be more often positive in compartments (49% vs. 44%). Within compartments, fecal samples were always negative if the sock was negative. Only in two samples the fecal sample was positive but the dust sample was negative (however in those cases the sock was positive as well). Dust was positive in 4 compartments where the sock was negative and the sock was positive in 7 compartments where the dust was negative.

We conclude that sock and dust samples are good matrixes to detect *Salmonella* in sow herds. Taking a pooled fecal sample, when taking a sock and dust sample from the same compartment, has no added value.



## BBD-044 - DETECTION OF BRACHYSPIRA HYODYSENTERIAE AND OTHER BRACHYSPIRA SPP. IN INGESTA-MUCOSA SAMPLES AND FAECES FROM SLAUGHTERED PIGS

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### Background & Objectives

The diagnosis of *Brachyspira hyodysenteriae* (BH) using pig faeces from herds with subclinical swine dysentery (SD) is challenging, thus requesting optimized sampling strategies. Use of ingesta may increase the detection rate of BH and hence improve diagnosis. The aim of this study was to determine the detection rate of BH and, additionally, of other *Brachyspira* spp., in the colon, caecum, and rectum of slaughtered pigs and to correlate findings to intestinal lesions, ingesta/ faecal consistency and specific time parameters.

### Material & Methods

Ten intestines per herd were randomly selected from 20 herds with previous detection of BH. Macroscopic lesions and ingesta/ faecal consistency were scored. Three samples per intestine (ingesta-mucosal scraping from the apex coli (A), the caecum (C), and faeces from the rectum (R)) were obtained, cultured for selection of *Brachyspira* spp. finally identified by MALDI-TOF MS. The times (T) between slaughter, pathological examination, and seeding the first *Brachyspira* selective agar, and previous detection of BH (D) were recorded. Differences with a  $p < 0.05$  were considered being significant.

### Results

BH was detected in 16 pigs (1-5/ herd) originating from 7 herds (only with T to D  $> 2$  years,  $p < 0.01$ ). Within the 23 positive samples, more intestinal samples (A and/or C) were BH positive than faeces ( $p = 0.04$ ). In BH positive pigs, SD like lesions were detected only at A ( $n = 4$ ). In general, the lesions were correlated to BH detection ( $p = 0.04$ ).

In 63 pigs from 17 herds other *Brachyspira* spp. were isolated, in particular from colon samples ( $p = 0.02$ ).

### Discussion & Conclusion

Lesions were related to BH detection but were rare (subclinical infection). BH, as well as other *Brachyspira* spp., was detected more frequently in intestines than in faeces. Additionally, slaughter material is easily accessible circumventing necropsies. In conclusion, the use of colon samples will result in better SD diagnosis.



## BBD-045 - EVALUATION OF THE INCIDENCE OF ATROPHIC RHINITIS BY THE EVALUATION OF LESIONS IN THE NASAL TURBINATES

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### Background&Objectives

Atrophic Rhinitis (AR) is present in every country with pig production. Even so, it is not easy to diagnose as the symptoms are very unspecific and because of the limited significance of a negative laboratory result, which does not always equate to absence of the disease. For this reason, evaluation of the lesions in the nasal turbinates has become established as one of the principal methods of evaluating the disease as it is simple to perform and obvious when there are lesions. The objective of this study was to evaluate the incidence of AR in different countries by evaluation of the lesions in nasal turbinates.

### Material&Methods

During the period July 2015/Oct 2016, 25 farms in Europe (Spain, Italy and Portugal) and Latin America (Mexico and Peru) that had not been vaccinated against AR and that had a respiratory history consistent with AR were analysed. Thirty animals per farm on average were selected in the slaughterhouse and the nasal turbinates were evaluated in accordance with the guidelines of the European Pharmacopoeia.

### Results

Overall, 90% of the nasal turbinates that were analysed had lesions, with an average lesion grade of 4.36/18, with 11.05/18 being the highest grade observed and 0.5/18 the lowest. If the results are analysed separately by zone (Europe and Latin America) the results are very similar; in Europe, 90% of the samples had bone destruction in the turbinates, the average lesion being 4.43/18, the highest value 11.18% and the lowest 0.53/18. In Latin America, 92% of the samples were affected, with an average lesion grade of 4.87/18, the highest and lowest being 11.36/18 and 0.53/18, respectively.

### Discussion&Conclusions

The results obtained in this trial show that AR remains a topical and significant disease in PRDC, with evaluation of the nasal turbinates key to the detection of its presence.



## BBD-046 - AUTOGENOUS VACCINES AGAINST STREPTOCOCCUS SUIIS ADMINISTERED TO PIGLETS AT ONE WEEK OF AGE: A STUDY TO ASSESS SAFETY AND EFFICACY

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### Background & Objectives

Vaccination is a major strategy to reduce the impact of *Streptococcus suis* infection and to decrease antibiotics usage. In France, autogenous vaccines are widely used on sows. The passive protection it provides has been described but may not last long enough to cover all the post-weaning period. Therefore, an early and active immunization of piglets with a one-shot vaccine needs to be explored.

### Material & Methods

In 2015, the study was realized in a 210 sow farrow-to-finish farm where clinical cases (serotype 9) between 6 to 7 weeks of age (WOA) have been observed since 2000 and where prevalence had increased up to 8%. 551 piglets of 1 WOA were randomized, from each litter, into three groups A, B and C of 183, 187 and 181 animals respectively. Group A was vaccinated with a two-shot vaccine at 1 and 4 WOA, group B with a one-shot vaccine at 1 WOA and group C received physiological water at 1 WOA. Local and general safety was recorded after vaccination. From 1 to 12 WOA, clinical signs and mortality were monitored.

### Results

**Safety:** only 2 piglets vomited within the hour following the vaccination in group B and C. **Efficacy:** Only one mortality due to *S. suis* (serotype 9) was observed (group B) and no clinical cases have been recorded.

### Discussion & Conclusion

This study shows a good safety of autogenous vaccines on piglets at one week of age. The significant reduction of the number of clinical cases in the non-vaccinated group compared with the period before the study suggests that the vaccination of even only a part of the animals within a batch contributes to limit the horizontal contamination and thus the clinical cases.





## BBD-047 - EFFECT OF ORAL COBALAMIN SUPPLEMENTATION ON MITOCHONDRIAL VITAMIN B12 STATUS IN PIGS VACCINATED OR NOT AGAINST LAWSONIA INTRACELLULARIS

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### Background & Objectives

Pigs with subclinical ileitis have decreased production performance due to *L. intracellularis*-infection and a reduced nutrient (e.g., vitamin) absorption by immature enterocytes in the distal small intestine. The majority of cobalamin (vitamin B<sub>12</sub>) is absorbed at the ileum. Our aim was to evaluate vitamin B<sub>12</sub> status in vaccinated and non-vaccinated pigs with ileitis before and after oral cobalamin supplementation and fecal *L. intracellularis* genome fragments in pigs.

### Material & Methods

Fifteen pigs each from a selected farm with confirmed *L. intracellularis*-infection were randomly assigned to four different groups (vaccinated [vacc./non-suppl.], non-suppl., vacc./suppl., non-vacc./non-suppl., and non-vacc./suppl.). Corresponding pigs were administered an avirulent *L. intracellularis* live vaccine or cobalamin orally (0.2 mg/kg feed) from day 8 to 21. Fecal and serum samples were obtained on day 0, 7, 14 and 21. *L. intracellularis* concentration was determined by qPCR test on feces. Cobalamin and methylmalonic acid (MMA) concentrations, which reflects the availability of cobalamin within the mitochondria, were measured in serum. A MANOVA model was used to evaluate vitamin B<sub>12</sub> status among the four groups of pigs and correlation analysis between serum cobalamin, MMA concentration and fecal *L. intracellularis* genome fragments.

### Results

Concentrations of serum cobalamin and MMA differed between non-vacc./suppl. (increasing) and remaining three groups of pigs ( $p=0.0113$ ) and between non-vacc. and non-vacc./suppl. (increasing) and remaining two groups of pigs ( $p=0.0131$ ), respectively. A correlation was observed between serum cobalamin and MMA concentrations ( $\rho: -0.38$  [95%CI: -0.49 to -0.27];  $p<0.0001$ ) and serum MMA concentrations and fecal *L. intracellularis* genome fragments ( $\rho: 0.16$  [95%CI: 0.03 to 0.29];  $p=0.0106$ ).

### Discussion & Conclusion

Cobalamin supplementation over two weeks affects serum cobalamin and MMA concentrations in non-vacc. and non-vacc./suppl. groups of pigs. Whether supplementation of cobalamin can be beneficial for non-vacc. pigs or serum MMA a predictor for subclinical *L. intracellularis* infection in pigs warrants further investigation.



## BBD-048 - ACTINOBACILLUS PLEUROPNEUMONIAE ERADICATION IN A FARROW-TO-WEAN FARM, USING MARBOFLOXACIN THERAPY AND STRICT BIOSECURITY MEASURES

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### Introduction

*Actinobacillus pleuropneumoniae* (APP) is often present in a sub-clinical form in production farms, but outbreaks may occur regularly, causing major economic losses. In France, 2 serovars of biovar 1 cause the most severe damage: serovars 1 and 2. Complete eradication of the disease is difficult due to the long-term persistence of the bacteria in tonsil crypts of infected animals. This clinical case aims to show that APP eradication is still possible, in specific conditions, without depopulation.

### Material and methods

This eradication project was conducted in a farrow-to-wean farm. 28 day-piglets were weaned every 3 weeks, and sent to fattening units at 5 weeks of age. Gilts had come from a new origin for 2 deliveries. As the farmer wished to produce his own gilts, sanitary status of the farm was investigated: the farm turned out to be APP B1S1 positive (as well as other serovars except serovar 2), yet at a sub-clinical level, justifying its eradication from the herd. All the animals of the farm (gilts, sows, and weaned piglets) were treated with a one-shot marbofloxacin. Sow herd was divided in 3 groups regarding their supposed sanitary status towards APP. APP monitoring was conducted monthly, sampling 20 sentinel gilts from groups 1 and 2, until only group 1 sows remained. 30 pigs older than 140 days were then sampled on each fattening unit.

### Results

During all the monitoring, all the gilts remained seronegative towards APP B1S1. Moreover, the other serovars present at the beginning were also eradicated. At the end of the monitoring, a last sampling was done, on 30 randomly selected sows: all remained seronegative, and fattening units were tested negative too. Eradication of APP was concluded to be a success.



## BBD-049 - DEVELOPMENT OF A NEW SWINE MYCOPLASMA MULTIPLEX QPCR

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### Background & Objectives

In swine 3 Mycoplasma species are deemed to be important, *Mycoplasma hyopneumoniae*, *Mycoplasma hyosynoviae* and *Mycoplasma hyorhinis*. This paper is describing the development of a new multiplex Mycoplasma qPCR which detects all 3 species.

### Materials and methods

#### Primer/Probe

Specific primers for *M.hyopneumoniae*, *M.hyosynoviae* and *M.hyorhinis* were designed. To these specific primers probes were designed for detection of qPCR products. An internal control primer/probe set was used for excluding inhibition.

#### Samples

For evaluation of sensitivity and specificity, reference strains for the specific Mycoplasma's, other Mycoplasma species, other common agents in swine and field samples with a known qPCR status were used. Specific plasmids were used to determine the Lower Limit Of Detection (LLOD).

#### Methods

DNA extraction was done by using a spin column based method. qPCR was performed according to BioChek's protocol. Data was quantified by using C<sub>q</sub> values.

### Results

In total 516 tests were performed to check specificity, no false positive results were obtained.

68 Field positive samples (oral fluids) were used for evaluating sensitivity. Based on these results sensitivity for *M.hyopneumoniae* was 98.2%, for *M. hyosynoviae* 100% and for *M.hyorhinis* 82.4% respectively. For the LLOD plasmids were used and LLOD's were for *M.hyopneumoniae* <100 copies/reaction, for *M.hyosynoviae* <50 copies/reaction and for *M.hyorhinis* <50 copies/reaction.

### Discussion and Conclusion

The new developed multiplex qPCR makes it possible to detect all 3 relevant Mycoplasma species in one single run. Both specificity and sensitivity are high. Sensitivity itself will depend on extraction methods used. With the low LLOD values the qPCR will start detection below clinical relevant levels. Combining the 3 Mycoplasma species in one qPCR will save time and effort for evaluation of Mycoplasma in swine.



## BBD-050 - QUANTIFYING THE GROWTH OF CHLAMYDIA SUIS IN CELL CULTURE USING HIGH-CONTENT MICROSCOPY

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*Chlamydia suis* is spreading in pig farming. Isolation of *Chlamydia suis* in cell culture is crucial for the generation and characterization of new isolates. However, isolation of *Chlamydia suis* from field samples is fastidious. Therefore, we exploited high-content microscopy to quantify the growth of 3 *C. suis* strains (H7 conjunctival isolate, R24 respiratory isolate and S45 intestinal isolate) in different cell lines being McCoy (Mouse fibroblast cells, CRL-1696 American Type Culture Collection), Vero (African Green Monkey kidney cells, CRL-1586, ATCC), BGM (Buffalo Green Monkey kidney cells, ATCC), IPEC-J2 (Intestinal porcine epithelial cells) and SK-6 (Swine kidney cells, both obtained from Eric Cox, Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University), and Caco-2 cells (Human colon adenocarcinoma cells, HTB-37, ATCC). Cells were fixed with methanol and stained for *Chlamydia* using the Imagen™ immunofluorescence staining, containing an anti-LPS monoclonal antibody directly conjugated to fluorescein isothiocyanate (FITC), Evan's Blue pan cellular counterstain and DAPI. A fully automated inverted Nikon Ti widefield fluorescence microscope (Nikon Instruments) was used, equipped with motorized XYZ stage, filter cube turret and shutters. Samples were magnified with a 403 Plan Fluor oil objective (numerical aperture of 1.3) and images were acquired with an Andor Ixon EM-CCD camera, yielding a pixel size of 0.276 mm/pixel.

We found that the cell line yielding optimal propagation of *Chlamydia suis* differed among isolates, and we identified cell lines outperforming those routinely used for chlamydial isolation. We conclude that adaptation of the propagation procedure to the origin of the putative field isolate is highly recommended to improve the recovery rate. The high-content approach that has been optimized in this study lends itself for fast screening of cell lines to validate the obtained results on clinical samples and to cultivate characterized *C. suis* isolates.



## BBD-051 - THE IMMUNE RESPONSE AGAINST CHLAMYDIA SUIS GENITAL TRACT INFECTION PARTIALLY PROTECTS AGAINST RE-INFECTION

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### Background

*Chlamydia suis* is spreading in commercial pig production and causes important economic losses. Currently, *C. suis* infections are mainly associated with conjunctivitis and reproductive disorders in sows and boars.

### Objectives

The aim was to reveal the characteristics of genital *C. suis* infection and re-infection in female pigs by studying the immune response, pathology, replication of chlamydia in the genital tract and bacterial excretion.

### Methods

Pigs were intravaginally infected and re-infected with the *C. suis* reference strain S45.

### Results

S45 is pathogenic for the urogenital tract. *Chlamydia* replication occurred throughout the urogenital tract, causing inflammation and pathology. The infection elicited both cellular and humoral immune responses. Compared to the primo-infection of pigs with *C. suis*, re-infection was characterized by less severe macroscopic lesions and less chlamydial elementary bodies and inclusions in the urogenital tract. This indicates the development of a certain level of protection following the initial infection. Protective immunity against re-infection coincided with higher chlamydia-specific IgG and IgA antibody titers in sera and vaginal secretions, higher proliferative responses of peripheral blood mononuclear cells (PBMC), higher percentages of B lymphocytes, monocytes and CD8+ T cells and upregulated production of IFN- $\gamma$  and IL-10 by PBMC.

### Conclusions

Although *C. suis* is often still considered as an insignificant pathogen of pigs, it was demonstrated to be a primary pathogen of the urogenital tract. Furthermore, we established an experimental challenge model, suitable for further pathological and immunological investigations and will probably also be useful for studying vaccine development.



## BBD-052 - ASSESSMENT OF CHLAMYDIA SUIIS INFECTIONS IN PIGS AND PIG FARMERS

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*Chlamydia suis* infections are spreading in pig farming in Europe. The infection can lead to conjunctivitis, pneumonia, enteritis and reproductive failure. Currently, the knowledge on the zoonotic potential of *C. suis* is limited. Moreover, the last decades, porcine tetracycline resistant *C. suis* strains have been isolated, which interfere with treatment of chlamydial infections. In this study, the presence of *C. suis* was examined on nine Belgian pig farms, using *Chlamydia* culture and a newly developed *C. suis* specific real-time PCR in both pigs and farmers. In addition to diagnosis for *C. suis*, the farmers' samples were examined using a *Chlamydia trachomatis* PCR. Additionally, the *Chlamydia* isolates were tested for the presence of the *tet(C)* resistance gene. *C. DNA* was demonstrated in pigs on all farms, and eight of nine farmers were positive in at least one anatomical site. None of the farmers tested positive for *C. trachomatis*. *Chlamydia suis* isolates were obtained from pigs of eight farms. Nine porcine *C. suis* isolates possessing a *tet(C)* gene were retrieved, originating from three farms. Moreover, *C. suis* isolates were identified in three human samples, including one pharyngeal and two rectal samples. These findings suggest further research on the zoonotic transfer of *C. suis* from pigs to humans is needed.



## BBD-053 - EDEMA DISEASE IN CZECH REPUBLIC

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Higher occurrence of edema disease(ED) of swine in Czech Republic was observed over the last 2-3 years. We can't precisely define the exact causes of high incidence of ED, it's a polyfactorial matter, but significant role plays the import of asymptomatic pigs, especially gilts. In last 5 years about 70% of Czech farms were repopulated, especially with Danbred and then with Topigs and PIC. I estimate that in last 2-3 years approx. 15% of farms were affected.

ED was tracked on 7 farms. The farms are marked A-G, farms A-F are one site or two site production system. The biggest farm has 1500 sows, the smallest one 6 sows, the other farms have 250-450 sows. Farm F is a fattening house with capacity of 3600 finishers. The diagnosis was made through clinical examination, autopsy and laboratory analysis-microbiology, ATB sensitivity and E.coli virulence factors determination.

Farms A-G: the outbreaks of ED were during the nursery period, at the earliest in 2 weeks and at the latest in 6 weeks after weaning depending on the time of ZnO withdrawal. In some cases there were changes of feed formulation or feed supplier. Farm F: the outbreaks occurred 10-14 days after pigs' arrival, on this farm were mixed pigs from E.coli Stx2e positive and negative farms. Clinical signs and pathological findings were typical of ED; in all cases were confirmed strains of E.coli F18 producing shigatoxin (Stx2e). Colistin was the medicine of first choice however the efficiency was after 2-3 days, we observed some relapses and there was resistance to colistin on one farm. Vaccine with Stx2e toxoid (one dose) was very efficacious. On one farm was administered simultaneous vaccination of this vaccine and PCV2 vaccine and it proved to be less effective.





## BBD-054 - EFFECT OF ILEITIS ORAL VACCINATION AGAINST LAWSONIA INTRACELLULARIS ON ANTIBIOTIC USE REDUCTION AND PERFORMANCE IMPROVEMENT IN AN IBERIAN PIG FARM

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### Introduction

Porcine Proliferative Enteropathy (PPE) caused by *Lawsonia intracellularis* is an enteric disease of pigs affecting most of the Spanish farms. Different antimicrobials can be used for the treatment of PPE, but nowadays the meat industry and its costumers ask for a reduction of antibiotic use in animals. The aim of this study was to evaluate the efficacy of Enterisol<sup>o</sup> Ileitis (Boehringer Ingelheim Vetmedica GmbH) on antibiotic use reduction and performance improvement in an Iberian pig farm.

### Materials and Methods

This study was conducted in a farm with 2.500 Iberian sows. Pigs at fattening were suffering subclinical Ileitis confirmed by ELISA (IgG). A total of 2,880 fattening pigs were included in the study (1,440 non-vaccinated and 1,440 vaccinated with the oral live vaccine Enterisol<sup>o</sup> Ileitis Boehringer Ingelheim Vetmedica GmbH). Thus 4 weekly batches were vaccinated and 4 consecutive batches were kept unvaccinated. The pigs were orally vaccinated 3 weeks after weaning in the nursery unit. Pigs were raised under the same conditions and housed in pens with 40 pigs and were individually weighed weekly.

Data was analyzed using ANOVA with SPSS v 15.0 (SPSS Inc, Chicago, IL, USA) software.

### Results

The reduction on antibiotic use in the vaccinated group represents 74.6% compared to those animals that were not vaccinated ( $p < 0.05$ ).

The weight at the end of the fattening was 3.97 kg more in the vaccinated group ( $p < 0.05$ ); the ADG was 18g/day better in the vaccinated group too. The percentage of light animals was 35% less in the vaccinated group. There were no statistical differences in mortality between groups.

### Conclusions and Discussion

In this field experience, it was demonstrated that antibiotic use can be reduced with vaccination with Enterisol<sup>o</sup> Ileitis. Growing parameters were also better, so the vaccination is an alternative to the use of antibiotics in the growing of Iberian Pigs.



## BBD-055 - CAN WESTERN JACKDAWS BE A RISK TO FINNISH PIG FARMS BY TRANSMITTING BRACHYSPIRA HYODYSENTERIAE?

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### Background

During this millennium, western jackdaws (*Corvus monedula*) have become common in some urban and agricultural areas in Finland. This has raised a question whether they act as vectors of production animal pathogens. *Brachyspira*, including *B. hyodysenteriae*, have been isolated in many bird species. In Finland, dysentery caused by *B. hyodysenteriae* is very rare in pig farms thanks to the national control program. *B. pilosicoli* and some strains of *B. intermedia* can also cause diarrhea to pigs.

### Material & Methods

Total of 212 fecal samples were collected in 2014-2015 (September to February) in rural cities of Seinäjoki and Lahti. The fecal samples were obtained from eight separate flocks of jackdaws by disturbing them to take flight from their resting trees, and collecting the fresh droppings immediately in transport medium for anaerobic bacteria.

The samples were cultivated the next day on selective medium for *brachyspira*, and incubated for 4 - 12 days in anaerobic atmosphere at 42°C. Gram-stain confirmed *Brachyspira* sp. were isolated in 112/212 fecal samples (53%). Three PCR-tests specific for *B. hyodysenteriae*, *B. pilosicoli* and *B. intermedia* were performed on all primary cultures and isolates

### Results

None of the *Brachyspira* sp. isolates from jackdaws belonged *B. hyodysenteriae*, *B. pilosicoli* or *B. intermedia* according to the PCR-tests.

### Conclusion

In this study we found no evidence that the studied populations of western jackdaws could transmit pathogenic *brachyspira* to pig farms.



## BBD-056 - DIFFERENTIAL GROWTH KINETICS BETWEEN PATHOGENIC AND NON-PATHOGENIC MYCOPLASMA HYOPNEUMONIAE STRAINS: A COMPARISON BETWEEN TITRATION TECHNIQUES

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### Background and Objectives

Little is known on the growth kinetics of different *M. hyopneumoniae* strains differing in pathogenicity. Hence, the aims of the present work were twofold: (1) to describe the growth kinetics of *M. hyopneumoniae* pathogenic and non-pathogenic strains and (2) to follow their growth by different titration methods.

### Material and Methods

Reference strains 11 (ATCC®25095™) and J (ATCC®25934™) and 232 strain were used. Whereas strains 11 and 232 are pathogenic, strain J is considered non-pathogenic. An initial culture of each strain (resulting from a single colony) was 100-fold diluted in duplicates in a final volume of 45 ml of ATCC® Medium 1699. Cultures were grown statically at 37°C until reaching the senescence phase. Once the dilution was done, 1 ml of fresh culture was taken (D0), next at 12 hours after (D1/2) and afterwards, every 24 hours until the end of the study (D14). Samples were daily used for ATP luminometry, colony forming units (CFU) and colour changing units (CCU).

### Results

All strains showed growth curves with logarithmic, stationary and death phases, and reached similar maximal ATP titres ( $13.5 \pm 1.7$   $\mu$ M ATP/ml). Strain J reached earlier the stationary phase (D5) than 11 and 232 (D7). All strains culture's colour change (from red to orange) occurred at the middle-end of the logarithmic phase. The first colonies appeared along the logarithmic phase for J, 232 and 11 strains. Once in the stationary phase (D5-8 in J strain and D7-11 in 11 and 232 strains), all cultures turned yellow.

### Discussion and Conclusion

*M. hyopneumoniae* J strain grew faster than 11 and 232 strains. Both the ATP and CCU assays resulted in similar growth response curves whereas CFU probably undervalued the number of living cells.

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## BBD-057 - CINNAMALDEHYDE AS A FEED ADDITIVE IN THE CONTROL OF SWINE DYSENTERY

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Swine dysentery (SD), caused by *Brachyspira hyodysenteriae*, is characterized by a severe muco-hemorrhagic diarrhea in grower and finisher pigs. Treatment is complicated by high levels of antimicrobial resistance. Alternative ways of controlling SD are therefore needed. The essential oil compound cinnamaldehyde showed *in vitro* a marked antimicrobial effect against *B. hyodysenteriae* with inhibitory concentrations of 0.16 – 0.31 mM. This study investigated the effect of cinnamaldehyde on SD using experimental transmission.

A seeder model of *B. hyodysenteriae* infections in pigs was used to mimic infections as they occur on farm. Twelve diseased seeder pigs were selected from a herd with clinical SD. They were equally allocated in 4 pens. Per pen 3 seeder pigs were mixed with 7 receiver animals originating from a SD-free herd. All animals weighed approximately 25 kg and were fed a control diet. This diet was supplemented with 450 ppm cinnamaldehyde in 2 pens. The animals were monitored during 30 days. Excretion of *B. hyodysenteriae* and occurrence of clinical signs were monitored 3 times a week for all animals. Animals were then euthanized and the intestines were scored for macroscopic lesions.

Seeder animals excreted *B. hyodysenteriae* for an average period of 9 days. Five out of 14 receiver pigs on control feed developed clinical disease and excreted detectable amounts of *B. hyodysenteriae* for an average period of 5.6 days whereas 4 receiver animals on cinnamaldehyde supplemented feed developed clinical signs and excreted bacteria for an average period of 5 days. The amount of bacteria excreted, did not differ between groups. Colitis was observed in all pigs shedding *B. hyodysenteriae* but not in healthy pigs.

No significant effects of cinnamaldehyde on the clinical signs and shedding of *B. hyodysenteriae* were observed. These *in vivo* results do not confirm the *in vitro* observed antibacterial effect against *B. hyodysenteriae*.



## BBD-058 - CLINICAL IMPACT OF DEOXYNIVALENOL ON THE SEVERITY OF AN EXPERIMENTAL MYCOPLASMA HYOPNEUMONIAE INFECTION IN PIGS

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### Background & Objectives

The mycotoxin deoxynivalenol (DON) is highly prevalent on cereal in moderate climates and therefore pigs are often exposed to a DON-contaminated diet. Pigs are highly susceptible to DON. The maximum guidance EU level is 900 µg/kg, the lowest compared to other species. The symptoms upon DON intake depend on many factors such as dosage, duration of intake and animal factors. It is well known that intake of DON contaminated feed may lead to an altered immune response. This study aimed to determine the effect of oral administration of DON on the course of an experimental *M. hyopneumoniae* infection in weaned piglets.

### Material & Methods

Fifty *M. hyopneumoniae*-free piglets were assigned at 33 days of age (D0) to: 1) negative control group (NCG; n=5): not experimentally infected+control diet, 2) DON (DON; n=15): not experimentally infected+DON-diet (1800 µg/kg), 3) experimentally infected with 7 ml 10<sup>7</sup> CCU of highly virulent F7 (D8) and low virulent F1 (D9) *M. hyopneumoniae*+DON-diet (1800 µg/kg) (DONMHYO; n=15), 4) *M. hyopneumoniae* (MHYO; n=15): experimentally infected with both strains+control diet. Parameters under investigation were: respiratory disease score (RDS), macroscopic lung lesions (MLL), histopathology (HISTO), log copies of *M. hyopneumoniae* DNA detected by qPCR at D35/36 (euthanasia). The data were analysed with a non-parametrical Kruskal-Wallis test.

### Results

The results for each group are given in the order: NCG, DON, DONMHYO, MHYO. Values marked with a different superscript are significantly different (P<0.05). The results for RDS and MLL were 0<sup>a</sup>, 0<sup>a</sup>, 0.83<sup>b</sup>, 0.91<sup>b</sup> and 0<sup>a</sup>, 0<sup>a</sup>, 4.02<sup>b</sup>, 5.55<sup>b</sup>. For HISTO and log copies of *M. hyopneumoniae* DNA were 1.75<sup>a</sup>, 2.00<sup>a</sup>, 2.44<sup>b</sup>, 2.54<sup>b</sup> and 0.736<sup>a</sup>, 0.410<sup>a</sup>, 3.98<sup>b</sup>, 4.08<sup>b</sup>, respectively.

### Discussion & Conclusion

Under the present conditions, no effect was seen from oral administration of DON on the severity of an experimental *M. hyopneumoniae* infection. These results need to be confirmed under field conditions.



## BBD-059 - POST-WEANING DIARRHOEA IN THE CZECH REPUBLIC, HUNGARY, POLAND AND ROMANIA: CHARACTERIZATION OF ESCHERICHIA COLI VIRULENCE FACTORS

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Post-weaning diarrhoea (PWD) caused mainly by enterotoxigenic *E. coli* (ETEC) remains an important swine disease resulting in economic losses. The objective of our study was to determine the prevalence of ETEC and its virulence factors in PWD cases in the Czech Republic (CZ), Hungary (HU), Poland (PL) and Romania (RO).

The study was conducted from September 2015 to December 2016. A total of 79 pig herds (22 in CZ, 16 in HU, 35 in PL, 6 in RO) showing clinical signs of PWD were selected for the study. Rectal swab samples from 5 diarrheic nursery pigs in acute phase were collected and submitted to the laboratory. Analysis consisted of routine bacteriology for *E. coli*, followed by DNA extraction and PCR testing for the presence of genes encoding adhesins (F4, F5, F6, F18, F41) and toxins (LT, STa, STb, EAST1 and Stx) using a multiplex PCR.

*E. coli* isolates were identified in 94% samples, ETEC isolates that carried genes for both fimbriae and toxins (LT, STa, STb or EAST1) were detected in 43 herds with PWD (54.4%; including 10 herds in CZ, 9 in HU, 18 in PL, 6 in RO). In 9 herds F4-ETEC was found as the cause of diarrhoea (20.9% of ETEC positive farms), whilst in 25 herds (58.1%; 6 herds in CZ, 5 in HU, 12 in PL, 2 in RO) *E. coli* isolates were classified as F18-ETEC. In 9 herds (20.9%) ETEC carrying both F4 and F18 genes was detected.

From our study we can conclude that both F4-ETEC and F18-ETEC were identified as a cause of PWD, but F18-ETEC isolates were more prevalent in these four monitored European countries. 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.



## BBD-060 - EFFECT OF INFECTION BY MYCOPLASMA SUIIS ON BLOOD PARAMETERS ON WEANED PIGLETS

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### Background & Objectives

*Mycoplasma suis* is epicellular pathogen affecting red blood cells with worldwide prevalence and growing importance. Infection is frequently subclinical and associated with weakness and anaemia in suckling and weaned piglets. Aim of this study was to investigate the effect of subclinical infection by *M. suis* on red blood cell parameters at weaning.

### Material & Method

5 litters from different parity sows (39 piglets) in total were included. Piglets were injected 1 ml by gleptoferron (200mg/ml) first day after born. *M. suis* infection was detected by species specific PCR protocol at weaning (28 days).<sup>1</sup> Selected parameters were established at the same time (hemoglobin- HGB, erythrocytes- RBC, haematocrit- HCT, mean corpuscular volume- MCV, mean cell hemoglobin- MCH, mean corpuscular hemoglobin concentration- MCHC and plasma iron- Fe (Mindray Hematology Analyzer BC-2800 VET). Two groups of piglets according the *M. suis* status were compared (A negative, B positive).

### Results

Fourteen piglets were identified as *M. suis* positive (B). Remaining 25 piglets were negative (A). Based on laboratory evaluation all selected red blood parameters were negatively affected in group B except MCHC and plasma iron level. Statistically significant difference was confirmed for Hb, HCT, MCV and MCH in positive piglets.

### Discussion & Conclusion

In this study we have confirmed negative effect of *M. suis* infection on important red blood parameters, despite the fact, that positive piglets did not show clinical signs of Iron deficiency anaemia (IDA). Hb levels of 80 g/L were categorized as borderline for IDA.<sup>2</sup> Ninety g/L is accepted as minimum for optimal development of fast growing genetics, therefore we expect negative effect *M. suis* subclinical infection on piglet performance.

### References

1 Wittenbrink et al., Vet Microbiology 2003

2 Gaddy et al., AASV 2012





## BBD-061 - COMPARISON OF AMOXICILLIN (VETRIMOXIN LA<sup>®</sup>) AND CEFTIOFUR TREATMENT PROGRAM OF EARLY *S. SUIIS* INFECTION

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### Background & Objectives

*S. suis* is considered as re-emerging pathogen and serotype 2 is associated with high mortality of piglets during the pre-weaning and nursery period. Early treatment is necessary in order to control clinical disease. The aim of the study was to compare the efficacy of injectable long acting amoxicillin (Vetrimoxin<sup>®</sup> LA) with long acting ceftiofur.

### Materials & Methods

Four hundred piglets randomly selected after birth were treated by amoxicillin (A) and ceftiofur (B) according with manufactures instruction. Farrow-to-finish farm with bacteriologically confirmed history of early clinical infection by *S. suis* serotype 2, sensitive to both antibiotics was selected. Mortality and additional injectable treatments were recorded and economic impact was calculated as cost of the treatment per piglet in the whole pre-weaning period (26 days). Production parameters: average weight increase (AWI) and average daily gain (ADG) were individually measured in 3 randomly selected litters per group (40 piglets from group A, 43 piglets from group B).

### Results

The total mortality in different groups was similar, in the group A- 5 piglets and group B- 4 piglets.

Examination excluded *S. suis* infection. No additional injectable treatment was applied. Final cost of the treatment per piglet was lower in group A (0,035 €) in comparison with group B (0,241 €). AWI and ADG in group A and B were 5.655 and 218 g, respectively 5.232 and 201 g. AWI was statistically significant in favor of group A ( $p=0.0385$ ).

### Discussion & Conclusion

We have confirmed that both antibiotics amoxicillin and ceftiofur are effective option for control of mortality and clinical disease. Amoxicillin was more cost-effective product. According the rational use of antimicrobials and ban of cephalosporin's of 3<sup>rd</sup> and 4<sup>th</sup> generation in some important swine producing countries, we can consider amoxicillin as first line treatments against *S. suis*.



## BBD-062 - STUDY OF THE BACTERIAL FLORA INHABITING THE LUNG AND BRONCHI OF PNEUMONIC AND SLAUGHTER PIGS

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### Background & Objectives

Little is known about bacteria inhabiting the lower respiratory tract in pigs, and its role in respiratory disease. This study aimed at identifies bacteria present in slaughtered stocks and diagnostic specimens both in lung and in bronchi.

### Material & Methods

Lung and bronchi (n=93) from diagnostic (n=42 from 32 herds) and slaughterhouse (n=51 from 3 herds) samples were analyzed. Diagnostic lungs (DL) and bronchi (DB) were submitted to Hipra's Diagnostic Center (Spain) for routine diagnosis. Slaughterhouse lungs (SL) and bronchi (SB) were collected after slaughter.

After gross lesion evaluation, swabs from the apical lobe bronchus and lung parenchyma (all samples) were aseptically collected and inoculated on chocolate and Columbia blood agar plates (along with an NAD-producing *Staphylococcus aureus*). After incubation (37°C/overnight) bacteria known as pathogenic for swine were fully identified. The remaining ones were classified as non-pathogenic.

### Results

No bacterial growth was recorded in 2 DB (5%), 20 DL (48%), 7 SB (14%), and 30 SL (59%). Pathogenic bacteria were isolated from 39 DB (93%), 18 DL (43%), 31 SB (61%), and 13 SL (25%). *Streptococcus suis* (26% DB; 14% DL), *Pasteurella multocida* (19% DB; 10% DL; 8% SB; 4% SL), *Bordetella bronchiseptica* (14% DB; 2% DL; 35% SB; 12% SL) and *Haemophilus parasuis* (14% DB; 2% DL) were identified as the main ones.

### Discussion & Conclusion

This study revealed a higher proportion of both pathogenic and non-pathogenic bacteria in bronchi than in lungs, regardless of the health status. Unexpectedly, a high percentage of pathogenic bacteria were retrieved from slaughter animals. Likewise, a remarkably high percentage of *Bordetella bronchiseptica* isolates were obtained. These results indicate that routine bacteriology for pneumonia diagnosis in swine should include both lower bronchi and lung parenchyma, although interpretation of results should include lesions and clinical history.

*Acknowledgements: HIPRA Diagnostic Staff for sample processing.*



## BBD-063 - REVEALING NEW STREPTOCOCCUS SUIIS VACCINE CANDIDATES: EXOPROTEOME ANALYSIS OF THE MOST PREVALENT SEROTYPES BY IMMUNOPROTEOMICS

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### Background & Objectives

*Streptococcus suis* is a major swine pathogen which is associated with a wide variety of diseases. There are no vaccines available for disease control yet because of lack of common cross-reactive antigens against different serotypes of this specie. Currently the focus has shifted towards surface proteins which are in contact with host cells and accessible to antibodies. For that, the analysis of secreted proteins from bacteria is also interesting. To identify secreted immunoreactive proteins common to the most prevalent serotypes of *S.suis*, which can be used in further studies as vaccine candidates, is the goal of this study.

### Material & Methods

Extracellular proteins from 21 strains belonging to 11 *S.suis* serotypes were isolated and separated by two-dimensional electrophoresis. Proteins from a replicate gel were transferred onto nitrocellulose membranes and incubated with serum from a convalescent pig infected with *S. suis*. Proteins were identified by MALDI-TOF/TOF-MS and using Mascot v 2.2.

### Results

A total of 67 proteins were identified distributed among the 11 different serotypes. The amidase membrane protein (SSU0020) was identified in more than 50% of the studied strains and more than 70% of analyzed serotypes. Among cell wall proteins, the SSU0706 was identified in more than 60% of studied serotypes and in the 33.3% of the analyzed strains. The SSU0186 was only identified in 36.6% of serotypes and 19% of the strains but was common to the 2 and 9 serotypes. The SSU0934 lipoprotein was identified in more than 50% of the serotypes and in 38% of the strains.

### Discussion & Conclusion

The SSU0934 lipoprotein, the SSU0186 cell wall protein and the amidase were commonly identified among the most important serotypes of *S.suis*. These immunoreactive proteins may be interesting targets for vaccine development since they could provide possible cross-reactivity among different serotypes of this pathogen.



## BBD-064 - DETECTION OF CLOSTRIDIUM NOVIYI TYPE B ALPHA TOXIN ANTIBODIES IN SWINE SERA IN SPAIN

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### Background & Objectives

*Clostridium novyi* is an anaerobic, spore forming, gram-positive rod in which the lethal and necrotizing  $\alpha$ -toxin is considered to be the principal toxin of the type B strain in pigs. Cases of sudden death in sows have been reported in intensive swine-breeding units in Europe and, in which *C. novyi* type B has been isolated. The aim of this trial was to detect and demonstrate the presence of specific antibodies of *C. novyi*  $\alpha$ -toxin in healthy swine sera on Spanish farms.

### Material & Methods

The statistical design of the study based on Spanish official census, determined the number of farms per region and the total number of farms (n=142), sows (n=852) and the number of sows per farm (n=6) to be sampled. The primary inclusion criterion was that the animals had not been previously immunised with any vaccine containing the  $\alpha$ -toxoid of *C. novyi*.

The serum samples were analysed in Diagnos, with an "in house" ELISA test (Hipra, Spain), adapted from previous studies, for the detection of *C. novyi*  $\alpha$ -toxin antibodies.

### Results

The prevalence of *C. novyi* found in the population of sows was 30.31% (95% confidence interval (CI): 27.23% - 33.39%). The prevalence of *C. novyi* on the breeding farms was 74.23% (95% CI: 67.07% - 81.39%); finally, the prevalence of *C. novyi* in the population of sows on positive farms was 52.04% (95% CI: 48.04% - 56.05%).

### Discussion & Conclusion

These results demonstrate the large number of individuals that have developed antibodies to the  $\alpha$ -toxin of *C. novyi* naturally, which undoubtedly involves a high risk factor for sudden death in breeders. Although these results cannot be linked directly to the incidence of sudden death in breeders, they shed more light on the research into the prevalence of *C. novyi*.



## BBD-065 - DETECTION OF THE DIFFERENT PATHOGENICITY FACTORS OF ENTEROTOXIGENIC ESCHERICHIA COLI AND CLOSTRIDIUM PERFRINGENS TYPE C IN EUROPE

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### Background & Objectives

Infection with enterotoxigenic *Escherichia coli* (ETEC) is a common cause of diarrhoea in suckling pigs worldwide and is becoming an important and devastating disease for swine producers because of the substantial economic impact on farms, due to high morbidity, mortality and reduced growth rates. The objective of this study was to evaluate which antigenicity factors of ETEC and *Clostridium perfringens* type C (CpC) are most commonly detected in Europe by laboratory techniques.

### Material & Methods

A total of 928 faecal samples from 315 farms with high prevalence of neonatal diarrhoea coming from 17 European countries, collected between February 2012 and November 2016, were sent to Diagnos through FTA® ELUTE cards. A multiplex PCR test was performed to detect genes encoding F4 (K88), F5 (K99), F6 (987P) adhesion factors and heat-labile toxin (LT) of ETEC, and the  $\beta$ -toxin of CpC.

### Results

Of the total of 928 samples (FTA ELUTE cards) analysed, 666 (71.77%) samples were positive for some of the antigenicity factors and 262 (28.23%) were negative for all the antigenicity factors. Of the 666 samples that were positive, PCR was carried out for each of the antigenicity factors mentioned and the following results were obtained: F4 fimbriae, 483 positive samples (52.4%); LT toxin, 325 positive samples (35.2%); F5 fimbriae, 60 positive samples (6.5%); F6 fimbriae, 35 positive samples (3.8%) and  $\beta$ -toxin of CpC, 19 positive samples (2.1%).

### Discussion & Conclusion

The detection of F4 (52.4%) fimbriae and the LT (35.2%) toxin in faecal samples from neonatal diarrhoea outbreaks was very common. Nowadays, not all the commercial vaccines on the market have the same composition, so that prevention by means of vaccines that contain the main adhesion factors of ETEC, especially protection against the toxins that these produce, appears to be essential for the control of diarrhoea in suckling piglets.



## BBD-066 - HIGH LEVEL OF ACQUIRED ANTIMICROBIAL RESISTANCE IN BRACHYSPIRA HYODYSENTERIAE ISOLATES FROM BELGIAN PIG HERDS

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### Background and Objectives

Swine dysentery (SD) caused by the anaerobic spirochete *Brachyspira hyodysenteriae*, causes substantial economic losses in all major pig producing markets. Acquired antimicrobial resistance is increasing for *Brachyspira hyodysenteriae*, making treatment of SD progressively more difficult. The aim of this study was to evaluate the current minimum inhibitory concentration (MIC) patterns of Belgian *B. hyodysenteriae* isolates, and to associate phenotypes with relevant gene mutations. Strain diversity was estimated using Multilocus Sequence Type (MLST) analysis.

### Materials and Methods

The MIC values for thirty-three *Brachyspira hyodysenteriae* isolates were determined using a broth dilution method [Karlsson and Franklin, 2000]. Isolates were recovered from fecal samples collected on 32 different farms. Six antimicrobial compounds were used at the following concentration ranges: lincomycin (0.063-128 µg/ml), doxycycline (0.016-16 µg/ml), valnemulin (0.004-32 µg/ml), tiamulin (0.002-4 µg/ml), tylosin (0.25-1024 µg/ml), and tylvalosin (0.016-128 µg/ml). Sequences for 23S rRNA gene, 16S rRNA gene and ribosomal protein L3 gene were determined. MLST analysis was performed according to the scheme developed by Råsbäck et al. [2007].

### Results

For doxycycline, 91% of strains had a MIC-value above the wild type cut-off value [Pringle et al., 2012]. Analysis of their 16S rRNA sequence consistently showed a mutation at position 1058 (G→C). All but 2 isolates (94%) had a MIC value above the wild type cut-off value of lincomycin. These two isolates had none of the mutations in the 23S rRNA gene described to decrease the susceptibility for lincomycin, and shared a unique sequence type (ST173). Results for pleuromutilins, macrolides and further MLST analysis will be presented as well.

### Discussion and Conclusion

The vast majority (> 90%) of the isolates tested showed acquired resistance against doxycycline and lincomycin. These high levels of acquired antimicrobial resistance emphasize the need of alternative measures to mitigate swine dysentery.



## BBD-067 - EFFECT OF TREATMENT WITH DOXYCYCLINE AND TYLOSINE ON EXCRETION OF *E. COLI* F18 IN FAECAL PEN FLOOR SAMPLES

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### Background & Objectives

Enteric diseases account for the majority of antibiotic treatments of nursery pigs in Denmark. F18 positive *Escherichia coli* (*E. coli*) detected in faecal pen floor samples (PFS), are often found as cause of diarrhoea outbreaks. The objective of this study was to assess the efficiency of five days of treatment with tylosine (TYL) or doxycycline (DOX) measured on *E. coli* F18 excretion in PFS analysed by qPCR sampled at the day of treatment and two days after treatment.

### Material & Methods

PFS were collected at day 14 and 21 post weaning from 75 pens in three Danish nursery facilities. Pen-level diarrhoea prevalence was assessed by faeces scoring of 15 randomly selected pigs per pen. The PFS were analysed for *E. coli* F18 genes by qPCR. 13 *E. coli* F18 positive pens were treated five days with 12.5 mg/kg doxycycline hydrate. 14 *E. coli* F18 positive pens were treated five days with 7.5 mg/kg tylosine. 48 *E. coli* F18 positive pens did not receive any treatment. The change in excretion levels for *E. coli* F18 was calculated by subtracting the paired qPCR results originating from the same pens. The effect of treatment on reduction of excretion of F18 was tested by chi<sup>2</sup> test.

### Results

Mean pen-level diarrhoea prevalence at the time of sampling was 22.5% (CI95%, 17.0-27.0). Reduction of excretion of *E. coli* F18 was observed in 66 of 75 pens. The proportion of pens with reduction in *E. coli* F18 excretion was not statistical significantly different between pens treated with TYL (93%), DOX (92%) ( $p=0.50$ ) or pens receiving no antibiotic treatment (85%) ( $p=0.58$ ).

### Discussion & Conclusion

The results of the study show that reduction of excretion of *E. coli* F18 was not associated with treatment with antibiotic. This indicates that pigs can stop excreting *E. coli* F18 without any antibiotic treatment.





## BBD-068 - DESCRIPTION OF LOW AND HIGH PATHOGENIC DIARRHEIC OUTBREAKS IN NURSERY PIGS

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### Background & Objectives

Mean bacterial load determined by qPCR testing of a pooled faecal sample for *Escherichia coli* F4 (F4) and F18 (F18), *Lawsonia intracellularis* (LI) and *Brachyspira pilosicoli* (PILO) from a group of nursery pigs can be used to determine the prevalence of bacterial enteritis/colitis. This has made it possible to classify outbreaks as high or low pathogenic diarrhoea. The objective of this study was to describe diarrhoea prevalence in pens with nursery pigs classified as high and low pathogenic diarrhoeic outbreaks.

### Material & Methods

A pen floor faecal sample was collected at day 14 and 28 post weaning from 169 pens in three nursery facilities in Denmark. In each pen, diarrhoea status of 15 randomly selected pigs was determined by visual inspection of rectal samples. The pen floor samples were analysed for F4, F18, LI and PILO gens by qPCR and the total excretion level per gram faeces was calculated. Furthermore diarrhoeic faecal droppings in the pen floor were counted. Pens was classified as high pathogenic when bacterial excretion levels was  $\geq 35.000$  total bacteria/g faeces and  $\geq 1.5$  diarrhoeic droppings per pen were present. Difference in pen level diarrhoea prevalence were tested by student's t-test between pens classified as having high or low pathogenic diarrhoea outbreaks.

### Results

In 66 pens (39.1%) classified as high pathogenic outbreaks the average diarrhoea prevalence was 0.292 (CI95%: 0.247, 0.337) which was significantly higher (t-test,  $p < 0.001$ ) compared to pens classified as low pathogenic outbreaks with an average diarrhoea prevalence of 0.145 (CI95%: 0.118, 0.172).

### Discussion & Conclusion

The results of this study show that analysing faecal pen floor samples by qPCR and counting diarrhoeic droppings can be used to identify flocks of diarrhoeic pigs. This finding supports the established definitions of high pathogenic diarrhoea relates well to the clinical disease severity.



## BBD-069 - OUTBREAK OF CASEOUS LYMPHADENITIS IN OUTDOOR PIGS

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### Background & Objectives

An outbreak of caseous lymphadenitis in an outdoor farm of 400 Iberian sows is described. Gross submandibular nodules were observed in a new group of Duroc boars born within the unit, with a morbidity rate of 60 %. Six months later the same lesions were observed in gilts and sows (morbidity of 7.5 %). No mortality was recorded.

### Material & Methods

Gross lesions were recorded in three humanely killed affected sows and samples from nodules were obtained and fixed in 10% buffered formalin and routinely processed for the histopathological analysis. Swabs and tissue samples from these nodules were plated on selective media and identified by biochemical tests. Antimicrobial susceptibility was determined by the broth microdilution method (CLSI, 2013).

### Results

At necropsy, nodules of 6 to 15 cm in diameter, in the form of clusters and with a yellowish to greenish, creamy content, surrounded by a thick connective tissue (abscesses) were observed in the submandibular area, and also in the subcutaneous tissue close to the mammary gland in one animal. No lesions were observed in internal organs. Microscopically, these lesions were characterised by abundant necrotic material with cellular debris, surrounded by a thick capsule of connective tissue and abundant infiltration of leukocytes. Microbiological analysis revealed the presence of *Trueperella pyogenes*, *Streptococcus suis*, *Streptococcus porcinus* and *Streptococcus dysgalactiae* subspecies *equisimilis*. All the isolates were susceptible to amoxicillin and gentamicin.

### Discussion & Conclusion

An outbreak of caseous lymphadenitis in growing animals associated with pyogenic bacteria is here described. The treatment with gentamicin and the culling of animals with spread abscesses allowed controlling the outbreak. Six months later, new cases have not been reported.



## BBD-070 - MOLECULAR AND ANTIBIOTIC SUSCEPTIBILITY CHARACTERIZATION OF AEROCOCCUS VIRIDANS ISOLATED FROM PORCINE URINARY INFECTION IN KOREA

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### Background & Objectives

*Aerococcus viridans* has been reported as a human and animal pathogen causing urinary tract infection (UTI), arthritis, pneumonia, meningitis and endocarditis. *A. viridans* is not usually surveyed in clinical diagnostic laboratories, rather commonly misidentified as other bacteria. In the present study, *A. viridans* strains from porcine UTI samples in Korea were isolated, identified molecularly, and characterized with antibiotic susceptibility.

### Material & Methods

Twelve of presumptive *Aerococcus* strains were isolated from sow urine samples in Korea. The urine samples suggestive of the UTI were selected based on the dipstick test screening results (leukocyturia, nitrite presence, proteinuria and pH > 7.5). The partial amplification of the 16S rRNA gene and the minimal inhibitory concentration (MIC) by the broth microdilution technique using Sensititre1 Standard Susceptibility MIC Plates BOPO6F (TREK Diagnostic Systems/Thermo Fisher Scientific, Waltham, MA, USA) was conducted. *S. pneumoniae* ATCC 49619 and *S. aureus* ATCC 29213 were used as an internal quality control. The MIC50 and MIC90 values for the respective antimicrobials were determined.

### Results

A total of 12 isolates were identified as *A. viridans* by 16S rRNA gene sequencing. The MIC values also presented variability especially for ceftiofur, fluoroquinolones and aminoglycosides. Sulphonamides, tetracyclines, macrolides and fluoroquinolones presented the highest MIC values.

### Discussion & Conclusion

The high MICs of aminoglycosides, tetracyclines, fluoroquinolones and macrolides seen among the *A. viridans* corroborate previous reports. The widespread veterinary use of those antibiotics calls attention for the implication of *A. viridans* infection to human as well as animal health. Especially, fluoroquinolones, widely used in UTI of pigs in Korea increased MICs. Our study strongly suggests that *A. viridans* be recognized as a pathogen responsible for the UTI in human as well as animals, and present the great genetic variation with an alarming antimicrobial susceptibility profile despite going unnoticed in routine laboratory diagnostics.



## BBD-071 - ASSOCIATION OF RESISTANCE TO AMPICILLIN AND OTHER ANTIBIOTIC AGENTS AS EVIDENCE OF CO-SELECTION IN *E. COLI* ISOLATES FROM DIARRHEIC NURSERY PIGS

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### Background-Objectives

Ampicillin resistance in *E. coli* isolated from human bacteraemia is occurring frequently and concerns about a porcine reservoir for ampicillin resistant *E. coli* which may be transferred to humans, has been raised.

Direct selection of resistant *E. coli* by ampicillin treatment of pigs and co-selection of ampicillin resistance by tetracycline and other antibiotic classes has been suggested. Statistical association between resistances to different antibiotic agents is a strong indicator of co-selection. The objective of this study was to evaluate the association between resistances to commonly used antibiotic agents such as tetracycline and resistance to ampicillin in *E. coli* isolates from diarrheic nursery pigs.

### Material-Methods

Rectal samples from diarrhoeic pigs two-four week post weaning and faecal pen floor samples were collected from three nursery facilities in Denmark. Samples were cultured on blood agar and analysed by PCR for adhesion factor-, and enterotoxin-genes to determine ETEC status. Resistance testing of 380 *E. coli* isolates was performed by the Sensititre system. Association between resistance to tetracycline, sulphamethoxazole, trimethoprim, spectinomycin, streptomycin and ampicillin resistance was tested by Chi<sup>2</sup>-test and odds ratios were calculated.

### Results

In 89 ETEC isolates tetracycline and streptomycin resistance was statistically associated to ampicillin resistance ( $p > 0.001$ ,  $0.002$ ). For TET-resistant isolates a lower level of ampicillin resistance (OR = 0.09) was observed compared to TET-sensitive whereas STR-resistant isolates had a higher level of ampicillin resistance (OR = 5.57) compared to STR-sensitive isolates. In 291 Non-ETEC isolates all tested antibiotic agents except spectinomycin was positively associated to ampicillin resistance with the following odds ratios: tetracycline=2.33, sulphamethoxazole =40.77, trimethoprim =17.23, streptomycin =27.67.

### Discussion-Conclusion

The results of the study showed evidence of co-selection of resistance to ampicillin and other antibiotic agents in *E. coli* isolates from diarrheic nursery pigs. Surprisingly tetracycline was found to have a lower co-selecting capacity for ampicillin resistance than other antimicrobial agents.



## BBD-072 - EFFICACY OF COGLAPIX® AGAINST THE INFECTION WITH A. PLEUROPNEUMONIAE SEROTYPE 8

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### Introduction

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 distribution of various serotypes differs substantially among countries in Europe. Serotype 8, showing high virulence, has been the most prevalent serotype in the UK, but has been isolated also in other countries. The aim of this study was to determine the efficacy of CoglapiX® (Ceva Sante Animale), a toxoid+bacterin vaccine, against the challenge with this A.p. strain and compare its efficacy with a largely used toxoid+OMP vaccine (vaccine A).

### Materials and methods

Twelve weeks old pigs, vaccinated either with CoglapiX or vaccine A in a prime-boost regime at six and nine weeks of age, and non-vaccinated controls were challenged with  $\sim 10^5$  CFU/pig of the challenge bacterium suspended in PBS by the aerosol route. Five pigs served as non-vaccinated non-challenged negative controls. After one week of observation the surviving pigs were euthanized and their weighted lung and pleura lesions scored (LLS) were calculated and compared to each other.

### Results

The mortality was 10% for CoglapiX®, 20% for the vaccine A and 20% for the positive control. The clinical scores were 1 for CoglapiX, 1.5 for the vaccine A and 3.67 for the positive control group. The weighted mean lung and pleura lesion scores were 1.06 for both vaccines and 1.62 for the positive control. The differences between the two vaccines were not statistically significant.

### Conclusion

In this experimental challenge trial CoglapiX® confirmed high level of efficacy even against the serotype 8 of A.pleuropneumoniae, isolated in the UK. No mortality and the lowest rate of clinical symptoms were obtained in the group vaccinated with CoglapiX®. The mean lung lesion did not differ in the two vaccinated groups.



## BBD-073 - MICROBIOLOGICAL STUDY OF TOTALLY CONDEMNED PIGS WITH DISSEMINATED TUBERCULOSIS-LIKE LESIONS

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### Background & Objectives

Disseminated tuberculosis-like lesions (TBL) in pigs are responsible for total condemnation at the postmortem inspection. Members of *Mycobacterium tuberculosis* complex (MTC) and *Mycobacterium avium* complex (MAC) and pyogenic bacteria, such as *T. pyogenes*, *S. suis*, *S. porcinus* and *S. dysgalactiae*, can be isolated from these lesions. The main objective of this study was to determine the role of these microorganisms in the occurrence and organic dissemination of TBL in pigs.

### Material and Methods

A total of 261 samples from 37 totally condemned pigs were aseptically removed at the slaughterhouse: submandibular (37), inguinal superficial (37), gastrohepatic (36), and popliteal (36) lymph nodes, lungs (37), liver (34), spleen (24) and tonsils (21). The presence of MTC and MAC was tested from tissue by a duplex qPCR. *T. pyogenes* and *Streptococcus* species were isolated on selective media (Columbia CNA with 5% sheep blood) and identified by biochemical methods. The genetic similitude of these isolates was evaluated by PFGE analysis.

### Results

MTC was detected in the 93.5% of animals, whereas pyogenic bacteria were isolated from 80.1% of them at different organic locations. All microorganisms showed a wide organic distribution. MTC was more frequently detected in submandibular and gastrohepatic lymph nodes, whereas pyogenic bacteria were also isolated from others organs, such as liver, lung or spleen. *T. pyogenes* was isolated from two or more organs in the 50% (8/16) of positive animals. *S. porcinus* and *S. dysgalactiae* were isolated from two or more organs in 33.3% (5/15) and 42.7% (6/14) of positive animals, respectively. PFGE analysis showed a high similitude among *T. pyogenes* isolates obtained from different organs; however, higher variability was detected in *S. porcinus* and *S. dysgalactiae* species.

### Discussion & Conclusion

The importance of pyogenic bacteria in the production of disseminated TBL lesions in pigs is showed, with special attention to *T. pyogenes* species.



## BBD-074 - IMPACT OF HYOGEN® VACCINE ON LUNG LESION PARAMETERS IN SWINE FARMS IN BRAZIL WITH HIGH INCIDENCE OF MYCOPLASMA HYOPNEUMONIAE

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### Introduction

The use of vaccines against *Mycoplasma hyopneumoniae* (M.hyo) is recognized as an important tool to control enzootic pneumonia (EP) in pigs. The aim of this study was to determine the efficacy of Hyogen® vaccine, containing inactivated high pathogenic *Mycoplasma hyopneumoniae* (BA2940) strain together with Imuvant™ against EP under field conditions in Brazil.

### Materials and Methods

The study was conducted on three different farrow-to-finish farms belonging to one producer. All farms reported intensive clinical symptoms despite the use of the M.hyo vaccine and strategic treatments for PRDC. The analysis included groups vaccinated with two doses of the commercial Mhyo vaccineA at 21 and 42 days, which were compared to groups vaccinated with a single dose of Hyogen® at 21 days. In each farm 5-8 batches of each treatment group were analyzed at slaughter using Ceva-Lung-Program on 100 pigs per batch.

### Results

The % of bronchopneumonia in Hyogen® groups were 22.7%\*, 38.3%\* and 41.0%\* compared to VaccineA with 40.4%, 53.4% and 63%. The % of affected lung parenchyma in the Hyogen® group was 4.5%, 4.6% and 1.6%\* compared to 5.0%, 7.0% and 5.4% in the VaccineA group. The percentage of scars in the Hyogen® pigs were 6.8%\*, 6.6%\* and 3.2%\* compared to 14%, 31% and 11.8% in the vaccine groups (\*indicates  $p < 0.05$ ).

### Conclusions

Regardless of the type of farm, number of animals and the initial severity of the lesions, the results suggest that Hyogen® improved the percentages of bronchopneumonia, lung-lesion area and percentage of lungs with scars, compared to the two shot M.hyo vaccine. The reduction of both fresh lesions indicating the late *M.hyo* infections and scars indicating the early infections may have a significant impact on the growth performance of commercial pigs.





## BBD-075 - ANTIMICROBIAL SUSCEPTIBILITY (MIC) OF *TRUEPERELLA PYOGENES* ISOLATED FROM PIGS WITH LYMPHADENITIS

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### Background and objective

*Trueperella pyogenes* can be responsible for different animal diseases, being one of the most important pathogens related to porcine lymphadenitis outbreaks. The control of that diseases is based on antimicrobial treatments, but actually there are not there are not defined cutoffs for this species. The objective of this study was to determine the CMI values of different antimicrobials commonly used in swine livestock.

### Material and methods

The susceptibility of 97 isolates obtained from pigs with lymphadenitis against 11 antimicrobial agents was determined by the broth microdilution test according to CLSI guidelines (2013). All strains were classified as susceptible, intermediate or resistant using the cutoffs available for other Gram positive bacteria (with the exception of apramacina, neomycin, streptomycin and tylosin, as there are no published cutoffs). Minimum inhibitory concentration (MIC), MIC50 and MIC90 of every antimicrobial in study have been defined.

### Results

The 97 isolates were susceptible to amoxicillin, and a high percentage of them were susceptible to penicillin (98.97%), gentamicin (98.97%) and ceftiofur (93.82%). The highest values of resistance were obtained against oxytetracycline (53.6%). Values of MIC90 varied among 0.06-1mg/ml for amoxicillin, penicillin, gentamicin, ceftiofur, enrofloxacin, respectively. MIC90 values from 2-8 mg/ml were obtained for trimethoprim-sulfamethoxazole, neomycin, apramycin, streptomycin. MIC90 values of 16 mg/ml were obtained for oxytetracycline.

### Discussion and conclusion

The beta-lactams, gentamicin and ceftiofur could be selected for empirical treatment of swine lymphadenitis. We also recommend further analysis with more isolates to define the behavior of this species against different antimicrobials, and to propose cutoff points.



## BBD-076 - COMPARISON OF INTESTINAL LESIONS BETWEEN ENTERISOL® ILEITIS AND PORCILIS® ILEITIS USING A LAWSONIA INTRACELLULARIS MUCOSAL HOMOGENATE SEEDER PIG CHALLENGE MODEL

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The objective of this study was to determine differences in ileocecal lesions between Enterisol® Ileitis and Porcilis® Ileitis vaccinated pigs.

Three week old weaned pigs were placed in a wean to finish barn managed by Pipestone Applied Research. Three treatment groups (non-vaccinated controls (NVC), Enterisol® Ileitis and Porcilis® Ileitis) were randomly assigned within block to construct a randomized block design. Additionally, three non-vaccinated, non-treatment group pigs were added to each pen to serve as seeder pigs. Porcilis® Ileitis pigs were vaccinated at three weeks of age with 2ml IM. Enterisol® Ileitis pigs were vaccinated orally using a Stenner pump five weeks post-weaning. Seeder pigs in each pen were orally challenged with 40 mL *Lawsonia intracellularis* (~log 10x8) at nine weeks post weaning. Four weeks post-challenge thirty indirectly challenged pigs from each treatment group showing clinical signs of ileitis were euthanized, necropsied and lesions grossly scored. Immunohistochemistry and Hematoxylin & Eosin staining of ileum and cecum were analyzed.

Enterisol® Ileitis had significantly less IHC positives (6.67%) in ileum sections compared to the NVC (63.33%) and the Porcilis® Ileitis (43.33%) treated pigs. Both Enterisol® Ileitis and Porcilis® Ileitis treatments had significantly less percentage of pigs affected on ileum H & E sections than the NVC. However, the Enterisol® Ileitis group had numerically less pigs affected on ileum H&E than Porcilis® Ileitis. Cecal microscopic lesions were evident in NVC and Porcilis® Ileitis. The Enterisol® Ileitis had lower numbers of *Lawsonia* PCR positives and lower levels from cecal content compared to Porcilis® Ileitis and NVC at necropsy.

Previous work has shown a strong correlation between increased prevalence and severity of ileocecal lesions there is a decrease in pig performance. In this challenge model, pigs that were vaccinated with Enterisol® Ileitis then indirectly challenged showed significantly lower IHC scores when compared to both NVC and Porcilis® Ileitis.



## BBD-077 - CHARACTERIZATION OF MYCOPLASMA HYOPNEUMONIAE STRAINS FROM VACCINATED AND NON-VACCINATED ANIMALS IN SPANISH SLAUGHTERHOUSES

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### Background and objectives

*Mycoplasma hyopneumoniae* (*Mhyo*) causes a pneumonia characterized by cranioventral pulmonary consolidation (CVPC). Different *Mhyo* strains have been detected at farm, animal and sample levels. However, information on *Mhyo* strains circulating in Spain is still limited. The purpose of this study was to detect and characterize *Mhyo* strains in vaccinated and non-vaccinated slaughtered animals showing CVPC lesions.

### Material and Methods

Lung samples from 3-5 animals showing CVPC from 7 vaccinated (V) and 7 non-vaccinated (NV) farms were collected at different Spanish slaughterhouses. Extension of CVPC was individually scored prior to the sampling. To confirm *Mhyo* as the cause of the lesions, these samples were firstly tested by qPCR. Within each farm, those qPCR with Ct value lower than 30 and more extensive lung lesion score were selected to be genotyped by sequencing different loci (p97, p146, H1 and H5). Characterization was based on counting the variable number of tandem repeats for each locus. Two reference strains (11 and J) were also genotyped as internal controls.

### Results

From the 65 samples analyzed (14 farms), 46 (22 from V and 24 from NV animals, respectively) were qPCR positive and 19 (12 from V and 7 from NV animals, respectively) qPCR negative. Mean Ct values for qPCR positive samples were 27 ( $\pm$  2.9) and 24 ( $\pm$  3.7) for V and NV animals, respectively. So far, three farms (1 V and 2 NV) were genotyped and 1-3 *Mhyo* variants (different from the reference strains) were detected per farm.

### Discussion and conclusion

Results obtained showed inter-farm and limited intra-farm *Mhyo* genetic heterogeneity in Spain. Further analyses are needed to elucidate the influence of the treatment received on such variability.

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## BBD-079 - ASSESSMENT OF DIFFERENT SAMPLES TO DETECT MAIN SWINE RESPIRATORY PATHOGENS

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### Introduction

The porcine respiratory disease complex (PRDC) is a multifactorial disease which increases morbidity and mortality rates causing remarkable economic losses. Some pathogens such as PRRSV, PCV2, SIV, *Mycoplasma hyopneumoniae* (MHYP) or *Actinobacillus pleuropneumoniae* (APP) are considered as primary agents but other microbes such as *Haemophilus parasuis* (HPS), *Streptococcus suis* (SSUI) or *Mycoplasma hyorhinis* (MHYR) have been described with an important role as secondary agents. Detection of all these parameters is sometimes problematic and therefore our aim was to assess if any difference between bronchoalveolar lavage fluid (BALF) and bronchial scraping (BS) exists.

### Material and Methods

A group of 63 animals coming from three different farms was selected for this study. That collection included animals from main production stages such as suckling piglets, weaned off animals, fattening pigs and sows. BALF and BS were taken from each animal and later their respective nucleic acids were isolated and tested by Exopol designed qPCR assays for all the parameters mentioned above. Fisher exact test was used to find differences in the detection of each parameter between BALF and BS.

### Results Discussion

All pathogens were detected in both kind of specimen (BALF/BS): PRRSV(31.1%/30.7%), PCV2(3.9%/4%), SIV(2.6%/5.3%), MHYP(26%/29.6%), HPS(44.2%/46.7%), APP(16.9%/22.7%), SSUI(50%/87.84%) and MHYR(62.3%/77.3%). Detection in BS was significantly better for MHYR ( $p=0.049$ ) and SSUI ( $p=1 \times 10^{-6}$ ).

### Discussion and Conclusion

Many practitioners reckon that BS might be less invasive for the animal and overall easier to collect, hence, these data present BS as an equally effective or even better alternative to BALF for detecting main PRCD agents.



## BBD-080 - IMPROVED WEIGHT GAIN AND REDUCED MORTALITY AND ANTIBIOTIC USE FOLLOWING ORAL LIVE COLIPROTEC® F4 VACCINATION IN PIGLETS AGAINST POST-WEANING DIARRHEA

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### Introduction

Post-weaning *Escherichia coli* diarrhea (PWD) is due to enterotoxigenic *Escherichia coli* (ETEC). Therapy to control PWD typically consists of antibiotic treatment, addition of zinc oxide (ZnO) and changes in feed composition. The objective was to compare vaccination with Coliprotec® F4 to two standard therapeutic approaches, namely ZnO and safe feed formulation (30% barley) in combination with individual antibiotic treatment in a F4-ETEC PWD clinical case.

### Materials and methods

Piglets from a 600-sow farm with F4-ETEC PWD were vaccinated at 18 days of age with Coliprotec® F4. At weaning, piglets (n=640) were randomly distributed into 5 groups with different treatments: (A) 1-phase diet (0-50d) + vaccination; (B) 2-phase diet (0-7d, 8-50d) + vaccination; (C) 3-phase diet (0-10d, 11-25d, 26-50d) + vaccination; (D) safe feed formulation without antibiotic; (E) ZnO. During the 7-week post-weaning period several technical production parameters were recorded.

### Results

Although group E had a significantly better fecal clinical score and a lower treatment incidence, the vaccinated piglets (A, B and C) had a significantly higher average daily gain, better feed consumption, lower mortality and a higher overall financial ROI (€ 5.31 compared to group D). The type of diet (1-, 2- or 3-phase diet) did not have a significant effect on performance parameters. The control group (D) has the highest mortality in combination with low average daily growth and very high antibiotic treatment incidence.

### Discussion and conclusion

This comparative study clearly shows that vaccination against PWD with Coliprotec® F4 has several advantages on technical performance parameters. Overall, vaccination with Coliprotec® F4 resulted in better growth with a lower antibiotic use. In conclusion, control of PWD through oral vaccination is a good option in order to prevent piglets from the negative clinical effects of F4-ETEC infection in the post-weaning period.



## BBD-081 - EFFECT OF ORAL COBALAMIN SUPPLEMENTATION ON CYTOPLASMIC VITAMIN B12 STATUS IN PIGS VACCINATED OR NOT AGAINST LAWSONIA INTRACELLULARIS

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### Background & Objectives

Subclinical ileitis caused by *Lawsonia intracellularis*-infection has been described affecting average daily weight gain (ADWG) in pigs. Cobalamin is primarily absorbed at the ileum and has been shown to be decreased (together with an increase in homocysteine, the metabolism of which by methionine synthase is cobalamin-dependent) in pigs with *L. intracellularis*-infection. However, it is unknown whether the cytoplasmic cobalamin-dependent enzyme (i.e. methionine synthase) is affected in pigs either vaccinated or not against *L. intracellularis*. Our aim was to evaluate if oral supplementation of cobalamin affects methionine synthase in pigs that are either vaccinated or not against *L. intracellularis*.

### Material & Methods

Eight pigs each from a selected farm with confirmed *L. intracellularis*-infection were randomly allocated to five different groups (vaccinated [vacc./non-supplemented [non-suppl.], vacc./suppl., non-vacc./non-suppl., non-vacc./suppl., and tylosin [non-vacc./non-suppl.]). Administration of an avirulent *L. intracellularis* live vaccine, tylosin orally (4.5 mg/kg body weight) throughout the study period or cobalamin orally (0.2 mg/kg feed) from day 8 to 21 was conducted in accordance to the groups. Serum samples were obtained on day 0, 7, 14 and 21. Cobalamin and homocysteine concentrations were measured. An ANOVA or MANOVA model was used to compare ADWG, cobalamin and homocysteine among the five groups of pigs.

### Results

The ADWG differed among the groups ( $p < 0.0001$ ), with the lowest ADWG found in the non-vacc./non-suppl. group compared to the remaining four groups (at least:  $p < 0.05$ ). Concentrations of serum cobalamin differed between the non-vacc./suppl. (increased) and remaining four groups of pigs ( $p = 0.0449$ ), whereas no differences were found for serum homocysteine ( $p = 0.0867$ ) concentrations among the groups.

### Discussion & Conclusion

Cobalamin supplementation over two weeks affects serum cobalamin concentrations in pigs that are not vaccinated against *L. intracellularis*. The insufficient response of the serum homocysteine concentrations could be related to cytoplasmic methionine synthase having more than one co-factor or cobalamin not being supplemented long enough.



## BBD-082 - CONTROL OF MYCOPLASMA HYOPNEUMONIAE IN A GROUP OF FARMS USING AIVLOSIN® AND VACCINATION

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In this study, a group of five commercial farrowing pig farms, all *Mycoplasma hyopneumoniae* (*Mh*) positive with piglets vaccinated for *Mh* at weaning and taking in *Mh* negative gilts, tried different methods to control *Mh*.

In April 2013, cough was observed on all farms. Influenza and *Mh* were diagnosed. After few weeks, cough stopped in 3 out of 5 farms.

In September 2013, tracheobronchial swabs from 28-day-old piglets were analysed by PCR for *Mh* in all farms. Herds were considered stable if all samples were negative and unstable if any samples were positive.

The three herds no longer coughing were stable. In December 2013, *Mh* vaccination was administrated to sows, 6 and 3 weeks before farrowing and piglets were vaccinated later at 9 weeks of age.

The two farms still showing clinical signs were unstable. Therefore, all sows were medicated with tylvalosin (Aivlosin®), followed by the same treatment at the entrance in farrowing room (January to August 2014).

In the stable herds, no respiratory problems were observed since July 2013. In one herd, *Mh* vaccination of piglets was stopped in all-in all-out external fattening units. In another herd, *Mh* was eradicated using an Aivlosin® protocol.

In the unstable herds, cough did not reappear after Aivlosin® treatments. Lung lesions decreased and performance were improved. In September 2014, PCRs from 28-day-old piglets were all *Mh* negative. Therefore, Aivlosin® was stopped; sows and piglets began to be vaccinated the same way as in stable herds.

This study highlights the interest of *Mh* diagnosing via PCRs on tracheobronchial swabs from weaned piglets to classify herds as stable or unstable. It also shows Aivlosin® treatments can decrease *Mh* transmission in unstable herds. *Mh* vaccination of sows seems to have an interest to maintain a sanitary stability in association with the introduction of *Mh* negative gilts.



## BBD-083 - DISTRIBUTION OF SENSITIVITY OF STREPTOCOCCUS SUIIS STRAINS AGAINST VARIOUS ESSENTIAL OILS

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### Background & Objectives

The control of *Streptococcus suis* infections is currently based on the application of antimicrobials at therapeutic and prophylactic doses. However, as commonly observed with other animal pathogens, it has been detected a higher therapeutic failure associated with the increasing bacterial resistance. Several essential oils have shown a remarkable antimicrobial activity against streptococci isolated from human and against enteric pathogens isolated from swine. An *in vitro* assay has been performed to evaluate the antimicrobial activity of cinnamon, oregano, common thyme and red thyme essential oils as well as to determine the distribution of sensitivity of 52 strains of *S. suis* of different serotypes, obtained from sick pigs.

### Material & Methods

Broth microdilution method was carried out by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of each strain against oils dilutions (39.062-5000 µg/mL), according to the guidelines of CLSI (2013). We estimated the MIC<sub>50</sub>, MIC<sub>90</sub>, MBC<sub>50</sub> and MBC<sub>90</sub> from the frequency distribution. Finally, the bactericidal power (rate MBC<sub>50</sub> and MBC<sub>90</sub> / MIC<sub>50</sub> and MIC<sub>90</sub>) was calculated, being considered as bactericides those oils showing a rate ≤4.

### Results

All the oils tested demonstrated antimicrobial activity with bactericide character (rate ≤2) against *S. suis*. Oregano and common thyme essential oils showed the highest antimicrobial activity, inhibiting and eliminating the 90% of the tested strains at a concentration of 312.5 µg/mL.

### Discussion & Conclusion

The results highlight the bactericidal potential of oregano and common thyme even when used at concentrations below the limit of cytotoxicity described by some authors (500 µg/mL), and support their possible use for the control of *S. suis* infections.

This experiment represents the first study on the distribution of the sensitivity of *S. suis* against essential oils. It also provides important information for future synergy studies with antibiotics to determine the administration dose in *in vivo* assays.





## Herd Health Management and Economy

### HME-001 - PREVALENCE OF DIFFERENT LUNG PATHOGENS AT PIG SLAUGHTERHOUSES IN GERMANY

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#### Background & Objectives

The severity of lung disease in pigs is supposed to correlate -among other things- with the variety of different pathogens involved and the quality of each pathogen (primary vs. secondary). The aim of this study was to monitor abattoir pigs on viral and bacterial antigens to create an overview of amount, prevalence and combinations of different pathogens on farms with respiratory problems.

#### Materials & Methods

From 2015 to 2016, 220 lungs from 46 different farms were taken from abattoirs in Germany. All farms had a history with both respiratory problems in the fattening period and increased incidence of pneumonia or pleurisy in previous slaughtering. After creating a sterile lung surface by using a Bunsen burner, all further samples were taken from the same inner part of the lung (between apical and middle lobe). From the bacteria found, MALDI-ToF analysis was performed and sensitivity to antibiotics was examined. In addition, lung tissue pool samples were analyzed by real-time (rt) PCR on APP, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, Influenza and PRRS-Virus. Lymph node tissue was checked using quantitative real-time PCR on PCV-2.

#### Results

Frequency of *Mycoplasma hyopneumoniae* detection and PCV-2 detection was significantly higher than any other primary respiratory pathogen found (82.6 % and 54.3 %, respectively). When it comes to secondary pathogens, *Pasteurella multocida* showed the highest incidence, with 43.2 % of the farms positive.

#### Discussion & Conclusion

Interpreting tools based on CT-values (=approx. antigen content) and on-farm-situation are proposed. The farms with the lowest CT-values for *Mycoplasma hyopneumoniae*, for example, had not used Mycoplasma vaccination. Sensitivity of APP culture was only ~30 % of the APP PCR, underlining the higher sensitivity of PCR and the value of using PCR for screening purposes.



## HME-002 - DISTRIBUTION AND FREQUENCY OF BIOCHEMICAL PARAMETERS OF BOARS FROM COMMERCIAL FARMS

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Boars used for the collection of semen for artificial insemination on commercial farms are subject to regular health control. The aim of this study is to determine values of certain biochemical parameters from blood and their possible contribution to the diagnosis of health status of the boars. In samples from farm A (23 boars), concentration of phosphorus, calcium, bilirubin, total protein, glucose, triglyceride, albumin and urea were measured. In samples from farms B and C (27 and 28 boars, respectively) AST concentration was tested. Results from farm A are as follows (mean  $\pm$  standard error): phosphorus  $1.4 \pm 0.04$  mmol/l; calcium  $2.8 \pm 0.03$  mmol/l; Ca / P relation  $2.0 \pm 0.05$ ; bilirubin  $6.7 \pm 0.32$  mmol/l; total proteins  $74.9 \pm 1.39$  mmol/l; glucose  $4.0 \pm 0.51$  mmol/l; tryglyceride  $0.6 \pm 0.02$ ; albumins  $47 \pm 0.80$  g/l, and urea  $4.0 \pm 0.51$  mmol/l. The boars have been most differing in values of urea and at least in calcium levels in the blood. Determined values of AST were  $43.67 \pm 13.2$  IU/L (farm B) and  $57.07 \pm 15.4$  IU/L (farm C). Knowing the value of AST is useful in assessing the functional status of the liver, although this enzyme can originate from another source. In 20% of boars AST value was above 60 IU/l, which is usually taken as the upper reference value in the literature. Results of biochemical analysis justified the invested funds, representing a significant contribution to the diagnosis of the health status and production of pigs on commercial farms.

Key words: boar, biochemical parameters, commercial farm

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## HME-003 - INVESTIGATE THE COMPOSTING CONDITIONS OF LIQUID BY-PRODUCT OBTAINED FROM DEGRADATION OF PIG CARCASS

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### Introduction

Globally, concern about emerging infectious diseases of livestock is growing. For the disposal of the animal carcass, it is necessary to recycle the carcass into an agriculturally usable product. The objective of this study was to investigate the composting conditions of liquid by-product obtained from degradation of pig carcass for agriculture recycling.

### Method and Results

Optimum conditions of liquid fertilizer were investigated using different microorganisms, pHs, and volumes of microorganisms (*Lactobacillus rhamnosus* + *Pichia deserticola*). Based on the results from the optimum conditions, compost maturity and quality of liquid fertilizer were evaluated for 112 days. The compost maturity of liquid fertilizer were higher in the order of LP (*Lactobacillus rhamnosus* + *Pichia deserticola*) > BC (*Bacillus cereus*) > BS (*Bacillus subtilis*). The optimum condition under different volumes of LP was injection of 0.5 mL/100 mL. The compost maturity under different pHs were higher in the order of pH 7 > 5  $\cong$  9  $\cong$  11. The liquid by-product at 56 days after composting was completely decomposed. The concentrations of T-N, T-P and K<sub>2</sub>O in liquid fertilizer at 56 days were 0.94, 0.17 and 3.78%, respectively, and the sum of those concentrations was 4.89%.

### Conclusion

Liquid fertilizer of by-product using pig carcass was decomposed with optimum conditions (LP, pH 7, injection of 0.5 mL/100 mL) in 56 days after composting, and was suitable for official standard of commercial fertilizer.



## HME-004 - SNEEZING IN WEANED PIGS DUE TO NON-INFECTIOUS CAUSES; A CASE REPORT

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### Background

Sneezing is induced by chemical, mechanical or allergen factors, either of infectious or non-infectious origin. We present a diagnostic protocol and the findings in a field case of recurrent extensive sneezing in weaned pigs on a 400 sow head farrow-to-finish farm.

### Material and Methods

A cohort study was conducted to determine the incidence of disease and the course of clinical signs. Every week sneezing and other signs in each room were assessed. In addition, 4 representative pigs were submitted for post mortem investigations and PCR testing. Furthermore, nasal swabs of 60 pigs were submitted for PCR on SIV. Finally, environmental and biosecurity risk factors were assessed.

### Results

Prevalence of sneezing increased from 2% in 3wk old piglets to 80% in 6 wk old pigs. Epiphora was observed in sneezing pigs, but already present in 80% of pigs at 4wks. Mild nasal discharge was of serous nature at 4wks and purulent in pigs >5 wks old.

Post mortem investigations returned no gross abnormalities, but a multifocal mild subacute histiocytic interstitial pneumonia and a multifocal acute mild purulent and eosinophilic rhinitis was found on histology. PCR tests for PCV2, PRRSv and SIV on lung tissue were negative. ISH for *Pneumocystis* spp was negative as well. 1/60 nasal swabs for SIV tested positive. Assessment of environmental risk factors returned low biosecurity scores, aerial NH<sub>3</sub> of 30 ppm in farrowing rooms and high dust levels in weaned pigs rooms.

### Discussion & conclusion

Based on the absence of infectious agents tested for and the presence of an eosinophilic inflammation, it was concluded that an allergic reaction was the primary cause of sneezing and purulent rhinitis was considered as a secondary bacterial infection. High dust levels, climatic errors and poor biosecurity were the likely cause of the allergic reaction. Dust levels nor exact dust composition were measured, but is recommended in future cases.



## HME-005 - IMPACT OF PRRS WHOLE HERD VACCINATION ON WEANING WEIGHT AND GROWTH PERFORMANCE IN NURSERY PIGLETS UNDER FIELD CONDITIONS

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### Background and objective

Due to the nature of porcine reproductive and respiratory syndrome virus (PRRSV) the control and elimination of PRRS is still problematic. Long-term successful control strategies involve whole herd vaccination programs together with strict biosecurity and management measures. This study investigated the impact of whole herd vaccination with a PRRSV-1 modified live virus vaccine on weight at weaning and weight gain in nursery piglets under field conditions.

### Material and methods

The study was conducted in Austria on a farrow-to-finish farm with 230 sows producing in a three week batch farrowing system. The PRRS status of the farm was PRRS positive, field virus circulation was repeatedly detected in the nursery and in the fattening unit. Before the start of the study no PRRS vaccination was implemented on the trial farm. The study was initiated with a double mass vaccination of the breeding as well as the growing pig herd with ReproCyc® PRRS EU and Ingelvac PRRSFLEX® EU, respectively. Subsequently, the breeding herd was vaccinated every 4 months and piglets were vaccinated around weaning concurrently with vaccinations against PCV2 and *Mycoplasma hyopneumoniae*. Body weight at 4 weeks (weaning age), 13 and 22 weeks of age of 3 consecutive batches before and 6 consecutive batches after implementation vaccination was recorded. Recording of data after PRRSV vaccination was started 12 weeks after the second mass vaccination. Mean weaning weight and mean average daily weight gain in nursery piglets before and after PRRSV whole herd vaccination were compared.

### Results

Mean weaning weight could be significantly increased in piglets of vaccinated sows. Additionally, a significant increase in the average daily gain of nursery piglets could be achieved after implementation of PRRS vaccination.

### Discussion and conclusion

The results indicated positive effects of PRRSV whole herd vaccination on the weaning weight as well as the growth performance in nursery piglets.



## HME-006 - USE OF PADRAP- PRODUCTION ANIMAL DISEASE RISK ASSESSMENT PROGRAM- IN 167 FARMS IN SPAIN

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### Introduction and objectives

Identifying and controlling internal and external risk factors for the introduction and spreading of PRRSV are a key factor in disease control and prevention. The PADRAP -Production Animal Disease Risk Assessment Program- was developed to support the risks evaluation and management. The objective of this study was to evaluate the biosecurity level of Spanish farms using PADRAP and to evaluate the differences between regions.

### Materials and Methods

In Spain, 167 sow farms completed PADRAP between 2014 and 2016 to assess the current biosecurity status. The results were compared to other sow farms round the world in the PADRAP database. Three Spanish regions, Central and Northwest (CNW), Northeast (NE) and Southeast (SE) were also compared.

Commercially available software (Minitab 17 for windows) was used for statistical analyses.

### Results

Both external and internal risks mean scores were higher in Spain than the global mean scores. For internal risks the mean was 24.3 vs 19.2 and for external risks the mean was 28.3 vs 22.1.

Comparing the three regions, the lowest mean scores, for both internal and external risks, were obtained in farms in the CNW region while the highest scores were obtained in SE region.

The median of both scores for internal and external risks are also lower in the CNW region and higher in the SE one.

### Conclusions and Discussion

PADRAP is a tool to measure risks and to rank the farms. In Spain most of the analyzed farms have scores above the global mean scores as well as clear differences between regions. The results are worse in high pig density regions, which have a negative effect on the external risks. Therefore as the internal risks are not influenced by the pig density, all the farms, even the ones in the most high density areas, can make efforts to improve their biosecurity.



## HME-007 - A STATUS UPDATE OF THE HUNGARIAN NATIONAL PRRS ERADICATION PROGRAM IN MSD PARTNER FARMS

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### Background and Objectives

In 2014, Hungary enforced a mandatory national PRRS eradication program and each infected farm had to set up a PRRS eradication plan (PEP). Until 2016, depop-repop method (DRM) was the preferred PEP according to the legal provisions. This paper gives a status report of the PEP of MSD partner farms.

### Materials and Methods

In 2014, ¼ of sows in large commercial farms were infected with PRRSV and half of them had MSD as a technical partner for PEP set-up, allowing continuous production. MSD and partners use project-based approach to achieve the goals. ResPig and ReproPig tools were often implemented during planning & designing. PEPs focused on project team member requirements and critical success factors. They were used in 8/19 counties, in 21 farrow-to-finish, 1 farrow-to-nursery and 3 fattening farms. A total of 34,350 sows were involved across all projects.

### Results

To ensure PEPs success, 22/25 farms were audited in ResPig and ReproPig was implemented in 5/25 farms to improve reproduction and batch management (BM). Batch management was not used in 7/22 farms, but 11/22 applied 1-week, 3/22 3-weeks and 1/22 5-weeks BM as PEP element. Two farms applied only biosecurity measures, 12 farms vaccinated their piglets every 4 months and 1 every 3 months. One farm used 6+60 vaccination, 4 farms finisher vaccination only, 5 farms double mass vaccination, and one of them already finished vaccination. By November 2016, 19 breeding, 20 farrowing, 12 nursery and 4 finishing units became PRRS free (PCR negative).

### Discussion and Conclusions

Vaccination, BM, strict biosecurity measures and laboratory monitoring are necessary and effective elements of PEPs. This project-based approach gives an opportunity for continued production which is more economical than DRM. Based on MSD-related eradication results, the authorities changed the legal background resulting in DRM no longer as the most preferred PRRS eradication method.



## HME-008 - PRODUCTION IMPACT OF 3 WEEKS BATCH MANAGEMENT AS ELEMENT OF PRRS ERADICATION PROGRAM – A CASE STUDY

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### Background and Objectives

Many commercial swine farms cannot implement all-in-all-out (AIAO) because of different capacities of farrowing, nursery and finishing units. However, breaking the infection cycle is essential in disease eradications, thus creation of a sustainable batch management system (BMS) gives an opportunity to implement AIAO. This study explored the production impact of 3-week BMS implementation as element of a PRRS Eradication Plan (PEP).

### Materials and Methods

A farrow-to-finish Topigs genetics swine farm had 800 sows and their progeny. The farm was obliged to set up PEP following initiation of a mandatory national PRRS eradication program. Due to different capacities of consecutive farm production units, a 3-week BMS was launched with estrus synchronization for grouping sows starting April 2015. The farm production indices were surveyed from 2014 April to 2016 March (one year before and after BMS launch) to measure impact. The data were processed with Microsoft Excel®.

### Results

After launching BMS monthly farrowing decreased by 10%, artificial insemination by 12%, but AI/F increased by 2% with an extra 0.5 live piglet/sow/year. Although litters/sow/year and weaned piglets/sow/year indices slightly decreased, number of slaughter pigs/sow/year increased by more than 1.5 times. Mortality and culling rate were improved; in farrowing unit the combined index decreased from 9.6% to 6.5%; in nursery from 6.9% to 1.6% and for finishers from 10.5% to 7.5%. Feed conversion ratio improved in the nursery from 2.27 to 2.17 and for finishers from 4.33 to 3.88. Annual total drug cost decreased by 25%, of which antibiotics by 34% and vaccination by 16%, contrary to an increased cost of disinfections by 241%.

### Discussion and Conclusions

The results support that 3 weeks BMS as element of PEP is an inevitable tool for the implementation of AIAO. BMS helps to break PRRSV infection cycle, thus, accelerates PRRS eradication and improves swine production indices.





## HME-009 - THE IMPACT ON MORTALITY OF ALTERATION OF FOSTERING AS ELEMENT OF A PRRS ERADICATION PROGRAM – A CASE STUDY

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### Background and Objectives

Fostering gives a possibility to grow more piglets and to increase milking capacity and fertility of first parity sows. In disease eradications, fostering alteration can play a role in breaking infection cycle and cross-infection prevention. This study reports impact on suckling piglet mortality of altered fostering methods as element of a PRRS Eradication Plan (PEP) in a Hungarian farrow-to-finish farm.

### Materials and Methods

The 2,250 sow farm applied cascade fostering (CF) and piglet rescue desks (RD), but in February 2015 a PRRS outbreak was observed. Afterwards, according to PEP, pigs were vaccinated twice against PRRSV and in April 2015, CF and RD were changed to McRebel Management System (MMS). After 6 months, additional PRRS negative nursing sows entered the repopulated farrowing pens and fostering was allowed again (but no piglet movement). In February and May 2016, CF and RD were re-introduced. Suckling piglets mortality was surveyed (February 2015 to August 2016) and impact on mortality of fostering alteration was analyzed.

### Results

After PRRS outbreak (February 2015 to August 2015), the number of live born piglets significantly decreased (-11%), but there were more piglets than available sow nipples. MMS reduced the infection pressure and weaned piglets became field virus PCR negative, but piglet mortality almost tripled (22%) because of no fostering. From November 2015, nursing sows' extra nipples improved piglets' survival and mortality decreased to 13%. Re-introduction of CD further reduced mortality to 10% and fell to 8% after RD introduction.

### Discussion and Conclusions

Efficient operation of intensive swine farms with hyper productive breeds requires effective fostering methods. CF and RD are good tools to receive as many weaned piglets as possible. During disease eradications, free fostering needs to be stopped to avoid piglets' cross-infection. MMS and then fostering alteration according to the epidemiologic situation in the farrowing unit can help reduce production losses.



## HME-010 - CASE REPORT: IDENTIFICATION OF MYCOPLASMA HYOPNEUMONIAE INFECTION IN A BREEDING HERD THROUGH TRACHEA-BRONCHIAL SWAB MONITORING

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### Background & Objectives

A dedicated program to monitor for freedom of several economically important diseases is present within most of the breeding companies that currently deliver high health breeding animals to their end customers. Serology is therefore the preferential approach in order to screen for most of these diseases, such as *Mycoplasma hyopneumoniae* (*M.hyo*). However, in case of positive serology, further decisions on farm health status and the related consequences should be based on additional confirmation tests. The objective of the current case report is to show the added values of trachea-bronchial swab (TBS) sampling in the confirmation of a dubious *M. hyo*-seropositive situation.

### Materials & Methods

A cluster of 2 high health breeding farms in Europe were shown positive for *M.hyo* using the conventional ELISA serology. Looking for confirmation with a second ELISA test, however, samples showed negative serology. Moreover, throughout the entire monitoring period, no coughing, necropsy lesions or lesions at slaughter could be detected in order to obtain a definite confirmation on the *M.hyo* health status. Since health status of breeding farms should be reliable in order to guarantee a continuous outflow of high health animals, other techniques such as TBS were used to confirm the health status for *M. hyo*. In total 186 samples were collected at different ages: piglets at 4 and 8 wk, gilts at 22 and 26 wk, and gilts and sows in the breeding location.

### Results

Collected TBS samples were negative until 8 wk of age, but older gilts before and after introduction in the breeding herd were shown *M.hyo*-positive using TBS sampling.

### Discussion & Conclusions

The collection of TBS samples to confirm the *M.hyo* health status of a breeding herd has been shown to create additional information in order to take crucial decisions concerning health management within a breeding company.



## HME-011 - RETURN-ON-INVESTMENT OF PORCILIS® PCV M HYO VACCINATION COMPARED TO OTHER VACCINATION PROGRAMS IN 15 BELGIAN PIG HERDS

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### Background & objectives

As economics are a big driver in the decision of piglet vaccination, return-on-investment (ROI) of vaccination with Porcilis® PCV M Hyo (PPMH), a ready-to-use combination vaccine for Porcine Circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhyo), was investigated in a large field study.

### Materials and methods

Porcilis PCV M Hyo vaccination at 3 weeks of age was compared to traditional vaccination programs which included vaccination against Mhyo (A, n=8), PCV2 (B, n=1), separate injections of Mhyo and PCV2 (C, n=2) or no vaccination (D, n=4). Herd factors including herd size, genetics, batch production system and the presence of clinical signs (dry cough, lung lesions at slaughter, heterogeneity and wasting) were recorded in the control. The extra cost of vaccination with PPMH (vaccine and labor) per pig was estimated at 1€, 0.85€, 0€ and 1.90€ for group A to D. The benefit after vaccination was calculated based on 0.025€ per +1 g/pig/day in average daily weight gain and 0.23€ per -0.01 in feed conversion rate from 10 weeks of age until slaughter. A weighted average profit per group was calculated based on the number of pigs per herd.

### Results

The average profit of Porcilis PCV M Hyo vaccination was 1.73€, 0.42€ and 1.57€ per pig compared to single Mhyo vaccination, single PCV2 vaccination and no vaccination. The ROI was 173%, 49% and 83%, respectively. Profit in group C was an extra 0.33€ per pig. Higher profit margins were recorded in herds with clinical signs in the control.

### Discussion & conclusions

Field data of 15 Belgian farms showed a positive ROI of Porcilis PCV M Hyo vaccination compared to vaccination against Mhyo only, PCV2 only, Mhyo and PCV2 as separate injections and no vaccination. Additional research will focus on herd factors that correlate with the profit margin.



## HME-012 - PIGTRACK® REAL-TIME MONITORING PROVIDES NEW INSIGHTS TO IMPROVE ON-FARM SOW MANAGEMENT STRATEGIES: A PRACTICAL CASE ON NEONATAL PIGLET MORTALITY

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### **Background & Objectives**

PigTrack® real-time data collection and analysis is able to create early alerts that are available to all stake-holders (farm manager, personnel and farm veterinarians) related to the sow herd. They can use the data in order to optimize intervention strategies at all levels of sow farm (gestation, transition, farrowing) and more specifically farrowing room management.

### **Materials & Methods**

A specialized swine veterinarian equipped a large sow farm (n=1300 sows) with PigTrack® in order to obtain real-time data collection and improved data analysis. The advantage of PigTrack® system is that clinical and technical production data can be analyzed real-time. Therefore, the PigTrack® system collects various important production and related health data, such as number of live born, dead born and mummified piglets, day of farrowing, piglet quality score (light weight piglets), mortality with specific reason or pathology, disease in piglets (diarrhea, coughing, joint illness, ...) and clinical status of the sow (fever, agalactia, ...).

### **Results**

Analysis revealed that piglet vitality and survival during the first days of life were issues that should be tackled. Specific management actions were determined: 1. sow feeding in the last weeks of gestation to improve piglet birth weight and overall vitality; 2. management measures to improve neonatal piglet care in the first 3 days of life to increase overall piglet survival; and 3. specific actions towards sow treatment following reduction of feed consumption frequently observed (code: sow does not eat) during the first week post-farrowing.

### **Discussion & Conclusions**

The first experience with broader use of the PigTrack® system on large sow farm in Belgium shows that the tool can contribute to improved farrowing house management. In conclusion, improved registration of neonatal piglet mortality resulted in immediate practical interventions to correct the situation and to improve piglet production in the sow herd.



## HME-013 - TECHNICAL PERFORMANCE COMPARISON IN FINISHERS VACCINATED WITH PORCILIS® PCV M HYO OR OTHER VACCINATION PROGRAMS IN 15 BELGIAN PIG HERDS

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### Background & objectives

Porcine Circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhyo) play a primary role in porcine respiratory disease and are endemic in Belgium. This field study compared the ready-to-use combination vaccine Porcilis® PCV M Hyo (PPMH) to other vaccination programs.

### Material & methods

Vaccination with PPMH at 3 weeks of age was compared to the traditional vaccination program (control) in 15 Belgian herds. The control varied between herds: single Mhyo vaccination (n=8), single PCV2 vaccination (n=1), Mhyo and PCV2 as separate injections (n=2) or no vaccination (n=4). Average daily weight gain (ADWG), feed conversion rate (FCR), antibiotic treatment and mortality were evaluated between 10 weeks of age and slaughter. In each herd, finishers of both groups were housed in the same location.

### Results

PPMH compared to single vaccination against Mhyo resulted in an average increase of 27 g/pig/day [range 3–68], a decrease in FCR of 0.09 [0.02–0.28] and a decrease in mortality of 0.3% [-0.8–2]. PPMH compared to single PCV2 vaccination resulted in an ADWG of 747 versus 724 g, a FCR of 2.63 versus 2.66 and a mortality of 1.65% versus 1.95%. Technical parameters were similar following vaccination with the ready-to-use combination vaccination as with two vaccines by separate injection. Compared to no vaccination, PPMH improved ADWG by 19 g [14–60] and FCR by 0.13 [-0.01–0.26]. Antibiotic use was not different across herds.

### Discussion & Conclusion

Vaccination with Porcilis PCV M Hyo improved ADWG and FCR compared to single vaccination against Mhyo and PCV2 and no vaccination. Porcilis PCV M Hyo vaccination performed as good as vaccination with two separate injections. The finding that additional vaccination against one or two respiratory pathogens did not reduce antibiotic treatment might be explained by a more preventive than curative use of antibiotics in this study.



## HME-014 - PIGTRACK® REAL-TIME MONITORING PROVIDES NEW INSIGHTS TO IMPROVE ON-FARM SOW MANAGEMENT STRATEGIES: PRACTICAL CASE ON DIAGNOSTICS AND GILT INTRODUCTION MANAGEMENT

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### Background & Objectives

PigTrack® real-time data collection and analysis is an early alert monitoring device aiming to provide immediate data output and analysis to all stake-holders related to the sow herd, such as farm manager, personnel and farm veterinarians, in order to improve monitoring and intervention strategies at all levels of sow farm and more specifically farrowing room management.

### Materials & Methods

A specialized swine veterinarian equipped its large sow farms with PigTrack® in order to obtain real-time data collection and improved data analysis. Using the PigTrack® system, several data points can be collected on a real-time basis: number of live born, dead born and mummified piglets, day of farrowing, piglet quality score (light weight piglets), mortality with specific reason or pathology, disease in piglets (diarrhea, coughing, joint illness, ...) and clinical status of the sow (fever, agalactia, ...). The advantage of PigTrack® system is that clinical and technical production data can be combined and analysed real-time.

### Results

Specific management actions resulting from farm data analysis were: 1. improved diagnostic effects for neonatal piglet diarrhea; 2. better treatment options for affected piglets; 3. changes in the piglet adaption management to specific sow parities (e.g. 1<sup>st</sup> parity sows vs. 3<sup>rd</sup>-4<sup>th</sup> parity sows); and 4. homogenization of gilt adaptation and subsequent introduction.

### Discussion & Conclusions

The first experience with broader use of the PigTrack® system on large sow farms in The Netherlands shows that the tool can contribute to improved farrowing house management through thorough analysis possibilities between clinical data on piglets and sows and the relevant technical production data of the same batch. In conclusion, direct availability of clinical scores of piglets and sows and detailed mortality registration result in immediate practical interventions to correct the situation and to improve piglet production in the sow herd.



## HME-015 - PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) HERD CLASSIFICATION OF UK BREEDING HERDS THAT USE DIFFERENT VACCINATION PROGRAMS

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### Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most important diseases in the global swine industry. Weaning non-viraemic pigs is desirable from a disease control point of view and gives the breeding herd a stable PRRSV status. The objective of this study was to assess the PRRSV status of serologically positive pig breeding units that vaccinate in the UK.

### Methods

To assess if PRRSV is present in pigs at weaning, blood samples were collected at the time of weaning and before PRRS piglet vaccination. The serum samples were individually tested by a PCR assay (orf 5; Lifetech PCR) and the herd was classified based on the result. A farm was classified as unstable if one sample was PCR positive.

### Results

Twenty nine (29) breeding units were evaluated (average herd size 883 breeding sows). Sixteen (55%) of these units were stable. Twenty-two (22) of the breeding units vaccinated with Porcilis® PRRS and 14 (64%) of them were classified PRRSV stable. There was no difference in outcome between Porcilis PRRS vaccination programs, i.e. sows being re-vaccinated either at pregnancy check (8 farms) or at weaning (13 farms). One farm, that re-vaccinated sows at both times, also tested stable. Of the seven (7) farms that used a competitor vaccine and mass-vaccination program only 2 (29%) were stable.

### Conclusions

Porcilis PRRS demonstrated to be a valuable tool for PRRS control regardless of vaccination program, with a higher percentage of farms being PRRS stable (Holtkamp PRRS classification 2) than competitor PRRS vaccines. Based on the results in this study, Porcilis PRRS tended to control PRRS better in breeding units than competitor vaccines ( $p=0.09$ ).



## HME-016 - COMPARATIVE FIELD EFFICACY OF AN INTRADERMAL PCV2 VACCINE AND A LICENSED INTRAMUSCULAR PCV2 VACCINE

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### Introduction

As intradermal vaccination has many advantages over intramuscular, we compared the efficacy of a new PCV2 intradermal vaccine, Porcilis® PCV ID, against a licensed intramuscular PCV2 vaccine.

### Methods

An indoor farrow-to-finish unit with 500 sows randomly allocated 141 pigs to group A, vaccinated with Porcilis PCV ID (0.2 ml) with the IDAL device or group B, 139 pigs, vaccinated with a licensed intramuscular PCV2 vaccine (1 mL). Farm management and study monitors were masked to the treatments and treatment groups were commingled.

All pigs were individually weighed at the time of vaccination, end of nursery, grower stage and before slaughter. Blood samples were collected to assess PCV antibody titres and viraemia at the same time points.

### Results

At weaning (W), end of nursery (N), grower (G) stage and finishing (F), live weights were not significantly different between groups: **A:** W - 8kg, N - 17.24kg, G - 42.49kg, F - 95.6Kg; **B:** W - 7.97kg, N - 17.18Kg, G - 42.49kg, F - 94.4kg.

PCV2 antibody titres were significantly higher for Porcilis PCV ID group at the end of nursery, grower and slaughter stage.

### Conclusions

Under the conditions of this study, ID vaccination resulted in significantly higher antibody titres at the end of nursery and grower stage, which may explain the 1.24 kg higher average slaughter weight (though not significant) of the Porcilis PCV ID pigs. Reduced handling stress may also have impacted this result. Both vaccines controlled PCV2 viraemia.





## HME-017 - ASSESSMENT OF PCV2 AND MYCOPLASMA HYOPNEUMONIAE INTRADERMAL VACCINATION ON SWINE PRODUCTION PARAMETERS

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### Introduction

Pig production depends on control of major pathogens to ensure health, welfare and sustainability of pig producing enterprises. This study assessed impact of PCV2 and *Mycoplasma hyopneumoniae* (Mhyo) intramuscular or intradermal vaccination on pig health and performance.

### Methods

An indoor farrow-to-finish unit with 200 sows vaccinated 61 pigs with Porcilis® PCV ID (0.2 ml) and Porcilis® M Hyo ID ONCE (0.2 ml) (intradermal) and 58 pigs intramuscularly with a competitor PCV2/Mhyo vaccine (2 ml) at 28 days of age. All pigs were weighed at vaccination and before slaughter. Blood samples were collected for PCV2 viraemia at the same time points. Respiratory tracts were assessed at slaughter and scored for Mhyo like lesions (EPL).

### Results

Live weight at vaccination (SD) was not significantly different between groups: ID 6.3 kg (1.3) and IM 6.4 kg (1.2). PCV2 ELISA titres were not significantly different between groups and all samples were negative for PCV2 PCR.

The average live weight (SD) before slaughter was significantly higher in ID than IM group: ID - 74.2 kg (6.73) and IM - 70.9 kg (7.31). Average daily live weight gain from wean to slaughter was 601 for ID and 571 g / day for IM group.

EPL was not significantly different between ID (n=55; 3.8) and IM groups (n=57; 3.6) and IM group had 18% pleurisy versus 11% for ID.

### Conclusions

Under the conditions of this study, ID vaccination resulted in higher live weight at slaughter. The better performance of the ID group may have been due to the lower handling stress and/or better immune response due to more consistent antigen delivery. Both administration routes resulted in good control of PCV2, with no viraemic pigs at slaughter.

The extra 3.3 kg of live body weight represents a significant increase in profitability of £1.9 (including feed cost).



## HME-018 - PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) DIVERSITY ASSESSMENT IN A REGION OF THE UK

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### Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most important diseases in the global swine industry. Sequencing of the orf5 region is a valuable tool to monitor PRRSV strains within a farm or region over time. The objective of this study was to assess the diversity of PRRS circulating in East Anglia (EA), UK.

### Methods

Blood samples and oral fluid samples individually tested and positive by a PRRSV orf5-specific commercial PCR were sequenced. Nucleotide sequences were aligned with proprietary software (MEGA 6.06) to construct phylogenetic trees and assess mean distances.

### Results

Ten samples from pigs from 2 pig production companies within a 90 km radius in East Anglia were sequenced. The orf5 sequences were all closely related with a within group mean distance of 0.0162. The mean distance between 26 orf5 sequences from samples taken from pigs in other UK regions and the EA clade was 0.1395. The mean distance between the EA clade and the DV strain of Porcilis® PRRS was 0.099 whilst it was 0.137 to other commercially available MLV vaccines. The samples in the EA clade originated from farms belonging to different swine production companies.

### Conclusions

The fact that orf5 sequences in such a large geographical area and in farms with different ownership were so closely related raises questions about the in-between farm transmission. As many farms are outdoors, the role of wildlife as carriers warrants further investigation. Biosecurity should be improved to ensure that PRRS control programs have a good chance of success. The EA clade was closer to Porcilis PRRS than to other vaccine viruses and the importance of this in PRRS control needs to be assessed. Sequencing of PRRS samples will prove key to any regional control program to assess virus spread and (re)-introduction within swine production companies and regions.



## HME-019 - A DESCRIPTIVE STUDY OF INTERNAL AND EXTERNAL BIOSECURITY LEVELS IN BELGIAN PIG HERDS

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### Background & Objectives

Biosecurity describes the measures applied to prevent the introduction of pathogens into a herd (external biosecurity, ExBS), as well as the spreading of those pathogens within a herd (internal biosecurity, InBS). Improvement of biosecurity at herd level can result in lower morbidity and mortality rates, making biosecurity a crucial tool in the daily herd health management. This study aims to describe the current biosecurity situation in Belgian pig herds.

### Material & Methods

A biosecurity audit (MSD AH Belgium) was used. It consists of a total of 24 multiple-questions subdivided in 2 subcategories for ExBS [location (L); purchasing policy/quarantine (PQ)] and 4 subcategories for InBS [All-in/All-out procedures (AIAO); hygiene (H); management of diseased animals (MD); housing/climate (HC)]. The answer to every question is a score between 1 (lowest risk of disease) when this measure is implemented and 4 (highest risk of disease) when the measure is not implemented. The average score for ExBS and InBS represents the total biosecurity score. A lower score is indicative of a better biosecurity and, hence, lower disease risk.

### Results

In total 30 Belgian swine farms were included in this study (1 farrow-to-wean, 17 farrow-to-20kg, 12 farrow-to-finish). The average amount of sows/herd was 616 [200-1700]. The average total biosecurity score was 1.77 [1.38-2.33]. The average score for each category were ExBS: 1.99 [1.29-2.71] and InBS: 1.68 [1.29-2.29]. The average score for each subcategory were L: 2.95 [1.00-4.00], PQ: 1.61 [1.00-2.20], AIAO: 1.98 [1.00-3.50], H: 1.63 [1.00-2.67], MD: 1.89 [1.00-3.67] and HC: 1.28 [1.00-2.00].

### Discussion & Conclusion

This study confirmed previous observations such as high pig density in Flanders. However internal biosecurity measures, such as all-in/all-out procedures and management of diseased animals, remain below desirable levels in Belgian pig herds. Attention should be focused on unidirectional workflow using separate material per age groups, and isolation of sick pigs.



## HME-020 - ECONOMIC IMPACT OF SURGICAL CASTRATION IN THE SUCKLING PERIOD

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### Background & Objectives

Available evidence shows that surgical castration has a detrimental influence on health, resulting in an economic impact. This study evaluated the economic impact of surgical castration with pain relief in the suckling period.

### Material and methods

A total of 3696 male pigs from 721 litters were included in the study. Within each litter, half of the males were kept as entire males (EM) and half were surgically castrated (SM), using a non-steroidal anti-inflammatory drug before castration and a topical antibiotic spray immediately after the surgical procedure. Economic calculations were done based on difference between treatments in number of piglets weaned per sow per year (WSY) and cost of castration (cost of medications used during castration and labour cost). 1.5 h time was assumed as time required to castrate 100 piglets, and cost of worker was 25 EUR/h. Comparison of weaned pig production cost of EM vs SM was done assuming cost per sow (677 EUR; SIP Consultors, 2015) and WSY production in Spanish farms in 2015 (26.2 WSY; PigCHAMP Pro Europa dataset).

### Results

Pre-weaning mortality (PWM) was higher in SM than in EM (6.3% vs 3.6%;  $P < 0.001$ ), but in this study percentage of pigs treated with antibiotics was not affected by castration. As a result, based on Spanish farms data in 2015, 0.33 male pigs less would be weaned in farms rearing SM compared with farms rearing EM. Castration cost was 0.19 EUR per pig and then the cost of one weaned pig per sow per year is 0.52 € higher in sows where surgical castration is applied in 3-6 days old pigs.

### Conclusions

Surgical castration promotes productive losses in the suckling period because it causes an increase in PWM. This implies an economic cost in the suckling period, worsening in later productive phases by a higher feeding cost.



## HME-021 - ASSESSMENT OF THE SALMONELLA STATUS IN 85 FARROWING FARMS IN GERMANY FROM JANUARY 2015 TO NOVEMBER 2016

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For over ten years the German *Salmonella* control program has focussed on assessing the prevalence in slaughter pigs via serology. However, this program has not managed to reduce the number of positive fattening herds. Previous studies have shown, that the *Salmonella* status of the fattening farm is strongly related to the *Salmonella* status of the sow farm (Hill 2015).

In order to assess the role of sow farms on the *Salmonella* introduction into fattening farms with a high *Salmonella* prevalence, 1925 samples from 85 farrow-to-grower farms (sized 80 to 10 000 sows) were analyzed for *Salmonella* via microbiological culture. In all the farms sock samples and environmental swabs were taken; the number depended on the size of the farm with at least one sample taken in all stages of production. For each sample new gloves and overshoes were used in order to prevent cross contamination. The samples were analyzed at the Institute for Microbiology at the University of Veterinary Medicine Hannover. All detected *Salmonella* strains were typed.

An average of 12 sock samples and 11 swabs per farm resulted in a total of 1015 socks and 910 swabs. On 79 farms (93%) *Salmonella* spp. was detected in at least one sample. 46.1% (n=439) of the socks and 32.6% (n=265) of the environmental swabs were positive for *Salmonella*. The most frequently detected serotype was *Salmonella* Typhimurium in 69.7% (n=306) of the socks and 66.9% (n=172) of the swabs.

These results show, that *Salmonella* control should start early in the piglet's life with a stronger focus on the prevention of the transmission from the sows to the piglets. Each farrowing farm should have knowledge about their *Salmonella* status, which should not be determined via serological sampling alone but rather on the analysis of sock and environmental swabs via microbiological culture.



## HME-022 - THE ASSOCIATION BETWEEN CAUSE OF DEATH AND THE OCCURRENCE OF STOMACH ULCERS IN PIGS SUBMITTED TO AUTOPSY FROM 114 HERDS

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### Background & Objectives

Previous studies indicate that infections, e.g. with PCV2 or *App*, might be associated with an increased risk of stomach ulcers. The aim of this study was to determine if there was an association between the cause of death and the presence of stomach ulcers in weaners and growing-finishing pigs submitted for autopsy at a laboratory.

### Material & Methods

All pigs (10-100 kg) sent to the Laboratory for Pig Diseases, Denmark, during a period of 13 months in 2015-16 were included in the study (max. five pigs per submission), a total of 302 pigs from 114 herds. Based on the autopsy findings, each pig was allocated to one of five diagnosis groups (estimated cause of death/euthanasia): respiratory disease, intestinal disease, septicaemia, arthritis, and no findings. Fifty-five pigs with other causes of death (including stomach ulcers) were excluded from the study. Any ulceration and fibrosis in the oesophageal part of the stomachs was recorded. A logistic mixed model analysis was carried out (SAS, EG version 7.1). The dependent variable was the presence of ulcers and/or fibrosis. The explanatory variables were diagnosis group, bodyweight and gender. The journal number was included as a random effect. No pairwise interactions between the explanatory variables were found.

### Results

An increased risk of having an ulcer/fibrosis was found in pigs that had died of respiratory disease (43%) compared to pigs that had died of intestinal disease (14%,  $p=0.017$ ) or arthritis (11%,  $p=0.049$ ). The risk of having an ulcer/fibrosis increased with increasing bodyweight ( $p=0.01$ ), although ulcers were also found in the smallest pigs.

### Discussion & Conclusion

The results indicate that respiratory disease can be a contributory factor in the development of ulcers. However, the results should be interpreted with caution since the study is based on selected material which is not necessarily representative of pigs that have died in the herds.



## HME-023 - A CASE REPORT ON DOUBLE NURSING; AN ALTERNATIVE NURSING STRATEGY FOR HYPER-PROLIFIC SOW HERDS

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### **Background and objective**

In hyper prolific sow herds the number of live born piglets easily exceeds the number of available teats and farmers use several strategies to feed the surplus piglets; e.g. cross fostering, supplying milk replacers or early weaning. This abstract describes the design and application of a so called 'double nursing' (DN) strategy to nurse the surplus piglets. A retrospective study on herd performance data, from a three year period was performed to assess the effect of DN on preweaning mortality in DN litter and reproduction parameters of the subsequent litter.

### **Material and Methods**

DN sows nurse two matched groups of piglets (by number and age) from  $\pm 12$  to 24 hours after parturition until weaning. Group 1 is the litter born from the DN sow and group 2 consists of an entire litter from one donor-sow. The donor-sow is then available as foster sow for surplus piglets from the same farrowing batch. DN sows must meet a number of criteria. Multivariable analyses, correcting for parity and year of farrowing, were used. Since a three year period was analyzed, multiple observations per sow were present. This was taken into account in the analyses, using a random sow effect

### **Results**

Preweaning mortality was lower in DNS (5.1%) than in single nursing sows (7.1%). A small negative effect of DNS on litter size in subsequent litters is observed (-0.95 Total Born Piglets). No negative effect on interval weaning to conception was found.

### **Discussion & conclusion**

In this herd, DNS proved to be a successful strategy. The authors want to articulate that the effects of DN that were found in this study only apply when sows meet the specific criteria.



## HME-024 - EVALUATION OF HEMOCUE® HB 201+ SYSTEM IN DIAGNOSING ANEMIA IN SOWS

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### Background and Objective

Assessment of hemoglobin (Hb) is a common method for diagnosing anemia and iron deficiency. Automated analysers are widely used for this purpose and are considered gold standard. HemoCue® Hb 201+ system (HemoCue®) is a recently introduced pen-side method of Hb determination in swine practice. Therefore, our study aimed at assessing the accuracy of HemoCue® in diagnosing anemia in sows compared to Advia 2120i Haematology System (Advia).

### Materials and Methods

Blood samples from 247 sows from five Danish commercial herds were analysed. Blood samples were collected from jugular vein at 56 to 70 days of gestation in EDTA tubes and Hb was determined simultaneously using HemoCue® and Advia. Anemia was defined as Hb value below 110 g/L based on literature. Sensitivity and specificity of HemoCue® in diagnosing anemia was calculated using Advia as the standard.

### Results

The mean Hb value using HemoCue® ( $112.1 \pm 16.6$  g/l) was significantly lower than measured using Advia ( $119.1 \pm 12.4$  g/l) ( $p < 0.0001$ ). Hb measurements from the HemoCue® and Advia were moderately correlated ( $r = 0.59$ , 95 % CI: 0.50-0.66). The sensitivity of HemoCue® in diagnosing anemia was 96.9 % (95 % CI: 92.8 - 99.6 %) with a specificity of 77.3 % (95 % CI: 70.0 - 83.2 %).

### Discussion and Conclusion

Although the HemoCue® measurements are generally lower than that of Advia, it is highly sensitive in diagnosing anemia in pregnant sows. Because of the moderate specificity, the consequence with this test might be that few sows which do not have anemia get iron treatment. The results should be weighed against the advantage of simple, cheap, quick and on-farm measurement of Hb concentration.





## HME-025 - IMPACT OF PIGLET PRRS VACCINATION WITH INGELVAC PRRSFLEX® EU ON FINISHING PERFORMANCES IN A FRENCH FARROW TO FINISH FARM

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Porcine Reproductive and Respiratory Syndrome virus (PRRSv) is a major pathogen associated with swine disease. PRRSv infection causes respiratory disease, slower growth and increased mortality in growing pigs. Biosecurity measures, management measures and vaccination are implemented in infected farms in order to control PRRSv. The objective of this study was to evaluate the impact of a PRRS control plan on medication and production parameters in growing pigs in a farrow to finish farm.

This "before and after" study was carried out in a 450-sow farrow-to-finish farm in Brittany, France. The herd was positive for PRRSv, PCV2, Swine Influenza virus, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*. At the time of inclusion only gilts and sows were vaccinated against PRRSv with a PRRS MLV vaccine and PRRSv was isolated on pigs of 6 and 9 weeks of age. As a prerequisite biosecurity and management measures were so far as possible reviewed and corrected. A whole herd vaccination on the same day with Ingelvac PRRSFLEX® EU and ReproCyc® PRRS EU was first carried out. Then a mass vaccination was implemented on the sow herd every 3 months and the growing pigs were vaccinated around weaning. The selected criteria to assess PRRS vaccination efficacy on piglets were recorded on the finishing phase. They include: ADG, loss rates, slaughter condemnations for pleuritis and antibiotic treatments.

PRRS vaccinated pigs performed better resulting in an ADG moving from 725g to 745g and a loss rates from 6,2 to 5,2%. The carcass respiratory lesions dropped from 3 to 1,3%. The medical treatment cost was reduced from 1,30 to 1,10€/pig only due to less antibiotic used. The calculated ROI was 3,9.

Under the conditions of this study, vaccination of the piglets around weaning with Ingelvac PRRSFLEX® EU improved the health status and performances in finishing.



## HME-026 - STUDY OF VALIDATION OF THERMOGRAPHIC CAMERA: PRELIMINARY RESULTS

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### Background & Objectives

Fever as clinical sign in pigs is measured through individual control by different kinds of thermometers. It is a time consuming procedure. Usage of thermography for assessing temperature in groups/herds is unexplored and can be a useful tool for vets.

So, the objective of this work was to demonstrate that thermal imaging, obtained through a smartphone adaptable device, and combined with specific software is a quick and non-invasive alternative to reflect body temperature in pigs.

### Material & Methods

The validation was made by comparison of rectal temperature of sows with images made with thermographic camera and using specific software, analyzing factors that influence deviation of results. Such factors are: distance pig to camera, room temperature, point of measurement, relative humidity, speed of air currents and dirtiness level. Four body areas of comparison were established for each image. The data were compared by means of Spearman's correlation.

### Results

It was demonstrated that data provided by thermographic camera is accurate and corresponds to rectal temperature if measured between 2 and 3 meters of distance. The temperature and humidity revealed poor influence on measurements. There was a correlation with rectal temperature between  $r=0.7$  ( $p=0.23$ ) for back area and  $r=0.95$  ( $p<0.001$ ) for vulvar area.

### Discussion & Conclusion

Early detection of possible alterations in pig herds is vital for sanitary control of the farm, as well as for prevention of complications in clue moments of production. This tool can be of great practical application to improve animal welfare.



## HME-027 - COMPARISON OF AVERAGE DAILY WEIGHT GAIN IN PIGLETS VACCINATED AGAINST *M. HYOPNEUMONIAE* AND SIMULTANEOUSLY OR TWO WEEKS LATER AGAINST PCV2

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### Background and objectives

Two vaccination schemes against Porcine circovirus type 2 (PCV2) were compared with regard to piglets average daily weight gains (ADWG) until the end of the rearing period. The background of the study is the discussion about stress during vaccination around weaning by handling and immune stimulation with a potential negative impact onto piglets growth rates.

### Material and methods

A total of 204 piglets from two farrowing batches were assigned to two groups according to their weight and gender. All pigs were vaccinated against *M. hyopneumoniae* in the first week of life and on the 21<sup>st</sup> day of life. Half of the piglets was vaccinated against PCV2 simultaneously on day 21 of life on the other side of the neck (groups B and D), while the other half of the piglets was vaccinated against PCV2 two weeks after weaning on day 35 of life (groups A and C). ADWGs in the suckling, rearing and whole study period were compared between both vaccination groups.

### Results

No significant difference in the ADWG was observed between groups A+C and groups B+D for the whole study period (1<sup>st</sup>-77<sup>th</sup> day of life,  $p=0.09$ ). However, in the rearing period the ADWG in the group with concurrent vaccine application was significantly higher ( $p=0.04$ ).

ADWGs differed significantly between the two examined farrowing batches in summer and autumn, with higher ADWGs in autumn ( $p<0.01$ ).

### Discussion and Conclusion

A concurrent application of vaccines against *M. hyopneumoniae* and PCV2 in different injection sites around weaning had no negative effect on ADWG compared to timely separated vaccinations. A tendency towards a higher ADWG was found in concurrently vaccinated piglets. This effect can be superimposed by seasonal and batch effects. The concurrent vaccination against *M. hyopneumoniae* and PCV2 has the advantage of reduced workload and less handling stress of pigs.



## HME-028 - RAISING PIGS WITHOUT ANTIBIOTICS, THANKS TO ALGAE

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Antibiotic resistance is one of the top five public health concern according to WHO 2015 report. Consequently, Olmix has developed a complete program aiming at accompanying producers to decrease the use of antibiotics and thus limit the development of antibiotic resistance.

The program was implemented in a farrow-to-finish pig operation of 840 sows in France. The farm was submitted to a detailed audit in December, 2015, from which an action plan was prepared and agreed with all collaborators. The action plan aimed at reducing antibiotic use from 100% of the pigs being systematically treated with one or several antibiotics, to a maximum of 10% of the pigs being treated with only one antibiotic.

Several recommendations constituted the action plan. An improved cleaning and disinfection protocol was implemented. Adjustments were made in the farrowing room in order to improve the comfort of newborn piglets. Biosecurity management was reinforced by a stricter quarantine program, more control of inputs to the farm and an action against rodents. The use of 5 innovative marine algae-based products, used in environment, feed or drinking water, completed these different measures. The action plan was implemented step-by-step from February to June 2016.

The first results of this case study (July to October 2016) show that the use of coccidiostat could be stopped, and antibiotic use was reduced by 94% (6.07% of the pigs underwent one antibiotic treatment between July and October 2016). Moreover, global performance in maternity was improved. The study continues in this farm in order to confirm these results and further improve performance. The one-year feedback already shows that it is highly important to adapt the strategy to each situation, and confirms the need of a global approach to reduce the use antibiotics in farms.



## HME-029 - ROOM DISINFECTION IN PIG HERDS: COMPARISON OF CHECKING PROCEDURES

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### Background and Objectives

Internal biosecurity in pig breeding is threatened when cleaning and disinfection operations are not properly carried out.

This study compares four methods of sampling and analysis to establish a standardized technical control for assessing the effectiveness of pig housing disinfection after the application of a disinfectant (Spectragen®).

### Material and Methods

The study was carried out in France (2015), in pig farms (9 farrowing, 3 post-weaning and 8 fattening rooms). Controls were realized in two times after the exit of animals: before disinfection and 24 hours after disinfection.

Samples were collected from 5 different areas (soils, partition walls, feeders, walls at the level and above animals).

Four different samples were realized on each area: 3 Petri dish for total flora research (PDTF), 3 Petri dish for fecal streptococci research (PDS), 3 swabs for total flora research (SWTF) and 3 swabs for fecal streptococci research (SWS).

### Results

Before disinfection, 98% PDS and 24% SWS showed results between 0 to 10 colonies of fecal streptococci. These analyses could not be interpreted.

After disinfection, the colonies of bacteria were greater than 300 colonies for 22% of PDTF.

The swabs (SWTF) results were most easily quantifiable (CFU/ml) and can be interpreted.

We could also compare the impact of disinfection depending on sampled areas. Despite the disinfection, feeders remained the most contaminated surface.

### Discussion and Conclusion

Total flora counting by swab (SWTF) is the most appropriate technique to assess the quality of cleaning disinfection in different breeding room areas, allowing to classify the quality disinfection and to point risk areas, by comparing data from the interpretation grid established by Corrége (2003).



## HME-030 - ATPMETRY: FAST AND EFFECTIVE TOOL ASSESSING CLEANING AND DISINFECTION QUALITY IN PIG HERDS

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### Background and Objectives

ATP-metry is a quick method of biological contamination measure of the surfaces.

This hygiene control is part of the HACCP process in food industry.

This field study aims to compare the results of ATP-metry and the counting of total flora, by swabbing surface, to validate the quality of disinfection after the disinfectant (Spectragen® or Virugen®) application in pig rooms.

### Material and Methods

This comparative study has been conducted in 20 French farms. Swab samples were collected from five areas (soils, partition walls, feeders, walls at the level and above animals), 24 hours after disinfection.

Analyzes were carried out using two types of swabs: one for total flora count (UFC/ml) and one for ATP measurement (in RLU).

The results were compiled according to the interpretation grids proposed by Azevedo Araujo (2002). The percentage of results for the same interpretation classes was observed. The correlation of the results was tested by khi2 test and ANOVA test.

### Results

There was a correlation between these two methods ( $p$ -value  $< 0.001^{***}$ ). Results of the two measurements were classified in the same category for 70% of the samples.

ANOVA showed these methods were statistically linked for walls at the level ( $p = 0,001^{***}$ ) and above animals ( $p = 0,0453^*$ ), but not for soils, partition walls and feeders.

### Discussion and Conclusion

ATP measures the bacterial population, but also the fungal population and viable somatic cells, which explains the differences of results for soils, partitions and feeders. ATP-metry cannot replace surfaces bacteriological control, but it has the advantage of being very fast (sampling and results within five minutes), effective and easy to implement. Thanks to this work, Synthèse Elevage® proposes a checking procedure, including ATP measures, to assess cleaning and disinfection quality in pig herd.



## HME-031 - ASSESSMENT OF BIOSECURITY ON PIG FARMS IN NORTHWEST GERMANY – EVALUATION OF PARAMETERS

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### Background & Objectives

To assess biosecurity, animal health, and the farm management various evaluation forms are in use which e.g. differ in the kind and number of parameters. To save time farmers and vets are looking for a small number of relevant and informative parameters to describe the situation on farm level.

### Material & Methods

On 51 pig farms, 102 parameters for farms biosecurity, animal health, and farm management were assessed. The assessment was repeated a year later on 37 farms. Regarding the issues internal and external biosecurity, feeding, production, clinical impression, and stable climate the impressions were independently assessed using a grading system from “1” (very good) to “6” (inadequate).

Different statistical analysis were conducted to evaluate the importance of the 102 parameters referring to the general impressions.

### Results

Regarding the 37 pig farms with repeated visits the assessment of parameters didn't differ between the first and second assessment a year later. Highly-rated farms were rated high again as well as poorly-rated farms were rated poorly in the second wave.

### Discussion & Conclusion

The statistical analysis reveals that biosecurity, animal health and farm management can be described by a small number of parameters. Based of those factors farmers and vets can launch measures to improve biosecurity and to increase animal health. Taking into account the correlations between parameters, the costs of the assessment of biosecurity on pig farms can be reduced.



## HME-032 - POSTPARTUM DYS GALAKTIA SYNDROME - EFFECT OF HERD HEALTH MANAGEMENT ON THE PREVALENCE AND THE TREATMENT INCIDENCE

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### Background and Objectives

The Postpartum Dysgalaktia Syndrome (PPDS) is considered as the most common disease in sows after farrowing. PPDS is a problem not only from the animal health and economic point of view, but also because of the possible development of antibiotic resistance. The aim of this study was to decrease the PPDS prevalence and the treatment incidence (TI) on problem farms with a veterinary herd health management (VHHM).

### Material & Method

VHHM was carried out during 12 months in 28 pig farms with a PPDS prevalence of more than 10%. The VHHM was divided into 2 sections: Assisted Intervention Program (regular farm visits) and independent intervention program (checking prevalence and implementation of measures by telephone). The antibiotics used were calculated with the TI according to Timmerman et al. (2006).

### Results

In 20 of 28 problem farms the PPDS prevalence could be decreased from 37.4% ( $\pm 21.8\%$ ) to 24.5% ( $\pm 14.1\%$ ). The strongest reduction of the PPDS prevalence was during the assisted intervention program (10.7%  $\pm 11.2\%$ ). VHHM had no significant effect on the TI. The most effective procedures to reduce the PPDS prevalence were the use of a prepartal transition feed, optimizing the PPDS diagnostic and the use of nonsteroidal antiinflammatory drugs (NSAID) and Oxytocin in the PPDS treatment.

### Discussion and Conclusion

The decrease of the PPDS prevalence clearly shows that a long-term improvement in animal health can only be achieved with perseverance and intensive veterinary herd health management. In order to avoid misdiagnosis and thus unnecessary antibiotic treatment, apathy, coprostitis, redness and swelling of the udder, hypogalacty, vaginal discharge, inappetence, and piglet behavior must be taken into account alongside the body temperature measurement. The use of "Highest Priority Critically Important Antimicrobials" should be avoided. In the treatment of PPDS administration of NSAID and Oxytocin is recommended.





## HME-033 - STORAGE OF PORCINE ORAL FLUID SAMPLES USING GENOTUBES

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### Background

Oral fluid samples (from pigs) are frequently used for prognostic monitoring of respiratory pathogens. Currently the samples need to be transported and stored in a cooled or refrigerated environment, which adds to the cost of shipping and also has a significant environmental impact. In this experiment, we evaluate GenoTubes as a method of transportation/storage of Oral Fluids from the producer to the diagnostic lab.

### Materials & Methods

The GenoTube is a non-invasive nasal swab for collection of DNA from the nostril of cattle, horses and other animals. With its proprietary drying system, it enables easy collection of animal diagnostic samples. Due to fast drying, the collected sample is conserved and helps send a high quality sample to the lab. Once dried, collected samples can be transported and stored without refrigeration, which makes transportation simple and cost effective. In the lab the samples can be easily reconstituted and used for numerous analyses.

Thirteen oral fluid samples (North American PRRSV positive and negative) were acquired and tested. A comparison between the standard Oral Fluid protocol and one using GenoTubes, was performed.

For each sample, the GenoTube was dipped in the sample and swirled around for at least 5 seconds. It was then placed back into its containing tube with desiccant and stored at room temperature for 24 hours. It was processed as a swab sample using a proprietary MagMAX protocol.

### Conclusion

Results show that using Genotubes will make it easier and more convenient to send samples to the laboratory and get the same reliable test results.

In conclusion, the GenoTube seems to be a viable way to collect Oral Fluid samples has some distinct advantages including room temperature shipment and storage. Future experiments will be performed to test the stability of GenoTubes at room temperature for several days as compared to the Oral Fluid sample stored at 4°C.



## HME-034 - FACTORS INFLUENCING CROSS-FOSTERING AND NUMBER OF PIGLETS ALIVE WITHIN SEVEN DAYS OF FARROWING

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### Background & Objectives

Cross-Fostering (CF) is implemented in intensive pig production in order to equalize litter sizes. This management procedure is crucial for reduction of piglet losses during the suckling period, considering the intake of colostrum that is necessary for their proper growth and development. The study aimed to assess the effect of CF and associated factors on the number of piglets alive within seven days of farrowing (PA7d) as the most critical period of their raising.

### Material & Methods

The study was conducted in Croatia from July to mid-November 2016 in the conditions of commercial production and included 316 farrowings in high-fertile hybrid sow lines, LW x L cross-breeds, from 1<sup>st</sup> to 8<sup>th</sup> Parity (P) with a total of 3993 Piglets Born Alive (PBA). Piglets were equalized upon completion of the colostrum period. Data analysis was performed using GLM model with six effects included in the model of calculation: PBA, Farrowing Group (FG), Parity Group (PG) with three levels (first group: primiparous; second group: secundiparous to quadriparous; and third group: ≥quintiparous), Average number of Piglets Alive *per* Group (APAG), CF effect and gender (G); the FG\*APAG interaction was also observed.

### Results

Results were expressed as LSM±SE. Significant effects were recorded for CF ( $P=0.04$ ), PG ( $P<0.01$ ) and FG\*APAG ( $P<0.01$ ). A significantly higher PA7d ( $12.02\pm 0.28$ ) was recorded in CF litters as compared with litters that were not equalized ( $11.40\pm 0.23$ ), as well as in second PG sows ( $12.15\pm 0.23$ ) *versus* third group sows ( $11.31\pm 0.21$ ).

### Discussion & Conclusion

Study results revealed that PA7d was influenced by CF and PG. Therefore, equalizing litters, as well as the potential selection of piglet nursing sows is important to reduce variability in the number of piglets alive *per* sow on day seven of farrowing, while at the same time increasing the sow productive efficiency.



## HME-035 - RESULTS OF A QUESTIONNAIRE ON MYCOPLASMA HYOPNEUMONIAE GILT ACCLIMATIZATION IN EUROPE

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### Background and objectives

Gilts are considered key in the *Mycoplasma hyopneumoniae* (*Mhyo*) transmission chain. An effective gilt acclimatization program should reduce *Mhyo* shedding at first farrowing and in consequence should decrease pre-weaning colonization prevalence and potential respiratory problems in fatteners. However, information on gilt acclimatization is scarce in Europe. The aim of this study was to identify current acclimation strategies for *Mhyo* used in Europe.

### Material and Methods

Information was collected using a survey consisting of 15 questions focused on gilt replacement status, acclimatization strategies and methods used to assess its efficacy. The survey was submitted by email to European swine veterinarians, advisors and producers. One survey counted for one farm.

### Results

A total of 218 surveys were voluntarily completed by 107 responders (from 18 European countries) representing 483,591 sows and 139,059 gilts. From these responders, 81.7% were aware of the health status of gilts on arrival, being 66.3% *Mhyo* seropositive replacement. Moreover, 69.3% of responders performed an acclimation process by different strategies, being the use of vaccine (68.9%), and combination of vaccine and cull sow (12.6%) or pig (8.6%) exposition, the most commonly used. Notwithstanding, only 16.9% of responders performing acclimatization strategies for *Mhyo* verified the success of the acclimatization by methods as ELISA (67.5%), PCR (10.8%) or both (18.9%).

### Discussion and conclusion

This study showed that only 2/3 of the farms represented in this survey do *Mhyo* acclimatization strategies for gilts, and less than 1/6 of them verify to some extent the efficacy of the process. Considering that the assessment of acclimation efficacy should optimize the ideal moment of replacement introduction into the breeding herd, it looks like these practices for *Mhyo* are still poorly developed in Europe.

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## HME-036 - EVALUATION OF THE EFFECT OF DOUBLE GLEPTOFERRON (GLEPTOSIL®) INJECTION ON GROWTH PERFORMANCE

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### Background & Objectives

Iron reserves in piglets are limited. Additional injection of iron increased the hemoglobin (Hb) concentration but limited information exists about effect on growing performance.

The aim of the study was to investigate the effect of the second injection of gleptoferron (Gleptosil®) on growth performance on SPF farm in the Czech Republic.

### Material and Methods

Four randomly selected litters from third parity sows were compared, two whole litter per group: group A = 29 piglets and group B = 27 piglets. Average birth weight in both groups was without statistical difference (A: 1,580.0 ± 56.2 and B: 1,597.0 ± 28.0 g), no specific disease occurrence was reported during the study. No cross fostering was applied. Group A was injected by gleptoferron two times, 2<sup>nd</sup> and 16<sup>th</sup> day after birth and group B once 2<sup>nd</sup> day according the manufactures recommendation: 1 ml (200 mg iron/piglet). Piglets were individually tagged and weighed (weaning- 24 days of age, end nursery 72 days of age). Blood parameters were investigated at the end of nursery period -RBC, HTC, MCV, MCH, MCHC, Hb and plasma Fe (Mindray bc 2800).

### Results

No statistical difference in weight at the end of nursery (A= 26.8 ± 0.8<sup>a</sup> and B= 26.6 ± 1.0<sup>a</sup> kg) and finishing period (127.0 ± 3.0<sup>a</sup> and B= 126.3 ± 2.3<sup>a</sup>) was detected. Numerical difference of weight in favor of group A was 0.7 kg.

Statistically better parameters HCT, MCV, MCH and Hb ( $p < 0,01$ ) were detected at the end of nursery in group A.

### Discussion & Conclusion

Our study confirmed beneficial effect of two application of gleptoferron on red blood parameters, but without significant effect on final weight at slaughter. More data and further studies of performance with larger number of animals will be needed to prove possible effect, particularly on SPF farms without presence of major important infections.



## HME-037 - PREVENTION OF CLINICAL AND PRODUCTIVE EFFECTS OF IRON DEFICIENCY ANAEMIA USING A NEW ANTI-ANAEMIC PREPARATION, PREVIRON®

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### Background & Objectives

Intensive housing system and genetic selection for improved production have had a negative impact on the iron requirements of suckling piglets. Therefore, iron supplementation is necessary to prevent iron deficiency anaemia (IDA) and its effects. This study aimed to evaluate the clinical and effects on production of a new anti-anaemic preparation in newborn piglets during farrowing.

### Material & Methods

A controlled study was performed in a Spanish farm housing 500 sows. One hundred and fifty four piglets born from sows with similar number of parities were randomly assigned to group 1 (G1) or control group (CG). PREVIRON® was administered intramuscularly to 3-day-old piglets in G1. Piglets in CG did not receive any iron supplement. Clinical effects were measured by the clinical signs of anaemia and the analysis of haematic parameters, while the effects on production were evaluated by body weight and average daily gain (ADG).

### Results

At study baseline, all animals showed a pre-anaemic state determined by haematological parameters. Subsequently, the CG showed clinical signs of anaemia at 14 and 21 days post-inoculation (dpi) and severe IDA assessed by haematological parameters. However, iron administration prevented the clinical signs of anaemia in all animals and allowed their recovery from the pre-anaemic state observed in 3-day-old piglets to normal haematological parameters. Regarding productive parameters, the mean body weight per piglet at 21 dpi was significantly higher after treatment inoculation with a rise of 21.9 gr/day (ADG) in G1 compared to CG ( $p$ -value<0.05).

### Discussion & Conclusion

The results of this study showed that the new anti-anaemic preparation had a beneficial effect in all parameters evaluated satisfying all the iron requirements of suckling piglets in a commercial farm. Therefore, this treatment could be used to prevent IDA as well as its clinical and productive effects in piglets.

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## HME-038 - INFLUENCE OF EARLY AND LATE CROSS-FOSTERING MOVEMENTS ON PIGLET GROWTH IN LACTATION

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### Background and Objectives

This study aimed to evaluate the influence of the cross-fostering day on piglet growth during lactation.

### Material and Methods

The study was conducted in a 1900-sow farm working with one week farrowing batches. One hundred and eighty-eight litters (n=2274 piglets) were followed up during lactation. Cross-fostering movements were done according to the farmer criteria based on litter size and body weight visual estimation the day of movement. Animal movements done at the first or second day after birth were considered early adoptions. Movements done from the third day of life until weaning (21 days of age) were deemed as late adoptions. All piglets (non-cross-fostered; early and late adopted) were weighed at birth and at weaning and average daily weight gain (ADWG) was calculated. Comparisons among the ADWG of the three groups took into account the birth weight.

### Results

ADWG values from non-cross-fostered (n=1428; 208.90 g ± 52.53) and early adopted (n=692; 194.08 g ± 50.70) animals were significantly different than the ones from late adopted piglets (n=154; 129.51 g ± 39.76). Moreover, a negative correlation between average ADWG and the day of late adoption movement ( $y = -6.3503x + 171.48 / R^2 = 0.81$ ) was found. In fact, this negative effect was more evident from the fifth day of life onwards, achieving the lowest ADWG (<100g) values for piglets adopted from the tenth day of life onwards.

### Discussion and Conclusion

The present study showed that the cross-fostering day is crucial for the piglet growth. In conclusion, the latter the farmer moved smaller pigs, the lower their overall ADWG during the suckling period.

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## HME-039 - IMPACT OF WHOLE HERD VACCINATION AGAINST PRRSV-1 ON SOW AND PIG PERFORMANCE ON A FARROW-TO-FINISH FARM IN HUNGARY

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### Background & Objectives

PRRS is considered to be one of the most costly diseases in pig production. However, vaccination with new PRRSV-1 modified live virus vaccines has shown to improve sow and pig performance under field conditions. This study investigated the impact of whole herd vaccination with those new vaccines on sow and pig performance on a farrow-to-finish farm in Hungary.

### Material & Methods

The study was conducted on a farrow-to-finish farm with 580 sows. Pigs on farm were positive for PRRSV antibodies and field virus circulation was detected at previous investigations in gilts and fattening pigs. However, PRRS was not considered to be of significant impact on farm performance. Therefore, no PRRS vaccination was implemented prior to the start of this study. The study was initiated with a double mass vaccination including all breeding stock, suckling piglets from 17 days of age and all pigs from weaning to slaughter. Vaccination was performed with ReproCyc<sup>®</sup> PRRS EU and Ingelvac PRRSFLEX<sup>®</sup> EU, respectively. Subsequently, the breeding herd was vaccinated every 4 months and piglets were vaccinated around weaning. Performance data regularly collected on the farm was compared before and after the implementation of the described vaccination program by the use of statistical process control charts with Minitab 17.

### Results

Preliminary results show a numerical improvement in pregnancy rate, farrowing rate, feed conversion ratio and average daily weight gain (ADG) in nursery/growing, as well as ADG during the fattening period. Already after the short period of observation, losses in fattening were significantly reduced. Final data will be presented at the conference.

### Discussion & Conclusion

The results indicate an overall positive impact of PRRSV whole herd vaccination on the performance of sows and pigs in a farrow-to-finish system.



## HME-040 - COMPARISON OF PERFORMANCE OF NURSERY PIGS VACCINATED AGAINST PCV2 WITH DIFFERENT COMMERCIAL VACCINES IN A LARGE PRODUCTION SYSTEM IN HUNGARY

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### Background & Objectives

Vaccination of pigs against PCV2 has shown to improve performance in clinically affected, but also in sub-clinically infected herds. Several commercial vaccines are available on the market, which have individually proven to be effective against PCV2 and therefore improve pig performance. However, reliability of this improvement may differ between vaccines and a reduction in batch-to-batch variation is economically beneficial under commercial conditions. This study aimed to compare the efficacy of different commercial vaccines against PCV2 over time in a large pig production system in Hungary.

### Material & Methods

The study was conducted at a farrow-to-finish system with 2800 sows. Due to insufficient space in nursery, an additional site 2 is in use. Pig performance was analysed between weaning and transfer to the fattening barns. The farm vaccinated piglets before or latest on the day of weaning consecutively with four different commercial vaccines (A-D), using each vaccine for several month over a period of approximately 14 month. Performance data of pigs was reported separately for every batch. Data was presented and analysed by the use of process control charts with Minitab 17.

### Results

Batches vaccinated with vaccine C had the highest weaning weight, average daily weight gain, weight at end of nursery and lowest mortality when compared to the other groups. A clear reduction in variation of mortality was seen during the period pigs were vaccinated with vaccine C, which was significantly lower in the mean ( $\bar{x}$  = 1.68 (C) vs. 2.95 (A), 2.66 (B), 2.09 (D)) and the standard variation, compared to vaccine A and B ( $p \leq 0.018$ ).

### Discussion & Conclusion

Beyond the overall better results in performance in nursery batches vaccinated with vaccine C, special consideration was given to the clear reduction in variation of mortality. Therefore the decision was to continue PCV2 vaccination with vaccine C.





## HME-041 - IMMUNOCRIT (RATIOS): A NEW TOOL TO ASSESS COLOSTRUM UPTAKE IN 1 DAY OLD PIGLETS ?

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### Background and Objectives

Passive immunity (Immunoglobulin/IgG), transferred from the mother to the newborn - is extremely important to ensure a good protection of the piglets during the first weeks of their life. Until recently it was very difficult to assess colostrum intake and consequently the transfer of IgGs. The objective of this study was to determine whether the Immunocrite Ratio (IR) could be a good practical tool to evaluate passive immunity in one-day-old piglets.

### Materials and Methods

502 litters were selected from 9 different sow herds during a period ranging from May to December 2016. From each litter six 24 hours old piglets were selected to collect a blood sample (approx. 1 ml) from the jugular/vena cava vein. From each litter 2 Light ( $\leq 1,1$  kg), 2 Medium (1,2-1,5 kg) and 2 Heavy ( $>1,55$ kg) were sampled using a sterile disposable syringe. Sow parity and litter size were recorded. Refrigerated blood samples were submitted to Laboratorio Tre Valli to determine the individual IRs according to Vallet's method (Vallet et al. 2013).

### Results

Average IR for first parity (IR 0,067) and pluriparous (0,062) sows was very similar with gilts showing more variability (Dev.st 0,065491 vs 0,032427). IR values for Light (0,062), Medium (0,061) and Heavy (0,067) were very close too although Light pigs showed more variability (0,008-0,172). Under the conditions of this study almost no correlation was found between IR and litter size and very little correlation between IR and PWM%. The largest source of IR variation was definitively the farm itself with the highest IR values recorded in farm 7 (0,098) and the lowest in farm 4 (0,040).

### Discussion & Conclusions

Although more data will be needed to build a more solid database we hope to be able to use IR values to set benchmark values for individual farms which will enable staff to improve colostrum management.



## HME-042 - CONTROL OF PORCINE PLEUROPNEUMONIA BY VACCINATING WITH COGLAPIX®

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### Introduction

Treatment with susceptible antibiotics as well as prevention with vaccines are suitable methods for tackling *Actinobacillus pleuropneumoniae*. The aim of study was to compare three different approaches by monitoring consumption of antibiotics, mortality and lung lesions.

### Materials and methods

The trial was performed in an Upper-Austrian fattening farm with A.p. problems. Three groups of pigs of same origin were examined in parallel. Group 1 (n=60) received no prophylactic treatment. Group 2 (n=224) was prophylactically vaccinated with Coglapix® (toxoid-bacterin vaccine against A.p., Ceva). Group 3 (n=75) was prophylactically treated with oral antibiotics via feed (amoxicillin). Injectable antibiotics for the treatment of respiratory disease and lameness were used in group 1 and 3 (amoxicillin, penicillin-streptomycin) and for lameness in group 2 (amoxicillin). Clinical signs and mortality were recorded and the consumption of antimicrobials was calculated. Pigs were blindly scored at the slaughterhouse for lung and pleurisy lesions using Ceva Lung Program Scoring Methodology.

### Results

Regarding the antibiotic consumption, group 3 had the highest value with 29 mg of active substance per animal and day (Th index 10,16), followed by group 1 (1,8 mg, Th index 0,383) and group 2 (70 µg, Th index 0,018). Mortality was highest in group 3 (1,33%), followed by group 2 (0,89%) and group 1 (0,00%). Dorsocaudal pleurisy prevalence was the lowest in group 2 (23,5%, APPI 0,72), followed by group 1 (59,3%, APPI 1,78) and group 3 (60,7%, APPI 1,68). Relative frequency of dorsocaudal pleurisy was significantly lower in group 2 ( $p < 0,0001$ ) compared to both groups 1 and 3.

### Conclusions

These results showed that the Coglapix® vaccinated group had significantly lower dorsocaudal lung pleurisy lesions as well as decreased antibiotic consumption compared to both group 1 and 3. Furthermore, Coglapix® was more efficient in reducing clinical signs of pleuropneumonia compared to prophylactic and therapeutic antimicrobial treatment.



## HME-043 - BIOSECURITY STATUS OF 236 SOW FARMS IN SIX EUROPEAN COUNTRIES

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### Background & Objectives

In veterinary medicine, the term biosecurity is used to describe measures taken to prevent pathogens entering a farm (external biosecurity) or spreading within the farm (internal biosecurity). Improving biosecurity helps to improve pig health and reduce antimicrobial use. In many pig producing countries the biosecurity status of pig farms is not known. The aim of this study was to score biosecurity in sow farms from six European countries in a standardized manner and find reasons for different scores between farms and countries.

### Material & Methods

In six countries (Belgium, Denmark, Finland, Germany, the Netherlands and Spain), the biosecurity was measured with an online questionnaire, based on Biocheck.ugent<sup>®</sup>, in 236 sow farms. This system allowed to score external & internal biosecurity, and both consisted of six subcategories with 115 questions. Scores ranged from 0 (absence of biosecurity measures) to 100 (presence of all biosecurity measures). To compare the external and internal biosecurity scores between countries, analysis of variance was performed (ANOVA, post hoc Bonferroni, IBM SPSS<sup>®</sup>).

### Results

The average scores for external and internal biosecurity were 76.3 and 56.9, respectively. The external and internal biosecurity scores were highest in Denmark (87.5 and 64.6 respectively). The best scoring external biosecurity category in the overall sample was 'purchase of animals and semen' (91.9), the best scoring internal biosecurity category was 'disease management' (71.3).

### Conclusion

In accordance with other studies, this study also showed that improvements are still possible regarding biosecurity on pig farms. External biosecurity scored higher than internal biosecurity in every country. This shows that it's either more difficult to implement internal biosecurity measurements on farms or the benefit of maintaining strict external biosecurity measures is considered higher. The reason for the large variation between countries is currently studied.

This work has received funding from the European Union under FP7 grant agreement No. 613574 (PROHEALTH).



## HME-044 - BIOSECURITY STATUS OF 173 FATTENING PIG FARMS IN FOUR EUROPEAN COUNTRIES

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### Background & Objectives

In veterinary medicine, the term biosecurity is used to describe measures taken to prevent pathogens entering a farm (external biosecurity) or spreading within the farm (internal biosecurity). Improving biosecurity helps improving pig health and reduce antimicrobial use. In many pig producing countries the biosecurity status of pig farms is not known. The aim of this study was to score biosecurity in fattening farms from four countries in a standardized manner and compare between countries.

### Material & Methods

In four participating countries (Belgium, Finland, Poland and the UK), the biosecurity was measured by means of an online questionnaire, based on Biocheck.ugent<sup>®</sup>, in 173 fattening farms. This system allowed to score external & internal biosecurity, and both consisted of six subcategories with 115 questions. Scores ranged from 0 (absence of biosecurity measures) to 100 (presence of all biosecurity measures). To compare the external and internal biosecurity scores between countries, analysis of variance was performed (ANOVA, post hoc Bonferroni, IBM SPSS<sup>®</sup>).

### Results

The average scores for external and internal biosecurity were 67.4 (sd. 8.4) and 59.2 (sd. 17.6), respectively. The external biosecurity score was highest in the UK (73.9) and the internal biosecurity score was highest in Poland (80.3). External biosecurity scored higher than internal biosecurity in every country, except for Poland. The best scoring external biosecurity category in the overall sample was 'purchase of animals and semen' (82.4), the best scoring internal biosecurity category was 'disease management' (74.5).

### Conclusion

In accordance with other studies, this study showed there is still room for improving biosecurity. The results showed that farms implemented proper biosecurity measures related to purchase of breeding animals and semen, and most of them applied health control programs. Further research will be done to explore differences between countries.

This work has received funding from the European Union under FP7 grant agreement No. 613574 (PROHEALTH).



## HME-045 - THE EFFICACY OF HYOGEN® IN FINISHING PIGS COMPARED TO A COMPETITOR VACCINE ON A FARM INFECTED WITH MYCOPLASMA HYOPNEUMONIAE

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### Introduction

*Mycoplasma hyopneumoniae* (M. hyo) is the etiological agent of enzootic pneumonia (EP) and causes chronic dry cough, retarded growth and reduced performance in swine. Several vaccines are available with varying results in terms of clinical signs and performance losses. The aim of this retrospective study was to test the efficacy Hyogen® vaccine (Ceva) in comparison with a competitor vaccine.

### Materials and Methods

A farm with 520 sows and 5.000 finishing pigs runs in a two-week batch management. Until February 2016 piglets were vaccinated in the second week of life with a one-shot M. hyo vaccine (G1). Since February 2016 the animals are vaccinated with Hyogen® in the third week of life (G2). The amount of medication, average daily weight gain and lung lesion scoring (Ceva Lung Program scoring methodology) was recorded and performed.

### Results

In total 528 lungs were scored, 166 for G1 (1 batch) and 362 for G2 (3 batches). The frequency of EP-like lesions differed significantly ( $p < 0.05$ ) between the groups, where 54,8% (91 of 166) lungs in G1 and 40,3% (144 of 362) lungs in G2 were affected. Moreover, G2 had a lower ( $p < 0.05$ ) median number of the sum of scores of each lobe separately (0, range: 0-9) compared to G1 (1, range: 0-11). Cranial pleurisy showed nonsignificant differences between the groups (12,05% in G1 and 9,11% in G2). However, G2 had a significantly lower ( $p < 0.05$ ) frequency of scars (3,32%) compared to G1 (11,45%).

### Conclusions

The vaccination of three-week-old pigs with Hyogen® significantly improved the frequency and severity of EP-like lesions compared with a one shot competitor vaccine, suggesting that Hyogen, can efficiently contribute in controlling Enzootic Pneumonia in swine farms.



## HME-046 - GUT MICROBIOME RESPONSES OF PIGS UNDER DEOXYNIVALENOL AND ZERALENONE CONTAMINATION IN FEED

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### Background & Objectives

With the close links between gut microbiome and animal health, an impaired balance could have deleterious effects on general health condition and mycotoxins are known to contribute toward the imbalance of gut microbes and their communities, favoring a shift toward intestinal aerobic bacteria. When pigs were exposed to T-2 toxin in feed, aerobic gut bacteria increased but Fumonisin B<sub>1</sub> did not alter the *in vitro* growth of them. The aim of this study is to investigate how the gut microbiome shifts when pigs are fed with DON or ZEN.

### Material and Methods

Fifteen of pigs, 20kg in weight were randomly divided into DON treated, ZEN treated and CTL (control) groups. DON and ZEN was purchased from Biomin and administered for 30 days at 8 mg/kg in feed, respectively. Microbial DNA was extracted from gut samples to be used as a template for PCR. Sequencing was performed on Ion PGM. After sequencing, sequence reads were filtered using the PGM software to remove low quality and polyclonal sequences. Operational Taxonomic Units (OTUs) were clustered using the QIIME and MicroSEQ 16S Reference Library database.

### Results

The DON and ZEN groups presented variable patterns of gut microbiome compared with that of the CTL group. The DON group had 321 OTUs and three families, which were Pasteurellaceae, Clostridiaceae and Lactibacillaceae while 340 OTUs and Lactibacillaceae, Clostridiaceae and Acholeplasmataceae.

### Discussion & Conclusion

Administration of DON and ZEN reduce the abundance and variability of the porcine gut microbiome. It is still not clear how variable and abundant intestinal microbiome functions but the CTL group presented obviously more abundant and variable gut microbiome pattern than the DON and ZEN groups. The data showed microbial population shifts representing both microbial succession and changes in response to DON and ZEN administration. The metabolomic analysis correlate with microbiome analysis is needed for further study.



## HME-047 - EFFICIENT PREVENTION OF ENZOOTIC PNEUMONIA WITH HYOGEN® IN HIGH HEALTH STATUS FARM

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### Introduction

Infections to *Mycoplasma hyopneumoniae* can result in substantial losses in the growth performance. Even subclinical disease can reduce ADG and feed efficiency. Efficient prevention of such mild infections might reduce economic losses due to suboptimal growth. The aim of the study was to assess the efficacy of Hyogen®, vaccine against *M.hyo*, Ceva in a high health status farm.

### Materials and methods

The trial was conducted on a 250 sow high health nucleus farm. Altogether 800 pigs were vaccinated at weaning with Hyogen® in four consecutive batches. The results of lung lesions at slaughter and growth performance were compared with four previous non vaccinated batches. The incidence and extension of the EP-like lesions were recorded using Ceva Lung Program. The average daily weight gain was measured in the period of weaning to slaughter. A potential profit was calculated and extrapolated using Respinomics™ application.

### Results

Vaccination with Hyogen® resulted in lower incidence (38,45% vs 16,58%,  $p < 0.05$ ) and extension (Madec index 1,53 vs 0,2;  $p < 0.05$ ) of the EP-like lesions with significantly reduced variability among different batches (The SD for the incidence was 18.26 in non-vaccinated groups and 6.10 in Hyogen groups and for the extension of lesions 0.57 vs 0.07 in non-vaccinated vs Hyogen group).

As a consequence of the improved respiratory health the growth performance was better in the Hyogen group, which had on average 34g more in the daily weight gain (818g vs 852g).

Calculation of a potential economic benefit using Respinomics™ application resulted in the 1429€ net profit per each 1000 pigs.

### Conclusion

This study confirmed that mild *M.hyo* infections might be underestimated in commercial swine farms and the vaccination with Hyogen® can improve the lung respiratory health and growth performance resulting in higher profit even in farms with a high health status.



## HME-048 - EVALUATION OF THE ACTINOBACILLUS PLEUROPNEUMONIAE INDEX DISTRIBUTION IN FRANCE USING CEVA LUNG PROGRAM

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### Introduction

Ceva Lung Program (CLP) offers the methodology which assists in evaluating the presence, incidence and impact of Enzootic Pneumonia (EP) and *Actinobacillus pleuropneumoniae* (A.p) infections using adapted lung scoring of slaughter pigs.

The French CLP Database, operating since 2014, is a very useful tool to establish a real prevalence of EP and A.p on a farm, regional or even a national level.

### Material and methods

In the CLP method, dorso-caudal pleuritis suggestive for previous A.p infections is scored using the

SPES point system based on the presence, extension and position of pleuritis observed on lungs directly on the slaughter line. *Actinobacillus pleuropneumoniae* Index (APPI) provides the information regarding the prevalence and severity of the dorso-caudal pleuritis.

The aim of this survey is to determine the distribution of APPI in France by using a high number of controls coming from one of the main pig producer organizations in France.

From 2014 to 2016, dorso-caudal pleurisy prevalence and APPI index were calculated from 278 715 lungs from 4016 controls coming from 1263 different farms.

All these farms were located in the dense pig production area in the west of France.

### Results

The CLP database made possible to establish a national reference APPI for France. The median APPI for all lungs is 0,05 ranging from 0 to 2,098.

It has been found that dorso-caudal pleuritis prevalence was higher in the areas with relatively higher density of swine production such as 5% in Côtes-d'Armor or 4.81% in Finistère.

### Conclusion

Although APPI median was rather low, a high variability among farms and areas was found. The results of the slaughter-check with the APPI index > 0,125 could reflect a high level of *Actinobacillus pneumoniae* infections in the farm.

Further assessment of the correlation between the APPI and the performance data will be needed.





## HME-049 - CLINICAL AND ECONOMICAL IMPROVEMENTS DUE TO BETTER CONTROL OF ENZOOTIC PNEUMONIA WITH HYOGEN®

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### Introduction

*Mycoplasma hyopneumoniae* (*M.hyo.*) is the primary pathogen causing enzootic pneumonia (EP). The infection often appears to have a subclinical course with only reduced growth. The presence of the infection is then confirmed in the slaughterhouse by lung scoring and other diagnostic methods. Clinical picture of EP includes dry cough, anorexia and dyspnea. Dynamic and duration of coughing are important for the estimation of the impact on growth retardation.

### Materials and methods

A commercial pig farm with the history of recurring coughing of fatteners was selected. Hyogen® and a competitor One-shot VaccineA were used. In total 220 pigs were randomly divided into 2 groups: G1 vaccinated with Hyogen® and G2 vaccinated with VaccineA at 28 days of age. The individual coughs were recorded using the Pig-Caugh-Monitor, Fancom, daily the whole fattening period. Weight gain was measured in the fattening period. The economic benefit was calculated using Respinomics™ application. The EP-like lesions were scored at the slaughterhouse using the Madec score method.

### Results

The average number of recorded coughs in the whole fattening period was significantly lower in the Hyogen® group compared to G2 (2033 and 2944 respectively). The % of healthy lungs in slaughter pigs was higher in Hyogen® group (45%vs35%), the % of severely affected lungs (3%vs7%) and the final Madec index (1,3vs2,4) were lower in G1. The difference in overall Madex index was statistically significant in favor of Hyogen®. Hyogen® group had the ADG 829g which was 44g more than the G2 pigs with ADG 785g ( $p < 0,05$ ). The potential financial benefit was 2450€ profit in favor of Hyogen®.

### Discussion and conclusion.

The results of this study showed that Hyogen® provided better protection of pigs than one shot Vaccine A, measured by the clinical course of the disease and the lung lesion score. Higher ADG corresponded to the improvements in the respiratory health.



## HME-050 - LUNG LESION SURVEY USING CEVA LUNG PROGRAM IN FINISTÈRE, BRITTANY, FRANCE

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### Introduction

Ceva Lung Program (CLP) offers the methodology which assists in evaluating the presence, incidence and impact of Enzootic Pneumonia (EP) and *Actinobacillus pleuropneumoniae* (A.p) infections using adapted lung scoring of slaughter pigs.

### Materials and methods

In this survey based on the CLP French Database analysis, the prevalence and importance of EP-like lesion have been quantified on a sample of 57 farms coming from the most important pigs production area in Brittany, the Finistère (29) and belonging of one of the leading pig producer in France.

### Results

Lungs were scored according to the CLP method following a modified Madec methodology.

Between 2015 and 2016, 12080 lungs were scored from 128 batches of 57 farrow-to-finish farms.

Bronchopneumonic lesions were detected in 54,60 % of the controlled lungs, and the mean Madec index was 2,19, ranging from 0,26 to 7,59. The mean % of lung surface affected by broncho-pneumonia was 7,43% and ranged from 0,01% to 24,45%.

Those results have been analyzed regarding the size of the farrow-to-finish unit and the batch management system used. It was found that as the herd size was bigger, the lung scores were smaller. This is explained by specialization to exclusively swine production on larger farms (>200sows) compared to mixed-species small size farming. That allows skilled personnel dedicated to swine only and also separation of farrowing/postweaning/fattening compartments, which is considered as one of the major elements to reduce EP.

### Conclusion

In conclusion, the CLP Database analysis of those 57 Finisterian farms shows that EP-like lesions are still a problem despite the high M.hyo vaccination rate in those pig production areas. It also demonstrates the relation between the farm size and the impact of enzootic pneumonia in fattener pigs.

Further investigations should be conducted to confirm those results at the national level.



## HME-051 - LUNG LESION SURVEY USING CEVA LUNG PROGRAM IN RUSSIA, UKRAINE AND BELARUS: COMPARISON OF PERIODS 2015 AND 2016

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### Introduction

Lung scoring at the slaughterhouse is an effective way how to evaluate respiratory health status and economic impact of respiratory disease on the swine farms. Ceva Lung Program (CLP) scoring methodology allows for rapid lung scoring and is used for evaluation of real prevalence of enzootic pneumonia (EP)-like and *Actinobacillus pleuropneumoniae* (A.p.)-like lesions.

The aim of the study is to evaluate the prevalence and severity of those lesions in Russia (RU), Ukraine (UA) and Belarus (BY) and compare with recently published country data (Sperling et al., 2016).

### Materials and Methods

In the period of 2016 a total number of 126 batches and 8713 lungs were scored according the CLP method: bronchopneumonia lesions, cranial pleurisy and fissures for the (EP)-like lesions; dorsocaudal pleurisy for A.p.-like lesions. Lungs were scored from the herds in Russia (78 batches), Ukraine (29 batches) and Belarus (19).

### Results

- All countries showed a similar rate of affected lungs by EP- like lesions, which was in RU, UA and BY- 50 %, 48 % and 49 % respectively. In case of Russia there was a significant increase of EP-like lesions in comparison with 2015 (+ 20 %).
- The highest % of lungs affected by A.p.-like lesions was evaluated in Belarus (57 %). On the opposite, the decreasing prevalence was recorded especially in Russia (42 % in 2015 and 11% in 2016).

### Discussion

The prevalence of EP- like lesions remains high in all countries and EP in Russia has apparently a growing tendency. On contrary, A.p.- like lesions decreased in Russia while remaining at the similar level in the rest of the countries. Some of the reasons might be the implemented repopulation strategies in Russia and also the improved control measures including increased vaccination rate against A.p.



## HME-052 - LUNG SCORING SURVEY IN EUROPEAN COUNTRIES IN 2016

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### Introduction

Scoring of lung lesions in the slaughter pigs belongs to major diagnostic methods to evaluate the presence, incidence and severity of respiratory diseases in commercial pigs. To some extent the results are indicative for previous infections by specific pathogens and can reflect the etiology. Lesions suggestive for previous M.hyo or A.p. infections and their scoring were described before. The aim of this survey was to collect the results of lung scoring performed in most of swine producing European countries in 2016.

### Materials and methods

Ceva lung program scoring methodology was implemented to score the lesions at the slaughterhouse. The results were collected from 19 European countries in the period of January till beginning of December 2016. The mean values and quartiles were calculated for % of lungs with bronchopneumonia (%BP), % of affected lung parenchyma out of sick lungs (% pneumonia), % of dorso-caudal pleurisy (%DP) and APP index (APPI). For the two latter indicators the results from France were not included, because they were not scored on the routine basis.

### Results

The total number of scored lungs was 186186 from 1937 reports with the average of 96 lungs per batch. The median value of %BP was 50.0% with the Q1=27.93% and Q3 68.72%. The median for % of pneumonia was 6.05% with the Q1=3.24% and Q3=9.25%. For % DP the median, Q1 and Q3 were 10.64%, 4.05% and 26.4% respectively and for APPI the corresponding values were 0.3, 0.11 and 0.72 respectively.

### Conclusions

The results of lung scoring at the slaughterhouse from 19 European countries indicate a high rate of EP-like lesions which is consistent with recently published data from Germany and Italy. The percentage of A.p.-like lesions was relatively lower in this study, probably due to inclusion also of the countries with lower incidence of pleuropneumonia in commercial pigs.



## HME-053 - AN ALTERNATIVE APPROACH TO REDUCE SYSTEMATIC ORAL MASS ANTIMICROBIAL USE IN PIGS FOR A PRUDENT USE OF ANTIBIOTICS

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### Background & Objectives

Systematic oral mass antimicrobial use in pigs is facing increasing public scrutiny for its potential role in antibiotic resistance development and risk of antibiotic residues in meat. A restricted and prudent use of antimicrobials has to be put in place. The aim of the present study was to compare oral prevention of Porcine Respiratory Disease Complex (PRDC) versus a therapeutic injectable approach in weaners.

### Material & Methods

The study was performed on a multi-site farrow-to-finish farm with a documented history of PRDC in the nursery. A total of 1950 weaned piglets aged 4 weeks were allotted in rooms with a central corridor: on the left side the animals received tilmicosin at expected period for PRRSv seroconversion (Pulmotil<sup>®</sup> ac – Elanco; 20mg/kg bw) in water for 7 days (Prevention group = 975 pigs). The animals on the right side (Individual group = 975 pigs) were left untreated and received injectable gamithromycin (Zactran<sup>®</sup> - Merial; 1.0ml/25 kg bw once) based on appearance of clinical signs of PRDC. Nursery growth, mortality, culling, additional treatments were monitored for 7 weeks.

### Results

The number of clinical PRDC cases were similar between groups (resp. 46 versus 50,  $P=0.67$ ). No difference between groups was observed in daily gain, mortality or culling rate ( $P>0.05$ ). The use of antibiotics in the nursery unit was reduced by 61% compared to the Prevention group (1269 vs 3291g/group; 1.3 vs 3.3g/head;  $P<0.05$ ). This reduction resulted in a decrease of treatment costs (1,06€/weaner).

### Discussion & Conclusion

The present approach to a prudent use of antibiotics in weaners addresses the needs of both public opinion (reduction of treatments) and farmers (reduction of costs). However, it is fundamental to highlight the key role of the stakeholder in the prompt recognition of sick animals, focusing attention on the importance of trained personnel in the farm.



## HME-054 - A SEVERE PRDC CHALLENGE AND THE EFFECT OF A TRIVALENT PRDC VACCINE FOR PCV2, MHP AND PRRS

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### Background & Objectives

Porcine respiratory disease complex (PRDC) is a significant challenge for the global swine industry. PRDC includes both viral and bacterial respiratory pathogens, mainly Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Porcine Circovirus type 2 (PCV-2), *M. hyopneumoniae* (*M.hp*) and secondary bacterial agents. Vaccine derived immune protection against clinical disease associated with PRDC pathogens is one control intervention. The purpose of this controlled-experimental study was to evaluate pigs vaccinated with 3FLEX<sup>®</sup> (A trivalent PCV2, M.hyo and PRRS vaccine) compared to a non-vaccinated group following a severe PRDC challenge.

### Materials and Methods

Group 1 was a non-vaccinated challenged control group of 20 pigs. Group 2 was a 3FLEX vaccinated and challenged group of 20 pigs. Group 3 was a non-vaccinated, non-challenged group of 10 pigs. Groups 1 and 2 were simultaneously inoculated with PCV2d (intranasal and intramuscular), *Mhp* strain 232 (intra tracheal) and PRRSV strain SDSU-73 (intranasal and intramuscular). The challenge incorporates a well referenced virulent heterologous PRRSV isolate, a contemporary virulent PCV2 field isolate, given simultaneously with *Mhp*, representing a severe PRDC challenge.

### Results

Significant differences ( $P < 0.05$ ) in respiratory clinical signs, gross lung lesions and lymphoid tissue damage were demonstrated. A reduction in post-challenge PRRSV viremia, and increased average daily weight gain was demonstrated in vaccinates compared to non-vaccinates.

### Conclusion

The results of this trial demonstrate that 3FLEX<sup>®</sup> provides protection to pigs simultaneously challenged with PRRS, PCV2 and *Mhp*. In the face of severe PRDC challenge, use of a trivalent vaccine mixture is an option for mitigation of the biologic impact of PRDC.



## HME-055 - ARE WE UNDERESTIMATING THE COST OF ANIMAL DISEASE BY USING COMMON ESTIMATION METHODS?

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### Introduction

Food animal disease creates multiple inefficiencies, death loss, increased weight and quality variation and impaired output quality. In addition, food animal disease often significantly degrades animal welfare and negatively affects farm morale. A survey of the literature reveals the most common method of assessing these losses, both for a farm and for regions or nations is the partial budget. The partial budget assembles the mean impacts on revenue and costs in a logical framework to measure net impact of the disease or any change. Recent availability of individual animal data (such as slaughter data) combined with information from experiments where feed intake and other variables were individually measured suggests that partial budgeting methods systematically underestimate the cost of disease.

### Materials and Methods

Epidemiological models of swine disease reveal they often emerge over time and create sub-populations of differently affected individuals. This creates multi-modal and typically left-skewed distributions of production values and metrics. In combination with asymmetric harvest payment schemes (light and heavy weight animals not equally penalized) and highly skewed hog price and feed ingredient cost distributions, this results in serious estimation errors when production process and price averages are used to gauge economic impact.

### Results

We demonstrate through stochastic simulation modeling that partial budgeting systematically *underestimates* the cost of disease. We illustrate the case modeling a Porcine Respiratory and Reproductive Syndrome Virus infection utilizing data from an experiment where different groups of piglets were challenged post-weaning with log-level escalations of PRRS virus and individually assessed in multiple ways over time through grow out.

### Conclusion

We report the difference in partial budgeting estimates and estimates derived from the more realistic simulation of the non-normal distributions and asymmetric harvest payment schemes and provide practitioners simple tools to improve estimates even when using partial budgeting.



## Vaccinology & Immunology

### VAC-001 - FALSE POSITIVE SEROLOGICAL REACTIONS FOR ACTINOBACCILUS PLEUROPNEUMONIAE SEROTYPE 2 AND MYCOPLASMA HYOPNEUMONIAE IN SOWS VACCINATED AGAINST LEPTOSPIROSIS

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#### Background & Objectives

A part of the SPF health control, is annual serological surveillance of the SPF diseases, App2 and Mycoplasma hyopneumoniae. In 2016 a number of SPF-herds, had an unusually high prevalence of seropositive reactions for App2 and Mycoplasma, without corresponding clinical or pathological findings. The common denominator for these herds, was that they were sow herds, that had started vaccination against Leptospirosis, using Izovac Lepto 3 (IL3), prior to the blood sampling.

#### Material & Methods

Serological testing for Mycoplasma and App2 is typically done by using a specific antibody ELISA test. The results of the blood tests are collected in the SPF-SuS database. Clinical or pathological findings are reported to SPF-SuS from veterinary practitioners assigned to the herds. The Vetstat database provided information about which SPF-herds used the IL3-vaccine.

#### Results

It was found that 79 SPF-herds used the IL3-vaccine. Of these 73 was declared negative for either Mycoplasma, App2 or both. 19 out of 68 App2 negative herds, experienced seropositive reactions for App2 (28%) over the course of one year. For Mycoplasma 13 out of 22 previously negative herds (59%) experienced seropositive reactions. The annual average re-infection rate in Danish SPF-herds is 2,3% for App2 and 11,5% for Mycoplasma, respectively.

#### Discussion & Conclusion

Based on the data it was found that there is a strong indication, that vaccination with the IL3-vaccine can cause false positive serological reactions for App2 and Mycoplasma in ELISA antibody test. This should be taken into consideration when diagnosing pulmonary infections. As a consequence of the increased rate of seropositive samples, 30% of the previously Mycoplasma negative herds that used the vaccine, was declared positive for Mycoplasma hyopneumoniae. This deterioration in SPF health status, had serious economic consequences for the individual farmers.





## VAC-002 - EFFICACY IN GILTS OF PORCILIS® ERY+PARVO+LEPTO FOLLOWING CHALLENGE DURING PREGNANCY WITH THE HIGHLY VIRULENT PORCINE PARVOVIRUS STRAIN 27A

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### Introduction

The objective of the study was to demonstrate the efficacy of the new octavalent inactivated ready-to-use vaccine Porcilis® Ery+Parvo+Lepto against reproductive problems associated with porcine parvovirus (PPV) infection of pregnant gilts. Porcilis® Ery+Parvo+Lepto contains *Erysipelothrix rhusiopathiae*, porcine parvovirus and six serovars of *Leptospira*.

### Materials and Methods

Two groups were included in the study. Animals in Group 1 (n=9) were vaccinated intramuscularly with Porcilis® Ery+Parvo+Lepto when they were approximately 5 months old and were revaccinated four weeks later. The gilts in Group 2 (n=8) were not vaccinated and served as challenge controls. Gilts were artificially inseminated four weeks after the second vaccination. At 36-39 days, in pregnancy gilts were challenged with virulent PPV strain 27a. On day 87 and 90 of pregnancy, gilts were euthanized and the offspring was analysed. Protection from PPV challenge was evaluated by determining the vitality of the offspring and also the absence of PPV (by PCR and haemagglutination assays) or PPV-specific antibodies (by haemagglutination inhibition assay) in the foetuses.

### Results

No clinical signs were observed in the gilts after vaccination or PPV challenge. A complete reduction in PPV-specific mortality (death or mummification of foetuses) was observed in Group 1 (0% affected) compared with Group 2 (60% affected). Likewise, PPV and/or PPV-specific antibodies were detected in 14% of the Group 1 offspring, while 90% of the Group 2 offspring contained PPV and/or PPV-specific antibodies.

### Conclusion

Vaccination with Porcilis® Ery+Parvo+Lepto significantly reduced transplacental infection, viral load and foetal mortality caused by PPV strain 27a infection of gilts.



## VAC-003 - PORCILIS® PCV IMPROVES FEED CONVERSION RATE IN WEANERS

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### Background & Objectives

Between 2012 and 2014, MSD Animal Health acquired production data from a number of swine herds that implemented Porcilis® PCV to measure the effect of vaccination on average daily gain (ADG), feed conversion ratio (FCR) and mortality rate (MR) in weaners.

### Material & Methods

Production data were obtained from 40 herds that produced weaners. To minimize bias of season and variation in data precision, only reports containing data 12 months prior to initiating vaccination and 12 months after were considered valid. Data from a 6 month implementation period as well as data sets from herds experiencing outbreaks of severe disease or eradication programs were not included. Comparisons of means were done by a non-parametric test.

### Results

Out of the 40 herds, 18 data sets were excluded due to insufficient observation time and five (5) herds due to a PRRSv infection during the observation periods. Out of the 17 remaining herds, nine (9) herds previously vaccinated with another PCV2-vaccine and eight (8) herds had not vaccinated at all. One of these did not provide data for FCR.

For all 16 herds, the mean FCR prior to vaccination was 1.99 FU/kg gain (FU: feeding unit=7.88 MJ) compared to 1.89 FU/kg gain during the vaccinated period ( $p=0.03$ ). For herds that did not previously vaccinate, the results were 2.01 FU/kg gain and 1.89 FU/kg gain respectively ( $p=0.03$ ). ADG and MR were not significantly different.

### Discussion & Conclusion

An improvement in FCR of  $\geq 0.1$  FU/kg gain for weaners was demonstrated following implementation of Porcilis PCV vaccination. Although other factors might have influenced the result, the genetic improvement in the period was less than 0.05 FU/kg gain.



## VAC-004 - IN VITRO INVESTIGATION ON THE COMPATIBILITY OF COLIPROTEC® F4/F18, LIVE ORAL E.COLI VACCINE AGAINST POST-WEANING DIARRHOEA, AND AVIBLUE® WATER STABILIZER

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Coliprotec® F4/F18 (Prevtec Microbia) is a live *E. coli* vaccine for immunisation of pigs against F4-ETEC and F18-ETEC, the most prevalent cause of Post-weaning diarrhoea (PWD) in Europe (Luppi et al., 2016). Coliprotec® F4/F18 is administered via drenching or drinking water and should be consumed within 4 hours after reconstitution. In order to neutralize disinfectants in drinking water (e.g. chlorine), the addition of skimmed milk powder is recommended. Aviblu® (Elanco) is a stabilizer with blue dye specifically formulated for drinking water in animals. This study investigates the stability of Coliprotec® F4/F18 with Aviblu® for drinking water administration as an alternative to skimmed milk powder to neutralize chlorine.

Lyophilised Coliprotec® F4/F18 vaccine was reconstituted in water and aliquots were mixed with chlorinated tap water containing Aviblu® at 0.125, 0.25, 0.5, 6.25 and 25 g/l (1x, 2x, 4x, 50x and 200x the recommended dosage in drinking water, respectively). The vaccine was added at a rate of 1 dose/130 ml of final drinking water (Aviblu® 1x, 2x and 4x groups) or 1 dose/2.2 ml of stock solution (Aviblu® 50x and 200x groups). Viable bacterial count was performed at 0, 4 and 6 hours. Control solutions using reverse osmosis water and chlorinated tap water were included.

No reduction in live bacterial counts was detected over the 6-hour tested period in the drinking water containing Aviblu® at 0.5 g/l (4x) and in the stock solution containing both tested concentrations (50x and 200x) of Aviblu®. However, a reduction was observed for 0.125 g/l (1x) and 0.25 g/l (2x) Aviblu® concentrations at 4 and 6 hours.

The results of this study indicate that Aviblu® at 0.5 g/l (4x the recommended dosage in drinking water) or higher concentrations required for stock solutions for administration via dosing pumps do not affect the stability of Coliprotec® F4/F18 and protects the vaccine against chlorine.



## VAC-005 - IMPROVEMENT OF PRODUCTION DATA IN A SOW HERD AFTER IMPLEMENTATION OF A NOVEL PRRS VACCINATION PROGRAM TOGETHER WITH BIOSECURITY MEASURES

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### Introduction

In Swine Herds PRRS outbreaks can lead to massive clinical symptoms with reproductive and respiratory disorders. Also in subclinical cases the disease can still have a clear impact on performance data. In this case report a novel vaccination program accompanied by improved biosecurity measures helped to improve performance.

### Materials and methods

A sow herd with 400 sows and 2275 nursery piglet with weekly farrowings used to vaccinate sows and piglets against PRRSv. Since spring 2015 more losses and respiratory problems in nursery occurred. Also PRRSv positive piglets were born, indicating an unstable breeding herd. In response to this the sow herd was vaccinated against PRRSv twice within 4 weeks as a mass vaccination with Reprocyc<sup>®</sup> PRRS EU. Piglet vaccination was also changed to PRRSFLEX<sup>®</sup> EU. All sow vaccinations were done as a mass vaccination. In addition a Biocheck.ugent<sup>®</sup> analysis (Ghent University) was done twice to improve biosecurity. Based on the identified risks changes were implemented that resulted in optimized piglet flow, improved hygiene and lowered stocking density.

### Results

Data were compared for 2 different time periods: A: 01.03.15 until 01.09.15, B: 01.09.15 until 01.03.16. Repeat breeders were reduced from 15,03 % to 12,41 %. Live born piglet increased from 16,36 to 17,14. Losses in nursery were reduced from 2,5 % to 1,74 %. Marketed piglets/sow/year increased from 32,7 to 33,83.

### Conclusion

Although production parameters were already at a very good level, improvement of the PRRS vaccination program together with improvement of biosecurity helped to raise production performance to a higher level. Other pathogens like *S. suis*, Influenza, *H. parasuis* were also found in necropsies and would also have an influence on production data. By improving biosecurity and controlling PRRSv the impact of the pathogens is tending to be lower than before. This Case Report was financially supported by Boehringer Ingelheim Vetmedica GmbH.



## VAC-006 - IMPACT OF AN PRRS OUTBREAK IN A SOW HERD WITH IMPROVED RESULTS AFTER WHOLE HERD VACCINATION

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### Introduction

In swine herds PRRS outbreaks can provide massive clinical symptoms with reproductive disorders in the sow herd and respiratory disorders in nursery and fattening. This case report describes an outbreak of PRRS and the results of a whole herd vaccination protocol for PRRS implemented in response.

### Materials and methods

A 2 site farm system with 125 sows, 400 nursery piglets and 550 fattening places had been vaccinating sows only against PRRS. A PRRS outbreak occurred in June 2015 with massive reproductive disorders, coughing and increased losses in nursery and fattening. PRRSv was diagnosed by blood samples and necropsies. In response to the outbreak a new vaccination protocol for PRRS was set up with a double mass vaccination of the sow herd (Reprocyt<sup>®</sup> PRRS EU) Piglets vaccination (Ingelvac PRRSFLEX<sup>®</sup> EU) was started in July 2015 in the weaning period.

### Results

Data were compared for 3 different time periods: A: 1.7.14 until 1.7.15 (before outbreak), B: 01.07.15 until 01.12.15 (outbreak) and C: 01.12.15 until 07.04.16 (after outbreak). Reproductive performance decreased massive during the outbreak and turned back to normal again. In the nursery unit losses increased to 5 % during the outbreak. After implementing piglet vaccination, mortality went down to 1 %. In the fattening unit losses and FCR increased dramatically, ADWG decreased massively during the outbreak. Performance returned back to normal after vaccination.

### Conclusion

Due to a PRRS outbreak in June 2015 massive reproductive and respiratory disorders appeared in this herd. In November 2015 and March 2016 Influenza was also diagnosed in the herd, this might have potentiated the reproductive disorders at the sow farm. When a whole herd vaccination program against PRRSv was implemented, clinical symptoms and losses were reduced dramatically. Reproductive performance stabilized again after 6 months.

This Case Report was financially supported by Boehringer Ingelheim Vetmedica GmbH.



## VAC-007 - CONCURRENT USE OF PORCILIS® PCV M HYO AND A LIVE VACCINE IS EFFICACIOUS AGAINST MYCOPLASMA HYOPNEUMONIAE

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### Background & Objectives

*M. hyopneumoniae* and PCV2 are major pathogens involved in the Porcine Respiratory Disease Complex. Although vaccines against a number of pathogens are available, safety and efficacy always need to be demonstrated in case of concurrent use of vaccines. The objective of the present studies was to test in a laboratory challenge the efficacy against *M. hyopneumoniae* of associated non-mixed use of Porcilis® PCV M Hyo and a live vaccine. Efficacy against PCV2 was evaluated by serology.

### Material and Methods

Groups of 25 SPF pigs were vaccinated at 3 weeks of age with Porcilis® PCV M Hyo alone or concurrently with a live vaccine (intramuscular and intradermal). A group of 25 unvaccinated pigs served as challenge control. *M. hyopneumoniae* challenge was performed intratracheally on two consecutive days with a culture of a Danish field isolate at 7 weeks of age. Three weeks after challenge, the pigs were necropsied to evaluate lung lesions. Blood samples were taken just before vaccination, prior to challenge and at necropsy. Serology testing following vaccination was done with a commercial assay (*M. hyopneumoniae*) or an in-house ELISA (PCV2).

### Results

In the vaccinated groups, the median lesion scores were 100% (Porcilis® PCV M Hyo only) and 95% (concurrent use) lower than in the control group ( $p < 0.05$ , Wilcoxon Rank Sum test). Lung lesion scores were not different between vaccinated groups. PCV2 serology results were not different between vaccinated groups. No post-vaccination clinical signs were observed in the experiment.

### Conclusion

This experimental challenge study indicates that associated non-mixed use of Porcilis® PCV M Hyo and a live vaccine is safe and efficacious against *M. hyopneumoniae*. In addition, a significant PCV2 serology response was also measured.



## VAC-008 - EFFECT OF ONE AND TWO DOSE PORCINE CIRCOVIRUS VACCINES ON PCV2 PREVALENCE AND VIREMIA LEVEL

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### Introduction

Porcine circovirus type 2 (PCV2) is the cause of Post weaning multisystemic wasting syndrome, a multifactorial disease in swine farms. The depletion of lymphoid cells and a high viral load in lymphoid tissues are characteristic of PCV2 infection. This study evaluated the effect of different commercial vaccines on PCV2 viremia measured by qPCR.

### Materials and Methods

Pigs originated from three different PCV2 positive Brazilian farms. Two commercial PCV2 vaccines were compared: Circumvent<sup>®</sup> PCV M (PCVM) (Merck) and Foster<sup>®</sup> PCV (FOST) (Zoetis). Farm A used PCVM and farms B and C FOST. PCVM pigs were vaccinated twice (3-6 weeks of age) and FOST pigs once (3 weeks). Ten (10) pigs per farm were tagged before first sampling and were sampled 6 times (27-63-95-112-134-154 days of age). Animals were housed in three different nurseries and finishers. PCV2 viremia was measured by qPCR (DNA copies log<sub>10</sub>/ml blood) at UNESP, Brazil.

### Results

Viremia prevalence was higher in fattening (53.33%) than nursery (13.33%) and viral load was higher in FOST animals compared with PCVM. None of the samples from 27 day old pigs were positive. At 63 days of age, 20% of PCVM samples were positive compared to 30% of FOST. PCVM viremia level was 0.86log<sub>10</sub> and FOST was 1.02log<sub>10</sub>. The prevalence of positive samples and viral load in fattening phase was: 95 days (40% PCVM, 0.91log<sub>10</sub>; and 75% FOST, 3.94log<sub>10</sub>), 112 days (0% PCVM and 85% FOST, 3.13log<sub>10</sub>), 134 days (10% PCVM, 0.57log<sub>10</sub>; and 55% FOST, 2.08log<sub>10</sub>) and 154 days (40% PCVM, 1.79 log<sub>10</sub> and 75% FOST, 2.49log<sub>10</sub>).

### Conclusion

PCV2 challenge was higher in fattening than nursery pigs. Circumvent<sup>®</sup> PCV M was able to control viremia better than Foster<sup>®</sup> PCV as indicated by lower % positive pigs and lower level of viremia. These results suggest that there are efficacy differences across PCV2 vaccines.



## VAC-009 - FULL SERVICE ON TARGET: A SCREENING SYSTEM FOR MONITORING PNEUMONIA AND BIOSECURITY IN HERDS WITH INTRADERMAL MYCOPLASMA HYOPNEUMONIAE VACCINATION

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### Background & Objectives

*Mycoplasma hyopneumoniae* is one of the main respiratory pathogens in swine and vaccination and optimization of management and housing conditions are key control measures. "Full Service on Target" is a monitoring tool for Mycoplasma-like lesions at slaughter and biosecurity level in herds with intradermal *M. hyopneumoniae* vaccination. This field study compares intramuscular vs intradermal *M. hyopneumoniae* vaccination in farms with different biosecurity level.

### Material & Methods

A total of 10 herds were included. The traditional *M. hyopneumoniae* vaccination scheme in each farm (intramuscular; IM) served as a historical control and was compared to intradermal vaccination (ID) (Porcilis Mhyo ID Once, MSD). Prevalence of Mycoplasma-like lesions (pneumonia) recorded at slaughter (Bollo et al., 2008) was the main comparison parameter. The biosecurity of each herd was scored by means of an audit (previously described by the author) before and after implementation of intradermal vaccination. Immediately after the first audit, an improvement of biosecurity (BS+) was advised and implemented in half of the herds (5/10). The remaining five herds did not make any biosecurity improvements (BS-).

### Results

The average prevalence of pneumonia was 16.3%<sup>A</sup> [3.6–46.4] and 4.5%<sup>B</sup> [0.5–13.3] in IM and ID groups, respectively ( $P < 0.05$ ). An average reduction of pneumonia of 72% was observed after ID vaccination. The average prevalence of pneumonia for each group was 18.9%<sup>A</sup> (IM/BS-), 13.7%<sup>A</sup> (IM/BS+), 6.8%<sup>B</sup> (ID/BS-) and 2.2%<sup>B</sup> (ID/BS+) ( $P < 0.05$ ). An average reduction of pneumonia of 64% and 84% was observed after ID vaccination in groups BS- and BS+, respectively. Biosecurity score was improved only in BS+ group. This increased biosecurity had only a numerical effect on pneumonia.

### Discussion & Conclusion

A reduction of Mycoplasma-like lesions was demonstrated after intradermal *M. hyopneumoniae* vaccination when compared with intramuscular vaccination. Improvement of biosecurity contributed to a numerical reduction of pneumonia. Further research is needed to confirm this observation.





## VAC-010 - DIFFERENCE IN EFFICACY OF DIFFERENT COMBINATIONS OF PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES

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### Background and objectives

Recently, ready-to-use (RTU) vaccines containing both PCV2 and *Mycoplasma hyopneumoniae* (M hyo) antigens were marketed in the EU. This study compares the efficacy of an RTU vaccine with a Freshly mixed combination.

### Material and methods

Piglets were randomly allocated to 3 vaccination groups at weaning: Group 1 vaccinated with Placebo, group 2 with RTU vaccine and group 3 with Freshly mixed vaccine. Efficacy was measured by ADG and lung lesion scores.

### Results

Totally, 1308 pigs were followed until slaughter. PCV2 and M hyo were absent in the nursery, but during finishing, all groups seroconverted to M hyo, and PCV2 was detected in pigs from all groups, but in a significantly lower number from group 2 and 3. Group 2 had a significantly lower ADG in the nursery (372 g/day) compared to group 1 and 2 (382 and 383 g/day). The finisher ADG was highest for group 2 and 3, but the overall wean to slaughter ADG was even in group 1 and 3 (710 g/day) and lower than in group 3 (718 g/day). The number and extension of M hyo like lung lesions was significantly lower in group 2 and 3 compared to group 1. The number of pleurisy lesions was highest in group 1 and lowest in group 3, and the extension of pleurisy lesions were significantly lower in group 3.

### Discussion and conclusion

The positive effect on finisher ADG was even in the vaccinated groups, but in group 2, the overall performance did not exceed that of non-vaccinates due to the nursery set-back. The vaccines had a comparable reduction of M hyo like lung lesions, but pigs vaccinated with the freshly mixed combination were significantly better protected against secondary infections, revealed as pleurisy lesions.



## VAC-011 - VACCINATION BY TATTOO WITH DNA IN NANOPARTICLES

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### Background and objectives

DNA vaccination in the skin is a new vaccination method. We eventually want to assess the efficacy of DNA vaccination against Human Papilloma Virus infections in the skin of pigs as a translational model for humans.

### Material and Methods

The experiment was done in 4 phases:

Selection of promising nanoparticles based on transfection in ex vivo pig skin with pDNA encoding firefly luciferase

Selection of the optimal nanoparticles based on transfection in vivo, one day and three days after application of pDNA encoding for firefly luciferase by tattoo.

Selection of penetration enhancer based on uptake of antigen encoded in the pDNA vaccine by pAPC's in the draining lymph nodes

Assessment of the best two formulations on their ability to stimulate T-cells after triple application of HPV16 E6E7 DNA vaccine, 14 days apart.

### Results

Tests a and b revealed that a formulation of pDNA in polyplex HA37 consistently performed better than other formulations including naked pDNA at a concentration 1mg/ml in dermal transfection efficiency in living pigs. Regarding the penetration enhancers, 5% DMSO in lanette oil in water emulsion improved penetration of the antigen from the skin to the draining lymph node. Further results from c. and d. will be shown at the congress.

### Discussion and Conclusion

Historically, mice have been used as a preclinical model for dermal vaccine development, but the results could not always be extrapolated to humans. Pig skin better resembles human skin and may therefore be a better preclinical model for human vaccine development.



## VAC-012 - OBSERVATIONAL STUDY ABOUT PRRS SOW VACCINATION EFFICACY AND VACCINATION SCHEDULES IN THE NETHERLANDS

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### Background & Objectives

Porcilis® PRRS sow vaccination reduces PRRSV infections as cause of weak born piglets and early farrowing. This study investigated: 1. effect of sow vaccination on proportion of farms with early farrowing/weak born piglets (E/W) and/or abortion (A); 2. Proportion of PRRS diagnoses (vertical transmission diagnosed via PCR saliva testing of piglets at farrowing) on Porcilis® PRRS or KV PRRS sow vaccinating farms and non-sow vaccinating farms with E/W or A; 3. Effect of MLV sow vaccination schedules on %PRRS diagnoses described in 2.

### Material & Methods

Data from MSD-AH ResPig® system (2013-2015). Every 6 months, the PRRS status is studied via cross-sectional blood sampling of gilts, sows, weaners and nursery pigs (saliva PCR testing since 2014). Weaners PRRS PCR results determine PRRS-stability of the sow herd. Investigations with clinical and vaccination anamneses evaluate the protective effect of PRRS sow vaccination and -schedules.

### Results

1. Proportion of farms with E/W or A: no sow vaccination 16% (9/57), Porcilis® PRRS sow vaccination 12% (53/421) and PRRS inactivated sow vaccination 16% (1/6).
2. Proportion of PRRS diagnoses following no sow vaccination was 20%(1/5) and Porcilis PRRS sow vaccination 13%(3/23). Only one inactivated PRRS sow farm in the data and PCR on weaners was positive.
3. PRRS diagnoses following Porcilis® PRRS sow vaccination in mid-gestation and farrowing unit was 7%(1/15) and 14%(1/7) in herd vaccination (4/ year). The one farm with farrowing-unit vaccination only was pcr+ at weaning.

### Discussion & Conclusion

Despite the small data set, the trends were similar to observations made in 2004-2007. However, the % PRRS diagnosis between 2013-2015 was lower compared to 2004-2007:(14%vs27%). The proportion of PRRS signs was lower in Porcilis® PRRS sow vaccinating farms compared to non- or KV sow vaccinating farms. Porcilis® PRRS sow vaccination reduces vertical transmission and the mid-gestation-farrowing unit schedule is at least as effective as a herd vaccination.



## VAC-013 - DEVELOPMENT OF INACTIVATED VACCINE AGAINST NON-TYPHOID SALMONELLA INFECTIONS IN PIGS

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### Background & Objectives

Non-typhoid Salmonella infections in pigs are continuous problem in the current pig industry. Even sub-clinical infections have an impact on pig health, decrease average daily gain and thus prolong fattening period. The other issue is a risk of Salmonella transmission to consumers. Despite partial success in the vaccine development in the past, the vaccination is currently not widely accepted by pig industry as a way how to decrease the number of Salmonella-positive pigs on slaughter. We believe the successful vaccine could not only be effective, but also have to enable to differentiate vaccinated pigs from infected pigs and the vaccine must not present health risk for pork consumers. We thus concentrated on development such vaccine in a joint project with Bioveta a.s. company.

### Material & Methods

Various experimental preparations of inactivated vaccine based on *S. Typhimurium* only or on *S. Typhimurium*, *S. Derby* and *S. Infantis* were used for immunization of weaned piglets or pregnant sows. Immune response to the vaccine was measured with Salmotype Pig Screen ELISA or with various home-made ELISAs based on Salmonella lipopolysaccharides or recombinant proteins. After vaccination, weaned or suckling piglets were challenged with *S. Typhimurium*, *S. Derby* or *S. Infantis*. Three days after challenge, the bacterial load in various tissues was measured by direct plating of organ homogenates.

### Results

We found that vaccination induced high levels of antibodies against antigens used in vaccine preparations in both, weaned piglets and sows. Maternal immunity was successfully transferred to suckling piglets. After challenge, only weak protectivity was found in weaned piglets, but high protectivity was found in suckling piglets.

### Discussion & Conclusion

Developed inactivated vaccine have a potential to decrease the transmission of Salmonella infection from sows to suckling piglets. This work was funded from QJ1210115 and LO1218 projects.



## VAC-014 - ULVAN FROM *ULVA ARMORICANA* (CHLOROPHYTA) ACTIVATES THE PI3K/ AKT SIGNALLING PATHWAY VIA TLR4 TO INDUCE INTESTINAL CYTOKINE PRODUCTION

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### Background & Objectives

The biological activities of water-soluble sulfated polysaccharides of green algae (ulvans) have been explored for use as bioactive molecules for the benefit of human and animal health. Recently, a crude extract of marine sulfated polysaccharides (MSP) was prepared from the green macroalgae *Ulva armoricana* harvested from Brittany shores (France). This extract showed its capacity to inhibit the growth of pathogenic bacteria and modulate the intestinal immune response using *in vitro* model of porcine intestinal cell line IPEC-1. The objective of this study was to purify an ulvan fraction, to evaluate its capacity to stimulate the immune response, and finally elucidate the molecular mechanisms underlying this biological activity.

### Material & Methods

Green tide algae *Ulva sp.* were collected, a new MSP batch was prepared, and a ulvan fraction was purified. We investigated whether this ulvan fraction was able to stimulate cytokine expression using IPEC-1 cells and evaluated its interaction with HEK-293 reporter cell lines expressing a panel of PRRs to identify the target receptor. We have also undertaken the identification of the signalling pathway involved in cytokine expression after TLR stimulation.

### Results

RT-qPCR and ELISA analyses showed that the purified ulvan fraction increased cytokines expression such as CCL20, IL8, and TNF $\alpha$  to a similar extent as the MSP extract. Using HEK-293 reporter cell lines, the ulvan fraction was found to primarily stimulate TLR4. Western blot analyses of ulvan-treated HEK293-TLR4 cells showed an increase in the phosphorylation status of Akt and the p65 subunit of nuclear factor- $\kappa$ B. Inhibition of Akt phosphorylation with the specific inhibitor abrogated the ulvan-mediated enhancement of IL-8 secretion.

### Discussion & Conclusion

The overall results showed that ulvan fraction is an immunostimulatory compound by itself, and furthermore, it could be used to effectively complex and deliver TLR ligands to relevant immune cells in vaccination strategies.



## VAC-015 - ASPECTS OF INTRADERMAL APPLICATION OF DIFFERENT ADJUVANTS IN PIGS

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Intradermal immunization (i.d.) has more advantages than intramuscular/subcutaneous immunization as the skin is the perfectly equipped place for antigen processing and adjuvants used can influence quantity and quality of induced immune response. However, it is still seldom used method of vaccine delivery. To determine the effect of i.d. application of different adjuvants on porcine immune response, two *in vivo* experiments were performed.

Firstly, animals were immunized with model antigen KLH only or KLH combined with different adjuvants: complete or incomplete Freund's adjuvants, Montanide ISA Emulsigen Al(OH)<sub>3</sub>. Blood samples were taken before immunization, three weeks after immunization and two weeks after reimmunization. Levels IgG1 and IgG2 antibodies in sera were determined by ELISA. Oil-based adjuvants induced isotypical IgG1/IgG2 switch and increased IgG1 levels in sera, thus providing good Th2 response.

Secondly, local cytokine and chemokine response after i.d. was tested. Animals were immunized with the same antigen-adjuvant combination. Skin biopsies were taken 4 and 24 hours post-immunization for histopathological analyses and quantitative RT-PCR. As expected, high proinflammatory response was clear and strong in the site of application and it correlated to local reaction of the skin and high cell infiltration into the site of application.

Based on these studies, we can conclude i.d. application can elicit good and rapid immune response and should be considered as a good vaccine delivery route.

The work was supported by the Ministry of Agriculture of Czech Republic (RO0516, QJ1510218) Ministry of Education, Youth and Sport of Czech Republic (LO1218) and AdmireVet (CZ.1.05/2.1.00/01.006, ED0006/01/01).



## VAC-016 - EXPERIMENTAL STUDIES TO EVALUATE THE ONSET AND DURATION OF IMMUNITY OF COLIPROTEC® F4/F18, LIVE E. COLI ORAL-VACCINE AGAINST POST-WEANING DIARRHOEA

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Coliprotec® F4/F18 is a live non-pathogenic *E. coli* vaccine for active immunization of pigs against PWD caused by F4-ETEC and/or F18-ETEC. This investigation reports on the efficacy of Coliprotec® F4/F18 against PWD using F4-ETEC and F18-ETEC experimental challenge models.

Two GCP-studies were performed to investigate the Onset (Ool) and Duration (Dol) of Immunity of Coliprotec® F4/F18 (Prevtec Microbia) after an experimental infection with F4-ETEC (*Study A*) or F18-ETEC (*Study B*). In each study, piglets of 17 ( $\pm$ 1) days of age were randomly allocated to 4 groups: vaccinated and non-vaccinated groups for the Ool and Dol studies, respectively. Coliprotec® F4/F18 was administered on the day after weaning in the drinking water via bowls and pigs were challenged with an F4-ETEC or F18-ETEC strain at 7 (Ool) or 21 (Dol) days post-vaccination (dpv). Diarrhoea was scored daily and faecal shedding of the challenge strain investigated for 4 (*Study F4*) or 7 (*Study F18*) days post-challenge (dpc).

The incidence of pigs showing moderate to severe diarrhoea at any dpc was reduced in the vaccinated groups compared to non-vaccinated groups [*Study F4*. Ool: 0% vs 50%;  $p=0.025$  and Dol: 0% vs 22%;  $p>0.05$ ; *Study F18*. Ool: 0% vs 75%;  $p<0.001$  and Dol: 0% vs 67%;  $p=0.001$ ]. A significant reduction in the faecal shedding of the challenge strain was shown for the Ool and Dol vaccinated groups in both studies. No challenge-related mortality was observed for vaccinated pigs of both the Ool and Dol in the *Study F18*, as compared to 33% and 67% mortality for non-vaccinated pigs, respectively. No challenge-related mortality was observed in the *Study F4*.

Coliprotec® F4/F18 reduced the incidence of pigs showing moderate to severe diarrhoea and the faecal shedding of the challenge strain following experimental infection with F4-ETEC or F18-ETEC. A preventive effect on mortality caused by F18-ETEC was demonstrated.



## VAC-017 - PREVALENCE AND VIRAL LOAD OF PCV2-VIREMIC LATE FATTENING PIGS FROM GERMAN FARMS WITH DIFFERENT PCV2 VACCINATION STRATEGIES

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### Background & Objectives

PCV2-viremia in late fattening pigs contributes to extended lung lesions in farms suffering from porcine respiratory disease complex (PRDC). The following investigation was conducted to get information about prevalence of PCV2-viremic late fattening pigs in Germany taking PCV2 vaccination status in consideration.

### Material & Methods

A total of 1.680 individual serum samples from pigs between 16-20 weeks of age from 59 German fattening farms with different PCV2 vaccination strategies (41 farms with piglet vaccination (pv), 1.140 samples; 6 farms with sow vaccination (sv), 180 samples; 12 farms with no vaccination (nv), 360 samples) were examined for PCV2 DNA by q-PCR. All samples originated from routine animal health screenings that were conducted in these farms (n= 20 to 30 samples per farm).

### Results

In 25/59 (42.3 %) of the farms at least one pig was positive for PCV2 DNA. Considering the vaccination strategies, 9/41 (22 %) of the pv- farms, 6/6 of the sv-farms and 10/12 of the nv-farms were positive for PCV2-DNA. The average in-herd prevalence for PCV2 DNA positive pigs in pv-farms (2.5 %) was significantly lower than for sv-farms (36.7 %) and nv-farms (33.6 %) ( $p < 0.001$ ). Whereas the mean viral load for PCV2 DNA positive pigs was comparable for animals in all three groups (pv:  $1.3 \cdot 10^5$  genome copies/ml serum; sv:  $2.6 \cdot 10^5$  genome copies/ml serum and nv:  $2.4 \cdot 10^5$  genome copies/ml serum).

### Discussion & Conclusion

Prevalence of PCV2 viremic pigs in late fattening in pv-farms was negligible, while prevalence of PCV2-viremic late fattening pigs in sv-farms was unexpectedly high and comparable to the nv-farms. PCV2-viremia in late fattening in cases of PRDC may occur because passive immunity might not be sufficient to give long lasting protection against viremia.





## VAC-018 - POTENTIATION OF THE MYCOPLASMA HYOPNEUMONIAE VACCINE EFFICACY WITH A NOVEL ADJUVANT

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### Introduction

Protective immunity against *M. hyopneumoniae* is driven by both humoral and cell-mediated immune responses, with notable significance of the latter. Previous studies have demonstrated excellent protection induced by Hyogen<sup>®</sup>, the inactivated *M. hyopneumoniae* vaccine of Ceva, in which the antigen is formulated using a specific adjuvant Imuvant<sup>™</sup>. This adjuvant consists of the non-toxic LPS derived from *E. coli* J5 in an oil-in-water emulsion. This optimized combination stimulates both the innate immune system and the specific adaptive immune response to *M. hyopneumoniae*.

### Materials and methods

In the first study, the boosting effect of the J5 component of Imuvant<sup>™</sup> on the cellular immunity of piglets was assessed. A group of 20, 21-day-old piglets were vaccinated with Hyogen<sup>®</sup> containing the full adjuvant, including J5 LPS and another group with a test vaccine containing the *M. hyopneumoniae* antigen adjuvanted only with the mineral oil component, without J5. The third group was left non-vaccinated. At 21 days post-vaccination, the animals were blood-sampled and the cell-mediated responses were measured with ELISPOT.

In the second study, the efficacy of these vaccines was addressed, using 15 animals in each group, retaining the same study design. In this study, the animals were challenged 18 days after the vaccination and then, 28 days later, subjected to lung scoring.

### Results

In the first study the ELISPOT test showed a much stronger specific cellular immune response in the Hyogen<sup>®</sup>-vaccinated pigs compared to the test vaccine. In the efficacy test, Hyogen<sup>®</sup> conferred significant protection against lung lesions ( $p=0.028$ ), whereas in the case of the test vaccine it was not statistically significant ( $p=0.288$ ).

### Conclusion

These studies confirmed the beneficial effect of J5 component of Imuvant<sup>™</sup> by inducing markedly strong cell-mediated immune responses after vaccination and conferring a solid protection against lung lesions.



## VAC-019 - COMPARATIVE DYNAMICS OF THE HUMORAL IMMUNE RESPONSE ELICITED BY 3 INACTIVATED VACCINES AGAINST SWINE ERYSIPELAS AND PORCINE PARVOVIRUS: PART I

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### Background & Objectives

The protective role of the specific antibodies against Swine Erysipelas (SE) and Porcine Parvovirus (PPV) enhanced through vaccination is key in the control of infectious reproductive problems in sows. In this study, the comparative dynamics of post-vaccination antibodies elicited by three different commercial vaccines is evaluated in gilts during a whole reproductive cycle (170d).

### Material & Methods

A controlled, blinded experimental trial was performed with SE and PPV naïve gilts. Forty-two animals were randomly assigned to 4 groups. G1-G3 (n=12) were vaccinated twice intramuscularly (on days 0 and 21) with vaccines A (ERYSENG<sup>®</sup>PARVO), B and C, respectively. G4 (n=6) received PBS. SE serology was performed in serum using a commercial ELISA kit (Civtest<sup>®</sup>Suis SE/MR). The suitability of this kit to detect anti-SE antibodies without bias towards any of the vaccines was previously reported.

### Results

SE antibody titres in G1 were the highest throughout the study. Differences were statistically significant with titres in G2 from 21 dpv to the end of the study, and with those in G3 at 21 and 147 dpv (Mann-Whitney U Test; *p-value*<0.05). All vaccines showed the highest mean SE titres at 41 dpv. G4 remained SE-negative throughout the study.

The percentage of seropositive gilts in G1 reached 100% from 21 to 83 dpv, and ≥75% until 147 dpv. G2 and G3 did not reach 100% at any time point. G2 showed ≥75% seropositive animals at 41 dpv, and G3 from 21 to 83 dpv. Statistically significant differences were recorded among groups at several time points (Fisher test; *p-value*<0.05), always in favour of G1.

### Discussion & Conclusion

The humoral immune responses against SE elicited by the vaccines tested were different in intensity and duration. The seroconversion to SE after ERYSENG<sup>®</sup>PARVO vaccination was faster, more intense and lasted longer than that after vaccines B and C, covering the entire reproductive cycle.



## VAC-020 - COMPARATIVE DYNAMICS OF THE HUMORAL IMMUNE RESPONSE ELICITED BY 3 INACTIVATED VACCINES AGAINST SWINE ERYSIPELAS AND PORCINE PARVOVIRUS: PART II

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### Background & Objectives

The protective role of the specific antibodies against Swine Erysipelas (SE) and Porcine Parvovirus (PPV) enhanced through vaccination is key in the control of infectious reproductive problems in sows. In this study the comparative dynamics of post-vaccination antibodies elicited by three vaccines is evaluated in gilts during a whole reproductive cycle (170 days).

### Material & Methods

A controlled, blinded experimental trial was performed with SE and PPV naïve gilts. Forty-two animals were randomly assigned to 4 different groups. G1-G3 (n=12) were vaccinated twice intramuscularly (on days 0 and 21) with vaccines A (ERYSENG®PARVO), B and C, respectively. G4 (n=6) received PBS. PPV serology was performed in serum using the haemagglutination inhibition assay.

### Results

Although G4 remained PPV-negative throughout the study an unexpected PPV seroconversion was observed in G1-G3 from 126dpv onwards. Therefore, the dynamics of post-vaccination antibodies was compared until 107dpv only.

PPV-antibody titres in G1 were the highest throughout the study. Differences were statistically significant with titres in G2 from 21dpv to the end of the study, and with those in G3 at 21dpv (Mann-Whitney U Test; *p-value*<0.05). All vaccines showed the highest mean PPV titres at 41dpv.

Seropositivity in G1 reached 100% from 41 to 63dpv, and ≥75% until 107dpv. G2 did not reach 100% at any time point. At 107dpv, G2 and G3 showed 8.3% and 50% seropositive animals respectively. Differences were recorded among groups at several time points (Fisher test; *p-value*<0.05) always in favour of G1.

### Discussion & Conclusion

In this trial a suspected natural PPV infection limited the study time. However, the humoral immune responses against PPV elicited by the vaccines tested were different in intensity and duration until 107dpv. The seroconversion to PPV after ERYSENG®PARVO vaccination was faster, more intense and lasted longer than that after vaccines B and C.



## VAC-021 - MAGNETIC RESONANCE IMAGING IN SUCKLING PIGLETS

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Safety testing is one major part of the licensing procedure of veterinary vaccines and demands a lot of animals. Magnetic resonance imaging was tested as an alternative and allows a three-dimensional follow-up of the injection site. In comparison to pathologic examination the MRI findings revealed the same results. In previous pig studies the following questions arose: (1) whether MRI can be used in suckling piglets as well; (2) does the injection of a saline solution lead to a volume effect, and (3) is the local reaction size affected by the tattoo marking of the injection point for the final pathomorphologic examination? To answer these questions the following study was used, comparing two vaccines for suckling piglets (8 animals per vaccine; group A and B) with two control groups (4 animals per group). One control group was injected with a saline solution (C) and the other was tattoo marked only (D). The animals were examined using MRI at day 1, 8, 15, 22, 29, 36, and 43 after vaccination (group C and D only day 1 and 8). A final pathomorphologic examination was performed at the specific examination end of each group. Pathomorphologic examination showed different distributions of both vaccines and therefore confirmed MRI findings. Saline solution does not result in a local reaction as detected injecting vaccines. Tattoo marking has no effect on the local reaction if it is performed prior injection. MRI allows a three-dimensional evaluation of the local reaction in comparison to the pathomorphologic findings. Therefore, MRI can be used as an alternative method for pigs in different age categories (suckling piglets, farrowed piglets, fatterings pigs, and gilts).



## VAC-022 - SAFETY AND EFFICACY OF AN ACTINOBACILLUS PLEUROPNEUMONIAE VACCINE UNDER FIELD CONDITIONS IN GERMANY

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### Introduction

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (A.p.) is a contagious disease with high morbidity. In Germany 87.8% of farms with recurring respiratory disease are seropositive for A.p.. Pigs seropositive for A.p. serotype 2 have an increased risk to suffer from chronic pleurisy. The aim of the present trial was to evaluate the safety and efficacy of an A.p. vaccination in a finishing herd with high prevalence of pleurisy.

### Material & Methods

At the beginning of finishing, 599 pigs were randomly allocated to two vaccine groups (V1 CoglapiX<sup>®</sup>, n=255; V2 autogenous A.p., *Streptococcus suis* vaccine, n=252) and one non-vaccinated control group (CG, n=92). V1 and V2 animals were re-vaccinated after three weeks. All animals were weighed individually at the beginning and end of finishing. Dorsocaudal pleurisy (DP) was recorded according to the CEVA Lung Program methodology. 70 pigs of another batch were randomly included for the safety assessments.

### Results

The mean average daily weight gain (ADWG) was 895.6g (V1), 873.3g (V2) and 864.7g (CG). V1 animals had numerically higher ADWG compared to CG (p=0.061) and V2 (p=0.081). Dorsocaudal pleurisy was evident in 62.5% of the lungs of V1, 59.3% of V2 and 71.4% of CG with severe bilateral DP in 20.8% of the lungs of V1, 25.6% of V2 and 32.7% of CG. The prevalence and severity of DP did not differ significantly between the groups. The occurrence of local reactions was equally distributed between the groups. No increase in mean rectal temperatures after vaccination was observed.

### Discussion & Conclusion

Vaccination of pigs at the beginning of finishing with CoglapiX<sup>®</sup> was safe and increased numerically the ADWG during finishing compared to V2 and CG. The presence and extent of dorsocaudal pleurisy did not differ significantly between the groups; however, there was a tendency for lowest incidence of the most severe lesions in the CoglapiX<sup>®</sup> group.



## VAC-023 - ASSESSMENT OF SAFETY OF A READY-TO-USE VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE AND PCV2 UNDER FIELD CONDITIONS IN GERMANY

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### Background & Objectives

*Mycoplasma hyopneumoniae* (M.hyo) and Porcine Circovirus type 2 (PCV2) are two major infectious agents in the Porcine Respiratory Disease Complex (PRDC). A combined vaccine against both pathogens allows to reduce the number of injections thus reducing handling stress for the piglets and labor for the user. The objective of the present study was to assess safety of a ready-to-use vaccine against M.hyo and PCV2 (Zoetis Suvaxyn Circo+MH RTU) in comparison to a combined commercial M. hyo, PCV2 vaccine under field conditions.

### Material & Methods

The study was performed in a farrow-to-finish farm with 200 sows in southern Germany. A total of 300 healthy suckling piglets (21 (+4) days old) were randomly allocated to the treatment groups: V1 (n=149, 2 ml Zoetis Suvaxyn Circo+MH RTU), V2 (n=151, 2ml of the combined commercial M. hyo, PCV2 vaccine). 20% of the animals (V1=31, V2=29) were selected for the safety assessments. Clinical observations and local injection site reactions (ISR) were recorded 4 hours (h) after vaccination and daily for 6 consecutive days. Additionally rectal temperature of the safety animals was measured prior, 4h and 24h after vaccination.

### Results

No systemic side effects were observed in any of the vaccinated pigs. ISRs were observed in one animal of V2 with a maximum diameter of 0.5 cm. 4 piglets of V1 and 10 piglets of V2 showed an increased level of pain at the injection side 4h after vaccination. 4h after vaccination the mean rectal temperature in V1 was  $40.2^{\circ}\text{C} \pm 0.5$  and  $40.5^{\circ}\text{C} \pm 0.6$  in V2 respectively ( $p > 0.05$ ). The maximally observed increase per animal was  $1.7^{\circ}\text{C}$  in both groups. After 24h rectal temperature in all treatment groups returned to a physical range.

### Discussion & Conclusion

Vaccination of piglets at 21 (+4) days of age with Zoetis Suvaxyn Circo+MH RTU is safe under field conditions.



## VAC-024 - ESTABLISHMENT AND APPLICATION OF A CELL CULTURE ASSAY TO QUANTIFY NEUTRALIZING ANTIBODIES AGAINST CLOSTRIDIUM PERFRINGENS TYPE-C BETA-TOXIN IN PIGS

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### Background & Objectives

*Clostridium perfringens* type C induced enteritis causes high mortality in newborn piglets and continuous vaccination is required to prevent disease. Vaccines containing high amounts of inactivated beta-toxin are considered to induce sufficient neutralizing antibody titers in sow colostrum to protect piglets. However, no method to quantify such neutralizing antibodies is available. We established a novel cell culture based assay for the quantification of neutralizing anti-beta-toxin antibodies.

### Material & Methods

Blood and colostrum samples of 10 primiparous and 35 multiparous sows and 2 piglets per sow from three different farms were collected. Gilts were vaccinated using commercially available vaccines five and two weeks prior to farrowing and multiparous sows were boosted once before every further farrowing. Blood samples were collected three weeks ante and 24 hours post partum. Colostrum was collected 24 hours, piglet sera two days post partum. 100 ng of recombinant beta-toxin were incubated with a serial dilution of sera or colostrum for 1 h. Primary porcine endothelial cells, grown on 96-well plates, were incubated with this mixture for 24 hours at 37°C. Cell viability was determined using a redox dye and colorimetric quantification.

### Results

Low neutralizing antibody titers were detected after the second immunization of gilts and antibodies were detectable in 66% of their piglets. Multiparous sows showed significantly increased antibody titers in serum and colostrum. The mean antibody titer in piglets of multiparous sows was three times higher compared to piglets from gilts.

### Discussion & Conclusion

Continuous vaccination of sows during each pregnancy induces high neutralizing anti-beta-toxin antibody titers in colostrum resulting in good passive immunity of piglets. Piglets from gilts being vaccinated twice during their pregnancy however seem to be insufficiently protected. In herds with continuous problems an adaptation of the initial immunization of gilts should be considered.



## VAC-025 - FIELD SAFETY OF SUVAXYN® CIRCO+MH RTU UNDER FIELD CONDITIONS IN SPAIN

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### Introduction

The study objective was to assess the safety of the new vaccine Suvaxyn® Circo+MH RTU (Zoetis) for piglets, compared with two existing commercial vaccines, under field conditions in Spain.

### Materials Methods

288 pigs were housed in 48 identical pens of a nursery building. Four pigs from each pen of six were enrolled in the study (total 192 animals), and randomly allocated to one of these four treatment groups: T01-Saline (2mL), T02-Suvaxyn Circo+MH (2mL), T03-Ingelvac CircoFLEX + Ingelvac MycoFLEX (1mL+1mL) or T04-Porcilis PCV M Hyo (2mL). This resulted in 48 animals per treatment group that received the corresponding licensed dose of each vaccine at about 3 weeks of age (Day 0) via intramuscular route. The study lasted 4 weeks (Day 28) and the individual pig was the experimental unit. Animals were individually weighed (Days 0 and 28) and assessed through clinical observations, individual temperatures and injection sites (Days 1, 3, 7 and 14) using a scoring system. Data were analyzed by ANOVA using the GLM of SAS.

### Results

The Suvaxyn group showed a numerical advantage in daily weight gain (325g/d) vs. other groups competitors (315g/d and 317g/d respectively). Pigs vaccinated with Porcilis showed a significant ( $p < 0.0001$ ) temperature increase 1 hour after vaccination, compared with the other two vaccinated groups; no significant differences in temperature were found between treatments on Day 1 through Day 4. There were few local reactions in all groups 1 hour post-injection (diameter of swelling area  $< 0.5$  cm), all resolved quickly (scores were 0 on Day 1 through Day 14). No systemic reaction observed in any pig during the study.

### Conclusion

Suvaxyn® Circo+MH RTU is proven to be very safe for pigs from 3 weeks of age, as demonstrated by satisfactory post-vaccination productive performance, stable body temperature, and minimal to none injection site reactions.





## VAC-026 - EFFICACY OF A NEW PRRSV 2 LIVE VACCINE IN PIGS CHALLENGED WITH A HIGHLY PATHOGENIC FIELD STRAIN

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The objective of this study was to evaluate the efficacy of a new porcine reproductive and respiratory syndrome virus (PRRSV) 2 live vaccine in pigs challenged with a Vietnamese highly pathogenic (HP) PRRSV 2 field strain.

Ten PRRSV negative piglets (group 1) were vaccinated with the PRRSV 2 live vaccine at the age of 26 days. Another 10 piglets (group 2) remained unvaccinated. All study piglets received an intranasal challenge with HP PRRSV 2 on day 0 of the study (42 days after vaccination). Blood samples were taken on several occasions after vaccination and challenge. Viral load, development of antibodies and clinical symptoms were analyzed. On day 28 after challenge, all piglets were euthanized and pathologically examined.

On days 7 and 21 after vaccination, PRRSV 2 viraemia was seen in all piglets of group 1 which remained in seven piglets until 42 days after vaccination. On day 3 after challenge, all piglets from both groups were positive in PRRSV 2 RT-qPCR. From day 7 onwards, viral load and number of PRRSV positive pigs were lower in group 1 than in group 2. All pigs of group 1 seroconverted after PRRSV 2 vaccination. PRRSV antibodies were detected in serum of all study pigs from both groups on day 14 after challenge and onwards. In group 2, moderate respiratory symptoms with occasional coughing were seen following the challenge with HP PRRSV 2. Pigs of group 1 remained clinically unaffected. An interstitial pneumonia was found in four piglets of group 1 and in all ten piglets of group 2. Histopathological findings indicated more severe pathological alterations in group 2.

It can be concluded that the used novel PRRSV 2 live vaccine provided a good partial protection of pigs against challenge with a Vietnamese HP PRRSV 2 field strain.



## VAC-027 - PREVENTION OF ERYSIPELAS SYSTEMIC DISEASE IN SWINE 6 MONTHS AFTER VACCINATION WITH A NEW PARVOVIRUS AND ERYSIPELAS INACTIVATED COMBINATION VACCINE

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### Introduction

Suvaxyn<sup>®</sup> Parvo/E-Amphigen is a new inactivated vaccine which protects sows and gilts from porcine parvovirus (PPV) reproductive disease and *Erysipelothrix rhusiopathiae* (Ery) skin lesions and systemic disease. The adjuvant, Amphigen, allows the development of strong and long lasting immunity while maintaining a good safety profile.

The objective of the program was to demonstrate the 6-month duration of immunity of the vaccine in front of a challenge with two virulent serovar 1 and 2 Ery strains.

### Materials and methods

In three different studies, 3-4 month-old pigs were either mock-vaccinated (PBS) or vaccinated with Suvaxyn Parvo/E-Amphigen, 2 ml by IM route, two doses 21 days apart. Four or six months later, all pigs were challenged with Ery serovar 1 and serovar 2 by intradermal route. Rectal temperatures were monitored and pigs were observed for seven days for typical clinical signs.

### Results

A 100% infection of control pigs was achieved for both serovars, demonstrated by development of diamond skin lesions in 100% of the pigs, and by development of fever, dyspnea, prostration and end-point clinical condition (euthanasia). All the later clinical signs were absent in vaccinated pigs.

Skin lesions in control pigs spread all over the body, preceding septicemia and clinical end-point. These lesions were prevented in vaccinated pigs for serovar 2. In contrast, in serovar 1 challenge, lesions observed in vaccinated pigs were self-limiting and did not spread. Lesions resolved spontaneously in vaccinated animals before the end of the studies.

In the 6 month DOI studies the development of skin lesions was significantly lower in vaccinated pigs in 3-4 time points after challenge, and similar results were obtained in the 4 month DOI study.

### Conclusions

Suvaxyn Parvo/E-Amphigen is able to prevent erysipelas-related disease 6 months after vaccination after challenge with serovar 1, and prevents systemic disease after challenge with serovar 2.



## VAC-028 - EVALUATION OF VACCINATION AS THE PRINCIPAL METHOD OF PREVENTING ATROPHIC RHINITIS

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### Background & Objectives

Atrophic Rhinitis (AR) is a disease that is present throughout the world, with vaccination as one of the principal preventive measures. At the same time, evaluation of the nasal turbinates has become established as one of the principal methods of evaluation of the disease. The objective of this study was to analyse the development of the lesions in the nasal turbinates before and after establishing a vaccination plan against AR.

### Material&methods

Two farms were selected (A and B), situated in Spain, that were not vaccinated against AR and where there were appropriate respiratory symptoms. Thirty animals from each farm were selected randomly in the slaughterhouse, and the lesions in the turbinates were evaluated in accordance with the guidelines of the European Pharmacopoeia. All the sows were then vaccinated with RHINISENG® (HIPRA). A year after vaccination was started, the nasal turbinates were again evaluated.

### Results

In the initial evaluation, on farm A, 80% of the samples showed bone destruction, with an average lesion grade of 4.12/18, the maximum being 15/18. On farm B, 96% of the animals had lesions, with an average value of 5.7/18, and a maximum of 17/18. In the second evaluation (1 year after vaccination was introduced), 70% of the animals on farm A had lesions, with average and maximum values of only 1.59/18 and 10/18, respectively. The same was observed on farm B: 64% of the samples were affected, with an average value of 1.56/18, and a maximum of 6/18.

### Discussion & Conclusion

After vaccination was introduced, the lesions in the nasal turbinates were significantly reduced, both in terms of the percentage of animals affected and in the average lesion grade. These results show the key role played by vaccination in the prevention and control of AR.



## VAC-029 - SAFETY AND EFFICACY OF A PARVOVIRUS AND ERYSIPELAS INACTIVATED VACCINE UNDER FIELD CONDITIONS

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### Introduction

Suvaxyn<sup>®</sup> Parvo/E-Amphigen is a new inactivated vaccine designed to protect sows and gilts from porcine parvovirus (PPV) reproductive disease and *Erysipelothrix rhusiopathiae* (Ery) skin lesions and systemic disease. The adjuvant, Amphigen, allows the development of a strong and long lasting immunity while maintaining a good safety profile.

The objective of the program was to demonstrate the safety and efficacy of the vaccine under field conditions, in three Spanish farms.

### Materials and methods

On each farm 30 gilts and 30 sows were vaccinated with Suvaxyn Parvo/E-Amphigen, 2 ml by IM route, two doses 21 days apart; 15 control gilts received another commercial vaccine against PPV and Ery, while 15 control sows received PBS. Rectal temperatures, injection site and systemic reactions were monitored after vaccination. All animals were followed for a complete reproductive cycle to evaluate reproductive performance.

### Results

Injection site reactions observed after vaccination (up to 30% in gilts and 93% in sows) were mild and disappeared in a maximum of 4 days. A transient increase in rectal temperatures (up to 27% in gilts and 10% in sows) was observed 4 to 6h after vaccination, returning to normality after 24h.

The efficacy of the vaccine was difficult to assess, since clinical outbreaks of PPV or erysipelas are uncommon. Reproductive performance was similar in vaccinated and control groups. PPV antigen was detected in tissues from some piglets born dead or mummified, particularly from gilts; the rate was up to 55.5% in gilts receiving the control product, while in Suvaxyn Parvo/E-Amphigen vaccinated females it was up to 13.5%. In gilts, the serological response to vaccination was not significantly different between groups, while in sows the serological response to Ery was significantly higher in vaccinated animals.

### Conclusions

Suvaxyn Parvo/E-Amphigen demonstrated to be safe under field conditions. There were indications that the vaccine induced the development of good protective immunity.



## VAC-030 - EFFICACY OF STRATEGIC PIGLET VACCINATION TO REDUCE PRRS TRANSMISSION IN THE NURSERY IN A COMMERCIAL FARM

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### Background & Objectives

PRRS vaccination has epidemiological properties by reducing shedding and viral transmission. The objective of this study was to evaluate the efficacy of Strategic Piglet Vaccination to reduce PRRS recirculation in a commercial nursery.

### Materials & Methods

The study was conducted in a 950 sow farm (site 1+2). The farm was PRRS positive, following a classical vaccination program in sows (Porcilis® PRRS IDAL/4 months), and was classified as stable. During first semester of 2015, an increase in respiratory clinical signs, mortality and reduced growth in the nursery was observed. PRRS infection was confirmed by positive PCR and serology of diseased 5 and 6 week old animals. To ensure that PRRSv was not recirculating in sows, PCR testing of nursing piglets was done and tested negative. Strategic Piglet Vaccination was implemented by vaccinating 12 consecutive batches of 14 day old piglets with Porcilis® PRRS IDAL to control viral transmission and improve productive parameters.

### Results

First non-vaccinated piglets after vaccination period were PRRSv serologically and PCR negative at the end of the nursery period (9w), confirming no recirculation in the nursery. This situation was maintained at least 5 months post-piglet vaccination. Mortality rate was reduced from 3.9% in pre-vaccination batches to 2.7% in vaccinated piglets ( $p < 0.05$ ). Nevertheless, mortality increased again to 3.7% in subsequent batches due to non-PRRS related causes. A recovery in growing rates was also recorded with increased body weight at 9w of age from 19.7kg in the pre-vaccination batches to 21.3 kg in the post-vaccination batches ( $p = 0.06$ ).

### Discussion

PRRS vaccination in piglets can be used as a strategic tool to eliminate PRRSv circulation in the nursery, providing a clear improvement in production parameters and being therefore a true alternative to sanitary emptying of nurseries.



## VAC-032 - IMMUNIZATION WITH A FRENCH PRRSV STRAIN OR MLV VACCINES OF GENOTYPE 1 OR 2 INDUCE PARTIAL PROTECTION UPON LENA CHALLENGE

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### Introduction

PRRS virus (PRRSV) causes huge economic losses for the swine industry worldwide. For several years highly pathogenic strains that induce even higher damages have emerged. One of this strain (Lena, genotype 1.3), isolated in Eastern Europe is threatening the Western Europe pig industry. To prepare the possible emergence of this strain in Western Europe, we evaluated and compared the protection provided by the immunization with a French PRRSV field strain, a new genotype 1 or a genotype 2 modified live virus (MLV) vaccine, upon challenge with the Lena strain.

### Materials and Methods

Forty one, 4-weeks-old piglets coming from a nucleus herd were housed in our biosecurity level-3 animal facilities. At 6 weeks of age, 7 pigs were inoculated with the genotype 1.1 French strain "Finistère" (Fini); 9 piglets were vaccinated with Ingelvac PRRSFLEX® EU (MLV1) and 9 piglets were vaccinated with Ingelvac PRRS MLV (MLV2). Four weeks later the 3 groups of immunized animals as well as a non-immunized group were challenged intranasally with the Lena strain. The clinical, virological and immunological parameters were followed during 5 weeks after Lena challenge.

### Results

Immunization with Fini, MLV1 or MLV2 strains shortened the Lena-induced hyperthermia. In the Fini group a positive impact was also demonstrated for the growth performances. The level of Lena viremia was reduced for all the immunized groups (significantly for Fini and MLV2). This reduction of Lena viremia was correlated with the level of Lena-specific IFN $\gamma$ -secreting cells.

### Conclusion

A commercial MLV vaccine of genotype 1 or 2, as well as a field strain of genotype 1.1 could provide a partial clinical and virological protection upon a challenge with the Lena strain. The slightly higher level of protection provided by the field strain was associated with a better cell-mediated immune response.



## VAC-033 - IMMUNE RESPONSE INDUCED BY PORCILIS® PCV ID VACCINATION IN PIGLETS AT 3 WEEKS OF AGE

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The primary objective of this study is to assess the immune response to PCV2 vaccination in intradermally vaccinated 3-week-old piglets by the measurement of the Antibody Mediated Immunity (AMI) and the Cell Mediated Immunity (CMI).

A controlled, randomised and blinded study was done in an Italian pig herd with a history of PCVD-SI. Healthy 10-14-day old suckling piglets (600 in total) were randomly allocated to one of three groups. At 4 weeks of age, the weaners were treated as follows: group A) one-shot Porcilis® PCV ID via the intradermal (ID) route by using the IDAL® injector; group B) one-shot intramuscular PCV commercial vaccine and group C) control. Twenty-five piglets per group were blood sampled at regular intervals for determination of the PCV2 viral burden by qPCR and the measurement of AMI and CMI specific to PCV2 (ELISA, IPMA and IFN-gamma SC, respectively). Additionally, weight at three time points, mortality and lung lesions at slaughter were also recorded. The average daily weight gain (ADWG) and lung lesions scores were compared using mixed ANOVA model. The AUC (Area Under the Curve) was used in the PCV2 viremia analysis. Serological response was described using descriptive statistics.

At four and six weeks post-vaccination the intradermally vaccinated group showed a statistically significantly higher number of IFN-gamma SC compared to the intramuscularly vaccinated pigs and controls. At 18 weeks of age, PCV2 natural infection was detected and a prompt increase of the IFN-gamma SC as well as PCV antibodies (ELISA and IPMA) occurred. The PCV2 load in the blood from vaccinated animals (both groups) was reduced as compared to the control. Interestingly, the AUC of the course of viremia in ID vaccinated animals was lower compared to the IM vaccinated pigs. ADWG was not different between vaccinated groups.



## VAC-034 - ASSESSMENT OF THE IMMUNE RESPONSE OF A ONE-SHOT PCV-M HZO RTU VACCINE IN 3-WEEK OLD PIGLETS

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This study was done to assess the efficacy of Porcilis<sup>®</sup> PCV M Hyo administered to piglets at 3 weeks of age in comparison with a commercial vaccine combination and with a negative control group (placebo). The immune response to PCV2 and M. hyo was studied by measuring immunological parameters of the Antibody Mediated Immunity (AMI) and Cell Mediated Immunity (CMI) by serology (ELISA and IPMA) and INF-gamma SC specific for PCV2 and M. hyo.

The study was a controlled, randomized and blinded design in a conventional Italian pig herd. Six hundred (600) piglets at 3 weeks of age were enrolled and assigned to three equal sized groups: group A) vaccinated with Porcilis PCV M Hyo (PCVM group); group B) vaccinated with a commercial vaccine combination and group C) controls receiving placebo. Blood samples were collected at regular intervals during the study from 25 pigs per group for determination of the PCV2 level and the AMI (ELISA antibodies and IPMA) and CMI (IFN-gamma SC) specific to PCV2 and M. hyo.

Group A pigs developed a prompt post-vaccination immune response to both PCV2 and M. hyo as supported by both antibody and IFN-gamma SC results. Conversely, piglets vaccinated with vaccine B (positive control) had a slower response that remained below the level of group A throughout the study. It is worth mentioning that PCV2 natural challenge did not occur during the trial (no PCR positive blood samples to PCV2 throughout the study) and the level of IFN-gamma SC to PCV2 maintained over the threshold of positivity for the whole observation period in group A only. The results from this trial supported that Porcilis PCV M Hyo induced a stronger and long lasting antibody and cell-mediated immune response (memory T cells) compared to vaccine B and control.





## VAC-035 - INVESTIGATION OF FAS (APO-1)-RELATED APOPTOSIS IN PIGLETS VACCINATED (ID, IM) WITH A PRRSV MLV VACCINE

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### Background & Objectives

PRRSV induces apoptosis through the activation of death receptors, including cell-surface Fas receptor (apoptosis antigen 1-APO-1/CD95). The aim of this study was to investigate the impact of intradermal (ID) and intramuscular (IM) vaccination with a PRRSV MLV vaccine in piglets on Fas-related apoptosis.

### Material & Methods

The study included 104 suckling piglets (2 weeks of age) from a commercial farrow-to finish pig farm, suffering from positive unstable PRRSV status. Animals were assigned in 4 groups, 2 replicates (13 piglets/group/replicate): group A: IM vaccination with a MLV commercial PRRSV vaccine, group B: ID vaccination with the same vaccine, group C: ID of Diluvac Forte and group D: IM of Diluvac Forte. Blood samples were collected from the same 3 pigs/group/replicate at 4, 7 and 10 weeks of age. Sera were examined by qRT-PCR for PRRSV (type 1 and 2) and by ELISA for soluble Fas (sFas).

### Results

The results qRT-PCR for PRRSV at 4 weeks of age were negative in all groups, at 7 weeks only group A was negative and at 10 weeks all groups were positive from natural infection. No differences in sFas levels were observed in groups A and B during the study. sFas was increased in group C (4vs7 weeks-p=0.028, 4vs10 weeks-p=0.028, 7vs10 weeks-p=0.046) and D (7vs10 weeks-p=0.028). Significant differences among groups were noticed only at 10 weeks (AvsC-p=0.010, AvsD-p = 0.055, BvsC-p=0.010, BvsD-p=0.037).

### Discussion & Conclusion

In unvaccinated piglets, increased serum sFas levels reveal apoptotic suppression compared to vaccinated piglets. In the latter, vaccine-derived antibodies limit the infection and may attribute to the reduced Fas expression, suggesting a weak induction of lymphocyte-mediated cytotoxicity. Finally, higher sFAS levels were observed with ageing, possibly due to persistent PRRSV infection.



## VAC-036 - COMPARATIVE STUDY TO DETERMINE PCV VACCINATION IMMUNE RESPONSE FOLLOWING DIFFERENT ADMINISTRATION ROUTES (IM VS ID)

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### Background & Objectives

This abstract is part of a broader study on welfare benefits of intradermal needle-free vaccination in piglets. This study describes the immune response following PCV vaccination of pigs either intradermally with a needle-free IDAL injector or conventionally with a needle-syringe.

### Materials & Methods

A total of 339, 28 day old piglets were distributed in 3 groups : i) vaccinated with Porcilis<sup>®</sup> PCV ID intradermally with IDAL (IDAL); ii) vaccinated with Porcilis<sup>®</sup> PCV intramuscularly (IM); iii) control, not vaccinated (Control). At the time of vaccination, all pigs were picked up by their hind legs and vaccinated according to the treatment (control pigs were touched with the hand). IgG (Ingezim Circo IgG, Ingenasa) and  $\gamma$ -IFN SC levels (ELISPOT assay) were determined in 77 and 25 piglets, respectively, at day of vaccination and 21 days later.

### Results

Seroconversion at day 21 was detected in 24 out of 25 piglets from the IDAL group and 25 out of 25 piglets from the IM group; none of the animals of the Control group seroconverted to IgG. On day 21, IM piglets presented a greater log<sub>2</sub> IgG titre (mean  $\pm$  SE=3.3  $\pm$  0.17) than IDAL piglets (mean  $\pm$  SE=2.90  $\pm$  0.14) ( $p=0.001$ ). Cell-mediated immune response 21 days post-vaccination, measured as PCV specific  $\gamma$ -IFN SC (ID-17.89 SC/10<sup>6</sup> PBMC vs IM-26.41 SC/10<sup>6</sup> PBMC), was not significantly different between IDAL and IM groups, while both groups were statistically different from the control group ( $p<0.001$ ).

### Discussion & Conclusion

Both intramuscular and intradermal vaccination induced a humoral and cellular immune response based on IgG and  $\gamma$ -IFN SC values, indicating that both vaccination routes induce a solid immune response. Additionally the present data support that IgG levels are an easy and valuable test to confirm correct PCV vaccination.



## VAC-037 - SAFETY OF PORCILIS® PCV IN WEANED PIGS

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### Background & Objectives

Vaccines that contain potent adjuvants have been suggested to negatively impact production parameters of nursery piglets. Despite the need for potent adjuvants in order to induce a sufficient duration of immunity, specialized piglet producers may therefore be reluctant to use such vaccines. This study investigates impact of vaccination on production and health parameters of nursery pigs.

### Material & Methods

In a Danish SPF herd, 11,957 pigs in 34 consecutive batches were randomly allocated to two groups 3-4 days after weaning: 1) Vaccinated with Porcilis® PCV and 2) non-vaccinated. Pigs were commingled within pens three days after vaccination. Mortality and treatment with antibiotics were recorded. Two pools of serum from 5 pigs were obtained from each group in each batch at three and six weeks after weaning. In three of the batches, all pigs (490 vaccinated and 497 unvaccinated) were weighed at inclusion and at end of nursery.

### Results

Three and six weeks after weaning, viremia was detected in 4.4 respectively 10 % of unvaccinated- and 1.5 respectively 0 % of vaccinated pigs ( $p=0.01$ ). Average number of antibiotic treatments per batch was 91 for non-vaccinated and 87 for vaccinated pigs ( $p=0.45$ ). Fifty non-vaccinated and 39 vaccinated pigs died ( $p=0.29$ ). The ADG for pigs in non-vaccinated group was 501 g (64.3) and 498 g (SD =64.0) in vaccinated ( $p=0.43$  ( $p_{SD}=0.90$ )).

### Discussion & Conclusion

Antibiotic treatments, mortality or ADG were not significantly different between non-vaccinated and vaccinated pigs. The overall prevalence of viremia was low. The standard deviation of ADG was equal in the two groups indicating a similar distribution in weight gain. In conclusion, Porcilis® PCV vaccination 3 days after weaning, was found to be safe and without negative impact on production and health parameters during the post-weaning period.



## VAC-038 - COMPARATIVE STUDY OF POST-INJECTION PRODUCT LOSS AND LOCAL REACTIONS WITH HIPRADERMIC® AND ANOTHER COMMERCIAL NEEDLE-FREE DEVICE IN SOWS

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### Background & Objective

Needle-free injection devices have been designed as a new option for intradermal vaccination on swine farms. However, concerns about possible vaccine loss and local reactions have been raised by field users. The aim of this study was to compare product loss and safety of two different commercial needle-free devices and their respective vaccines.

### Materials & Methods

A total of 156 sows in the mating-control phase from a commercial PRRS-positive farm were randomly divided into two groups (G1-2) and vaccinated intradermally in the perianal area. G1 was vaccinated with UNISTRAIN® PRRS using Hipradermic® (0.2ml/dose) and G2 was vaccinated with a commercial PRRS vaccine using its associated needle-free device (0.2ml/dose). Non-injected vaccine volume was measured immediately after application by a volumetric (ml) method and a perception rating scale method. Local reactions were evaluated after vaccination and 4h later.

### Results

G1 showed a vaccine loss of  $0.019 \pm 0.14$  ml and G2 of  $0.026 \pm 0.14$  ml ( $p$ -value=0.011). The qualitative evaluation showed lower subjective loss perception in G1 based on percentage losses (G1 vs G2): no loss (18.9% vs 2.5%), low (56.8% vs 15.6%), medium (24.3% vs 16.9%) and high losses (0% vs 18.2%). Regarding local reactions, G1 showed a lower percentage of animals with blood loss after vaccination (46.5% less) or haematoma at 4h (24.5% less) than G2 ( $p$ -value<0.05). However, more papules were observed in G1 (90.9%) than in G2 (79.7%) ( $p$ -value=0.049).

### Discussion & Conclusions

Post-injection loss of product was lower in G1 compared to G2. Local reactions such as papules, bleeding or haematomas were observed with both vaccinations; however, the number of animals with bleeding or haematomas was higher in G2. Therefore, the UNISTRAIN® PRRS and Hipradermic® combination achieved a better user perception based on a lower loss of product and greater safety than the other combination of a commercial PRRS vaccine and its needle-free device.



## VAC-039 - SAFETY ASSESSMENT OF THREE WIDELY USED INACTIVATED SOW VACCINES AGAINST NEONATAL DIARRHOEA

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### Background & Objectives

Safety is a critical factor when a vaccine is injected into pregnant sows, due to the risk of abortion. However, maternal immunity is essential to protect piglets against infections during the suckling period, and vaccines contribute to enhance the colostrum protective effect. This study aimed to compare the safety of various commercial vaccines for preventing neonatal diarrhoea in piglets.

### Material & Methods

Safety was assessed in groups of 10 animals. Sows in G1 and G2 were vaccinated intramuscularly with Vaccine A (SUISENG<sup>®</sup>) and Vaccine B, respectively. G3 received Vaccine C injected subcutaneously. Vaccines were administered following the manufacturer's instructions. Control group received PBS (5 animals intramuscularly (G4A) and 4 animals subcutaneously (G4B)). Vaccine safety was measured by rectal temperature, systemic and local reactions at 4h<sub>pv</sub>, 6h<sub>pv</sub>, 1d<sub>pv</sub> and 2d<sub>pv</sub>.

### Results

After vaccination no changes were observed in the animals' behavior. Likewise, mean rectal temperature remained within the physiological range (<40°C) in most animals, except in 2 animals in G2 at 4 and 6h<sub>pv</sub> (repeated after each shot). Furthermore, G2 showed a significant rise in mean rectal temperature at 4 and 6h<sub>pv</sub> compared to G4A ( $p$ -value<0.05). No statistical differences were observed in the other groups when compared with the control group. Regarding local reactions, G2 and G3 had a larger number of animals (50% and 90%, respectively) with more inflammatory response and long-lasting (more than 3 days) local reactions than animals in G1.

### Discussion & Conclusion

Although the vaccines compared in this study have been authorized to be used massively in breeding sows, not all of them show the same degree of safety when injected under similar conditions. These differences should be considered when defining a vaccine program, due to the large number of vaccines administered that may potentially increase the risk of iatrogenic abortion.

*Acknowledgements: CEYC and UCAM staff.*



## VAC-040 - EVALUATION OF THE EFFECT OF TWO DIFFERENT VACCINE COMBINATIONS AGAINST PCV2 AND MYCOPLASMA HYOPNEUMONIAE (MHYO) ON PHYSIOLOGICAL PARAMETERS

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### Objectives

Body temperature, acute phase proteins (APPs) and weight gain are suitable indicators of inflammation and well-being of pigs. The aim of this study was to evaluate the physiological effects of 2 vaccine combinations against PCV2 and Mhyo by measuring the body temperature, APPs and the Average Daily Gain (ADG).

### Material & Methods

This trial was conducted in a farrow to finish, 450-sow, herd. Overall 1289 piglets were included in the study. One day before weaning, piglets were randomly allocated to Group 1 or Group 2, identified and weighed individually. Group 1 was vaccinated with 2ml of a freshly mixed preparation of Ingelvac CircoFLEX<sup>®</sup> and Ingelvac MycoFLEX<sup>®</sup> whereas Group 2 was vaccinated with 2 ml of a PCV2 and Mhyo fixed-combination vaccine. All piglets were weighed again 14 days after vaccination. Twenty-five piglets were randomly selected for the assessment of body temperature and APPs (Haptoglobin and C - Reactive Protein) within the 48 hours following vaccination. The serum concentration of Haptoglobin and CRP were measured using a Pig Haptoglobin ELISA kit (Life Diagnostics HAPT-9) and a Pig C-Reactive Protein Elisa kit (Life Diagnostics CRP-9) respectively. Statistical analyses were performed by a t-test.

### Results

Six hours post-vaccination, the body temperature was significantly lower in group 1 (39.9°C) than in group 2 (41.0°C) ( $p < 0.001$ ). Concerning APPs, a significant difference was observed between the 2 groups 24 hours post-vaccination, in favor of group 1, for both Haptoglobin and CRP ( $p < 0.001$ ). Fourteen days after vaccination, the ADG was significantly higher in group 1 (192.4 g/day) compared to group 2 (182.8 g/day) ( $p < 0.01$ ).

### Conclusion

The outcome of this study is consistent with other trials showing that vaccines against PCV2 and Mhyo lead to different local and systemic responses. It confirms that the selection of vaccines should certainly be based on efficacy but also on their effect on piglets' well-being.



## VAC-041 - EVALUATION OF INNATE AND ADAPTIVE IMMUNE RESPONSE IN PIGS NATURALLY INFECTED WITH MYCOPLASMA HYOPNEUMONIAE

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*Mycoplasma hyopneumoniae* (MH) is the main causative agent of enzootic pneumonia of pigs.

49 pigs, presenting lung lesions, from 5 different herds were sampled at slaughterhouses.

Lung lesions were individually scored according to Madec e Kobish (1982).

MH was locally RT-PCR detected from each lung lesion according to Marois e coll. (2010)

Lung samples were formalin-fixed. 5 µm paraffin sections were histologically evaluated with a score, ranging from 0 to 4, for BALT volume, severity of bronchi, bronchiolar disepithelization and subacute inflammation.

Immunohistochemistry was carried out using commercial antibodies anti-MAC387 to detect macrophages, anti-CD3 for T-cells, anti CD79α for B-cells, Foxp3 for regulatory T-cells. The amount of antigen detected was scored from 0 to 4 based on the number of positive T and B cells foci per 4, 200 magnification fields. T-reg cells were individually counted in four BALT per pig.

The average histological lung score ranged from 0.14 to 1.98. Results of RT-PCR were 32 positive and 17 negative samples.

Lungs showed catarrhal bronco-interstitial pneumonia with increased volume of peribronchial, peribronchiolar and perivascular lymphoid tissue.

10 samples showed subacute inflammation and 22 showed lesions MH induced without secondary bacteria irruption.

MAC387 positive cells were numerous in lung lesions (scored 4) affected by severe subacute inflammation while B, T and Treg cells were poorly represented. On the contrary, T cells were numerous in hyperplastic BALT MH induced (scored 3 to 4). B cells were scarcely represented in subacute inflammation; in MH induced BALT lesions were scored from 2 to 3.

This study shows that Treg cells are very rare in hyperplastic BALT, suggesting a less active role of Treg cells in maintaining a low activation of immune system. Macrophages are numerous in subacute inflammation, while less in MH BALT, suggesting a low grade of apoptosis of cells of the follicle center.



## VAC-042 - BENEFIT OF SUVAXYN CIRCO+MH RTU VACCINATION AGAINST WEIGHT GAIN LOSSES ASSOCIATED WITH A PCV2 SUBCLINICAL INFECTION UNDER FIELD CONDITIONS

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### Objective

The objective of this trial was to assess the safety and efficacy of Suvaxyn<sup>®</sup> Circo+MH RTU in 3-week old piglets under field conditions.

### Materials & methods

The trial was conducted at a commercial farrow-to-finish farm located in the The Netherlands. A total of 130 3-week old pigs were intramuscularly vaccinated with 2 mL of either Suvaxyn<sup>®</sup> Circo+MH (65 pigs) or saline solution (65 pigs). Safety parameters included clinical observations, rectal temperatures (RT), injection site reactions and body weight (D-1 to D30). As efficacy parameters, mortality, PCV2 viremia, PCV2 fecal shedding, serology (PCV2 and *M. hyo*), body weight and *M. hyo* lung lesions at slaughter were evaluated. The study design was a randomized complete block design.

### Results

No systemic reactions were observed after vaccination. A transient increase in RT was detected at 4 hours post-vaccination, but differences disappeared at 24 hours. Injection site reactions were present in 13% of vaccinated pigs, did not exceed 0.5 cm of diameter and lasted a maximum of two days. Non-inferiority testing for the ADWG from D-1 to D30 was demonstrated, indicating no negative effect of vaccination on growth.

None of the pigs was diagnosed with PCVD. However, PCV2 viremia, serology and the presence of PCV2-like lesions and PCV2 genome in lymphoid tissues of the pigs that died indicated the development of a PCV2 subclinical infection. PCV2 viremia was significantly reduced in vaccinated pigs. At slaughter, vaccinated pigs were significantly heavier (average 4.4 kg) and the ADWG over the full duration of the study was also significantly higher (0.04 kg/day) in the vaccinated group. The lack of seroconversion to *M. hyo* and the low level of lung lesions at slaughter indicated no *M. hyo* exposure during the study.

### Conclusions

Vaccination of 3-week old pigs with Suvaxyn<sup>®</sup> Circo+MH demonstrated an excellent safety profile and was able to reduce the body weight losses associated with a PCV2 subclinical infection.





## VAC-043 - MATERNAL ANTIBODIES DO NOT INTERFERE WITH THE EFFICACY OF VEPURED®

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### Background & Objectives

The VEPURED® vaccine has been developed to protect piglets against edema disease (ED) caused by *Escherichia coli* from the age of 2 days. At this age, the maternally derived antibodies (MDAs) could still be present. Thus, the objective of this laboratory trial was to assess the influence of MDA in the administration of VEPURED® vaccine against ED in 2-day old piglets.

### Material & Methods

In this study, two groups of 2-day old piglets were vaccinated with VEPURED® vaccine –one presenting field representative MDA levels and the other without MDAs. A third group of piglets was used as a control (non-vaccinated) and to follow the decay of MDAs. When MDAs disappeared in this control group, a challenge by an intravenous injection of VT2e toxin was performed in all the piglets from these groups. Additionally, a non-vaccinated non-challenged group with no MDAs was included as a sentinel group.

### Results

Vaccination with VEPURED® resulted in a statistically significant reduction in both the prevalence and the severity of ED-related clinical signs, even in the presence of field representative MDA levels. In addition, vaccinated piglets, with and without MDA, were fully protected against ED mortality. In contrast, most of the non-vaccinated piglets (7 out of 9) died after the challenge. Besides this, the weight gain in pigs in the vaccinated and challenged groups was equivalent to that of the sentinel group and it was therefore confirmed that vaccination can also help to control growth retardation associated with ED.

### Discussion & Conclusion

Intramuscular vaccination with VEPURED® of 2-day old piglets with field representative MDAs reduced the presence and severity of clinical signs of edema disease after an experimental challenge with VT2e toxin and also prevented mortality induced by edema disease.



## VAC-044 - ASSOCIATION BETWEEN THE PRESENCE OF VT2E NEUTRALISING ANTIBODIES PRODUCED BY VEPURED® AND PROTECTION OF PIGLETS IN VTEC INFECTIONS

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### Background & Objectives

Edema disease (ED) in piglets caused by Verotoxin 2e (VT2e) produced by *Escherichia coli* (E. coli) is an enterotoxaemic disorder that produces neurological signs and subcutaneous edemas. In the absence of treatment, the disease can progress very rapidly and death can occur within a few hours or days. VEPURED® vaccine has been developed to protect piglets against ED. VEPURED® activates piglets' immune systems, generating neutralising antibodies against VT2e before the infection.

In this study, the association between serological immune response and VT2e protection efficacy was analysed.

### Material & Methods

Piglets without VT2e neutralising antibodies were vaccinated with 1 mL of VEPURED® vaccine or with 1 mL of phosphate-buffered saline (PBS). Subsequently, the piglets were challenged with VT2e (w/t) intravenously and ED clinical signs and mortality were monitored daily. The presence of neutralising antibodies in sera was determined before challenge.

### Results

In order to examine the association between serology and protection, the association between the presence of neutralising antibodies and clinical signs and mortality was determined. The presence of neutralising antibodies was associated with a reduction in mortality ( $p < 0.001$ ), and also with a reduction in clinical signs ( $p < 0.001$ ).

### Discussion & Conclusion

The presence of neutralising antibodies in piglets vaccinated with VEPURED® is associated with protection against ED.



## VAC-045 - EFFICACY OF VEPURED® AGAINST EDEMA DISEASE IN PIGS UNDER FIELD CONDITIONS

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### Background & Objectives

A multicentre, randomised, placebo-controlled, clinical field trial was performed to evaluate the efficacy of VEPURED®, a new single-dose vaccine against Edema Disease (ED).

### Material & Methods

A total of 1769 clinically healthy piglets from five commercial farms in Belgium and France were included. The animals were either vaccinated with VEPURED® (n= 945) or given phosphate-buffered saline (PBS) (n=824) at 2-3 days of age and were monitored until the end of fattening.

### Results

Clinical outbreaks of Edema Disease were observed in four out of the five selected farms whilst the presence of verotoxin-producing *E. coli* was confirmed by PCR on all farms.

Vaccination with VEPURED® on those farms with clinical outbreaks of ED resulted in a statistically significant reduction in both mortality and the occurrence of clinical signs of the disease. In addition, at the end of fattening a statistically significantly higher average daily weight gain was observed in the VEPURED® group compared to that of the placebo group. Protective seroneutralising antibodies against VT2e were observed in most of the animals vaccinated with VEPURED® up to the end of fattening.

On the farm with subclinical ED, differences were also observed in the average body weight gain of the animals at the end of farrowing in favour of the VEPURED® group. Most of the animals in the VEPURED® group also presented protective seroneutralising antibodies against VT2e up to the end of fattening.

### Discussion & Conclusion

Vaccination of piglets with VEPURED® reduces mortality and the occurrence of clinical signs of ED on farms with clinical outbreaks of the disease. In addition, on farms with both clinical and subclinical disease, it improves growth performance of pigs and guarantees the presence of protective seroneutralising antibodies against VT2e up to the end of fattening.



## VAC-046 - HYOGEN® IS SUPERIOR IN LUNG PROTECTION IN COMPARISON TO A TWO-SHOT VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE

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### Background and Objectives

*M. hyo* is one of the major players in the Porcine Respiratory Disease Complex and causes enzootic pneumonia. Aim of the study was to compare efficacy and lung protective effects of the Hyogen® (Ceva) one-shot vaccine given at the age of 3 weeks according to its license against an alternative two-shot vaccine (control group) given at 1 and 3 weeks of age.

### Material and Methods

The study, which is part of a larger study including over 600 animals in total, was performed on a closed combined farm housing 84 sows and working in a 3-weeks rhythm in Lower Austria. Sows are vaccinated against PRRSV every 4 months. 74 weaners were included and assigned either to the Hyogen® (n=39) or the control (n=35) group. At day 185, pigs were slaughtered and a modified lung scoring according to Madec (Ceva lung scoring program) was performed.

### Results

At slaughter pigs of the Hyogen® group weighed 97.7 kg (mean) and those of the control group 96.8 kg. Daily weight gains over the total fattening period were 551 g and 546 g, respectively. Coughing and sneezing values (mean number of animals observed over a period of 2 min at a weekly basis throughout the fattening period) were 1.4 vs 3.6 and 7.3 vs 5.4. The respective lung score values were 1.62 vs 3.42 (p=0.007). In the Hyogen® group 46 % of the pigs showed a cranial pleuritis and in the control group 66 % (p=0.04). Formation of scarified lung tissue was observed in 27 % and 36 % of the pigs.

### Discussion and Conclusion

Although fattening data did not differ significantly between the two groups, a significantly superior effect on preserving lung health could be demonstrated for the Hyogen® vaccine.



## VAC-047 - SAFETY AND IMMUNOGENICITY OF VEPURED® CO-ADMINISTERED WITH PREVIRON® IN PIGLETS

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### Background & Objectives

Edema disease (ED) is an enterotoxaemia caused by verotoxigenic *Escherichia coli*. ED is mainly observed in recently weaned piglets, and it is prevented by immunisation of 2 day old piglets with VEPURED® vaccine. In the first days of life, iron is administered to piglets, and this is also the appropriate time to vaccinate animals with VEPURED®. In this study, the safety and immunogenicity of piglets vaccinated with VEPURED® administered concomitantly with PREVIRON® was evaluated.

### Material & Methods

Piglets without Verotoxin 2e (VT2e) neutralising antibodies were distributed between 3 groups: one group was vaccinated with 1mL of VEPURED®, co-administered but not mixed with 1mL of PREVIRON®, a second group was vaccinated with 1 mL of VEPURED® and given 1 mL of PREVIRON® one day after vaccination, and a third group (non-vaccinated group) was given 1 mL of phosphate-buffered saline (PBS) and 1 mL of PREVIRON® one day after administration of PBS.

General clinical signs, local reactions, and temperature were daily monitored from day 0 to day 3 post-vaccination. Weight was evaluated before vaccination and 28 days post-vaccination.

Blood samples were collected before vaccination, and 28 days post-vaccination in order to evaluate the presence of neutralising antibodies against VT2e.

### Results

There were no statistically significant differences between groups in terms of general clinical signs, local reactions, body temperature, and weight gain by piglets throughout the study.

At day 28 post-vaccination, none of the non-vaccinated piglets had neutralising antibodies whilst 90% of the piglets from each vaccinated group had neutralising antibodies against VT2e.

### Discussion & Conclusion

The results obtained in this study show that VEPURED is safe when administered concomitantly with PREVIRON, and demonstrate that this simultaneous administration does not interfere with the generation of VT2e neutralising antibodies by the vaccine.



## VAC-048 - PREVENTION OF EDEMA DISEASE IN PIGS BY VACCINATION WITH VEPURED® VACCINE

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### Background & objectives

Edema disease (ED) is an enterotoxaemia caused by certain *Escherichia coli* (*E.coli*) colonising the small intestine and produces verotoxin (VT2e). ED is mainly observed in recently weaned piglets, although it can also be observed during the growing and finishing phases. In this study, the safety and efficacy (onset and long-term duration of immunity) provided by VEPURED® against ED was evaluated.

### Material & Methods

Seventy-four healthy 2-day old piglets were distributed between 3 groups. One group (n=32) was vaccinated intramuscularly with 1 millilitre of VEPURED®. Another group (n=32) received 1 ml of phosphate-buffered saline. Ten pigs were included as a sentinel group. The onset and duration of immunity were evaluated by an intravenous administration of VT2e toxin at 21 days and 16 weeks after vaccination, respectively.

### Results

No clinical signs were observed in 2-day old piglets as a result of the intramuscular administration of VEPURED®.

Vaccination with VEPURED® resulted in a statistically significant reduction in both the prevalence and severity of clinical signs associated with ED. In addition, all the piglets vaccinated with VEPURED® were totally protected against mortality after the challenge. In contrast, in the non-vaccinated group, the mortality was over 70%. Protective seroneutralising antibodies against VT2e developed in all the piglets vaccinated with VEPURED® and it was demonstrated that this may persist for more than 16 weeks after vaccination.

### Discussion & Conclusion

In conclusion, the results obtained in the present study demonstrated that the active immunisation of 2-day old piglets with VEPURED® vaccine confers a safe and effective protection against ED 21 days after vaccination. This vaccine is able to reduce the presence and the severity of clinical signs and prevents mortality. Furthermore, VEPURED® gives long-lasting protection of at least 16 weeks after vaccination and thus, VEPURED confers protection during the fattening phase.



## VAC-049 - THE CAPSULAR POLYSACCHARIDE AS A POTENTIAL NOVEL SUBUNIT VACCINE WITH CROSS-PROTECTION AGAINST THE MOST PREVALENT SEROVARS IN THE CZECH REPUBLIC

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*Haemophilus parasuis* is a part of the normal flora of the respiratory tract of pigs. However, under certain conditions it can also induce severe systemic disease with high morbidity and mortality leading to gross economic losses in the pig industry. To date, 15 serovars of *H. parasuis*, differing in virulence, have been defined. The most prevalent serovars in the pig herds in the Czech Republic are virulent serovars 1, 4, 5 and 13. The currently available commercial vaccines are inactive vaccines with certain limitations, such as no or poor cross-serotype protection. In this study, we constructed a novel subunit vaccine with a capsular polysaccharide (CPS) isolated from *H. parasuis* 132 (serovar 5) and evaluated its immunogenicity in a mouse model. Results showed that intramuscular injection with the CPS resulted in the production of antibodies with high levels. On the basis of survivings, pathological findings, and bacterial isolations, mice immunized with CPS were protected against challenge with homologue serovar as well as with heterologue serovars (serovar 1, 5, 13) of *H. parasuis*. This cross-protection was also confirmed by western-blotting. Our findings indicate that CPS may provide the effective immunogenicity in preventing *H. parasuis* infection caused by the most prevalent serovars in the Czech Republic. The study was supported by Ministry of Agriculture of the Czech Republic (QJ1210120) and MEYS LO1218.



## VAC-050 - ATTENUATION OF A VIRULENT EUROPEAN PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) ISOLATE ON A CD163-EXPRESSING HAMSTER CELL LINE

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### Introduction

Monkey kidney cell line MA-104 and derivatives have been used almost exclusively for the propagation and attenuation of commercial modified live PRRSV vaccines. Discovery of the CD163 PRRSV receptor has permitted the development of new PRRSV-permissive cell lines. Attenuation on these cell lines yields vaccine viruses with properties that differ from existing vaccines. Here we describe the use of a CD163-expressing hamster cell line to attenuate and select a Genotype 1 PRRSV strain.

### Materials and methods

Genotype 1 viruses from several European countries were isolated on porcine alveolar macrophages and adapted to growth on BHK21-CD163 cells. After biologically cloning they were further attenuated by serial passage. High-titer viral clones were selected for safety/efficacy testing in a homologous challenge model. The clone with superior safety and efficacy was evaluated in a heterologous challenge model. Eighteen 3-week-old pigs were either vaccinated with the attenuated PRRSV virus (9 pigs), or mock-vaccinated (9 pigs). Four weeks later, all pigs were challenged with the Spanish PRRSV isolate Olot/91. Pigs were monitored for clinical signs, rectal temperatures, body weights and viremia. Ten days after challenge, all pigs were euthanized and necropsied, and lungs evaluated for macroscopic lesions.

### Results

Vaccination significantly reduced post-challenge viremia at days 3, 5, 7 and 10, while duration of viremia (percent viremic days) was reduced from 69.57% to 28.17%. Vaccination significantly reduced both the percentage of lung showing macroscopic lesions and the percentage of pigs showing typical PRRSV macroscopic lung lesions (100% control pigs vs 44% vaccinated pigs). Vaccination was demonstrated to be safe, since no clinical signs were observed in the vaccinated group and significant differences in body weight were not observed 28 days after vaccination.

### Conclusions

The use of a BHK21-CD163 cell line for the attenuation of Genotype 1 PRRSV has been demonstrated to be a useful tool for the development of novel modified live vaccines.





## VAC-051 - A CASE REPORT: FALSE POSITIVE SEROLOGICAL REACTIONS FOR MYCOPLASMA HYOPNEUMONIAE

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### Background & Objectives

A SPF multiplying unit, consisting of a sow herd and two gilt units, in the spring of 2016 experienced multiple positive samples for *Mycoplasma hyopneumoniae* in ELISA antibody test. As a result, the 3 herds were declared positive for *Mycoplasma*. No clinical or pathological findings were present in neither the sow herd nor the connecting offspring units. Only the sow unit had positive samples, and only sows that were vaccinated with Izovac Lepto 3 (IL3), were positive for *Mycoplasma hyopneumoniae* in ELISA test.

### Material & Methods

The multiplying unit is undergoing surveillance for SPF diseases including *Mycoplasma*, through monthly blood sampling and clinical evaluation. Every month a minimum of 10 blood samples in the sow herd and 20 blood samples in each connected offspring unit. *Mycoplasma* testing is done by using ELISA antibody test. A total of 405 samples were analyzed between April and October 2016.

### Results

The first *Mycoplasma* positive samples appeared in the sow herd, in the end of April 2016. Over the next 5 months, between 10% and 56% of the samples taken from sows and gilts in the sow herd, were positive. In the offspring herds, all samples were negative. No clinical or pathological signs of *Mycoplasma* were present in either of the herds. The sow herd had started vaccination with IL3 in march 2016. Only vaccinated animals were positive for *Mycoplasma* in ELISA test. Following October 2016 all samples in the sow herd, have been taken from unvaccinated animals, and all are negative.

### Discussion & Conclusion

The multiplying unit, restored *Mycoplasma*-free status, after showing that only IL3-vaccinated animals presented *Mycoplasma* positive samples. Similar situations have been observed in at least 12 other SPF herds that use the IL3 vaccine. The SPF Health Department has no knowledge of similar findings, with other vaccines.



## VAC-052 - FIELD STUDY ON THE EFFECTIVE REDUCTION OF NON-PROGRESSIVE ATROPHIC RHINITIS IN PIGLETS AFTER SOW VACCINATION

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### Background & Objectives

The metaphylactic use of antimicrobials in the EU pig industry has been limited by legislation, with vaccination regarded as one of the alternative methods for the prevention of bacterial diseases such as the non-progressive atrophic rhinitis (NPAR) caused by *Bordetella bronchiseptica* (Bb). This study aimed to investigate the passive transmission of maternal Bb antibodies to newborn piglets, and the clinical outcome after sow vaccination with a vaccine indicated for the prevention of NPAR.

### Material & Methods

A Spanish farrow-to-nursery pig herd with respiratory problems caused by Bb in the nursery was selected for a controlled clinical trial. Four pregnant sows were primo-vaccinated with RHINISENG® (GV), while other four received PBS (GNV). Bb agglutinating antibodies were measured in colostrum and serum (piglets and sows). Serum IgG antibodies and NPAR nasal lesions in piglets were also assessed.

### Results

All sows were seropositive for Bb prior to vaccination. After vaccination, the agglutinating Bb-antibodies in sow serum and colostrum higher in GV compared to GNV ( $p$ -value<0.05). At farrowing, Bb-antibody titrations in sow serum and colostrum were 7 times higher in GV than GNV. Thus, piglet serum also showed higher levels of Bb antibodies in GV compared to GNV ( $p$ -value<0.001) from farrowing to 46-day-old (17- and 4-fold more, respectively). No differences in the IgG levels were observed between groups, neither in sows nor in piglets. Finally, a higher percentage of piglets in GNV showed NPAR lesions (30% more) and higher mean nasal lesion scores compared to GV (3.4 vs 0.4).

### Discussion & Conclusion

This study shows that immunization of pregnant sows against Bb allows the reduction of NPAR in their offspring, favored by a high concentration of specific antibodies against Bb transferred from sow colostrum to piglet serum.

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## VAC-053 - DELAYED TYPE HYPERSENSITIVITY RESPONSE TO PCV2 ANTIGEN IN PIGS BORN FROM UNVACCINATED OR CIRCOVAC-VACCINATED SOWS UNDER ITALIAN FIELD CONDITIONS

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### Introduction

Delayed-type hypersensitivity (DTH) is revealing cellular mediated immunity towards an antigen and can be adapted for vaccine compliance tests. This study aimed to confirm this potential for PCV2 vaccination under Italian field conditions.

### Material and methods

First farm: thirty three-week-old just-weaned piglets born from unvaccinated sows were either vaccinated (n=15) with CIRCOVAC IM 0.5mL or left unvaccinated (n=15). Five weeks later, the pigs were intradermally inoculated with 0.1-0.2mL of CIRCOVAC antigen solution in the lower abdomen area. The diameter of skin reaction (erythema) at the point of inoculation was visually inspected 24h post-inoculation. Second farm: 30 five-week-old piglets born from CIRCOVAC-routinely-vaccinated sows were either vaccinated with CIRCOVAC or left unvaccinated. The DTH reaction was analogously assessed, both before vaccination and 5 weeks post-vaccination. Piglets born from vaccinated sows were bled at weaning and all experimental animals at 5 weeks post-vaccination. Antibodies were titrated using an in-house ELISA or a seroneutralization technique.

### Results

First farm: only 33% of vaccinated piglets showed high anti-PCV2 ELISA titres following vaccination. Vaccinated pigs exhibited consistent and significantly higher seroneutralizing titres than unvaccinated pigs indicating a proper vaccine take. The proportion of pigs showing erythema (100% vs. 33%) and the average diameter ( $25\pm 15$  mm vs.  $4\pm 7$  mm) was significantly higher ( $p<0.01$ ) for the vaccinated animals. Second farm: piglets born from vaccinated sows consistently displayed high ELISA titres and a mild reaction ( $9\pm 4$ mm) to the DTH test before vaccination. Five weeks later, ELISA titres were significantly decreased ( $p<0.01$ ) in unvaccinated pigs compared to vaccinated ones. The diameter of the skin reaction strongly increased ( $p<0.01$ ) in vaccinated pigs ( $24\pm 4$ mm) whereas it tended to decrease in unvaccinated ones ( $8\pm 2$ mm).

### Conclusion

These results confirmed the potential of intradermal application of PCV2 antigen as a CIRCOVAC vaccination compliance test.



## VAC-054 - EDEMA DISEASE VACCINATION IN CHRONICAL WEANERS IMPROVES THE FEED CONVERSION RATE

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### Background & Objectives

Edema disease is an E.Coli enterotoxemia in pigs usually occurring after weaning. In positive herds, also subclinical or surviving pigs become unthrifty and show reduced daily weight gain and feed conversion rate. Aim of the present study was to determine if vaccination of pigs with a genetically modified recombinant Stx2e antigen could prevent both subclinical and clinical edema disease.

### Material & Methods

The study was performed in 490 piglets from a farrowing site with an history of recurring clinical edema disease and Stx2e positive laboratory results. Animals were randomly assigned to two groups: Vaccinated (n=244; intramuscular injection at 4days of age of Ecoporc Shiga, Chemifarma-IDT Biologika GmbH), and Non-vaccinated group (n=246). The preventive use of all drugs against E.Coli was banned. All pigs were weighed by group at weaning (28days) and 55days after. Within each group, 40 piglets were also individually weighted. Clinical signs, mortality, feed consumption and pharmacological treatments were recorded over the whole period.

### Results

No clinical signs were showed in both groups, nor any statistical difference ( $P>0,05$ ) for average daily gain (358 vs 350gr) and percentage of treated animals/day (1,6 vs 1,9). However, a greater mortality was showed in non-vaccinated pigs (2.5 vs 4.9%) as a greater percentage of pigs that did not reach the threshold of 20kg at 55days (28.5 vs 21.7%). Moreover, the feed conversion rate was improved in vaccinated pigs (1.55 vs 1.81). Improved parameters led to an estimating production cost of vaccinated pigs about 1.5€ lower than non-vaccinated.

### Discussion & Conclusion

Vaccination has positive effect on growth performance and feed conversion rate in case of edema disease even in absence of clinical signs. The study suggests the potential for using vaccination also in herds with a chronic edema disease with unspecific signs like unfavorable feed conversion rate and poor uniformity.



## VAC-055 - EFFECT OF VACCINE TEMPERATURE ON SAFETY AND EFFICACY OF PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES: A CASE STUDY

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### Background & Objectives

Pig farmers sometimes observe post-vaccination side effects like reduced feed intake. Such observations can be reduced by following vaccine leaflet instructions. Although self-vaccinating farmers in the Netherlands have to be certified, vaccinating staff is not always aware of the leaflet instructions. This case describes use of Porcilis<sup>®</sup> PCV M Hyo according to leaflet instruction.

### Materials and method

In a swine rearing farm (1800 gilts), 23-weeks-old breeding gilts were vaccinated with Porcilis PCV M Hyo\*. A drop in feed intake and some lethargic animals were observed after vaccination. The vaccine was administered immediately after removal from the refrigerator. When the farmer requested a different vaccine, the farm veterinarian decided to do a small field trial.

In three units, all gilts (50) on left side of corridor were vaccinated with warm (21°C) Porcilis PCV M Hyo and gilts (50) on right side with a competitor PCV2 and *M. hyopneumoniae* vaccine (also 21°C). Vaccine was removed from refrigerator at 7 AM for vaccination at 11 AM. Feeding was at 5 PM and clinical inspections at 6 PM. Ten random gilts from each vaccine group were blood sampled for PCV2 analysis (AlphaLISA PCV2) and compared with gilts that received cold vaccine.

### Results

At inspection, all feed troughs were empty regardless of vaccine and all gilts appeared healthy without any side effects. The average PCV titer in Porcilis-vaccinated gilts was 10.4 ( $\pm 1.4$ ) compared to 9.1 ( $\pm 1.1$ ) in competitor combo. In comparison, the average titer was 9.0 ( $\pm 0.5$ ) following cold Porcilis vaccination.

### Conclusions and discussion

Both vaccines were safe when administered at room temperature. The farmer is now convinced that the drop in feed intake before the trial was due to injecting vaccine directly out of the refrigerator. He continues vaccination with Porcilis PCV M Hyo due to the better humoral immune response.



## VAC-056 - COMPARATIVE SAFETY OF A FRESHLY MIXED PCV2 AND M HYO COMBINATION COMPARED TO A READY-TO-USE VACCINE

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### Background and objectives

Recently, ready-to-use (RTU) vaccines containing both PCV2 and *Mycoplasma hyopneumoniae* (M hyo) antigens were marketed in the EU. A study was designed to compare wean-to-slaughter performance of piglets vaccinated with freshly mixed, RTU and placebo vaccine in a Danish herd, where infection with PCV2 and M hyo occurred in the finishing pigs. This paper presents the data from the nursery period, showing the performance of vaccinated pigs in the absence of infection with PCV2 and M hyo.

### Material and methods

Design: Side-by-side study with random allocation stratified according to piglet weight within litters. Piglets were individually identified by numbered eartags, and grouping was blinded. Piglets were weighed at vaccination and at the end of nursery.

Piglets were vaccinated against PCV2 and M hyo at weaning at 4 woa according to label of the respective vaccines. After approximately 45 days in the nursery, they were moved to finishing barns.

Placebo (saline): 906 pigs

RTU vaccine: 901 pigs

Freshly mixed vaccine: 913 pigs

### Results

The mean weight was 5.9 kg at weaning and 22.9 kg end nursery. The placebo vaccinated pigs and pigs vaccinated with freshly mixed vaccine had comparable ADG (382 and 383 g/dag;  $p=0.955$ ), but the ADG of pigs vaccinated with the RTU vaccine was significantly lower (372 g/day;  $p=0.028/0.031$ ).

### Discussion and conclusion

The study shows that the RTU vaccine against PCV2 and M hyo significantly decreased weight gain of uninfected pigs in the nursery, compared to vaccination with saline or with freshly mixed vaccine. The reduced ADG was probably caused by vaccine reactivity.



## VAC-057 - COMPARATIVE EVALUATION OF TWO VACCINATION SCHEMES AGAINST PRRS, PCV2 AND MYCOPLASMA HYOPNEUMONIAE REGARDING CLINICAL, TECHNICAL AND SEROLOGICAL PARAMETERS IN FATTENERS

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### Objective

The aim of the study was to evaluate two vaccination protocols against PRRSv, PCV2 and *Mycoplasma hyopneumoniae* (Mhyo).

### Materials and Methods

This field study was conducted in a farrow to finish farm, weaning piglets at 21 days of age (doa), every 3 weeks, and where a circulation of a PRRS European strain, Mhyo and PCV2 was confirmed. In total, 1176 piglets from 4 batches were included around weaning. The two first batches (group A) were vaccinated the same day, 5 days after weaning, against Mhyo and PCV2 with two commercial vaccines (SUVAXYN PCV<sup>®</sup> and INGELVAC MHYO<sup>®</sup>) by intra-muscular (IM) route and vaccinated against PRRSv at 40 to 45 doa (PORCILIS PRRS<sup>®</sup>) by intra-dermal route. The two last batches (group B) were vaccinated the same day (around 25 doa) against the 3 pathogens with INGELVAC MYCOFLEX<sup>®</sup>, INGELVAC CIRCOFLEX<sup>®</sup> and INGELVAC PRRSFLEX<sup>®</sup>, by IM route. Internal biosecurity measures were applied to avoid direct and indirect contact between the two groups.

These different parameters were followed: respiratory counts every 3 weeks from weaning to slaughter, day of inclusion to slaughter average daily gain (ADG), mortality rate, veterinary interventions, serological and virological follow-up of Mhyo and PCV2.

### Conclusion

There was no clinical relevant difference regarding coughing counts (at 24 weeks of age, 3,4% in group A and 2,9% in group B), mortality rate (respectively 1,2% and 2%) and veterinary interventions.

ADGs were not different (749g/day in group A versus 744g/day in group B).

There was no evidence of circulation of PCV2 in any groups. The seroconversion against MHyo was more marked in group A (61% and 95% seropositives at 21 and 24 weeks of age) than in group B (respectively 16% and 21%).

We can conclude that the 2 vaccination protocols provided a similar level of protection against Mhyo, PRRSv and PCV2.



## Miscellaneous

### MIS-001 - THE DOMESTIC PIG AS A POTENTIAL MODEL FOR BORRELIA SKIN INFECTION

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#### Background and Objectives

Lyme borreliosis is one of the most frequent vector-borne diseases in the northern hemisphere in humans. A characteristic early manifestation of this disease is a skin lesion called "erythema migrans" (EM). So far, the pathomechanisms leading to the development of this particular skin lesions are not fully understood. The aim of this study was to induce EM lesions in experimental *Borrelia burgdorferi* sensu lato (Bbsl) infection in the skin of domestic pigs. Domestic pigs were considered as model species, as porcine skin shares a number of striking similarities with human skin.

#### Material and Methods

18 healthy Large White pigs weighing 20–30 kg were injected intradermally with 100 µL of an either high dose ( $1 \times 10^9$ ) or low dose ( $1 \times 10^6$ ) suspension of spirochetes. For the next 10 days the pigs were monitored on a daily basis, considering the following parameters: average behaviour, skin lesions, inner body temperature, as well as neurologic, cardiac, and orthopedic abnormalities. On days 0, 1, 3, and 10, skin samples were taken for histological and PCR analyses.

#### Results

The formation of EM-like lesions was observed after intradermal injection of these spirochetes, with the lesions forming very clearly in 2/6 animals, when a strain of *B. garinii* was used. However, no molecular or clinical proof of systemic infection of the pigs could be achieved.

#### Discussion and Conclusion

It could be shown that Bbsl are viable in porcine skin, although typical skin lesions developed only in the context of *B. garinii* infection. This outcome may form a basis for future studies considering porcine skin as a model for dermal *Borrelia* infections.





## MIS-002 - VACCINATION TIME IN PIGS IS REDUCED WITH IDAL COMPARED TO TRADITIONAL NEEDLE AND SYRINGE

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### Introduction

IDAL (IntraDermal Application of Liquids) is a device for applying vaccines intradermally (ID). This report focuses on the time spend on vaccination with IDAL compared to traditional intramuscular needle and syringe vaccination.

### Materials and Methods

The test included more than 4,700 pigs equally divided in two groups, which were vaccinated either with IDAL or needle attached to dosing syringe. Pigs were vaccinated approximately 30 kg lbw. One unit contained two pens around a liquid feed valve and one section in the slaughter pig herd contained 16 units. The vaccination was done in two different ways: 1) All pigs were moved to the end of the inspection hall, where they were vaccinated with either IDAL or dosing syringe. Vaccination time was measured in two different ways: a) Start at opening first pen and vaccination of pigs to return to second pen. b) Start at moving pigs in the hall back-end and vaccination to last pig vaccination including interruption of moving pigs back, and taking pigs out of second pen. 2) Pigs vaccinated by IDAL remained in the pen and were vaccinated during feeding.

### Results

Times spend on vaccination:

36 pigs in two pens measured from opening the gate to the first pen and close the gate in second pen: 4.16 min by IM and 3.57 min by IDAL.

36 pigs in two pens measured from pigs ready at the end of the hall until last pig vaccinated: 3.31 min by IM and 2.72 min by IDAL.

36 pigs vaccinated in the pen during feeding: 0.91 min by IDAL.

### Conclusions

The trial supports that vaccination with IDAL is faster than with needle and dosing syringe. Depending on the setup, IDAL was after receiving proper training in using IDAL, respectively 14%, 18% and 78% faster than the traditional needle vaccination.



## MIS-003 - PREVALENCE OF THE MOST IMPORTANT PATHOGENS IN GASTROINTESTINAL TRACT OF SUCKLING PIGLETS IN THE CZECH REPUBLIC

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### Introduction

Gastrointestinal tract of suckling piglets falls into the most frequently affected organ systems. Etiological reasons of infectious diarrhoea of piglets in a farrowing house use to be simple or mixed infections of viruses, bacteria and parasites.

### Material and Methods

A total of 31 herds of pigs bred in the Czech Republic with different health status and a different level of veterinary-prophylactic measures were included in the study. Their monitoring was performed in the years 2015 and 2016. The samples were taken from untreated suckling piglets with clinical manifestation of diarrhoea at the age from 2 to 28 days. All samples were tested for the presence of *C. perfringens* type A and C, *C. difficile*, ETEC, AEEC, rotavirus group A, coronavirus TGE and PED and parasite infection.

### Results

The presence of minimum one pathogen was detected in all the farms included in the study. *C. perfringens* type A was detected in 14 herds (45.2 % of herds), rotavirus group A in 13 herds (41.9 %). Less frequently detected were *Isospora suis* – 7 herds (22.6 %), PED and ETEC – both in 4 herds (each 12.9 %). AEEC was found in 2 herds (6.5 %). TGE, *Cryptosporidium* and *Strongyloides ransomi* were detected each in only one herd (each 3.2 %). *C. perfringens* type C and *C. difficile* were not detected in any of the herds.

### Discussion

The results indicate a persistent problem with infectious diarrhoea in suckling piglets. The most frequent detected pathogens are *C. perfringens* type A and rotavirus group A. As it follows from the study, other less frequent or even rare pathogens should not be omitted in diagnostics to be able to start with effective therapeutic-prophylactic measures.



## MIS-004 - ASSESSING THE DIAGNOSTIC PERFORMANCES OF TWO ELISAS TO DETECT PCV2 ANTIBODIES

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### Background & Objectives

PCV2 is one of the economically most important viral pathogens affecting the swine industry worldwide. The virus is associated with various disease conditions known as porcine circovirus-associated diseases. The availability of accurate and rapid to perform serological tests is necessary for epidemiological, diagnostic and control purposes. Several ELISAs ("in-house" and commercial) are available to detect PCV2 antibodies but the performances of all these tests have not been compared to date. The study aimed at assessing the diagnostic characteristics of two PCV2 ELISAs.

### Material and Methods

465 serum samples from finishing pigs (25 herds) not vaccinated against PCV2 were used. Samples were tested by two ELISAs ("in-house" ELISA-Anses and SERELISA<sup>®</sup>PCV2 Ab Mono Blocking (Synbiotics)). A sample was considered positive when the OD value was higher than 1.5 for the ELISA-Anses (cut-off previously determined by comparison to IPMA). A ROC curve was used to assess the optimal threshold of the SERELISA<sup>®</sup>PCV2 by taking the ELISA-Anses as reference. This led to an SERELISA<sup>®</sup>PCV2 result  $\geq 170$  considered as positive. The sensitivity and specificity of each ELISA were then estimated without a gold standard using a Bayesian approach.

### Results

The mean sensitivity and specificity of ELISA-Anses were 0.97 (Credibility Interval at 95% CI95%: [0.93-1.00]) and 0.91 (CI95%: [0.79-0.99]) respectively. SERELISA<sup>®</sup>PCV2 reached a mean sensitivity and specificity of 0.94 (CI95%: [0.91-0.97]) and 0.80 (CI95%: [0.72-0.88]) respectively.

### Discussion & Conclusion

Since the infection status of the pigs tested under field conditions was unknown, and no gold standard is available, the sampling methods were analyzed using a Bayesian approach. To the best of our knowledge, this is the first field study to use such an approach to evaluate two ELISAs assessing PCV2 infection in pigs. The results of the present study indicate that ELISA-Anses obtained higher diagnostic performances compared to SERELISA<sup>®</sup>PCV2, particularly on the specificity.



## MIS-005 - PHARMACOKINETICS OF BENZYL PENICILLIN IN PIGS

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### Background

Penicillin is an important drug for treatment of pigs, but data on how this antimicrobial is absorbed, distributed, metabolized and eliminated (i.e. the pharmacokinetic properties) are sparse in pigs. The objective of this study was to investigate pharmacokinetic properties of benzylpenicillin in growing pigs.

### Methods

The exposure profiles of Ethacilin vet (20 mg/kg, n=6) and Ultrapen vet (30 mg/kg, n=6) containing procaine benzylpenicillin in an aqueous and oily suspension, respectively, were determined after intramuscular (im) administration to pigs aged 12 to 13 weeks. The disposition of benzylpenicillin was also determined after intravenous injection of potassium benzylpenicillin (10 mg/kg, n=3 or 20 mg/kg, n=3). Logarithmically spaced blood samples (n=9) were collected over 24 hours from each pig. Plasma concentrations of penicillin were determined using UHPLC interfaced to LCMSMS. The exposure and disposition were analyzed according to standard non-compartmental approaches.

### Results

The plasma concentration of benzylpenicillin after im administration was highly variable for both im formulations ( $C_{max}$  910-3200 µg/L with  $t_{max}$  15-240 min) and probably with an absorption-rate limited terminal decline. The maximum plasma concentrations of Ultrapen had more than one peak. After intravenous administration plasma concentrations dropped 2-3 orders of magnitude within the first five hours after injections of either 10 or 20 mg/kg but in three animals a slow terminal phase was observed. There was no difference in decline related to dose after iv administration.

### Discussion & Conclusion

Due to the variability in plasma concentrations of benzylpenicillin after im administration at 24h intervals, with concentrations ≤500 µg/L for prolonged periods of time, there is an apparent risk for treatment failure when infections are caused by bacteria with minimal inhibitory concentrations exceeding 500 µg/L. Simulations of steady-state exposure however revealed that concentrations >500 µg/L may be obtained when either 20 or 30 mg/kg of the different formulations is given im at 12-hour intervals.



## MIS-006 - RELATION BETWEEN TREATMENT FOR ARTHRITIS DURING SUCKLING AND PERFORMANCE THE DURING FATTENING PERIOD

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### Background & Objectives

Arthritis in pigs is of major animal welfare concern, but difficult to study as records are needed on individual pigs. This study analysed arthritis and growth performance from birth to slaughter in pigs with documented pedigree.

### Material & Methods

At Lövsta research station (SLU), all piglets are given individual identity at birth and have individual records on health and growth performance from birth until slaughter. In 2012, the research station was rebuilt with a floor adapted to piglets (Piglet Floor<sup>®</sup>, Flowcrete Sweden AB, Perstorp, Sweden). Thereafter, all 12,744 piglets born alive during 3.5 years were studied.

### Results

In total 9,807 piglets were weaned at five weeks of age, and 9,569 were alive at 9 weeks. Of these, 208 (2.2%) had been treated for arthritis. Of these 208 pigs, 3 (1.4%) were again treated during the fattening period, compared to 28/9,361 previously non-lame pigs (0.3%). At slaughter 2/208 (1.0%) and 30/9,361 (0.3%) were recorded with arthritis.

The mean birth weight (1.5 kg) did not differ between groups, but the weight at 5 weeks, 9 weeks and the carcass weight were 1.3, 2.4 and 2.3 kg lower in pigs treated for lameness during suckling ( $P < 0.001$ ).

Pre weaning arthritis was recorded in 53 out of 249 (21.3%) pure bred Yorkshire litters compared to in 111 out of 626 (17.7%) Hampshire-Yorkshire litters ( $P > 0.05$ ).

### Discussion Conclusion

Piglets treated for arthritis grew slower than healthy pigs, confirming that arthritis may be very costly. Further, the results indicated a greater risk to be attended for lameness during the fattening period if treated during suckling (OR=4.7). However, the results also suggested that the negative impact of arthritis can be reduced by proper floor surfaces, since the incidence of arthritis pre-weaning was lower than the national average (2% vs 10%, OR=0.2). The somewhat lower incidence of lameness in HY-litters may also indicate a heterosis effect.



## MIS-007 - MALIGNANT LYMPHOMA IN A HIGHLY PREGNANT GILT – A CASE REPORT

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Although cancer is relatively uncommon in pigs, lymphoma is the most frequently reported cancer of swine. In the United States and some European countries lymphoma accounts for 23 to 41% of slaughtered pigs condemned due to neoplasia (1). C-type viruses have been associated with natural cases of malignant lymphoma in pigs. Moreover, a genetic predisposition could be proven for inbred herds. The multicentric form of the disease is found in about 2/3 of swine showing lymphoma. Commonly affected organs are lungs, heart, spleen, liver, kidney, bone marrow, the intestinal tract and lymph nodes (2). The onset of the disease is mainly one year or less. This case report describes a case of multicentric malignant lymphoma in an approximately 12 to 14 months old, highly pregnant gilt, having 212 kg body weight. The gilt died peracutely without showing clinical signs. The animal was in a very good body condition (BCS 4 out of 5). It was kept on a conventional sow breeding farm rearing 1,000 sows. Farrowing occurs in a one-week rhythm with 3 weeks suckling period. Multicentric lymphoma could be found in the small intestine, liver, spleen, kidneys, on the peritoneum and in several mesenteric lymph nodes. Tumorous alterations reached up to double fist-size and showed an inhomogeneous outer appearance and cut surface with necrotic lesions in the center. 18 mature piglets could be found in the uterus. The mammary gland was already fully developed.

### References

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## MIS-008 - STABILITY OF WHITE BLOOD CELL COUNTS OVER TIME

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### Background & Objectives

WBC counts mirror the infectious status of pigs, but time may affect the results due to cell lysis. If so, there is a risk for incorrect results in samples needing transportation to lab. We therefore compared the results of samples analysed at different times.

### Material & Methods

Blood samples with EDTA as additive were collected from crossbred pigs aged 12-13 weeks. The samples (n = 139) were analysed at 1, 25 and 49 h after collection with an automatic analyser (Exigo, Boule, Stockholm, Sweden) and stored at +4°C between analyses.

### Results

The mean concentrations of WBCs were  $25 \pm 11 \times 10^9$  per ml on all occasions. Mean percentages of lymphocytes were  $36 \pm 14\%$ ,  $37 \pm 12\%$  and  $36 \pm 13\%$  at 1, 25 and 49h, respectively. Granulocyte percentages were  $57 \pm 15\%$ ,  $49 \pm 13\%$  and  $52 \pm 13\%$ , respectively.

The samples were divided into four categories based on the percentage of lymphocytes at the first analyse: In high level pigs (>50%, n=30), the lymphocyte percentages were  $55 \pm 3\%$ ,  $51 \pm 7\%$  and  $52 \pm 4\%$  at 1, 25 and 49h, respectively, with a granulocyte percentage of 36% on all occasions.

In "semi-high" level pigs (40-50%, n=26), the lymphocyte percentages were  $43 \pm 3\%$ ,  $42 \pm 7\%$  and  $44 \pm 4\%$  at 1, 25 and 49h, respectively, with granulocyte percentages of  $49 \pm 3\%$ ,  $45 \pm 8\%$  and  $44 \pm 4\%$ .

In "semi-low" level pigs (30-40%, n=35), the lymphocyte percentages were  $35 \pm 3\%$ ,  $38 \pm 6\%$  and  $37 \pm 4\%$  at 1, 25 and 49h, respectively, with granulocyte percentages of  $57 \pm 3\%$ ,  $49 \pm 7\%$  and  $50 \pm 5\%$ .

In low level pigs (<30%, n=45), the lymphocyte percentage was  $19 \pm 6\%$ ,  $25 \pm 10\%$  and  $22 \pm 7\%$  at 1, 25 and 49h, respectively, with granulocyte percentages of  $75 \pm 7\%$ ,  $61 \pm 11\%$  and  $67 \pm 8\%$ .

### Discussion & Conclusion

WBC and differential counts were fairly stable over time when samples were stored at +4°C between analysis. This opens up for a more extensive use of WBC counts within porcine medicine as blood samples can be sent to a lab for analysis.



## MIS-009 - STABILITY OF AMOXICILLIN IN FEED ACCORDING TO DIFFERENT STORAGE CONDITIONS

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### Background and objectives

Amoxicillin remains a valuable agent for pig antimicrobial therapy by oral route. Objective of this study was to test the stability of three amoxicillin medicated premixes after feed pelletization and storage in various temperature and relative humidity (RH) conditions.

### Material and methods

The premixes contained 50% (A), 30% (B) and 10% (C) of amoxicillin. They were incorporated in a starter pig feed according to recommended amoxicillin concentrations for each product (400 ppm for A and B, 300 ppm for C). Medicated feeds manufacture and sampling were performed in a pilot plant (steam pressure : 1.6 bar and pelletization temperature : 85°C) : 6 samples before and 3 samples after pelletization per product. Three pelleted samples per product were also stored during one month for each storage condition (25°C/60% RH, 30°C/65% RH, 40°C/75% RH). Amoxicillin was assayed after extraction by HPLC with UV detection. Concentrations were compared between products by the Kruskal-Wallis test.

### Results

Mean concentrations before and after pelletization respectively ranged between 98% and 104%, and between 93% and 105% of theoretical concentrations, without statistical differences between products. Mean concentrations after storage were different between products in all conditions ( $P < 0.05$ ), product A being the only one with mean concentrations above 95% of theoretical concentration after storage at 25°C or 30°C.

### Discussion and conclusions

Amoxicillin is known as being susceptible to heat degradation (Derrieu *et al* 2000, Zemanova *et al* 2008). Differences observed here between products may be due to various amoxicillin coating processes aiming at protecting the molecule from degradation. The importance of maintaining antimicrobial activity till animal oral absorption is crucial both for efficacy and to limit the emergence of resistances.

### References

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Zemanova M. *et al* 2008. Acta Vet. Brno 77, 341-345.





## MIS-010 - STABILITY OF A LINCO-SPECTIN® SOLUTION IN WATER TREATED WITH BIOCIDES

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### Introduction

Drinking water in breeding farms often requires to be treated to improve bacteriological quality. It is also used for the administration of medicines and the biocides used for treatment may decrease their efficiency. The aim of this study is to investigate if sodium hypochlorite and hydrogen peroxide would be liable to a degradation of LINCO-SPECTIN® in solution.

### Materials and Methods

We compared the initial concentrations and after 24 hours of a solution with 150 mg/liter of LINCO-SPECTIN® and 1 mgCl<sub>2</sub>/l of chlorine or 50 mg/l H<sub>2</sub>O<sub>2</sub> of hydrogen peroxide.

The titration of antibiotics was performed by chromatography.

Biocides were brought by a sodium hypochlorite solution at 10.5% and a solution at 150 g/Kg of hydrogen peroxide associated with peracetic acid at 25 g/Kg.

The solutions were prepared with tap water that was analyzed for its main characteristics.

The manipulations were repeated twice.

### Results and Discussion

None of both antibiotics was degraded in the presence of sodium hypochlorite.

However in the presence of peroxide, decrease of concentrations for both antibiotics was observed, respectively by 7 and 8% for lincomycin and by 15 and 28% for spectinomycin.

Note the variability of measures for the spectinomycine.

These results do confirm previous tests run by Hemonic and al, assessing the impact of the same biocides on other major antibiotics (amoxicillin, doxycycline, colistin, tylosin and trimethoprim). In this study chloride had no impact on the stability of the antibiotic solution while peroxide and especially electrolysed water had a significant effect.

### Conclusion

This study demonstrated the stability of LINCO-SPECTIN® in the presence of chlorine at high concentration. However peroxide showed to have an impact especially on spectinomycin stability. Based on recent data, cautions should be taken when delivering LINCO-SPECTIN® in electrolysed water.



## MIS-011 - COLLOIDAL GOITRE IN SLAUGHTER PIGS

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Colloidal goitre, a pathological lesion characterized by an increase of the thyroid volume, is commonly associated with iodine deficiency, feeding of natural goitrogenic substances, exposure to endocrine disruptors or illegal assumption of thyreostatic drugs. Moreover, several studies performed in humans, highlighted that genetic can play a pivotal role in the goitre's evolution. This study aims at reporting the occurrence of goitre in slaughter pigs.

The first part of the research was conducted at the slaughterhouse and was based on randomly collecting and weighing ten thyroids per batch of slaughtered pigs. In total, 124 herds located in Northern Italy were considered. After the first screening, thyroids from 8 herds with a constant higher average thyroid weight were submitted to histological exams and to HPLC for the detection of the use of thyreostatics. In the last phase, two farms having significant differences in the average thyroid weight were selected and the thyroid of pigs died at different ages were histologically examined. Moreover, 18 blood samples per farm were collected and the concentrations of free thyroxine (T4), free triiodothyronine (T3) and thyroid stimulating hormone (TSH) were analysed by an ELISA Kit specific for swine (Abnova®).

The average thyroid weight was  $16,33 \pm 7,33$  g. and the use of thyreostatic drugs was excluded by HPLC exams. The thyroïdal hormones profile was not statistically different comparing different herds but the histological lesions referable to colloidal goitre in the farm with higher thyroid weight were evident.

This study demonstrates that the presence of colloidal goitre is frequently observed at slaughterhouse. This lesion, not associated with thyroid hormones disorders, seems to have a genetic predisposition interacting with environmental risk factors. Further studies are needed to understand the basis of the genetic susceptibility and the environmental influences that lead to goitre development.



## MIS-012 - RESTRAINING OF PIGS DOES NOT AFFECT GROWTH

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It is important that pigs keep still during collection of e.g. blood or nostril samples. Such samples are therefore collected from restrained pigs. However, restraining has recently been questioned from an animal welfare point of view by Swedish Ethical Boards authorising experiments on animals. As discomfort may reduce weight gain of pigs, we explored the possible long-term negative impact of restraining on the welfare of pigs by comparing the weight gain of repeatedly restrained pigs to unrestrained siblings.

Nine SPF pigs from three litters were restrained nine times during 24 hours, at 0, 15, 30, 60, 90, 120, 240, 720 and 1440 minutes. Each restraining lasted for around 20 seconds when a blood sample was collected. Sixteen control pigs from the same litters (5+5+6) were not restrained. All pigs were weighed the day before restraining, as well as 9 and 58 days thereafter.

The initial mean weights were  $39.4 \pm 1.2$  kg and  $38.9 \pm 3.2$  for restrained and control pigs, respectively. Nine days after the day of restraining, these pigs weighed  $48.1 \pm 2.1$  kg (DWG =  $963 \pm 111$  g/day) and the control pigs weighed  $47.5 \pm 3.9$  kg ( $951 \pm 128$  g/day). At day 58 post restraining, the mean weight of the restrained group was  $100.2 \pm 9.0$  kg ( $1063 \pm 156$  g/day) compared with  $103.1 \pm 10.9$  kg ( $1136 \pm 153$  g/day) for the control group.

Both groups had a high DWG, as would be expected from SPF pigs. There were no statistical differences between weight or DWG at any occasion (Student's t-test). The restrained pigs actually grew numerically better than control pigs during the first nine days after restraining. Thus restraining, when performed correctly, did not affect growth negatively indicating that the long-term well-being of pigs was not affected by restraining.



## MIS-013 - NEWBORN PIGLETS WITH LOW OR HIGH BLOOD-GLUCOSE LEVELS ARE ASSOCIATED WITH INCREASED MORTALITY

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### Background & Objectives

Piglets are born with limited energy stores and a not fully developed gluconeogenesis. This makes them prone to hypoglycemia. We hypothesized that if glucose-regulation mechanisms do not work properly the first days after birth, the piglets will have abnormal blood-glucose levels. The aim of this pilot-study was to investigate if, and how, blood glucose levels are associated with pre-weaning mortality.

### Material & Methods

The study included 111 crossbred piglets, conveniently chosen among 762 live born piglets from 53 sows, from one loose-house piglet producing herd in Norway. Blood was collected by vein puncture of vena subcutaneous abdominalis and blood-glucose levels were analyzed with the easy, handheld glucometer, Bayer Breeze 2, 15-36 hours after birth. Blood-glucose levels (mmol/L) were divided into three groups according to reference values at weaning (Egeli et al., 1998); <5.1, 5.1-8.3 and >8.3. Piglets were ear tagged, weighed, and litter size, cross-fostering and deaths until weaning was recorded. Logistic regression was used for the statistical analyses.

### Results

Mean piglet-weight was 1.50 kg (SD=0.38). Mean blood-glucose level was 5.40 mmol/L (SD=1.83). Of the 111 piglets 12 (10.8 %) died before weaning. Piglets with low blood-glucose levels had an increased risk of dying (OR=7.26, p=0.026). Although not statistically significant in such a small group, there was an indication that also piglets with high blood-glucose levels had increased risk of dying (OR=11.65, p=0.089).

### Discussion & Conclusion

Hypoglycemia in piglets is often due to starvation, and high blood-glucose may cause the piglet to be more active, and have a higher risk of crushing. But since this small pilot-study found that both low and high blood glucose levels seems to be a risk factor, this may indicate that some piglets die because they have trouble maintaining stable blood-glucose levels. Further investigation are being performed to explore how blood-glucose affects piglet mortality.



## MIS-014 - THE PREVALENCE OF SPLEEN ATROPHY IN SOWS

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### Background and objectives

Complete or partial spleen atrophy is frequently observed in slaughter sows as well as in sows submitted for necropsy to the Animal Health Service (AHS). The exact relevance of spleen atrophy as well as the association with mortality or sow health is unclear, but it may be relevant for sow immunity or as a sign of decreased sow welfare. The prevalence in slaughter sows in The Netherlands was estimated at  $\pm 2\%$  in 1984, 1986 and 1991 (*van Leen goed, ESPHM, 2014*), but risk factors nor exact pathogenesis are unknown. To enable future pathogenesis and epidemiological studies this study aims to estimate the current prevalence of spleen atrophy in Dutch slaughter sows and in sows submitted for necropsy.

### Material and methods

An observational study was performed in a Dutch slaughterhouse on three randomly chosen days. Spleen size and shape of slaughtered sows were scored as 1) being completely atrophied, 2) 0 to  $\pm 17$  centimetres long, 3)  $\pm 17$  to  $\pm 34$  centimetres long spleen or 4) longer than 34 cm. In addition, a retrospective data analysis was performed on necropsy results from animals submitted to AHS in 2015 - 2016.

### Results

22 of 994 (2.21%) scored spleens showed completely atrophy. 0.7% of the spleens were  $< \pm 17$  centimetres long and 5.6% of the spleens were  $> \pm 17$  and  $< \pm 34$  centimetres long. There was no significant difference in spleen scores per observation day. Occurrence of spleen atrophy could not be traced to the farm of origin. In total 10,0% of animals  $> 1$  year old showed (partial) spleen atrophy at necropsy.

### Conclusion and discussion

The prevalence of spleen atrophy in slaughter sows per day was very consistent, indicating that multiple farms experience this phenomenon. In sows submitted for necropsy (partial) spleen atrophy is diagnosed more often, suggesting a relation between sow mortality and spleen atrophy.



## MIS-015 - A CASE STUDY OF NEUROLOGICAL SYMPTOMS IN PREGNANT PUREBRED LANDRACE SOWS

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### Introduction

In February 2016 CNS-symptoms were observed in pregnant purebred Landrace sows in a multiplier (gilt producing) herd with approximately 250 sows in production. Approximately 8% of the sows developed CNS-symptoms during a period of 10 months. They were of all parities and in late pregnancy. The CNS-symptoms were manifested as body-shaking due to shivering of the muscles, difficulty of moving, frothing of the mouth, hyperventilation and nystagmus. The sows had increased body-temperature. They were treated with NSAID's and antibiotics with varying results. Most of the sows recovered except two which died during treatment.

### Material and Methods

Postmortem examination was performed at the National Veterinary Institute on 4 affected sows and samples from lung, brain, spleen, liver and gut were collected. Blood samples were drawn from pregnant non-affected sows and gilts in the herd and analyzed for Ca, Mg, P, and albumin content. Blood samples were drawn at acute stage of illness and accordingly analyzed for hematology. One shivering gilt was euthanized by the farmer and necropsy was performed on the farm. Samples for bacterial analysis were collected from the brain, as well as samples from spleen and heart. Analysis of hygienic and nutritional quality of the feed was performed.

### Results

The results from the postmortem examination of two of the sows showed chronic multifocal heart muscle degeneration, circulatory disturbances/ acute lung edema, reparative processes and acute bleedings of the heart. The findings in the two other sows showed osteochondrosis. Blood samples showed normal values of Ca, Mg, P and albumin. The analysis of blood drawn at acute stage of illness showed clear signs of acute infection.

### Conclusions

We conclude so far that our sows suffer from an infection, differential diagnosis could be *Streptococcus suis* or *Encephalomyocarditis virus*. The ongoing investigation will hopefully give us answers to solve this mystery, which will be presented at ESPHM2017.



## MIS-016 - HOW DO WELFARE AND BIOSECURITY RELATE TO EACH OTHER?

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Biosecurity and welfare are related to animal health but the link between them has not been elucidated. Here we examine the relationship between on-farm and abattoir welfare and biosecurity.

Twenty-five farrow-to-finish Irish pig farms were scored using Biocheck.ugent® (February to November 2016) and their scores were related to welfare assessments on-farm, based on the Welfare Quality® protocol, and at slaughter (July to November 2015). Spearman Rank correlations were used to analyse the data.

The % of pigs at slaughter with skin lesions (SL) was negatively associated with the external biosecurity score (ES) ( $r_s = -0.48$ ,  $P = 0.015$ ) and the internal score (IS) ( $r_s = -0.45$ ,  $P = 0.022$ ). The % of pigs at slaughter with SL was also associated with the age of the oldest building housing pigs ( $r_s = 0.46$ ,  $P = 0.020$ ). The % of pigs without tail lesions (TL) at slaughter was associated with the biosecurity scores at farrowing and suckling section ( $r_s = 0.59$ ,  $P < 0.001$ ) and at the fattening section ( $r_s = 0.59$ ,  $P = 0.013$ ). The % of pigs with poor body condition score (BCS) was positively associated with the farm manager's experience with pigs ( $r_s = 0.45$ ,  $P = 0.024$ ) but negatively associated with the number of workers ( $r_s = -0.41$ ,  $P = 0.041$ ) and with several biosecurity parameters, including ES ( $r_s = -0.51$ ,  $P < 0.001$ ), IS ( $r_s = -0.54$ ,  $P < 0.001$ ), and the overall score ( $r_s = -0.56$ ,  $P < 0.001$ ).

Results show higher internal and external biosecurity and newer facilities lower the proportion of pigs with SL and TL at slaughter, hinting welfare is better in farms with high biosecurity. The lower proportion of poor BCS pigs with higher number of workers, ES, IS and overall biosecurity scores also support this thesis. In contrast, the manager's experience in keeping pigs affected the proportion of pigs with poor BCS, suggesting desensitization for this issue. This study suggests there is a link between biosecurity and welfare indicators and further studies should be carried out to characterize it.



## MIS-017 - RELATING FARM BIOSECURITY SCORES TO THE IRISH NATIONAL HERD PERFORMANCE MONITORING DATABASE

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Biosecurity has shown moderate correlations to production performance in other countries. We aimed to investigate which biosecurity aspects were relevant for production performance in Ireland.

Fifty-seven farrow-to-finish Irish pig farms were surveyed using the Biocheck.ugent® scoring tool from February to November 2016 and their scores were related to their 2015 production parameters retrieved from the Irish national database (Teagasc e-Profit Monitor System). Spearman Rank correlations were used to analyse the data.

The average herd size was correlated to the external biosecurity score ( $r_s=0.38$ ,  $P<0.01$ ) and to the entrance of personnel and visitors into the farm ( $r_s=0.37$ ,  $P<0.01$ ). Positive correlations were identified between Average Daily Feed Intake (ADFI) and the following biosecurity parameters: personnel and visitors ( $r_s=0.39$ ,  $P<0.01$ ), vermin and bird control ( $r_s=0.39$ ,  $P<0.01$ ), external score ( $r_s=0.47$ ,  $P<0.001$ ), disease management ( $r_s=0.45$ ,  $P<0.001$ ), cleaning and disinfection ( $r_s=0.37$ ,  $P<0.01$ ) and the overall biosecurity score ( $r_s=0.47$ ,  $P<0.001$ ). Weaner mortality had a negative correlation with vermin and bird control ( $r_s=-0.36$ ,  $P<0.01$ ) and this parameter had a positive correlation with the number of pigs produced per sow per year ( $r_s=0.38$ ,  $P<0.01$ ). Lastly, the Average Daily Gain (ADG) was correlated with disease management ( $r_s=0.44$ ,  $P<0.001$ ).

Results suggest bigger farms had better external biosecurity, namely when regarding the use of hygiene locks and their location in the farm and the use of farm-specific clothing and shoes. Production parameters such as ADFI, ADG, weaner mortality and number of pigs produced per sow per year improve with higher biosecurity, meaning that refining some practices on farm, such as having rodent control programmes, checking herd health status regularly or treating sick animals after checking the healthy ones may have a favourable economic impact on Irish farms.





## Parasitology and Parasitic Diseases

### PPD-001 - EFFICACY OF PIGFEN® 40 MG/ G PREMIX AGAINST ADULT AND LARVAL STAGES OF ASCARIS SUUM IN PIGS

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The bioavailability of a product is depending on the quality of the active, like the crystal form and size, and the formulation. This is especially valid for molecules that are insoluble in water, like fenbendazole. Brand specific efficacy studies are required. In this study, the efficacy of different dose regimes of Pigfen® 40 mg fenbendazole/ g microgranulated premix for pigs against different stages of *Ascaris suum* was evaluated.

Helminth naïve pigs were artificially challenged with approximately 450 embryonated eggs of *Ascaris suum* over 1 or 3 consecutive days. Non-treated spare animals were included and euthanased either at the start, or during the administration, to collect small intestines and/ or lungs. This provided information on the parasitic stage at the time of the treatment. The adequacy of the infection was demonstrated across all treatment groups. Following challenge, Pigfen® was administered in feed over 1 day or divided over 7 or 14 days, totalling 5 mg fenbendazole/ kg bodyweight. At necropsy, adult worms, L4 larvae and intestinal L5 larvae were counted and compared to a control group for each category. The percentages of worm and larvae count reduction at necropsy for the treated groups compared to the corresponding negative control groups were calculated.

A significant reduction of 97.5 % and 95 % in adult worm counts for a 7 and 14 days treatment period was noted. L4 counts were significantly reduced by 99.9 % as a single dose and 100 % for a 7 days treatment. The L5 counts showed a 100 % reduction for all treatment regimes.

The efficacy of an anthelmintic treatment with a fenbendazole premix is product related. Pigfen® 40 mg/ g premix administered at 5 mg/ kg bodyweight has shown to be very efficacious against all tested stages of *Ascaris suum*, independent of the duration of treatment.



## Reproduction

### REP-001 - HISTOLOGY OF THE UROGENITARY SYSTEM OF SOWS WITH REPRODUCTIVE DISORDERS IS HIGHLY VARIABLE

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#### Background and Objectives

Inflammation of the urogenitary system is a substantial cause for reduced fertility in sows and plays a significant role in sow elimination. Consequently, there is a great impact on the economic profitability and a demand for adequate diagnosis.

#### Material and Methods

The major aim of this study was to analyze the histological appearances of endometritides and cystitides in sows with reproductive disorders. Therefore, samples of uteri and bladders from 42 sows with reproductive disorders from nine farms in Lower Austria were collected after slaughter and examined by light microscopy. Additionally, bacteriological analyses from urinary bladder swabs as well as from the endometrium were performed.

#### Results

In the animals studied a large range of morphological changes and a diffuse distribution pattern of inflammatory cells were present. Within the detected inflammatory cells, stromal lymphocytes dominated by number, followed by intraepithelial lymphocytes and stromal plasma cells in both the endometrium and the mucosa of the bladder. The quantity of immunocytes was highly variable. However, at the individual level the quantity of cell populations and the degree of inflammation correlated in both organs. The occasional additional presence of fibroblasts in both organs was indicative of a chronic inflammation. No correlation could be found between the detected pathogens (mainly *E. coli*, streptococci, enterococci, and *Candida* spp., but also pathogenic *Leptospira* and *Chlamydia* spp.) and histologic alterations.

#### Discussion and Conclusion

The high variability of inflammatory cell numbers and histological changes gives striking evidence of the importance of an exact clinical and microbiological diagnosis in the field of reproductive disorders.



## REP-002 - POST WEANING ALTRENOGEST USE IN SOWS DURING SUMMER

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### Background and objectives

Objective of this study was to assess the impact of delaying the return to service after weaning by one week on reproductive performances. Such design was implemented by using altrenogest (oral progestagen) during summer known as a season inducing infertility.

### Material and methods

Study was performed in a Spanish farrow to nursery herd owning 2500 sows managed as a 1-week batch system (average weaning at 24 days). During August 2015, 455 lactating sows were randomly assigned to a treated or control group. Treated sows were given an oral solution of altrenogest at the dose of 20 mg/d during 8 days, starting the day before weaning. Oestrus detection, artificial insemination and pregnancy diagnosis were managed according to farm procedures from weaning (control sows) or treatment stop (treated sows). Reproductive performances were compared between groups till subsequent farrowing by appropriate statistical tests.

### Results

Parity effect was seen on anoestrus, pregnancy and farrowing rates of weaned sows, performances being better for multiparous than for primiparous sows (2.2% vs 28.4%, 91.6% vs 51.9%, 88.2% vs 47.5%, respectively,  $P < 0.001$ ). Though non significant, numerical differences between treated and control groups were higher on primiparous sows (25.6% vs 31.0%, 57.5% vs 46.3%, 52.5% vs 42.5%, respectively for these criteria). Number of piglets born alive and weaned per litter were significantly higher in treated than in control group on pooled parities (16.4 vs 15.5,  $P < 0.05$  and 14.4 vs 13.2,  $P < 0.005$ , respectively). Parity effect on the number of weaned piglets per litter was close to significance ( $P < 0.1$ ), average value being lower in primiparous sows.

### Discussion and conclusions

In this high performance farm, second parity syndrome was evidenced on main reproductive criteria. Extended recovery period after weaning induced a higher prolificacy in treated sows during summer. Positive altrenogest effect on prolificacy has already been reported both in gilts and in sows.



## REP-003 - DOES FARROWING INDUCTION OF HYPERPROLIFIC SOWS WITH CLOPROSTENOL REDUCE STILLBORN PIGLETS?

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### Background & Objectives

The practical purpose of parturition induction is to synchronize farrowing and facilitate farrowing supervision. The present study investigated the effect of induced parturition in hyperprolific sows with four or more cycles, on the number of stillborns per farrow, depending on the timing of farrowing (morning, evening or night)

### Materials & Methods

The present study was performed in a commercial swine herd with Danbred genetic in the Northeast of Spain during 2016. A total of 875 farrows were investigated. The sows were randomly assigned in two groups: control (444 farrows) and induced parturition (431 farrows). The induction of parturition was done by administration of 2 ml (175 µg) of cloprostenol intramuscular (Planate®) on the due date of parturition in sows with four or more cycles. Farrowing process was carefully supervised. The timing of the farrowing, percentage of farrowing at each time, number of total born and stillborn was recorded and compared between induced and control group.

### Results

The average of total born was 16.64 in control group and 17.03 in induced group. The percentage of morning farrowings were 22.2% in control vs 54. % in induced group. In trial group with 431 farrowings, 153 farrowings were induced with Planate®, and 217 piglets more were born than in control group (in sows with >=4 cycle). Induction also reduced stillborns by 1.4 per farrow.

### Discussion & Conclusion

Parturition induction allows producers to manage their herds more effectively because parturition is synchronized which facilitates supervision of farrowing and increases the possibility to equalize litter size by cross-fostering. Induced parturition in hyperprolific sows is a management tool than can reduce piglet mortality. Induced parturition with Planate® helps to have a higher percentage of farrowings at times where they can be supervised, resulting in decreased stillborns even in hyper-prolific sows.



## REP-004 - REPRODUCTIVE PERFORMANCE AND OVULATION TIME DETERMINATION WITH SONOGRAPHY IN WEANED SOWS WITH OR WITHOUT PORCEPTAL TREATMENT UNDER ANESTROUS SEASONAL CONDITIONS

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### Background & objectives

Maintaining optimal reproductive performance during anestrus season is essential for meeting economic targets. Busereline, can be used to synchronize ovulation which can increase reproductive efficiency. This study determined wean estrus interval (WEI), estrus duration (ED) and Wean ovulation interval (WOVI) in two groups of sows with or without previous treatment with Porceptal under anestrus seasonal conditions.

### Material & methods

The study was conducted in a commercial farm in 62 selected weaned sows, randomly split in two groups, a control group (CG, n =20) Vs treatment group (TG, n=42). TG was treated with 2.5 ml of Porceptal 85 hours after weaning. Both groups were scanned from 84 hours after weaning to ovulation every 8 hours, the size of three follicles were measured every scanning. It was assumed that ovulation happened 4 hours before the presence of corpora hemorrhagica (CH) with or without follicles. Estrus was evaluated twice in a day from 72 hours after weaning to the end of estrus.

### Results

Twelve sows were excluded: 8 (14%) due to anestrus, 6 (13%) in the TG and 2 (10%) in CG; 3 (6%) sows presented CH in the first sonography control and 1 (2%) corpora lutea. Finally 16 in CG and 34 in TG finished the study. The WEI was in TG  $94 \pm 10$  [84-116] Vs  $103 \pm 17$  [84-140] in CG. DC was in TG  $51 \pm 10$  [36-80]h Vs  $53 \pm 11$  [36-80]h in CG. The WOVI was in TG  $126 \pm 8$  [116-148]h Vs  $139 \pm 16$  [116-168]h in CG.

### Discussion & Conclusion

Porceptal was effective on ovulation synchronization in weaned sows despite anestrus season, showing a 13 h reduction in the average of WOVI and also less variability (8h Vs 16h). Although the results were worse than in a more favorable reproduction season, 90% of the sows were inseminated in the most favorable interval for fecundation.



## REP-005 - BENEFITS OF PORCEPTAL® IN A CONTINUED FIXED TIME INSEMINATION PROGRAM IN A SPANISH PRODUCTION FARM

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### Background & Objectives

The aim of this trial was to demonstrate that continued use of Porceptal® in a FTI protocol gives equivalent technical reproductive results compared with classical breeding programs.

### Materials & Methods

This study was conducted in a 900 sow farm, located in Palencia. A total of 1351 multiparous, weaned sows (23 lactation days) were included in the study and were assigned to a control (C) (906 sows) between January-April 2014 and Porceptal group (P) (445 sows) between May-October 2015. The P group was treated with 2.5 ml of Porceptal® (10 µgr busserelin), 83-89 hours after weaning, while the control group was inseminated according to the standard post-cervical insemination protocol. The Porceptal group only used Fixed Time Insemination 30-33 hours after treatment with Porceptal®.

### Results

Fertility was not significantly different between the (P) group 90.6% and 86.1% (C) group ( $p>0.05$ ). The farrowing rate was P 89% vs 84.54% in C group. Prolificacy was also not different. Total born was 13.57 (P) vs 13.23 (C) ( $p>0.05$ ), born alive was in P (12.61) vs (12.25) in C ( $p>0.05$ ).

Under the study conditions, Porceptal® would give an ROI of 3.7 € per treatment and 21,233€ in total annual benefits, when extrapolating the results for a full year production.

### Discussion & Conclusion

Porceptal® allowed for synchronized ovulation, more efficient workforce, and better grouping at time of farrowing. This resulted in more homogeneous litters, improved management in the next production phases by grouping the farrowings and better use of elite boars. Porceptal could be a very useful tool for FTI programs, considering that fertility and prolificacy is similar to standard multiple inseminations programs.



## REP-006 - TREATMENT WITH CRYOPROTECTANTS AT 25°C IMPROVES POST VITRIFICATION DEVELOPMENTAL COMPETENCE OF PORCINE GERMINAL VESICLE STAGE CUMULUS-OOCYTES COMPLEXES

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Although offspring have been produced from vitrified porcine cumulus-oocyte complexes (COCs), embryo development rates have remained low. Because at lower temperatures the toxicity of cryoprotectants (CPA) will reduce, the temperature during equilibration can influence blastocyst development. To reveal the optimal temperature to treat immature COCs, we equilibrated at 38°C (38), at a decreasing temperature (decreasing) and at 25°C (25). Those groups were compared to a non-vitrified group (control). COCs were vitrified and warmed according to Somfai *et al.* (2015 J. Reprod. Dev.) with polyvinyl pyrrolidone. COCs were equilibrated in cytochalasin B and 4% (v/v) ethylene glycol (EG)+propylene glycol (PG) (1:1). The vitrification solution contained 35% (v/v) EG+PG (1:1). After warming COCs were subjected to IVM, parthenogenetic activated and cultured for 7 days. Survival, nuclear maturation, cleavage, blastocyst quantity and quality (number of cells) were compared among groups. All parameters, except blastocyst quality, were statistically analyzed by binary logistic regression. Blastocyst quality was analyzed by ANOVA with *post hoc* Tukey test. There were no significant differences among vitrified groups (38, decreasing and 25) in survival ( $67.20 \pm 7.51\%$ ,  $57.75 \pm 2.27\%$  and  $66.49 \pm 5.90\%$ , resp.), maturation ( $84.00 \pm 2.95\%$ ,  $82.41 \pm 1.60\%$  and  $82.11 \pm 4.01\%$ ), cleavage ( $71.43 \pm 4.03\%$ ,  $78.82 \pm 8.05\%$  and  $75.27 \pm 9.49\%$ , resp.), blastocysts on day 6 ( $18.37 \pm 1.75\%$ ,  $28.24 \pm 3.77\%$  and  $26.88 \pm 4.06\%$ , resp.) and blastocyst quality ( $67.75 \pm 10.97$ ,  $56.85 \pm 7.02$  and  $52.66 \pm 6.94$ , resp.). However the three groups were lower than the control concerning survival ( $99.34 \pm 0.61\%$ ), maturation ( $92.67 \pm 0.96\%$ ), cleavage ( $91.73 \pm 1.35\%$ ), blastocysts on day 6 ( $72.18 \pm 5.58\%$ ) and blastocyst quality ( $106.52 \pm 5.15$ ). The blastocyst rates on day 7 were significantly improved in 25 ( $34.41 \pm 7.76\%$ ) compared to 38 ( $21.43 \pm 3.04\%$ ) though they did not reach the level of the control ( $73.68 \pm 5.15\%$ ). It can clearly be concluded that treating COCs with CPA at 25°C is more beneficial to maintain the developmental potential of porcine immature oocytes than 38°C. Appeltant R. is an International Research Fellow of the JSPS Japan (P15402).



## REP-007 - INCREASE IN BACKFAT REDUCES THE THICKNESS OF THE UTERUS PREPARTUM

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### Background

High backfat values and large litter size can increase the farrowing duration. The aim of the study was to investigate if this may be due to impaired uterine contractions. It is known from other species, e.g., cattle and dog, that fatty infiltration or overstretching of the uterus due to large litter size can reduce contractions and therefore prolong parturition. Thus, the hypothesis is that backfat and litter size negatively correlate with prepartum uterine thickness, possibly representing overstretching or fatty infiltration of the uterus.

### Methods

An ultrasound examination of the uterus was performed transabdominally (4.3 MHz convex probe, Esaote) in 51 sows about 2 days before expected parturition. The thickness of the uterine wall was determined around the thorax of five different fetuses and averaged using IMPAX 6.5.5 picture archiving and communication system (Agfa Healthcare). The backfat was measured with ultrasound (10 MHz linear probe, Esaote) above the last rib of the standing sow (P2). Furthermore, weight, parity and litter size of the sows were recorded and analyzed as explanatory variables in a general linear model (PASW Statistics v.18.0.0).

### Results

Sows with a parity of  $3.6 \pm 2.4$  (mean  $\pm$  SD), a weight of  $286 \pm 25$  kg and a backfat thickness of  $13 \pm 4$  mm gave birth to  $14.3 \pm 3.1$  piglets. The thickness of the uterus, determined  $2 \pm 0.2$  days before parturition (day  $113 \pm 1$  of gestation), was  $6.0 \pm 0.7$  mm. The only variable related with uterine thickness was backfat ( $P = 0.049$ ). An increase by 1 mm decreased the uterine thickness by  $0.05 \pm 0.02$  mm.

### Conclusions

Only backfat effected negatively uterine thickness before parturition. This may be due to deposition of fat within the muscle or due to a reduced myometrial hypertrophy, therefore impairing muscle tone and strength and prolonging parturition.





## REP-008 - SERRATIA MARCESCENS IN BOAR SEMEN: SULFHYDRIC PRODUCTION FROM GLUTATHION

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Bacteriospermia is a frequent finding in freshly extended boar semen and can lead to detrimental effects on semen quality and longevity. The most frequently isolated bacterial family in porcine semen was Enterobacteriaceae, main strains were *Serratia marcescens* (12,55%), (Ubeda et al. 2013). In the search of new substances to improve the characteristics of the extenders or that preserve the quality of the semen doses, we discovered an unpleasant odor generated by the growth of *Serratia marcescens* in presence of glutathione.

Five ejaculates were fractionated and diluted into extender and stored at 10°C and 16°C. Base-extender was used as control (16°C). In the samples stored a 16°C, a strong odor was detected (in T1 and T3 samples) with no sperm movement. Bacteriological study of the samples, showed high number of *Serratia marcescens* (>3000 ufc/ml). Semen quality was maintained in the control samples.

**With the aim of identify the source of the problem**, extenders with possible combinations of antioxidants were developed: T1 (A2+A3), T2 (A1+A3), T3 (A1+A2) and T4 (control, no antioxidants). Five ejaculates were collected, extended into diluents and stored at 16°C. Semen was evaluated after 2 and 7 days. Next parameters were evaluated: total motility, progressive motility, hypo-osmotic swelling test (Host), osmotic resistance (Ort) and pH. T2 showed the highest percentages in semen quality. pH remained stable in T2 and no odor or bacterial growth was registered. Glutathione was termed as A2. It was the source of semen damage.

Glutathione is a potent antioxidant, preventing damage to important cellular components caused by reactive oxygen substances (ROS). Its benefits in semen conservation have been demonstrated in freezing extenders. However, GSH acidifies the medium and facilitates the growth of bacteria at higher temperatures. The odor is produced by action of anaerobic sulfure bacteria in sulfhydryl group.



## REP-009 - RISKS DERIVED FROM THE INTERACTIONS OF VARIOUS PLASTIC COMPOUNDS PRESENT IN MATERIALS USED FOR ARTIFICIAL INSEMINATION

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Studies have shown that some chemical compounds transferred from plastics to the semen cause reproductive failure in humans, animals ... and this fact has alerted the health agencies in recent years.

Artificial swine insemination involves many interactions where spermatozoa are in direct contact with plastics such as: semen collection bag with filter, storage and transportation of semen doses in heat sealing tubes or blisters, ...

The lack of control of the composition of plastic material has caused serious problems in reproductive indexes in swine worldwide (Europe, EE.UU, ...).

Analysis and characterization of toxic compounds of different plastic materials used in swine artificial insemination were studied with techniques that analyze volatile and semi-volatile compounds with high sensitivity and the migration study was performed too (to confirm the transference of these compounds to the semen solution). The results confirm that there are different toxins present in the plastics such as: lactones, erucamide, BADGE and phthalates.

Different trials have been performed in Magapor with different toxins found, the techniques used were: CASA system, flow cytometry (early apoptosis, mitochondrial activity, viability and reacted acrosomes) and in vitro fecundation tests.

Test with Badge, found in blisters, as primarily responsible for reproductive failures, 24 hours after the incubation the flow cytometry results showed differences in reacted acrosome (10% Control VS 22% Problem) and in vitro fecundation was 14% lower in the problem sample. Other toxic compound, phthalate, found in heat sealing tubes was evaluated, spermatozoa preserved in control and problem sample passed all the tests, and no differences were observed between storage in both but, in vitro fecundity tests showed differences in the Problem sample (22% lower respect to the control).

From the study it is clear that it is extremely important to carefully control the materials that come in contact with semen to avoid possible reproductive problems at farm.



## REP-010 - THE IMPACT OF INDUCED PARTURITION ON CELLS, CYTOKINES, COMPLEMENT AND LYSOZYME IN PORCINE COLOSTRUM

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Synchronizing parturition with prostaglandins can bring benefit of easier supervision of farrowing. It brings improved colostrum intake of whole litter with benefits of higher IgG concentration in piglets serum. However, the induction of farrowing negatively affects the colostrum yield, fat content and piglet growth rate. It is not known if the farrowing induction can affect the composition of colostrum immune components.

In this study, we determined immune cells composition (flow cytometry), cytokine levels (GM-CSF2, IL-1 $\beta$ , IL-4, IL-6, IL-10, TGF $\beta$ 1, TGF $\beta$ 2, TNF $\alpha$ ) (ELISA), lysozyme concentration (radial diffusion in agarose gel mixed with *Micrococcus luteus*) and complement activity (bioluminescence-based method using transformed *E. coli* K12 with luxABCDE gene) in colostrum of sows with induction of parturition and non-induced parturition. Samples of colostrum of 37 multiparous sows from which 19 were given 175  $\mu$ g of cloprostenol were collected in time interval between the birth of the first and fourth piglet.

A significantly higher proportion of lymphocytes (32,0%  $\pm$ 16,3) was found in colostrum from induced sows compared to colostrum from non-induced sows (13,8%  $\pm$ 10,5). There was not found any significant difference in numbers of granulocytes and macrophages. No significant differences were found in the proportions of lymphocyte subpopulations comparing colostrum from induced and non-induced sows. Significantly higher levels of IL-1 $\beta$ , IL-6, TGF $\beta$ 1 and TNF $\alpha$  were found in samples from sows with induced parturition. Significantly higher lysozyme concentration was found in samples from sows with non-induced parturition. There wasn't found any significant difference between samples from induced and non-induced sows in complement activity.

According to our results we can conclude that the induction of parturition can influence the immunological quality of colostrum.

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## REP-011 - COMPARISON OF TWO DIFFERENT PROTOCOLS OF INSEMINATION AND ITS EFFECT ON THOSE PARAMETERS REPRODUCTIVE

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### Introduction

There are different protocols of artificial insemination (IA), with different results. The objective of this study was to compare two protocols of IA, and evaluate the productive results as well as the cost of IA by sow.

### Materials and methods

The experience was realized in a farm located in Córdoba province (Argentina), with 1000 hyperprolific sows. They were two protocols of AI: the Traditional Protocol (PT), and an Alternative Protocol (PA). The PT was to pass to the male 2 times a day (8 am and 5 pm) and to detect zeal. The sow that manifested zeal was inseminated 10 hours after, and subsequent IAs were realized with 12 hours of interval while persisted the zeal. PA consisted of passing only once a day in the morning the male (8 am). The sow that presented zeal was inseminated 10 hours after. If in the next morning it demonstrated zeal it will be inseminated again. 24 hours post second dose if the sow continues demonstrating zeal, it will be inseminated again. In both protocols we worked with sow multiparous and nulliparous. Were evaluated 6 weeks of production (321 services) of which 159 services correspond to the PT, with 3 doses of I.A and 162 services to the PA with 2.28 dose of I.A. The cost per dose is of €4.16.

### Results

It was not observed differences in born live (BN) (PT: 12.80 and P.A: 12.78), and born dead (BD) (PT: 0.64 and P.A: 0.68), between those systems. The fertility PT was 92.45% (147/159) and the 93.20 (151/162) for PA.

### Discussion

The protocols not showed significant differences ( $P > 0.05$ ) in what respect to those parameters reproductive. Was observed a higher fertility rate and was needed less amount of IA in the PA, which a saving by week of € 153.



## REP-012 - EFFECT OF INTRAVAGINAL PROSTAGLANDIN E2 APPLICATION IN FARROWING SOWS DURING PARTURITION

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The duration of birth is an important parameter linked to piglets' survival and sow's health regarding parturition. Therefore, prolongations are frequently treated with oxytocin - a potent uterotonic agent - although several undesirable side effects including umbilical cord lesions, meconium staining and weak piglets have been described. In human medicine, Prostaglandin E2 (PGE2) is used, because less side effects have been observed. The aim of this pilot study was to test the influence of 2mg PGE2 gel applied intravaginally after birth of the fourth piglet on the birth process.

Three randomly selected sows in a pig herd were treated with PGE2-Gel (group T) and three other sows were treated with a placebo gel (group C). The total duration of birth (time between first piglet and last placenta) and the piglet interval were recorded, and each piglet was scored for meconium staining and vitality.

In group T the duration of birth was 386 min (average of 20.0 piglets per litter) compared to 439 min in group C (average of 14.3 piglets per litter). The piglet interval was 10.2 min in group T compared to 14.3 min in group C. No or slight meconium staining was observed in 53% and 35% of piglets in group T, and in 70% and 30% of the piglets in group C. Severe meconium staining was only found in 12% of piglets in group T. Moreover, 10% of piglets in group T showed an oedematous and haemorrhagic umbilical cord, lethargy and anoxia.

This study describes an impact of PGE2 gel on the birth process in sows. While duration of birth and the piglet interval tended to decrease, umbilical cord lesions, fetal distress and anoxia increased. Further investigations should focus on the optimal dosage of PGE2 in order to evaluate the use of this drug in farrowing sows.



## REP-013 - EFFECT OF ALTRENOGEST (ALTRESYN®) SYNCHRONISATION ON THE REPRODUCTIVE PERFORMANCE OF GILTS IN ONE WEEK BATCH MANAGEMENT SYSTEM

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### Introduction

Optimal use of the gilt pool has a major impact on reproductive efficiency and on the farm economy. Oestrus synchronization in gilts by using altrenogest (Altresyn®) is widely used because of proven effectivity for maintaining a constant flow of breeding animals, optimal age of first insemination and utilisation of farrowing space.

The aim of this study was to evaluate impact of synchronization on reproductive performance and optimal age of insemination on large scale farm with one week batch management in Germany. Two periods were compared- period before synchronisation and after its implementation.

### Materials and method

Performance of 357 not synchronised, cycling gilts (group A) was compared with 3177 gilts synchronised by altrenogest (group B). Treatment group was individually fed by 20 mg of altrenogest at the same time once daily for 18 days. Following parameters were evaluated: age at first insemination, total number of piglets (TBP), live born piglets (LBP). Length of lactation and gestation period was evaluated. Data were obtained from sow planner (Microsoft Excel) and evaluate by statistical programme SPSS 22.0.

### Results

The average age during first mating in group B was  $255 \pm 11$ , group A  $244 \pm 14$  (optimal parameter 250-260 days according breeding standard). TBP and LBP parameters were better in treatment group ( $14,6 \pm 3,5^b$  and  $13,4 \pm 3,3^b$  in comparison with  $13,6 \pm 3,4^a$  and  $12,6 \pm 3,1^a$ )(a;b;  $p < 0.05$ ). The average gestation period was shorter in group B with less variability and consequently lactation period was 1,3 days longer in comparison with group A ( $p < 0.05$ ).

### Discussion

Synchronisation of gilts in one-week batch management system was effective way of control of age at first insemination and provide better utilisation of farrowing crate which increased litter performance parameters (TBP, LBP). Less variable farrowing and consequently longer lactation period in average was observed for synchronised gilts.



## REP-014 - COMPARING A BREEDING SCHEDULE WITH BUSERELIN/DOUBLE FIXED AI AGAINST ECG/HEAT-ORIENTED AI: PRELIMINARY RESULTS

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### Background & Objectives

“Single fixed time insemination” (sFTI), a technology based on a treatment with the GnRH-analogue Buserelin (Porceptal®) in order to induce ovulation in gilts and sows, followed by only a single AI both at defined times, has been proven to yield good fertility results. However, farmers are reluctant to switch to sFTI when they have been using post-weaning (pw) eCG and double heat-oriented AI (d-hAI). The aim of this study was to compare d-hAI against a program consisting of a Buserelin treatment followed by a double AI at fixed times (B-dFTI).

### Materials & Methods

Study was performed in a 1,350 BHZP genetics sow farm with 4-wks lactation and weekly batch farrowing. A total of 55 sows (parity 2-4) were randomly allocated to either d-hAI (n = 29) or B-dFTI (n = 26). The d-hAI group received 800 IU eCG IM at 24 and B-dFTI 10 µg Buserelin at 84 hrs pw. Heat checking was done AM/PM. d-hAI were inseminated twice based on standing heat, while B-dFTI received AI twice at 30-33 and 12-14 hrs post GnRH. Conception (CR) and farrowing rates (FR), returns, fallouts as well as total (TB), live (LB) and stillborn piglets (SB) were recorded.

### Results

All d-hAI and 25/26 B-dFTI sows were in heat within 4 days pw. All sows were bred regardless of standing heat. All sows but the one B-dFTI sow without heat conceived (CR 100 vs. 96.1% for d-hAI and B-dFTI). Two d-hAI and two B-dFTI sows either aborted or died, respectively. The FR was similar for d-hAI and B-dFTI sows (93.1 vs. 92.3%). Groups did not differ in mean TB, LB and SB.

### Discussion & Conclusion

In summary, a breeding program with Buserelin treatment followed by two inseminations at fixed times performs as good as a traditional program with eCG and double heat-oriented AI.



## REP-015 - EFFECT OF DIFFERENT TREATMENT PROTOCOLS USING FERTIPIG® ON OVULATION AND WEAN-ESTRUS-INTERVAL OF WEANED SOWS: PRELIMINARY RESULTS

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### Background & Objectives

Gonadotropins (Go) are frequently being used post weaning (pw) to induce fertile estruses and thus reduce weaning-estrus interval (WEI). Different time schedules for the application of Go pw are available, with the most common being 0-24 hours pw. This study was aimed to test the effect of a combination of 400 IU eCG/200 IU hCG (Fertipig®) administered at different time intervals pw on ovulation and WEI.

### Material & Methods

Study was performed on a 4.500 farrow-wean sow farm with a mean lactation length of 26 days in Germany. Forty sows of different parities (2-8) were randomly allocated to 3 groups (n = 10/group) being different in the time Fertipig was administered: 24 hrs prior to (G-I), at (G-II) and 24 post pw (G-III). A fourth group (G-IV; n = 10) remained untreated and served as a control. Ultrasonography was performed to monitor ovulation on days 5 and 6 pw, and the WEI determined.

### Results

Fertipig accelerated ovulation, as 9,7 and 8 sows of G-I, G-II and G-III had ovulated on day 6 pw compared to 4 in G-IV; G-I performed best in this regard with 4 sows having already ovulated on day 5 pw compared to zero in the other groups. WEI was shortest in G-I with 103.2 hrs, but was not different if compared between G-II, G-III and G-IV with 115.2, 119.3 and 117.6 hrs, respectively.

### Discussion & Conclusion

In conclusion, application of Fertipig® 24 hrs prior to weaning more effectively accelerates ovulation and shortens WEI compared to a treatment at or 24 hrs pw, and even more if compared to untraded controls.





## REP-016 - OCCURRENCE OF BACTERIA IN MILK SAMPLES AND BIOPSY SAMPLES OF THE MAMMARY GLAND FROM HEALTHY SOWS

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### Background & Objectives

Mastitis of sows as a part of the postpartum dysgalactia syndrome is a common problem in pig production. Coliform bacteria are considered to play a predominant role, but also other species can be isolated from affected sows. Diagnostic investigation is complicated, because often even in milk samples from healthy sows a similar bacterial content can be detected. Contamination during sample collection is supposed to be a possible reason. Therefore the objective of the study was to verify by comparative bacteriological examinations of milk samples and mammary gland biopsies whether a better assessment of the bacteriological status is possible using biopsies.

### Material & Methods

Milk samples and biopsies for bacteriological investigation were taken from the same complex of 25 healthy sows one day postpartum. Teats and the mammary skin were cleaned and disinfected accurately. First spray of milk was rejected before sample collection. Biopsies were taken after local anaesthesia using a 7cm biopsy needle. Tissue samples of 18 sows additionally were examined histologically.

### Results

In 96% of milk samples and 92% of biopsies a low bacterial content with up to five different species could be found. Both Gram positive (mostly streptococci, staphylococci) and Gram negative bacteria (mostly *Escherichia coli*) could be detected. Histopathological examination revealed generally mild interstitial infiltration of inflammatory cells, mainly plasma cells and lymphocytes. In rare cases neutrophils were also detected (interstitium/secretory alveoli).

### Discussion & Conclusion

Biopsy samples of the mammary gland provided no advantages for bacteriological diagnosis compared to milk sampling. It is assumed that in the milk as well as in the mammary parenchyma of clinically healthy sows after parturition, ubiquitous bacteria are present regularly. As a consequence an increased inflammatory cell count in the mammary glands during lactation seems to be physiologically. In case of neutrophilic infiltration a subclinical mastitis cannot be excluded.



## REP-017 - IMPROVED FARROWING PEN UTILISATION AND EFFICIENCY THROUGH SYNCHRONISATION OF GILTS

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### Background & Objectives

Efficiency of achieving weekly output, in terms of sow and gilt farrowings, is a key component for effective reproduction management and efficiency. Ineffective flow management results in large variation both between and within weekly service numbers, deviating over 26% away from the target rate. This will directly cause problems with the utilisation of farrowing accommodation. The organisation of gilt introduction is a key driver for achieving consistent output, due to a direct beneficial effect on total number of services per week.

Opportunity exists using Altrenogest (AG) (Altresyn<sup>®</sup>, Ceva Animal Health) to effectively achieve gilt synchronisation and consistent gilt numbers. The potential for achieving consistent farrowing throughput can be linked to an economic growth algorithm, known as the nursing period utilisation rate (NPUR).

### Material & Methods

A 1000 sow unit with a weekly batch target of 46 services, including both sows and gilts, has utilised these management tools, resulting in a target of 41 farrowings per week. Altresyn<sup>®</sup> was used in a gilt management protocol for synchronising oestrus, altering progesterone concentrations. The product was administered following a tailored protocol, with 18 treatment days per animal. Data analysis was carried out in relation to the NPUR, allowing quantification of performance and economical benefits.

### Results

Data showed consistent gilt replacement rate, leading to controlled total service numbers, from 42-48 per week. Reduced variability in the number of total services per week, with corresponding target farrowings were achieved. A 50% reduction in NPUR was observed (from 26% to 13%), when following the Altresyn<sup>®</sup> protocol.

### Discussion & Conclusion

The use of the NPUR calculation is highly beneficial for bench-marking performance in relation to weekly service targets and aiming to achieve effective production throughput on farm.



## Residents' ECPHM Session

### RES-001 - SUPPLEMENTATION OF TALL OIL FATTY ACID AND RESIN ACID IN LATE PREGNANCY DIET OF SOWS CAN AFFECT COLOSTRUM QUALITY

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#### Background & objectives

Colostrum plays an essential role in piglet survival and growth, providing the piglets with a source for both immunoglobulins and energy. The neonatal piglets lack immunoglobulins, which makes them dependent on colostrum as the sole source of antibodies. The aim of this study was to examine whether tall oil fatty acid and resin acid (RA) derived as a co-product in pulp production, added to a late pregnancy diet affected colostrum composition and yield (CY) in sows.

#### Materials & Methods

44 sows were randomly allocated to two groups as follows: a negative control diet (n= 21) and the same diet supplemented with 5 ml RA/day (n=23) during the last week of pregnancy till farrowing. The RA used was Progres® (Hankkija Oy/Suomen Rehu, Hyvinkää, Finland). Within the first 2 hours from the beginning of farrowing, a 10 ml colostrum sample was obtained to check for nutritional composition (protein, fat, lactose, dry matter, with FITR analysis), and immunoglobulins content (IgA, IgM and IgG with ELISA analysis). All piglets were individually weighted at birth and 24 hours later in order to calculate CY.

#### Results

Colostrum content of protein, lactose, fat and dry matter did not significantly differ between the two groups. While RA fed sows had higher level of IgG ( $86.37 \pm 5.27$  mg/ml vs  $70.94 \pm 5.49$  mg/ml) in colostrum ( $p < 0.05$ ), IgA and IgM levels in colostrum remain same in the two groups. There was also no changes in the CY of the sows in two groups.

#### Discussion & Conclusions

In conclusion, adding RA to late pregnancy diet in sows did not affect the CY and protein, fat and lactose content in colostrum, but contributed to higher IgG content. Therefore RA added to sow diet seems to increase colostrum IgG ensuring better survival of neonate piglets.



## RES-002 - ASSESSMENT OF SWINE INFLUENZA VIRUS SUBTYPES IN SPANISH PIG FARMS DURING THE PERIOD 2015 TO 2016

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### Background & Objectives

Swine influenza (SI) is caused by type A influenza viruses (IV). Pigs can be infected by avian and human IV. The aims of present study were to assess the prevalence of the different subtypes involved in clinical cases from Spain where SI was suspected.

### Material & Methods

A total of 40 farms were studied during 2015-16; from each farm, submissions consisted of nasal swabs (5-15 samples) that were taken from animals with clinical respiratory/reproductive signs in which SI was considered in the differential diagnosis. An RT-qPCR for IV was carried out. Positive samples with a Cq value <30 were selected and investigated with subsequent RT-qPCRs to determine the subtype.

### Results

29 out of 40 submissions tested positive for IV. The most prevalent subtype was H1avN1 (13) followed by H1huN2 (8) and H1avN2 (7). Also, pandemic subtypes were detected from 3 submissions and H1huN1 from another one. An H3N1 was detected in a single farm, but not the classical H3N2. Five submissions yielded two combinations of IV subtypes (three with H1avN1/H1N2, one with H1avN1/H1huN1 and one with H1N2/H1avN2). One positive submission was not able to be subtyped.

### Discussion & Conclusion

Swine influenza viruses (SIV) are widespread in many countries all over the world, being considered endemic in Spain. This study characterized the different subtypes found in several clinical scenarios compatible with SIV infections, including respiratory (majority) and reproductive problems. Although different serological studies have indicated that all three subtypes are highly prevalent in Spain, detection of SIV in studied clinical cases accounted mainly for H1N1 and H1N2 subtypes. Also, this study represents one of the first descriptions of the H3N1 subtype in Spain, since to authors' knowledge this subtype was reported only once in 2008 in the country. Noteworthy, none of the cases were associated with infection by the H3N2 subtype.



## RES-003 - A CLINICAL APPROACH ON INVESTIGATING AND AUDITING SEMEN PRODUCTION PRACTICES IN AN ARTIFICIAL INSEMINATION CENTER FACING REDUCED SOW FERTILITY RESULTS

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This report describes a clinical case of reduced fertility in an artificial insemination (AI) center with Piétrain terminal sire boars. On October 2015, four herds receiving semen doses from the AI center reported repeat breeding rates between 60% and 100%. The anamnesis revealed that reduced fertility results concerned not only the older boars, but also young ones. Initially, extended semen doses from 11 boars were examined at the third day of storage. Results showed that semen from six boars (6/11) exhibited reduced motility and progressive motility, with an average of  $56.7 \pm 11.20\%$  and  $23.5 \pm 6.32\%$ , respectively. Also, 11/11 semen doses had a high percentage of dead sperm cells (average  $17.36 \pm 10.38\%$ ). Clumping was observed in 3 out of 11 doses. Additionally, 3/6 doses with low motility were used for bacteriological analysis. Standard culture and Maldi-Tof MS identified high concentrations of three bacterial contaminants (*Stenotrophomonas maltophilia*, *Oerskovia xanthineolytica* and *Ochrobactrum anthropi*) and profuse antimicrobial resistance to commonly used antimicrobials. Next, morphological examination of pre-diluted semen samples from 21 boars showed that 10/21 samples had a high percentage of dead sperm cells (average  $21.75 \pm 11.11\%$ ), and severe clumping in 9/21. All above mentioned results indicated severe bacterial contamination, happening already prior to semen dilution. Thus, it was decided to monitor all routine procedures and perform an audit. The audit measures concerned the process of semen collection and the practices of the personnel in the dummy area and the laboratory. After three weeks of implementation, the repeat breeding rates in all herds returned to the levels that existed before October 2015 (between 5% and 7%). To conclude, when severe bacterial contamination occurs, investigations should focus on: 1) assessing semen doses prior and after dilution; 2) bacteriological culture, typing and antimicrobial resistance testing; 3) following-up of all procedures, from semen collection to packing, to identify the sources of contamination.



## RES-004 - SUCCESSFUL USE OF AN AUTOGENOUS VACCINE AGAINST STAPHYLOCOCCUS HYICUS IN A HERD FACING SEVERE EXUDATIVE EPIDERMITIS PROBLEMS IN WEANED PIGLETS

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An autogenous vaccine against *Staphylococcus hyicus* (*S. hyicus*) was applied in a 1000-sow commercial herd facing recurrent problems with exudative epidermitis. The herd operated a four week-batch production system. On October 2014, a mortality increase (+4.2%) was reported during the third week of the nursery period, in addition to an already existing mortality of 2.5% in the nursery (~50% morbidity). Skin swabs were taken from five affected nursery pigs and the typing of the isolates revealed that they were infected with *S. hyicus* strains producing the type B exfoliative toxin. Consecutively, three out of five isolates were used to produce an oil-based autogenous vaccine against *S. hyicus*. Then, from four consecutive farrowing groups of sows, two groups were vaccinated against *S. hyicus* at five and two weeks before farrowing, and two sow groups remained non-vaccinated. Efficacy of vaccination was measured by weighing 20% of the piglets from each group of sows at weaning and at the end of the nursery period. Additionally, mortality and treatment data were collected. Results showed that piglets from the vaccinated sows were on average  $410 \pm 43$ g ( $P=0.000$ ) and  $1000 \pm 250$ g ( $P=0.011$ ) heavier than the ones from the non-vaccinated sows at weaning and at the end of the nursery period, respectively. The piglets of the vaccinated sows had a 2.20% lower mortality than the piglets of the non-vaccinated sows ( $P=0.003$ ). Injectable antimicrobials were applied in 0.33% of the piglets from the vaccinated sows and in 1.22% of the piglets from the non-vaccinated sows ( $P=0.000$ ). To conclude, vaccination significantly improved weights, and reduced overall mortality and the use of antimicrobials in the group of pigs from the vaccinated sows, when compared to the pigs from the non-vaccinated sows. The results also indicate that lack of maternal immunity can lead in recurrent exudative epidermitis outbreaks during the early nursery period.



## RES-005 - HAEMORRHAGIC BOWEL SYNDROME IN ROUTINELY TIAMULIN TREATED FATTENING PIGS

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### Introduction

Haemorrhagic bowel syndrome (HBS) is a sporadically occurring disorder in fattening pigs, characterized by sudden death in combination with severe abdominal distension and intense red colouration of the intestines. Deep understanding of aetiology and pathogenesis of HBS is still lacking, but several risk factors such as liquid feeding, whey feeding and specific bacteria have been described.

### Case Description

In a continuously stocked fattening farm with 1500 pigs and liquid feeding based on whey, the mortality rate increased from 1.7% to 3.5% during summer time. Sporadic sudden death of growing pigs occurred with severe abdominal distension as the main picture at that time. All new batches received in-feed medication with Tiamulin due to previous detection of *Brachyspira hyodysenteriae*, although a partial sanitation was conducted after that finding. No changes of the origins, housing and the feeding procedure were reported. A herd examination was conducted, revealing a hygienic problem in the feeding system. For further diagnostics a necropsy was performed, showing a pale carcass with a bloated abdomen and increased amounts of blood in the intestine. A feed analysis was conducted, revealing a pH-value of 5 in the feeding soup, and a severe contamination with *Enterobacteriaceae* was detected. Based on these examinations, HBS was diagnosed. Subsequently, the farmer controlled the pH-value of feeding soup with formic acid, improved the cleaning procedure of the liquid feeding system and stopped the in-feed medication. Due to these measures performance characteristics improved significantly.

### Conclusion

In the present case, it is likely that the causes of the clinical manifestation of the HBS were a contamination of *Enterobacteriaceae* in the liquid feed, a hygiene problem of the feeding system and a long lasting dysbacteria of the intestinal tract due to the non-justified routine use of antimicrobials. Speculatively, the prophylactic antibiotic treatment was not only unnecessary, but might even have increased clinical manifestation.



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Poster session

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RES-006 - WITHDRAWN





## Veterinary Public Health & Food Safety

### VPH-001 - TARGETED EFFORTS IN SWINE HERDS CAN REDUCE THE USE OF TETRACYCLINES OR REPLACE TETRACYCLINES WITH NARROW SPECTRUM ANTIBIOTICS

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#### Background & Objectives

As part of the political aim to reduce MRSA CC398 in Denmark, it was decided to lower the use of tetracyclines as they are believed to promote the presence of MRSA over MSSA.

The aim was to evaluate if the consumption of tetracyclines in swine herds could be reduced through targeted efforts (intervention) as suggested by the practicing veterinarian.

#### Materials & Methods

The practicing veterinarian selected 21 swine herds with a potential for lowering their use of tetracyclines. Herds were selected, when it was evaluated that interventions could reduce the use of tetracyclines. The exact interventions in each herd were not recorded. The consumption of tetracyclines was lowered either by reducing the amount of tetracyclines used or by replacing tetracyclines with narrow spectrum antibiotics.

Antibiotic use in each swine herd was evaluated for a period 9 months before and 9 months after the intervention on the basis of the number of prescriptions made by the practicing veterinarian for the herd. Data was collected from the national registry of antibiotic use on Danish farms (VETSTAT).

#### Results & Discussion

The consumption of tetracyclines was lowered in 14 out of 21 herds and in 9 herds a significant reduction (> 75 % reduction) was achieved. In most of these herds tetracyclines were replaced with narrow spectrum antibiotics.

The aim of the interventions was to eliminate the disease from the farm through changes in management or through less severe outbreaks, and the ultimate aim was to reduce consumption of tetracyclines. In herds where tetracyclines were replaced by narrow spectrum antibiotics, analyses generally revealed only one or few pathogens to be the causative agents.

#### Conclusion

Results show that through a strategic effort it is possible to either lower the use or to replace tetracyclines with narrow spectrum antibiotics in some, but not all herds.



## VPH-002 - STRONGYLOIDES RANSOMI AND TRICHURIS SUIS: INDICATORS FOR HYGIENE ON A SOCIAL DAY CARE FARM

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### Background and Introduction

Infections with the nematodes *Strongyloides ransomi* (threadworm) and *Trichuris suis* (whipworm) are, although ubiquitous, rarely found in modern pig farming. This paper describes the detection of these parasites in pigs on a social day care farm for disabled people. The farm was also open to the general public, and included 41 pigs, 7 cows, and a farm store selling vegetables, cheese and meat. The pigs showed clinical signs of chronic growth retardation, wasting and skin lesions suggestive for mange infection. Detection of *S. ransomi* was documented for the first time in the Netherlands.

### Material, Methods, Results

In April 2016 eggs of *S. ransomi* and *T. suis* were found in a fecal sample. Additional samples taken several weeks later from four pens with finishers confirmed the diagnosis: 5/7 fecal samples positive for eggs of *S. ransomi*, 1/7 positive for eggs of *T. suis*. In addition eggs of the roundworm (*Ascaris suum*) and mite eggs were found. The fecal samples were examined using the centrifugation-flotation method.

### Discussion & Conclusion

Risk factors for the occurrence of *S. ransomi* and *T. suis* were present: 1) use of straw bedding, 2) lack of cleaning and disinfection of the pens and 3) access to outdoor paddocks with soil. Recommendations given included: (a) treat the parasitic infections, (b) improve hygiene by regular cleaning and disinfecting pig compartments, and thus breaking the infection cycle of the parasites, (c) improve hygienic precautions for humans. Although *Strongyloides ransomi* and *Trichuris suis* do not pose a zoonotic danger, they do indicate lack of hygiene and insufficient healthcare highlighting the possible risks for the presence of zoonotic diseases. This is all the more important because an increasing number of patients is taken care of at social day care facilities on farms with animals.



## VPH-003 - EVALUATION OF ANTIMICROBIAL DRUG USE IN 128 PIG FARMS IN SWITZERLAND

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### Background & Objectives

In Switzerland the SuisSano Programme was implemented in order to monitor antibiotic use on pig farms. Data gained from 128 farms joining the SuisSano Programme were used to analyze antimicrobial consumption in the field.

### Material & Methods

The amount of antimicrobial consumption in 2015 was calculated on 128 farms based on veterinary prescriptions. For calculation the technical unit „Defined Course Dose Switzerland (DCD<sub>ch</sub>)“ was used in analogy to the DCD<sub>vet</sub> units defined and published by the EMA. The DCD<sub>ch</sub> are defined as the quantity of active ingredient necessary for a complete therapeutic course by multiplying highest dosis and maximum duration of treatment as published in the approved national Summaries of Product Characteristics (SPCs). The number of DCD<sub>ch</sub> was analyzed for four age groups with standardized weights (piglets: 2kg; weaning pigs: 10kg; fattening pigs: 25kg and sows 200kg) and antimicrobial classes.

### Results

88.13% of all prescribed antimicrobials in piglets (408'548 DCD<sub>ch</sub>) belonged to the class of the penicillins and 8.46% to fluoroquinolones (FQ). In weaning pigs (114'447 DCD<sub>ch</sub>) the proportion of penicillins was 39.25%, polypeptides 17.53%, tetracyclines 15.95%, macrolides 10.83% and FQ 3.68%.

65.16% of all prescriptions in fattening pigs (47'308 DCD<sub>ch</sub>) belonged to the class of the penicillins, 8.49% to sulfonamides, 7.73% to tetracyclines, 6.49% to aminoglycosides, and 5.55% to macrolides. In sows (6'713 DCD<sub>ch</sub>) the proportion of penicillins and FQ was 56.48% and 15.42%, respectively.

### Discussion & Conclusion

The number of DCD<sub>ch</sub> does not represent the effective number of treatments, but provides an useful approximation of the proportion of antimicrobial consumption for different antimicrobial classes. The proportion of prescriptions of Highest Priority Critically Important Antimicrobials (HPCIA) was moderate over all age groups, but still should be of concern. Further studies are required concerning the effective therapeutic intensity for these drugs.



## VPH-004 - NEW COMBINATIONS OF FEED ADDITIVES TO FIGHT SALMONELLA TYPHIMURIUM IN PIGS

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Salmonellosis is a common cause of intestinal disease in human. The EFSA attributes 15-23 % of cases to pigs and pig meat. Reduction of the level of Salmonella in the intestinal tract of pigs will reduce the number of human Salmonella cases. Anti-bacterial feed additive strategies against Salmonella include direct antibacterial effects, like reduction of bacterial membrane function or competitive binding of bacteria, and support of intestinal function to reduce colonization. Combining different strategies may increase the anti-bacterial effect of feed additives.

Several compounds and combinations were evaluated *in vitro* on both antibacterial activity (MIC) and binding ability to *Salmonella typhimurium*. The best performing compounds were selected for *in vivo* testing.

An optimized combination of organic acids (antibacterial activity) with either hydrolyzed copra meal (CM) or rye overgrown with selected mycelium (ROM) (both competitive binding to bacteria and modulating immune response) was administered after weaning during the whole study period. Piglets got infected with *Salmonella typhimurium* 10 days after weaning for 7 consecutive days.

In a second *in vivo* study a combination of the organic acid blend, CM, and ROM was administered after weaning during the whole study period. The animals got infected with *Salmonella typhimurium* 5 days after weaning for 7 consecutive days.

Combinations of the organic acid blend with either CM or ROM reduced Salmonella peak shedding during the first days of infection (3.8 or 3.9 log CFU/gram vs 5.0 log CFU/gram in the control group). In the second study the combination of the organic acid blend, CM, and ROM reduced *Salmonella typhimurium* peak shedding during the first days of infection (4.0 log CFU/gram vs 5.1 log CFU/gram in the control group).

Therefore, preventive use of combinations of feed additives using different strategies may reduce the Salmonella prevalence in the feed to food chain.



## VPH-005 - VACCINATION OF SOWS AGAINST SALMONELLA TYPHIMURIUM HAS A POSITIVE EFFECT ON THE LEVEL OF MATERNAL IMMUNITY TRANSFERRED TO PIGLETS

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Pig herds are frequently infected with *Salmonella* Typhimurium. Infections are mostly subclinical and difficult to control with the currently available control measures. Vaccination might be effective to control *Salmonella* infections at farm level and hence be a promising tool to reduce the risk for human salmonellosis.

In this study, the serological response of vaccination of sows against *Salmonella* Typhimurium was tested and compared to a non-vaccinated control group on three Belgian farrow-to-finish herds. An attenuated vaccine (Salmoporc<sup>®</sup>, IDT Biologika) was administered subcutaneously twice, 6 and 3 weeks before farrowing. Blood samples were collected before and after vaccination to evaluate the serological response of vaccination. Blood samples of 3-day old piglets were collected to evaluate the level of maternal immunity transferred from the sows to the piglets. The sera were analyzed by ELISA and the sample-to-positive-ratios (S/P-ratio) were assessed.

Prior to vaccination, the S/P-ratios of the vaccinated group and the S/P-ratios of the non-vaccinated group did not differ significantly (farm 1: 1.45 and 1.40, farm 2: 1.04 and 0.67, farm 3: 0.86 and 1.01). Three days after farrowing, the mean S/P-ratio of the vaccinated sows was significantly higher than the mean S/P-ratio of the non-vaccinated sows (farm 1: 2.53 and 1.11, farm 2: 2.26 and 0.45, farm 3: 1.85 and 0.96;  $p \leq 0.001$ ). At three days of age, piglets from vaccinated sows had a significantly higher mean S/P-ratio compared to piglets from non-vaccinated sows (farm 1: 2.67 and 1.33, farm 2: 2.69 and 0.40, farm 3: 2.02 and 0.45;  $p < 0.001$ ). A strong positive Pearson-correlation between the S/P-ratios of the sows and the S/P-ratios of the piglets was found on all farms (farm 1: 0.934, farm 2: 0.953, farm 3: 0.886;  $p < 0.001$ ).

Vaccination of sows induces a serological response and increases the level of maternal immunity which is transferred from the sow to the piglets.



## VPH-006 - DIVERSITY OF SALMONELLA SPP. AND LISTERIA MONOCYTOGENES ISOLATES FROM FREE-RANGE PIG PRODUCTION PLANTS

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### Background & Objectives

Zoonotic agents, such as *Salmonella* spp. and *Listeria monocytogenes*, are considered high-risk zoonotic pathogens by the European Food Safety Agency (EFSA). The aim of this study was to evaluate the diversity of *Salmonella* spp. and *Listeria monocytogenes* strains isolated along the pork chain production plants of two different slaughterhouses.

### Material & Methods

A total of 150 animals (fifteen animals/farm) were traced at both slaughterhouses to collect a total of five samples/animal (faeces, abrasive sponges at the pre-scalding point, ileocolic lymph nodes, tonsils and a pool of meat samples), that were analysed using specific ISO methodologies for the detection of *Salmonella* spp. and *Listeria monocytogenes*. Bacteria serotype was determined by means of an agglutination technique and by means a phage's panel provided by the International Reference Laboratory of phage typing. The genetic similitude of the isolates was evaluated by PFGE analysis.

### Results

The global prevalence of *Salmonella* (97 isolates) and *L. monocytogenes* (68 isolates) was of 12.93% and 9.07%, respectively. Thirteen different *Salmonella* serotypes were detected with monophasic *Salmonella* Typhimurium (mST) (21), Anatum (20), Typhimurium (17) and Hessarek (15), as the most frequent ones. The phage types 193 (17) and U302 (8) were the most common ones. Thirty-nine *L. monocytogenes* isolates were identified as serotype 4b and 28 samples identified as serotype 1/2a, with the three isolates recovered from meat samples belonging to the latter. Four different pulsotypes of *Salmonella* were identified, with the same clone being detected from meat and tonsil samples, which point out potential cross-contamination. Six different pulsotypes of *L. monocytogenes* were detected, but no evidence of cross-contamination was detected.

### Discussion & Conclusion

Our results highlight a high diversity in *Salmonella* and *L. monocytogenes* isolates recovered from free-range pig samples, with evidence of cross-contamination along the production chain in the case of *Salmonella*.



## VPH-007 - VIABILITY OF STREPTOCOCCUS SUIS, STREPTOCOCCUS DYSGALACTIAE AND TRUEPERELLA PYOGENES IN DRY-CURED PORK SHOULDERS AND LOINS

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### Background & Objectives

Dry-cured hams, shoulders and loins of autochthonous pig breeds reared in free-range systems are highly appreciated in national and international markets. The dry curing process is known to reduce the bacterial load of well-known foodborne pathogens; however, the viability of other pathogens has not been evaluated. Lymphadenitis, caused by pyogenic bacteria, is one of the main pathologies of pigs reared in these systems. Thus, the efficacy of the dry curing process on the viability of *Streptococcus suis*, *Streptococcus dysgalactiae* and *Trueperella pyogenes* along the dry curing of Iberian pork shoulders and loins was evaluated in this work.

### Material & Methods

Pork loins (28) and shoulders (17) were experimentally inoculated with 5-6 log CFU/ml of each selected pathogen and then were subjected to the routine dry curing process performed at the industry. A microbiological analysis to determine the viability of these microorganisms at different stages (T1: 24-h post refrigeration; T2: end of the post-salting stage / post-seasoning; T3: drying stage; T4: final product) was performed. In addition, pH and water activity ( $a_w$ ) values were recorded along the dry curing process.

### Results

A progressive reduction in the bacterial counts of all the analysed pathogens was observed, showing a statistically significant reduction from T1 to T4, where none pathogen was isolated. A significant positive correlation was observed between the dynamics of bacterial load,  $a_w$  and pH values along the dry-curing of loins.

### Discussion & Conclusion

The dry curing process allows the elimination of *T. pyogenes*, *S. suis* and *S. dysgalactiae*. Although salting of dry-cured shoulders had a moderate bactericidal effect, results of this study suggest that drying and ripening were the most important stages to obtain dry-cured products free of these microorganisms.

(This study has been already published in Food Microbiology: Cardoso-Tosef et al., 2017. 61; 66-71).



## VPH-008 - TRANSFER OF DEOXYNIVALENOL FROM A SOW TO PIGLETS

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Deoxynivalenol (DON), also known as vomitoxin, is produced by *Fusarium*. Pigs are one of the most sensitive farm animals to DON exposure. During years 2014 and 2015 vast part of feed was contaminated with moulds and simultaneously increased mortality of piglets occurred in many Czech farms. It is the reason why we pay attention to DON transfer from sow to an offspring. The aim of the present study was, besides describing the transfer, analyzing its potential impact on piglet's immune system.

Five sows and their litters were included in the study. Deoxynivalenol was applied to 3 sows intravenously daily within 2-3 days before delivery (300µg). Concentration of DON was analyzed using LC-MS/MS with high resolution. Maximum level of DON in plasma was 12 hours after application (19.3±2.7ng/ml) and decreased rapidly (2.5±2.1ng/ml during delivery). DON was nearly undetectable in colostrums. On the contrary, cord blood contained relatively high amount (11.8±5.8ng/ml). Deoxynivalenol concentration in piglet's plasma was (6.7±6.0ng/ml) 12 hours after delivery. This is the proof of significant intrauterine transfer. Furthermore, DON was still present in piglet's blood not only in the first week (1.8±2.1ng/ml) but also in the third week of the life (1.8±2.1ng/ml). It suggests lower capacity of piglets to metabolize DON in comparison to adult pigs.

Finally, we found significant differences between piglets affected by DON and control group in several immunological parameter such as lower production of reactive oxygen species, different ratio of CD4 and γδTCR lymphocytes, decreased level of regulatory T cells and lower plasma concentration of immunoglobulins.

In conclusion, short time exposure of sows to DON has strong impact on the offspring. Intrauterine transfer of DON occurred and seems to have much higher negative effect on piglets than transfer via colostrum. DON persists in piglet's blood up to three weeks and affects balance of immune system.

(MEYS LD15055)





## VPH-009 - SOFTWARE FOR MONITORING ANTIBIOTIC CONSUMPTIONS IN PIGS: FIRST RESULTS

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In the context of prudent use of antibiotics, the monitoring of consumption is indispensable to have better knowledge of practices in order to implement improvement actions and evaluate them. In this perspective, software for monitoring antibiotics consumption, INDICAVET, has been developed by the company DBM. This is an innovative tool that measures the real consumption through the prescriptions of veterinarians. It can be used by farmers and veterinarians and completes the current follow-ups done by ANSES in France: either monitoring of antibiotics sales declared by pharmaceutical laboratories, or consumption measured with a panel of farmers. The software collects production data (kg of meat produced) on the one hand, and prescription data from veterinarians on the other. Then, using official calculation methods, antibiotic consumption is evaluated through French and European official indicators (mg/kg, ALEA, DDDvet, DCDvet). In 2016, two years of monitoring and 165 swine farms were included in the database. The software can measure the efforts carried out by farmers and veterinarians and changes of use can be rapidly detected as proved by the two following examples. The first one concerns the use of colistin: due to EU referral, the SPC of premixes containing colistin were changed at the end of 2015. Indicavet measured a 42% reduction of the consumption of colistin in the next six months among the farmers' sample. Another example relates to the prescription of fluoroquinolones and cephalosporins, associated with strong constraints in France since March 2016: further to these measures, a 99% drop of the use of fluoroquinolones was observed over the last six months of the year. INDICAVET is a didactic tool that enabled farmers to have a reactive "diagnosis" of their antibiotics consumption. It helped veterinarians to make appropriate decisions through a personalized follow-up with target figures and measured the efficiency of conducted actions.



## VPH-010 - STABILITY OF REFERENCE GENES IN RELATION WITH STUDY OF GENES AFFECTING BOAR TAINT

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### Background & Objectives

It is essential to control variables by validation of normalization of the data when studying gene expression by the method real-time PCR. This validation of data is gained by comparison between expression profiles of studied genes and reference genes. Reference genes are constitutively expressed genes among different tissue. We focused on expression study of the genes involved in the degradation of skatole in porcine liver and thus reduction of boar taint in meat.

### Material & Methods

The aim of this study was to determine the most stable reference gene in liver of final hybrids of pigs. RNA was isolated from 20 boars and reverse transcribed to cDNA. The cDNA of each sample was analyzed using Rotor gene cycler and *Ct* (cycle of threshold) values were obtained from Rotor gene software. The mRNA stability of expression was studied for three genes: *TOP2B*, *TBP1* and *PPIA*, where the *Ct* value characterized the level of expression in liver for each gene. GeNorm application was used for determining the stability of analysed genes. **Results:** The obtained *M* value is the average pairwise variation of a particular gene with all other control genes. The most stable gene was *PPIA* ( $M = 0.823$ ), second most stable gene was *TOP2B* ( $M = 0.904$ ) and the least stable gene was *TBP* ( $M = 0.937$ ).

### Discussion & Conclusion

Our findings will serve as a tool for normalisation of expression data of genes of interest to test their association with the level of skatole and androstenone in meat and with the boar taint. We recommend using of *PPIA* gene as endogenous control in real-time quantitative PCR analysis for gene expression in porcine liver.



## Virology and Viral Diseases

### VVD-001 - NOVEL APPROACH TO EVALUATE THE SAFETY OF PRRSV VACCINES IN SOWS

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#### Introduction

Infections with porcine reproductive and respiratory syndrome virus (PRRSV) during late gestation are dangerous due to replication of PRRSV in the placenta and fetuses leading to late-term abortion, early farrowing and the birth of weak piglets. An ideal attenuated PRRSV vaccine given during gestation should not cross the placenta and if it does should not cause problems in piglets at birth.

#### Material and methods

In the present study, 3 x 2 PRRSV-negative gilts were purchased. Each time, one gilt was vaccinated at day 80 of gestation with an experimentally attenuated PRRSV subtype 1 strain while the other gilt was mock-vaccinated. Clinical parameters in gilts were followed for 14 days and blood was collected weekly. After farrowing, newborn piglets were subjected to clinical scoring after which they were euthanized and blood and organs were collected for subsequent virus titration.

#### Results

Side effects upon vaccination were not observed. Vaccinated gilts A and C had a viremia when titrated on Marc-145 cells at 7 days post vaccination (dpv); in gilt B, low virus titers were found in serum at 14 dpv when titrated on PAMs. The birth weights of piglets from control and vaccinated gilts were not statistically different. In contrast, the piglets from vaccinated gilts were statistically livelier than piglets from control gilts (time interval birth-standing and birth-making noise). Only in 50% of the piglets from vaccinated gilt A, PRRSV was found in serum and/or organs (lungs, thymus and spleen). The piglets from the 2 other gilts were all virus negative.

#### Conclusion

Although a viremia was detected in vaccinated gilt B, the vaccine virus was not transferred to the fetuses. In only one gilt out of three, 50% of the newborn piglets were PRRSV-positive. Vaccine virus infection in the fetuses did not have a negative effect on the course of pregnancy and liveliness of the newborns.



## VVD-002 - EXPERIMENTAL ATTENUATED PRRSV VACCINE IS SAFE AND PROTECTS PIGS AGAINST CHALLENGE WITH FLANDERS'13 BETTER COMPARED TO A COMMERCIAL PRRSV VACCINE

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### Introduction

PRRSV subtype 1 strains were, until recently, not causing huge respiratory disorders under field conditions in Western Europe. In 2013, a more pathogenic subtype 1 strain was isolated and designated Flanders'13. It was interesting to assess the efficacy of vaccines against this pathogenic strain. In this content, a study was designed with one commercial and one new experimental attenuated vaccine (AV<sub>com</sub> and AV<sub>exp</sub>).

### Material and methods

Safety was evaluated by the analysis of local and systemic side effects. Serum collected weekly after vaccination and contact, was titrated on porcine alveolar macrophages (PAM) and MARC-145 cells in order to follow the post-vaccination viremia and potential transmission. Pigs were challenged 35 dpv (intranasal, 10<sup>5</sup> TCID<sub>50</sub>/dose, 2 ml) and followed for clinical signs. Samples were collected to follow the course of viremia and nasal shedding.

### Results

No or only very mild side effects were observed. AV<sub>com</sub> and AV<sub>exp</sub> showed a strong level of viremia when titrated on MARC-145 cells and low acceptable titers were observed when titrated on PAM cells. AV<sub>com</sub>-virus spread to one contact animal and AV<sub>exp</sub> to two contact animals during 5 weeks of contact. Intranasal inoculation of Flanders'13 failed to induce clear clinical signs and as a result clinical protection could not be assessed. AUC values for viremia and nasal shedding in AV<sub>com</sub>-vaccinated group were reduced but were not significantly different from AUC values in control animals. In contrast, AUC values were significantly lower in AV<sub>exp</sub> compared to mock-vaccinated animals.

### Conclusion

AV<sub>exp</sub> was safe and protected pigs better than the AV<sub>com</sub> against a challenge with PRRSV strain Flanders '13.



## VVD-003 - PCV2 VIREMIA IN NEWBORN PIGLETS ON ENDEMICALLY INFECTED SOW FARMS IS EXTREMELY RARE

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### Background & objectives

Whereas PCV2 associated disease (PCVAD) occurs mostly in pigs of >10 weeks of age, the time of infection is usually earlier. The number of piglets born infected influences infection dynamics greatly and consequently the suitability of control strategies [1]. US based studies indicate a high PCV2 prevalence at birth [2,3,4], but studies under EU conditions are scarce [5]. Objective of this study: to estimate PCV2 prevalence in newborn pigs on endemically infected farms.

### Material & Methods

Assuming a beta binomial distribution of PCV2, sample size calculation resulted in sampling 8 piglets from 11 litters to obtain a prevalence estimate of  $0.3 \pm 0.1$  per farm, with a between litter variation of  $\rho=0.5$ . The study was repeated in four farms, to improve external validity. Farms met these inclusion criteria: breeding own replacement stock, no PCV2 sow vaccination, dynamic sow group housing, no clinical PCVAD. From piglets, EDTA blood samples were obtained from the umbilical cord directly at birth. From sows, serum samples were obtained after farrowing. All piglet and sow samples were analysed by qPCR for PCV2 DNA and serum samples were analysed for IgM and IgG antibodies by ELISA.

### Results

0/352 piglets and 0/44 sows tested positive for PCV2 DNA. None of the sows tested positive for IgM. In farm A, none of the sows tested IgG positive, whereas in farms B, C and D, 13/33 sows tested PCV2 IgG positive.

### Discussion & Conclusion

The prevalence of PCV2 in newborn piglets in the sampled herds is much lower than can be expected from literature [2,3,4]. The findings warrant strict implementation of hygienic measures to prevent infection of PCV2 free litters.

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## VVD-004 - IMPROVEMENTS IN GROWING PIGS PERFORMANCE AFTER PRRS CONTROL PROGRAM IMPLEMENTATION IN A LARGE FARROW TO FINISH HERD IN SPAIN

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### Introduction

Controlling the Porcine Reproductive and Respiratory Syndrome (PRRS) in large production systems is very challenging.

This is a summary of a large field trial designed to evaluate the impact of the 5 step process approach using Reprocyc PRRS EU<sup>®</sup> and PRRSFLEX EU<sup>®</sup> (Boehringer Ingelheim Vetmedica GmbH) a modified-live type I PRRSV vaccine, on control of heterologous PRRSV in commercial finishing pigs, measured by live animal performance.

### Materials and Methods

The study was conducted in a large PRRS positive farrow to finish production system in Spain. The resident field virus strain was 16,3% heterologous to the vaccine strain.

Following the 5 steps whole herd approach concept, since day 0 all pig population was double mass vaccinated 4 weeks apart. Sows were injected intramuscularly with 2ml of Reprocyc PRRS EU<sup>®</sup> and pigs were administrated 1ml IM of PRRSFLEX EU<sup>®</sup>. After the first mass vaccination, every weekly piglet batch was vaccinated on regular basis at weaning (28 days).

The setup of this study is a before and after treatment data analysis, comparing 71 batches before to 29 batches after. No feed changes were implemented during this period.

The key performance indicators were averaged daily weight gain (ADWG), standardized feed conversion ratio (FCR<sub>st</sub>), days on feed (DOF) and losses. For statistical process control (SPC chart) analyzing method, Minitab.17.1.0 software was used.

### Results

The means of ADWG and FCR<sub>st</sub> were better in the batches after PRRS control: 78 grams and 100 grams/kg respectively. The mean of DOF was 13 days less compared to the group before starting the program. These differences were statistically significant ( $p < 0,001$ ).

The reduction of the losses after the program was 0,16%.

### Conclusions and Discussion

The combination of the 5 step process approach and the whole herd vaccination program implemented, had a significant positive impact on the efficient pig growth. The calculated return on investment was 3:1 for the intervention.



## VVD-005 - DEVELOPMENT OF POSTPARTUM DYSGALACTIA SYNDROME IS ASSOCIATED WITH CHANGES IN WHITE BLOOD CELL COUNT AND INFLAMMATORY CYTOKINES

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### Backgrounds & Objectives

Postpartum Dysgalactia Syndrome (PDS) is one of the most common disorders in farrowing sows in Europe. Clinical signs are unspecific and the pathogenesis is not fully elucidated, increasing the risk of inefficient treatment and prevention. This study investigated inflammatory markers in sows developing PDS (PDS+) and healthy sows (PDS-) to examine the pathogenesis and to identify possible diagnostic biomarkers.

### Material & Methods

38 PDS+ sows and 38 matched PDS- sows were monitored during parturition. Clinical examination and blood sampling were performed every 24 h. Levels of cytokines (interleukin-1, interleukin-6, and tumor necrosis factor alpha), acute phase proteins (APPs) (serum amyloid A, C-reactive protein, and haptoglobin), leukocytes, albumin, iron, haemoglobin and prostaglandin F<sub>2</sub>-α (PGF<sub>2</sub>-α) were determined. Repeated measurements ANOVA was used to assess changes in biomarker concentrations over time and difference in levels between PDS+ and PDS- sows. A value of P < 0.01 was used to indicate significance.

### Results

In PDS+ sows level of cytokines, APPs, leukocytes, iron, albumin and PGF<sub>2</sub>-α varied significantly (P<0.01) over time with the largest changes occurring at 12-36 h postpartum. In PDS- sows, changes were less pronounced, but a significant change (P<0.01) was observed only for APPs and iron. Concentrations of cytokines, leukocytes and iron differed significantly (P<0.01) between PDS+ and PDS- sows.

### Discussion & Conclusion

Several inflammatory changes occurred in all sows, also those that remained clinically healthy postpartum. Farrowing thus seems to be an inflammatory event, which therefore may cause altered disease behavior. While APP responses were similar in the two groups, increased levels of cytokines and decreasing leukocyte counts may help in differentiating sows with and without PDS, showing promises for a future clarification of the pathogenesis of the disorder.



## VVD-006 - EFFECT OF PARTIAL DEPOPULATION AND EXPOSED IMMUNITY IN A PORCINE RESPIRATORY AND REPRODUCTIVE SYNDROME ENDEMIC FARM

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Porcine respiratory and reproductive syndrome (PRRS) is one of the most important diseases in the swine industry. It predominantly causes respiratory disorders in nursery and growing pigs and leads to reproductive failure in breeding populations. Due to the characteristics of quasispecies and various cross-protective effects among strains, immunity induced by wild or universal vaccine virus is usually considered to be insufficient protection against heterologous PRRSV. In this study, we monitored the effects of improved environmental conditions and the cross-protection of pre-exposure to homo- or heterologous PRRSV in field conditions. Data were collected from a growing-finishing farm, which suffered from severe endemic PRRS. Eight- to ten-week-old pigs were introduced from several farms into a continuous feeding unit and were bled within the first two days to perform PRRSV ORF5 sequence detection and serological examination. A total of 58 batches (approximately 300 pigs per batch) of pigs from 4 farms were included. During the monitoring period, partial depopulation was implemented in the growing unit, which also served as an analytic variable in this study. The results showed that implementation of de-/re-population was able to significantly improve the growth performance and decrease mortality. Compared to those with a large number of antibodies, populations with no or few antibodies showed higher mortality rates (7.1 vs 0.8%) and a greater percentage of weak pigs were retained when transferred to the finishing unit (13.8 vs 4.3%). The mortality rates of the population exposed to heterologous wild-/vaccine-strain virus were significantly greater than those of the population exposed to the homologous type (1.10 vs 0.39%;  $p < 0.05$ ), especially before depopulation (2.19 vs 0.83%). Taken together, although well-developed immunity could offer protection against PRRSV, it is often insufficient when encountering heterologous strains, particularly under high infective pressure. Therefore, strategies to improve managerial and environmental conditions are essential in PRRS control.





## VVD-007 - BATCH VARIATION IN PORCINE CIRCOVIRUS TYPE 2 VIRAL LOADS IN SERUM POOLS AND ORAL FLUID

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### Background & Objectives

Porcine circovirus type 2 (PCV2) herd diagnosis is often based on qPCR of serum pools (SP) and/or oral fluid (OF) collected on one day from different age groups belonging to different weekly production batches. Results are then interpreted 'longitudinally', assuming that herd infection dynamics only differ according to age group and that findings will represent present and future batches. The objective of this study was to evaluate this 'longitudinal' interpretation by assessing variation in PCV2 viral loads, in SP and OF, between production batches.

### Material & Methods

In 14 batches of finishing pigs from one herd, SP from 4 pigs and OF representing around 30 pigs in the same 2-5 pens per batch were collected at 3-week intervals, totally 4 times. PCV2 was analyzed by qPCR and mean viral load of the four samplings represented the overall PCV2 load at pen level. Batch variation of mean viral loads in SP and OF, respectively, was assessed by analyses of variance (ANOVA). Pairwise comparisons were done by Tukey's test. Statistical significance level was set at 0.05.

### Results

Range of batch means (SD) for SP and OF were 4.49-6.07 (0.03-1.31) and 6.74-7.46 (0.10-0.39) log<sub>10</sub>, respectively, PCV2 copies per ml sample. The analyses of variance indicated a significant effect of batch for both SP ( $p=0.003$ ) and OF ( $p=0.03$ ), but subsequent pairwise comparisons revealed only significant differences between some batches.

### Discussion & Conclusion

Due to the high between-batch variation for SP, one-day sampling of several age groups by SP cannot be considered a valid proxy for 'longitudinal' interpretation. For this purpose, OF results were more consistent with less within- and between-batch variation. Due to the larger sample size, highly viremic pigs are much more likely to be detected by OF than by SP, which should be considered when interpreting results.



## VVD-008 - SCREENING FOR PRRSV EXPOSURE WITH VIRUSCHECK VIA CROSS-SECTIONAL SAMPLING OF PIGS FROM 91 HERDS IN BELGIUM

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### Background & Objectives

PRRSV plays an important role in the porcine respiratory disease complex. Identifying the age of first exposure is essential for control and prevention strategies. A feasible way of screening for exposure to PRRSV at herd level is cross-sectional blood sampling and serological analysis on serum samples of non-vaccinated animals. The present study used VirusCheck, a service tool that includes herd information and serological investigation for PCV2 and PRRSV.

### Material & Methods

A total of 91 pig herds were screened for PRRSV between January 2015 and October 2016. For each herd, blood samples were collected from pigs of 3 to 5 different age groups, ranging from 4 to 28 weeks (W) (5 samples per group), individually analyzed via ELISA (Herdcheck PRRS X3<sup>®</sup>) and classified as unexposed (S/P <0.4) or exposed (S/P ≥0.4). Results were grouped by age: 7-10W, 11-14W, 15-18W and >18W. According to the age of first PRRSV exposure the herds were classified as unstable (≤10W) or stable (>10W). Herd information including clinical signs and vaccination strategies was recorded.

### Results

The clinical history of almost all participating herds included coughing and/or heterogeneity in growth. The prevalence of PRRSV-positive herds was 95%. In 55% of the herds the first age of PRRSV exposure was between 7-10W and so they were classified as unstable. In 17% of the herds, exposure was between 11-14W, 15% between 15-18W and 8% after 18W of age and these were all classified as stable. In this study, we did not find a significant association between vaccination strategy of sows and the PRRSV classification in a herd.

### Discussion & Conclusion

Based on this study, PRRSV infections are highly prevalent in Belgium with 55% of the herds classified as unstable and 40% as stable. Risk factors for first age of exposure should be further investigated.



## VVD-009 - SCREENING FOR PCV2 AND PRRSV INFECTIONS WITH VIRUSCHECK VIA CROSS-SECTIONAL SAMPLING OF PIGS FROM 91 HERDS IN BELGIUM

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### Background & Objectives

PCV2 and PRRSV infections play an important role in porcine respiratory disease complex. Knowing the age of first exposure is essential for control and prevention strategies. A feasible way of screening for these pathogens at herd level is cross-sectional blood sampling. The present study describes the results from VirusCheck, a service tool that includes herd information and serological investigation for PCV2 and PRRSV.

### Material & Methods

A total of 91 pig herds were screened for PCV2 and PRRSV between January 2015 and October 2016. For each herd, blood samples were collected from pigs of 3 to 5 different age groups, ranging from 4 to 28 weeks (W) (5 samples per group). For PCV2, samples from each group were pooled, analyzed via qPCR and classified as negative ( $<10^4$  PCV2 copies/ml) or positive ( $\geq 10^4$  PCV2 copies/ml). For PRRSV, samples were individually analyzed by ELISA (HerdChek PRRS X3<sup>®</sup>) and classified as unexposed (S/P  $<0.4$ ) or exposed (S/P  $\geq 0.4$ ). Results were grouped by age: 7-10W, 11-14W, 15-18W and  $>18$ W. Herd information, including clinical signs and vaccination strategies, was recorded.

### Results

The clinical history of almost all participating herds included coughing and/or heterogeneity in growth. Sixty four herds were infected with PCV2 (70%). In 27% of the herds, first age of exposure was between 7-10W, 18% between 11-14W, 19% between 15-18W and 6% after 18W of age. Fifty nine herds were infected with both pathogens (65%). In 42% of the herds, first exposure to both pathogens occurred simultaneously.

### Discussion & Conclusion

Based on this study, PCV2 and PRRSV infections are highly prevalent in Belgium with 65% of the herds infected with both viruses. First exposure to both pathogens occurred simultaneously in 42% of the herds. Risk factors for first age of exposure should be further investigated.



## VVD-010 - ATYPICAL PORCINE PESTIVIRUS ASSOCIATED OUTBREAK OF CONGENITAL TREMOR TYPE A II IN A CLOSED PIGLET PRODUCING FARM IN LOWER AUSTRIA

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Congenital tremor type A II (CT-AII) has been a mystery in swine medicine for a long period. Outbreaks of this disease have been observed in newborn piglets for decades and were suggested to be caused by an unknown virus. In 2015, a newly described, so-called atypical porcine pestivirus (APPV), was identified in the US and quickly associated with clinical signs of CT-AII. Further cases of CT-AII linked to APPV infections were subsequently reported in Germany, the Netherlands and in Austria. Hardly any information exists regarding pathogenesis and epidemiology of this virus. Infected gilts and boar semen were identified as potential sources of introduction and therefore can be considered as risk factors for naïve sow herds. In October 2016, an acute outbreak of CT-AII was observed in a closed piglet producing farm in Lower Austria. The farm was producing piglets with about 80 sows in a five week batch farrowing interval. In one farrowing batch, piglets from 100% of litters were affected showing typical signs of CT-AII. The within litter prevalence varied from 10 to 100%. In total, 49% of all newborn piglets from that batch showed signs of CT-AII. Pre-weaning losses peaked at 39%. In the consecutive farrowing batch 54.5% of litters were affected. In total, 12.3% of piglets showed signs of CT-AII in that batch. Presence of APPV genome was confirmed by RT-qPCR in affected piglets and genetically closely related to previously detected isolates from Austria.

In the first affected batch, two of the affected litters were supposed to produce replacement gilts. Female piglets from those two litters will be monitored for APPV infection over the breeding period in order to define if those animals are persistently infected and whether they can be used as breeding stock for piglet production or should be removed from the farm.



## VVD-011 - SEROPREVALENCE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN UKRAINIAN COMMERTIAL PIG FARMS BETWEEN 2009 AND 2016

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### Background and Objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) belonging to the family Arteriviridae is recognized as one of the most important agents of pig diseases all over the world. as it causes huge economic losses practically in all countries with established pig-breeding. The annual losses can reach 85.74€ per every sow and 3.50€ per every marketed pig. In order to determine the actual situation of PRRSV infection on the farms of Ukraine, it was necessary to determine the distribution of PRRSV antibodies in different pig farms and regions. Therefore, the aim of the study was to determine serological prevalence of PRRSV infection in Ukrainian pig populations over a period of the last 8 years.

### Materials and Methods

Blood samples were collected from 281 different pig farms in 25 regions of Ukraine in the period of 2009 to 2016. Commercial ELISA kits (IDEXX PRRS till 02/2010, IDEXX PRRS X3 from 03/2010) were used for detection of PRRSV antibodies in serum samples. The tests were performed according to the manufacturer's instructions. A sample was considered to be positive for the antibodies if the sample-to-positive (S/P) ratio was  $\geq 0.4$ . All the schemes of the research methods of PRRSV antibodies used were accredited according to the requirements of ISO/IEC 17025:2005 standards.

### Results

During this study we analyzed 54575 blood samples, 5715 were positive for PRRSV antibodies. 85 pig farms out of 281 were serologically positive for PRSSV. PRSSV circulation was confirmed for 21 regions of Ukraine. There was no PRRSV-positive serum detected in Lugansks, Mykolaiv, Ternopil and Kherson regions.

### Discussion and Conclusions

Results of the study represent actual situation on PRSSV spread in Ukraine. 21 out of 25 regions and 85 pig farms were positive for PRSSV. These results indicates that big improvement is needed in control and eradication of PRSSV in Ukraine.



## VVD-012 - FIELD EFFICACY OF INGELVAC CIRCOFLEX VACCINATION FOLLOWING PCV2 DETECTION IN ORAL FLUIDS BY QUANTITATIVE PCR

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### Background & Objectives

Numerous publications have shown that subclinical PCV2 causes production losses. As subclinical PCV2 is not readily observable, reliable methods to screen for the presence of PCV2 are necessary. qPCR of oral fluids for PCV2 is a convenient method to detect the pathogen in herds.

The objective of the study was to determine whether the detection of PCV2 in oral fluid from pigs without any clinical signs of PCV2 disease, is a) sufficient to diagnose subclinical PCV2 and b) whether vaccination will improve production parameters and decrease the viral titres.

### Material & Methods

The trial was done on a Mycoplasma positive, multi-site, 1500 sow unit without clinical PCV2. Groups aged 8, 12, 16 and 20 weeks were screened for PCV2 using oral fluids.

5848 pigs participated; divided into 8 groups with ~700 pigs/group. Four groups were vaccinated (V) with Ingelvac Circoflex at 21 days alternating with four control (C) groups left unvaccinated. Mortality, ADG and FCR was measured for all groups. Oral fluid was collected from two V and two C groups, three pens/group at 8, 12, 16 and 20 weeks of age.

### Results

Pre-screening indicated that PCV2 was absent at 8 weeks, but present in all older groups.

Significant differences were observed in a) post-weaning mortality of 1.1% in V vs. 2.4% in C ( $P < 0.015$ ) and b) ADG from 28-128 days of 661g in V vs. 631g in C ( $P < 0.015$ ). According to the linear mixed effects model, the V vs. C were 3.84kg heavier at 128 days ( $P < 0.001$ ).

Oral fluid PCV2 titres were lower in V vs. C at 8 weeks ( $P < 0.069$ ) and significantly lower at 12 ( $P < 0.001$ ), 16 ( $P < 0.033$ ) and 20 weeks ( $P < 0.003$ ).

### Discussion & Conclusion

Oral fluid qPCR proved useful in identifying a farm with subclinical PCV2. The vaccinated group benefitted significantly showing decreased post-weaning mortality, increased ADG and heavier pigs. There was also a significant decrease in viral shedding in V vs. C.



## VVD-013 - CASE REPORT: REPRODUCTIVE FAILURE DUE TO PCV2 IN A SPANISH FARM

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### Introduction

PCV2 can be associated with reproductive failure. A previous study carried out in Spain found that only in one out of 293 reproductive failure cases PCV2 DNA was detected.

The aim of this study is to evaluate different pathogens that can be involved in a reproductive failure in a Spanish farm.

### Materials and Methods

The case farm is a two-site, 500-head sow farm located in southeastern part of Spain. The farm was positive for PRRS (stable at the time of the reproductive disorders), Mycoplasma, and PCV2. The farm was vaccinating against PRRSv (Reprocyt PRRS EU; Boehringer Ingelheim Vetmedica GmbH) every 3 months, IAV-S (H1N1, H3N2) and PPV which was applied post farrow. During the summer of 2016 an increase in the abortion rate (5,1% to 48,3%) (Figure 1) and a decrease of total born pigs (14,31 to 10,43) was detected for the gilts (Figure 2). Also the viability of the piglets went down. The reproductive parameters of the multiparous sows remained stable. Blood samples and 6 aborted fetuses from 3 gilts were taken and tested via PCRs from lung samples for PRRSv, PCV2, IAV-S and via ELISA from thoracic fluid for PPV. Paired blood samples were taken from sows on the day of an abortion and 15 days later and tested via ELISA for Leptospira, PRRSv and IAV-S).

### Results

No seroconversion was observed in the sows.

All the diagnostics run in the fetuses resulted negative, except PCV2 that were positive in 5 out of 6 fetuses and with a mean viral load of  $1,17 \times 10^9$  copies per ml.

### Conclusions and Discussion

We must take into account that PCV2 epidemiology might have changed since piglet vaccination for PCV2 has been implemented in Spain and elsewhere. We suggest that in future PCV2 should be considered for differential diagnosis of reproductive disorders of sow herds.



## VVD-014 - LARGE SCALE PRRSV EXPOSURE DYNAMIC IN SPANISH FARMS

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### Introduction

The objective of this study was to determine PRRSV exposure pattern in Spanish farms.

### Materials and Methods

97 PRRS positive farms were identified across Spain represented 117,480 sows, 5.2% of the Spanish's census. An on-farm questionnaire was implemented at each farm capturing information about: Production System and vaccination program. At each farm, a cross sectional sampling was implemented: At weaning (30 piglets at the end of the lactation period); end of nursery (10 Blood samples and 2 Oral fluids (OFs) and mid finisher (same sampling as nursery). At each sampling point, the blood samples were analyzed in pools (1:5) by PRRS Rt-PCR, and individually in the case of OFs. All the samples were analyzed by ELISA individually. In total, 3,663 ELISAs and 1,289 pooled PCRs were analyzed.

### Results

According to the questionnaire, 17.5% of the farms were Farrow to finish (FF); 67% farrow to nursery (FN) and 14.5% multi-sites (MS). Median size: 1,221 sows, range from 300 to 5,000 sows. 90.7% of the farms were using Modified-live PRRS vaccines in sows, in contrast only 1% of the farms applied vaccine in piglets.

Prevalence to PRRS measured by ELISAs in the production flow was 100%. The overall percentages of positive PCR per production phase were 15.75% at weaning, 50% and 11.3% in blood and in OF at the end of the nursery, and finally in mid finisher only a 8.73% and 3.38% of the PCRs were positive in blood and in OF respectively.

### Conclusions

Wild type PRRSV prevalence by pig flow was 100%, having the highest circulation at end of nursery. These results highlighted PRRSV presence and circulation in nurseries piglets as an important epidemiological event. For that, it should be considered changes in internal biosecurity, management flow and piglet vaccination in control and eradication programs in FF and FN farms.





## VVD-015 - ULTRAVIOLET-C INACTIVATION OF PSEUDORABIES VIRUS (PRV), PORCINE REPRODUCTIVE RESPIRATORY SYNDROME VIRUS (PRRSV), PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) IN BOVINE PLASMA

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### Introduction

Spray dried plasma (SDP) is a functional protein source used in pig diets due to its beneficial effects on post-weaning performance and survival. Manufacturing process of SDP involves several safety features, additional safety steps should be investigated. Ultraviolet at 254 nm wavelength (UV-C) is a non-thermal process that disrupts cellular transcription and replication, leading to death of bacteria, viruses, and molds. Presently, a special UV-C designed process for large industrial volumes of turbid liquids (such as animal plasma) is now available. The aim of this study was to check the effectiveness of the UV-C irradiation using a proprietary system (SurePure SP1) on survival of PRRSV, PEDV and PRV inoculated in bovine plasma (BP).

### Material & Methods

A total of 24 L of BP were used for each virus (divided in three different sub-batches of 8 L each). On time zero, 15 mL samples were obtained and served as negative control before virus inoculation. A positive control sample was collected 5 min after the plasma was inoculated and homogenized. Each sub-batch was consecutively irradiated at 750, 1500, 3000, 6000 and 9000 J/L and sequential samples were taken at each UV-dose. Plasma recirculated under turbulent flow at 4000 L/h in a closed system of the SP1 device. Pre and post-UV irradiated samples were stored at -80°C prior to analysis of infectivity in target cell cultures, using the microtiter assay procedure.

### Results and Discussion

Four fold reduction was reached at 1728.90 J/L for PRV, 1500 J/L for PRRSV and 2826.68 J/L for PEDV. Overall results indicated a reduction of 4 log titers by an UV dose less than 3000 J/L. These results indicate the usefulness of the UV treatment to inactivate virus as an intermediate redundant safety step of fresh plasma in the manufacturing process of SDP.



## VVD-016 - VOMITING AND WASTING DISEASE IN A HIGH-BIOSAFETY LEVEL HERD: A CASE REPORT

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The case occurred in a farrow-to-finish herd of 140 sows in Brittany in France. The herd is conducted in 7 batches and piglets are weaned at 28 days. The herd is free of PRSS virus and of *Actinobacillus pleuropneumoniae*. Finishing pigs are seronegative against *Mycoplasma hyopneumoniae*. Sows are vaccinated against parvovirus infection and rhinitis and gilts against porcine circovirus type 2.

Clinical signs began in maternity and affected piglets of 8 days of age. They showed first vomiting and then wasting and the sows showed anorexia. Antibiotic treatment was unsuccessful to treat clinical signs. Five days after the beginning of symptoms, half of the litters were affected. After the outbreak, 45% of the total born piglets died and the morbidity reached 100%. Three affected piglets that did not receive any treatment were euthanized for autopsy, histology and bacteria and virus detection.

The clinical signs were consistent with a coronavirus infection and specifically with the vomiting and wasting disease caused by a beta-coronavirus named porcine hemagglutinating encephalomyelitis coronavirus.

Histological examinations revealed non suppurative encephalitis with lymphoplasmocytic infiltration of gastric mucosa..

Pan-coronavirus RT-PCR performed on brain samples were positive but not PHEV specific RT-PCR. Nevertheless next generation sequencing demonstrated fragments of Beta coronavirus RNA genomes in the same samples.

This case report suggests PHEV still occurs sporadically in our country, in poorly protected animals with no specific immunity.



## VVD-017 - COMPARISON OF THREE SEROLOGICAL TESTS FOR DIAGNOSTIC OF PORCINE RUBULAVIRUS INFECTION

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### Background & Objectives

Blue eye disease was first identified in early 1980 in Mexico. It is caused by porcine rubulavirus (PorPV) and characterized by infertility in sows and boars, nervous signs in young pigs, and corneal opacity in pigs of all ages. Several serological tests to detect specific antibodies to PorPV have been developed. The aim of this work was to determine sensitivity, specificity and predictive values of HI, SN and IPMA tests for serodiagnosis of PorPV.

### Material & Methods

29 sera towards PorPV PAC-3 and 9 hyperimmune sera (towards vaccine viruses and different isolates) were used as positive controls, 12 no immune sera were used as negative controls. Specificity, sensitivity, predictive values, Kappa coefficient, standard deviation and coefficient of variation (CV) were calculated for each test. Antigen used in all tests was PAC-3 strain.

### Results

CV of HI test with complete treatment (heat inactivated and adsorbed with chicken erythrocytes and kaolin) of sera samples and IPMA were 0.0% for negative reference sera, indicating high reproducibility of the tests. Low values of sensitivity were obtained when bovine erythrocytes were used in HI test (44.-80%) depending upon variables of each HI protocol. Sensitivity and specificity in IPMA were 85 and 100%, respectively. SN showed high values of sensitivity and specificity, 91 and 96 %, respectively.

### Discussion & Conclusion

HI tests performed with complete treatment of sera were able to completely remove nonspecific inhibitors of hemagglutination. High values for sensitivity and specificity in HI test obtained could suggest its use as a confirmatory test. IPMA has the advantage of using an inactivated antigen making it a suitable candidate to be adapted in diagnostic laboratories located in disease free areas. SN and IPMA tests were very sensitive and specific; however, it will be necessary to consider laboratory infrastructure, personnel trained and the costs for its implementation.



## VVD-018 - PCV2 GENOTYPE DISTRIBUTION IN GERMAN FATTENING PIG FARMS

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### Background & Objectives

Since 2013, PCV2d has been detected in cases of severe PCVD in Germany. A total of 59 German fattening farms were examined to understand PCV2 genotype prevalence, including PCV2d, in the German domestic pig population.

### Material & Methods

In total, 1,680 serum samples from 59 German fattening farms with different PCV2 vaccination strategies (41 piglet vaccination, 6 sow vaccination, 12 no vaccination) were examined for PCV2 DNA by q-PCR. All samples originated from routine animal health screenings that were conducted in these farms (n= 20 to 30 samples per farm). PCV2 DNA positive samples were further analyzed by whole genome sequencing. Comparison to reference sequences was conducted to determine the PCV2 genotypes in these farms.

### Results

In total, 86 complete PCV2 DNA sequences from 24 farms were obtained: 53.3 % of the PCV2 isolates were assigned to PCV2a, 34.9 % to PCV2b and 11.6 % to PCV2d. Thirteen isolates came from 9 farms with piglet vaccination (8x PCV2b, 3x PCV2a and 2x PCV2d), 17 isolates from 5 farms with sow vaccination pigs (16x PCV2a and 1x PCV2b) and 56 isolates from 10 farms with non-vaccinated pigs (27x PCV2a, 21x PCV2b and 8 x PCV2d). On farm level, 12 farms were positive for PCV2a, eight for PCV2b, one for PCV2d, one for PCV2a and PCV2b and two for PCV2b and PCV2d.

### Discussion & Conclusion

The prevalence of PCV2a was unexpectedly high since PCV2b was expected to be most prevalent. PCV2d was not as widespread as in USA and China. High PCV2 vaccination rates in Germany might decelerate the spread of PCV2d in German pig herds.



## VVD-019 - COMPARISON OF PCR METHODS FOR THE DETECTION OF PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV)

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Porcine epidemic diarrhea virus (PEDV) is an enteropathogenic coronavirus causing vomiting and acute watery diarrhea in pigs. After discovery in Europe in the 70's, the virus has emerged since 2010 in Asia, North and South America causing significant economic losses due to high piglet mortality and weight loss in fattening pigs. In 2014, PEDV was reintroduced in some European countries raising question about diagnosis preparedness.

Numerous RT-PCR methods are available to detect PEDV infection but less is known about their performance and their ability to fulfill today's diagnostic requirement.

In the present study, we compared performance of 6 gel-based RT-PCR, 2 published and 5 commercial real-time RT-PCR assays in term of their analytical sensitivity, inter-assay repeatability and diagnostic sensitivity.

Using serial dilutions of a virus culture, the limit of detection ranged from 10E-2.5 to 10E-3.5 TICD50/ml (10E1-10E0 copies/ $\mu$ l) for most of the real-time RT-PCR assays, but was more variable for gel-based assays. The inter-assay repeatability was estimated below 2.5% for published or commercial real-time RT-PCRs.

The diagnostic sensitivity of seven more convincing assays was evaluated on 66 fecal samples from experimentally infected animals and 19 positive field samples from Canada. The four real-time PCR assays consistently detected virus presence between 1 and 10 dpi and sometimes up to 18 dpi. The three gel-based assays were less sensitive and may present aspecific positive reactions. With the Canadian field positive panel qualitative and quantitative results were comparable between the assays behalf one that seemed to react suboptimal with this panel.

In conclusion, the different Taqman RT-PCR assays tested in this study presented a high correlation in qualitative and quantitative results and could be recommended for rapid and sensitive detection of PEDV. Only one gel-based assay had performance comparable to the real-time RT-PCR assays whereas other lacked of sensitivity or specificity.



## VVD-020 - COMPARATIVE STUDY OF SEROLOGICAL METHODS FOR DIAGNOSIS OF PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) INFECTION

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Porcine epidemic diarrhea virus (PEDV) is an enteropathogenic coronavirus causing vomiting and acute watery diarrhea in pigs. After discovery in Europe in the 70's, the virus has emerged since 2010 in Asia, North and South America causing significant economic losses due to high piglet mortality and weight loss in fattening pigs. In 2014, PEDV was reintroduced in some European countries raising question about diagnosis preparedness.

In the present study, we compared performance of four methods for serological testing for PEDV: (1) "in-house"-made immunoperoxidase monolayer assay (IPMA); (2) immunofluorescence assay (IFA) using commercially available reagents (VMRD); (3) indirect ELISA (Biovet) and (4) indirect ELISA (IDVet).

Using serial dilution of a hyperimmune serum obtained from a pig experimentally infected with CV777 strain, the IPMA assay demonstrated the highest analytical sensitivity which was slightly better than the IFA. Very poor sensitivity was observed for both ELISAs with this serum sample.

The diagnostic sensitivity of the different assays was evaluated on 96 sera from experimentally infected animals. Samples collected at 10 dpi and later were identified as positive with IPMA, IFA and Biovet ELISA. Earliness in PEDV detection was observed with IPMA at 7 dpi. Only 1/3 of the positive IPMA samples could be identified by IDVet ELISA.

Results were confirmed with additional panels of porcine sera collected in the United States, Canada and Belgium analyzed to measure the diagnostic sensitivity and specificity.

In conclusion, the IPMA method was the most sensitive method for detection of PEDV antibodies, while the IFA was slightly less sensitive. Between the two evaluated ELISA the Biovet ELISA kit performed better despite non-negligible proportion of non-specific positive results.



## VVD-021 - COMPARATIVE INVESTIGATION ON PCV2B AND PCV2D VIRULENCE AFTER INTRANASAL INFECTION IN A CONTROLLED PIG CHALLENGE MODEL

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### Background & Objectives

Recently PCV2d was detected in severe cases of PCVD in German pig herds. These observations raised concerns about a possibly higher virulence of this genotype compared to PCV2b. Challenge trial results from North America and China give inconsistent results concerning this topic. The present study was conducted to compare the virulence of PCV2b and PCV2d in a controlled pig challenge model.

### Material & Methods

Twenty (20) pigs were challenged intranasally at eight weeks of age with PCV2b (n=10) or PCV2d (n=10) (6ml of 5.0 log<sub>10</sub> TCID<sub>50</sub>/ml per animal). Viral loads in serum and fecal shedding (swab samples) were monitored (q-PCR) every 7 days until day 22 post infection (p.i.). After euthanasia of the animals 22 days p.i., tonsils, mesenteric lymph node, inguinal lymph node, and lung were given a histopathological- and an immunohistochemistry score (IHC) and viral loads in corresponding tissues were measured by q-PCR.

### Results

All serum and fecal samples were PCV2-DNA negative on the day of infection. After challenge, all animals became viremic and shed virus via the feces over time. No significant differences were observed among both challenge groups concerning viral loads in serum and feces as well as in viral loads of tissue samples. Furthermore, histopathological- and immunohistochemistry score of all examined tissue samples were comparable between animals in both groups.

### Discussion & Conclusion

Our study shows that PCV2b and PCV2d exhibit comparable virulence in an intranasal infection model. Our findings support the results of North American examinations concerning the virulence of PCV2b and PCV2d and are in contrast to findings from China where PCV2d exhibited a higher virulence than PCV2b.



## VVD-022 - EVALUATION OF SEROCONVERSION OF FETUSES SURGICALLY INOCULATED WITH BOVINE VIRAL DIARRHEA VIRUS

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### Background and objectives

The period of immunocompetence in swine fetuses occurs around 65-70 days of gestation. In order to evaluate the effects of Bovine Viral Diarrhea virus (BVDV) in the fetal immune system before the known immunocompetence period, intrauterine inoculation was performed in six sows with 45 days of gestation by laparotomy.

### Material & methods

Six sows were submitted to laparotomy, the uterus was exteriorized and each amniotic vesicle was inoculated with 0.5 ml with ncp BVDV-2. Four sows aborted up to three days after the procedure, the other two were observed clinically until delivery and gave birth totalizing 15 piglets alive, two mummified and three stillborn. All piglets had serum samples collected at birth and weekly, during 35 days. The blood serum underwent virusneutralization test to evaluate the seroconversion of the animals.

### Results

At birth, all blood samples were negative to BVDV-2 in RT-PCR. All piglets produced high antibodies titers against the virus, even though the inoculation was performed prior to the expected immunocompetence period. At birth, 6 piglets had antibody titres of 640, 1 of 320, 3 of 160 and 5 of 80. During the sampling, the titres varied between 640 and 5120 in 5 piglets, remaining high until the end. The other 9 animals had titres remaining at 640, decreasing at the end to 160.

### Discussion & Conclusion

The virusneutralization results showed that piglets were able to produce antibodies by exposing the fetuses to the agent before the pre-established immunocompetence period. The immune response was late and persistent in BVDV infection in pigs, since the first samples were taken about 70 days after inoculation and remained high until the end of the experimental period. This result is highly important to elucidate the capacity of depletion of infection by swine fetuses.

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## VVD-023 - PRRSV ERADICATION ON GROWING PIGS IN A FARROW-TO-FINISH FARM COMBINING MASS VACCINATION WITH MLV VACCINE AND STRICT BIOSECURITY MEASURES

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### **Introduction**

PRRSV stabilization proved to be efficient thanks to mass vaccination of the animals combined to strict biosecurity measures. In farrow-to-finish farms, where pig and human flows are sometimes difficult to respect, eradication of the virus in fattening units is a challenge. This clinical case highlights the benefits of reinforcing the vaccination protocol to control PRRSV circulation from weaning to slaughtering.

### **Materials and Methods**

This field study was implemented in 2015 in a farrow-to-finish farm, weaning 21 day-piglets every 2 weeks. Circulation of a PRRS European strain was confirmed shortly before, responsible for reproductive disorders and high loss rate. A PRRSV stabilization program was then established. As the farmer's objective was to produce his own gilts, eradication was aimed on fattening units. In June 2015, all the herd was mass-vaccinated (sows with REPROCYC<sup>®</sup>PRRS EU, and pigs of 21 days and older with INGELVAC PRRSFLEX<sup>®</sup>EU) twice 4 weeks apart. A 8-weeks herd closure was also implemented. Then, gilts were vaccinated twice: upon arrival and 4 weeks later. To strengthen the protocol, 20 batches of piglets were vaccinated at weaning and 4 weeks later. Strict biosecurity measures were implemented. The stabilization program ended when the last batch of piglets was vaccinated. Herd stability was monitored by PCRs on 2 batches of piglets at weaning and by ELISA on sentinel gilts sampled during 2 months. To control the status of fattening units, 2 successive batches were monitored. 10 sentinel pigs per batch were sampled monthly from 6 weeks of age to slaughtering.

### **Conclusion**

Results of the monitoring showed the absence of transmission of PRRSV from sows to their piglets. The absence of contamination of growing pigs, on the 2 successive batches confirmed the eradication of PRRSV in fattening units. Monitoring of sentinel gilts is on-going.



## VVD-024 - EVALUATION OF TRANSMISSION OF BOVINE VIRAL DIARRHEA VIRUS BY WATER LINE IN EXPERIMENTALLY INFECTED PIGS

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### Background & Objectives

Swine under natural conditions can become infected with BVDV. The prevalence of BVDV infection in pigs is increasingly frequent and causes economic losses in the world pig farming. The objective of this research was to verify the transmission of BVDV via water line in experimentally infected piglets.

### Material & Methods

Six piglets with 20 days of age were separated into three groups by isolators (control, infected and sentinel). The sentinel and infected group were connected only by a water line. The infected group was inoculated orally and nasally (BVDV-1, Singer strain), and the experimental period was performed in 25 days (daily collection of nasal swab and blood collection every 7 days); with euthanasia and necropsy at the end. Seroconversion was evaluated by Virusneutralization (VN). Detection of virus in nasal secretions was performed by RT-PCR.

### Results

The results demonstrate that one infected and one sentinel animal seroconverted on the 25<sup>th</sup> with antibody titer of 20, in both. Viral excretion was detected in the I2 swab samples at 6<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> days post inoculation. The infected animal (I1) did not present seroconversion during the sampled period, but viral excretion was detected on days 5, 7, 8 and 24 after infection. The sentinel animal S1 presented excretion at the 20<sup>th</sup> dpi.

### Discussion & Conclusion

These results demonstrated that there was excretion of the virus prior to seroconversion. Cattle eliminate the virus through nasal discharge, and 48-72 hours post-infection the virus can be isolated in various secretions. From the 5<sup>th</sup> to 8<sup>th</sup> dpi, the maximum viral concentration is reached. Epidemiologically, the present study shows that pigs can eliminate the virus in the environment and serve as a source of contamination for susceptible cattle.

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## VVD-025 - FACTORS THAT INFLUENCE MECHANICAL TRANSMISSION OF PORCINE EPIDEMIC DIARRHEA VIRUS AT THE TIME OF UNLOADING ANIMALS INTO SLAUGHTER PLANT LAIRAGE

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**Objectives:** To estimate the impact of environmental conditions and management practices on the likelihood of PED virus (PEDV) cross-contamination of a pig transport vehicle during market animal unloading. **Materials and methods:** An experimental model was developed to simulate an indirect contact involving footwear between an unloading dock and a pig transport vehicle. Two experiments were conducted. Experiment 1 evaluated temperature on the model trailer (4°C, 15°C, or 28°C) for 60 minutes after contact with the contaminated dock (32 contact replicates per temperature). In Experiment 2, conditions on the model dock were evaluated in a 2 × 2 × 2 factorial arrangement with repeated measures. Main effects were temperature (4°C, 32°C), UV light (ambient or supplemental UV light), and mechanical scraping (de-bulked or not de-bulked) with four contact events for each combination. Samples were collected using a "Swiffer" (Procter & Gamble, Cincinnati, OH), and all samples were tested using reverse transcriptase PCR at the Iowa State University Veterinary Diagnostic Laboratory. **Results:** Experiment 1: Temperature did not affect the amount of PEDV RNA recovered. If PEDV RNA was detected on the model dock, it was transferred and detected on the model trailer 80% (95% CI [70.0%, 90.0%]) of the time. Experiment 2: De-bulking resulted in a significant reduction in the likelihood of transfer (Odds Ratio = 0.14, 95% CI [0.06, 0.32]). **Implications:** Contact at the harvest plant lairage unloading is a risk factor for PEDV transmission with inadequate livestock trailer hygiene. This risk can be mitigated through mechanical removal of gross contamination of the dock.



## VVD-026 - MONITORING OF EUROPEAN PRRSV STRAINS USING SEQUENCING TECHNOLOGIES

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Porcine reproductive and respiratory syndrome (PRRS) is considered one of the most economically important infectious diseases of swine. PRRS is caused by a RNA virus with a high mutation rate. The aim of the present study was to monitor circulating PRRSV strains throughout Europe using sequencing technologies. Sequencing information enables veterinarians and farmers to monitor PRRS circulation in the herd, combined with ELISA and Real Time PCR assays. Sequencing PCR positive samples may give additional information about the origin of the sample.

Thermo Fisher Scientific established different partnerships to collect 102 PRRSV positive samples in 10 different countries. Sequencing strategy applied depends on PRRS viral load and quality of the sampling process (sample collection, storage, shipment). Sampling quality determines the strategy applied for sequencing: whole genome sequencing or only targeted sequencing. For 82 samples containing a high/medium PRRS viral load with a high quality sampling, RNA-Seq or Long Range protocols were applied in order to obtain whole PRRS genome sequence. For 20 samples containing a weak viral load or with a poor quality, target sequencing protocol was performed in order to obtain ORF7 sequence of PRRS genome.

For 82 samples containing a high/medium PRRS viral load, 46 complete sequences and 36 partial sequences (more than 50% of PRRS genome) were obtained from serum, culture and tissues samples. For 20 samples containing a weak viral load or with a poor quality, 20 ORF7 sequences were obtained from serum, oral fluids and tissues samples.

Compared to a Real Time PCR assays that enables the pathogen presence/absence, sequencing approaches offer the possibility to identify new PRRSV strains. The monitoring of circulating European PRRSV strains, using sequencing technologies enables to sequence RNA directly isolated from field samples. Thermo Fisher Scientific offers a range of adapted workflows from the sampling, extraction methods to the sequencing solutions.



## VVD-027 - PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME: IS THIS THE RESIDENT VIRUS OR IS IT A NEW INTRODUCTION?

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### Background and Objectives

Veterinarians are challenged with the question, “Is this the resident virus, or a new introduction?” following the receipt of Porcine Reproductive and Respiratory Syndrome (PRRS) positive test results. With sequencing and comparison to previous viral isolates, various rules of thumb (typically 2% or 3% heterology) are often applied to attempt to make the determination. This is a case report from a 5000 sow herd, in a high density area of the United States, which illustrates the importance of evaluating more of the contextual evidence.

### Materials and Methods

The herd was infected with a virulent PRRS virus in late 2010. In early 2011, the herd underwent elimination by loading the farm with gilts, closing the herd and mass vaccinating with a modified live vaccine. In late 2012, PRRS virus was detected in due to wean piglet and onsite finishing samples with 1.8% and 2.2% heterology to the 2010 isolate, respectively and 1% different from each other. Participation in the local PRRS area/regional control program (ARC) allowed additional information to be assessed for this determination.

### Results

Using the rule of thumb would result in determining that these 2012 isolates were the same as the previous resident virus. Other available evidence, including the locations of neighboring farms, the timeline of site status changes, and a comparison within the regional sequence database, give a compelling argument to conclude that there was a successful elimination and a new introduction of a similar virus from a neighboring farm.

### Discussion and conclusion

Without the information from the ARC, the rule of thumb alone allows the producer to conclude a failure of the elimination plan or in execution of the plan rather than a breach in biosecurity. This perception would no doubt inform future PRRS control decisions on the farm and in the area.



## VVD-028 - DETECTION OF MAMMALIAN ORTHOREOVIRUS TYPE 3 DURING A PED OUTBREAK IN NORTH EAST ITALY

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Mammalian Orthoreoviruses (MRV) are members of the Reoviridae family and are non-enveloped viruses with a segmented genome of 10 dsRNAs. Three MRV serotypes (1-3) have been identified up to date. MRVs can infect a variety of mammalian species, including humans, bats and swine. Clinically, MRV infections range from asymptomatic to respiratory or gastro enteric forms. MVR3 were detected in swine showing gastro-enteric signs and diarrhoea in China and South Korea. In 2014, MRV3 was detected in swine during porcine epidemic diarrhoea (PED) outbreaks in USA. The US MRV3 presented a high homology with European bat isolates. The first detection of swine MRV3 in Europe occurred in 2015 during severe PED outbreaks in Italy. Here we report a further the detection of MRV3 during a PED outbreak in North East Italy, Veneto region.

In February 2016 an acute episode of gastroenteritis was observed in a closed cycle farm of 180 sows in Treviso province. Diarrhoea was observed in fattening pigs for 5-6 days with no mortality. PED and MRV were detected in faeces samples. The MRV isolate was typed by PCR as type 3 and S1 sequence showed 97% nucleotide (nt) homology with MRV3 from Italian bats detected in 2012 and a MRV3 from a child with acute gastroenteritis detected in Slovenia detected in 2013. Interestingly the MRV3 detected in Veneto region showed a lower S1 homology with the bat and human MRV3 strains compared with swine MRV3/Italy/2015. Based on the aa sequence, the MRV3/2016 from Veneto showed closer relationship with the human strain. The role of MRV3 in causing enteric disease alone and in exacerbating the disease during PED outbreaks is still obscure. The detection of an MRV3 with high homology to human and bat isolates warrants further studies on interspecies transmission and zoonotic potential of such virus.



## VVD-029 - CONTROL OF SWINE INFLUENZA A VIRUS ENDEMIC PERSISTENCE IN FARROW-TO-FINISH HERDS: INSIGHTS FROM A STOCHASTIC METAPOPULATION MODEL

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### Background and Objectives

Swine Influenza has been shown to persist in an enzootic form in farrow-to-finish farms with recurrent occurrence in successive batches at a similar age. The specific population dynamics of farrow-to-finish pig farms, the immune status of the animals, the co-circulation of distinct subtypes and their reassortants leading to consecutive even concomitant infections have been evidenced as factors favouring swIAV persistence within herds. The aim of this study was to evaluate the efficacy of control strategies related to herd management and/or different vaccination schemes (batch-to-batch or mass vaccination of the sow herd and vaccination of growing pigs).

### Material and Methods

An event-driven stochastic metapopulation model has been developed to represent the co-circulation of two distinct swIAVs within a typical farrow-to-finish pig herd comprising two subpopulations - breeding sows and growing pigs -, interacting during lactating stage. A transmission experiment involving weaned piglets with or without maternal immunity, in direct or indirect contact with seeder-pigs was carried out to parameterize the model.

### Results

The introduction of one infected gilt in service room led to endemic swIAV within-herd persistence as observed in the field. The export of consecutive piglets batches at weaning was found as the most efficient measure facilitating swIAV infection fade-out (HR = 13.7 [8.0 – 23.4]). Although some vaccination schemes (batch-to-batch vaccination) had a beneficial effect in breeding sows by reducing swIAVs persistence within this subpopulation, none vaccination strategies achieved swIAV fade-out at the within-herd pig population level.

### Discussion and Conclusion

The most commonly-used prevention way (i.e. batch-to-batch vaccination), performed before farrowing to induce high antibody level in piglets, showed limited efficacy in controlling swIAV within-herd persistence alone. Introducing gaps in the growing pig population and a strict separation between the breeding and the growing part of the herd are key factors for swIAV control.



## VVD-030 - PORCINE ROTAVIRUS GROUP A AND C ARE ENZOOTIC ON SWINE BREEDING FARMS

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### Introduction

Rotaviruses are one of the major agents of gastroenteritis in young children and animals. So far eight serotypes are defined (A-H). Depending on the infectious pressure clinical and also economic consequences might be significant. The study focused on the detection of GARV and GCRV in field samples in Germany and the molecular characterization of GARV positive samples.

### Material and methods

In total 24 litters with and without clinical signs of diarrhea were included in the study. Fecal samples from piglets were investigated for clinical signs of diarrhea as well as GARV and/or GCRV shedding by RT-PCR. Corresponding sows were additionally investigated for GARV antibodies in serum and milk.

### Results

Molecular characterization of the diagnosed GARV positive samples in Germany revealed 4 different G-genotypes (G3, G4, G5, G9) as well as 4 different P-genotypes (P[6], P[7], P[23], P[32]) in different combinations. According to the literature they belong to the predominant genotypes of porcine GARV. No uncommon genotypes were present in the pigs included in this study. All piglets from sows having decent amounts of GARV specific antibodies in the milk were protected from GARV shedding. Some of the piglets, however, showed GCRV shedding supporting the observation that no cross protecting immunity exists between these two groups of porcine rotaviruses.

### Conclusion

Porcine rotavirus group A and C are constant companions in animal husbandry. Molecular characterization of the diagnosed GARV positive samples in Germany revealed the presence of at least 4 different G-genotypes (G3, G4, G5, G9) as well as 4 different P-genotypes (P[6], P[7], P[23], P[32]). These genotypes have also been reported in other countries to be present in pig populations. Hygiene and passive transfer of maternally derived antibodies can provide protection from GARV shedding as milk containing GARV specific antibodies showed to correlate best with the absence of GARV shedding.





## VVD-031 - SURVEY AND OUTBREAK OF LESS OBSERVED VIRUSES IN THE CZECH REPUBLIC

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Porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus (PCV2) and influenza virus are viruses very well known as the primary pathogens of pigs causing serious failure in production and reproduction of pigs especially in the respiratory tract. In our epidemiology study we have observed these viruses altogether with other viruses which are not very often diagnosed in the Czech Republic. Porcine cytomegalovirus, porcine coronaviruses both respiratory and enteral (PED-like TGE-like and respiratory form) and group of Teschoviruses which are known to cause different syndromes are observed in the epidemiological study in the representative number of farm in the Czech Republic.

We used both serum and salivary fluid and faeces samples for this study. We have looked for the virus (real time PCR) and antibody (ELISA) against this viral agents.

The result of this study which are still ongoing and which will end in the spring of the 2017 will be the subject of the outcome of this presentation.

The results were obtained with a financial support of the project LO1218 from the MEYS of the CR under the NPU I program and the project QJ1510108 of the Ministry of Agriculture.



## VVD-032 - IDENTIFICATION OF MULTIPLE EUROPEAN SWINE INFLUENZA GENOTYPES IN ITALY BETWEEN 2013 AND 2016

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Passive surveillance for Swine Influenza virus (SIV) in northeastern Italy (Veneto and Friuli Venezia Giulia regions) carried out between 2013 and 2016 identified in 9 infected farms in nine different municipalities covering 4 neighbouring provinces. In some farms repeated respiratory outbreaks were observed over the investigated period.

Watson et al. (2015) have recently described 25 different SIV genotypes in Europe (A-W). To explore the genetic characteristic of the SIVs circulating in the area of investigation in Italy, we isolated and genetically characterized the complete genome of 15 viruses collected from seven different farms using the Illumina MiSeq.

Topology of the maximum likelihood phylogenetic trees identified two pandemic H1N1 (A(H1N1)pdm09), 5 human-like H1N2 (H1<sub>hu</sub>N2) and 5 avian-like H1N2 (H1<sub>av</sub>N2) subtypes, 1 avian-like H1N1 (H1<sub>av</sub>N1) and 1 H3N2 subtype virus. A total of 6 different genotypes were detected: A, B, D, F and P genotypes. The human like H1N2 viruses belonged to 2 different genotypes: F (5 viruses), and 1 novel genotype, which had never been identified in Europe before and the avian like H1N2 belong to 2 different genotypes: A and D. Interestingly genotype D is mainly circulating in Denmark

The presence and geographical distribution of the two novel genotypes in other European countries, their antigenic characteristics and in vivo pathogenicity need to be further assessed. Considering that the A(H1N1)pdm09 matrix protein had previously been associated with increased transmissibility in guinea pig and ferret models, these new genotypes may pose a potential public health risk, therefore their persistence in pigs should be monitored closely.



## VVD-033 - HORIZONTAL TRANSMISSION OF A FRENCH INDEL STRAIN OF PORCINE EPIDEMIC DIARRHEA VIRUS

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### Background & Objectives

The porcine epidemic diarrhea virus (PEDV) causes severe diarrhea with deshydration. A case of porcine epidemic diarrhea occurred in France in 2014 and the InDel strain FR/001/2014 was isolated. The study aimed at evaluating the pathogenicity and spread ability of this strain within a pig population.

### Material and Methods

The study has been carried out in Anses animal facilities according regulations on animal experimentation. Two rooms were used with 10 weaned pigs per room separated in two groups of five. One pig was inoculated orally with 5ml of an inoculum of FR/001/2014 titrating  $10^8$  copies of viral genome/ml. This pig was in direct contact with 4 pigs and in indirect contact with the 5 other pigs housed in a neighbor pen. The virus genome load was quantified by RT-qPCR in blood, feces and air samples and seroconversion assessed by ELISA.

### Results

The inoculated and the direct contact pigs shed virus in the feces from 48-55 hours after inoculation and up to 49 dpi. Three contact pigs showed two distinct shedding periods (between 11 and 39 days) which were likely due to a second infection after the first infection. The seroconversion occurred in inoculated and in direct contact pigs. There were no clinical signs and no seroconversion observed in indirect contact pigs. The viral genome was detected in air samples from the second day after inoculation.

### Discussion & Conclusion

The results of this study evidenced direct contacts as the main transmission route for PEDV InDel strain within this small pig population. Although viral genome was detected in the air samples, airborne transmission of the virus to indirect contact pigs was not effective in our experimental settings. The estimation of transmission parameters will be used for modelling the PEDV InDel strain transmission in a larger population.



## VVD-034 - CONGENITAL POXVIRUS INFECTION IN TWO PIGLETS: A CASE REPORT

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Poxvirus infection in pigs is an acute, mild or subclinical disease characterized by typical skin lesions. Vertical transmission is sporadic, leading to congenital infection and abortion or stillbirth. The aim of this study was to describe a sporadic case and discuss about the pathogenesis of congenital poxvirus infection.

A sporadic case of poxvirus infection occurred in a 680 sows multi-site herd in Northern Italy involving 2 piglets out of 13, belonging to the same litter. One of the affected piglets was stillborn, while the second one was alive at birth, but died few hours later. The sow and the other littermates didn't show clinical signs. The piglets were sent to the laboratory for necropsy. Samples of skin, lungs, stomach, intestine, liver, kidney, spleen and brain were collected and fixed in 10% formalin for histological evaluation. Samples of skin were examined by electron microscopy.

The piglets showed multiple roundish cutaneous lesions (from 0,5 to 3 cm of diameter) over the entire body and in the oral cavity at different stages of development. Differential diagnosis included bacterial or fungal dermatitis, vesicular diseases, skin parasitosis and poxvirus infection.

Microscopically the skin lesions showed circumscribed areas of necrosis extending from the epidermis to the dermis. The keratinocytes showed eosinophilic intracytoplasmic inclusion bodies. A diagnosis of poxvirus infection was confirmed by electron microscopy that showed typical poxvirus particles in all the skin lesion specimens.

The pathogenesis of congenital poxvirus infection in piglets is not completely clear. Sow could become viremic after the spreading of the virus from skin lesions, developing low levels of viremia with a slowly spread of the virus through the uterus. The compartmentalization of the placental membranes could restrict the spreading of the infection from one fetus to another. This could explain why only two piglets of the same litter get infected.



## VVD-035 - EVALUATION OF PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV)-ANTIBODIES IN A 250 SOW HERD AFTER A PEDV OUTBREAK

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### Background & Objectives

Outbreaks of PEDV-infections have occasionally been reported in Germany. After an PEDV-infection time-to-baseline production takes approximately 6 weeks and immunity does not last for long. According to the veterinary literature data are missing that evaluate the duration of protective PEDV-specific antibodies in sows after an outbreak. Thus, our aim was to evaluate PEDV-antibodies and resulting ongoing PEDV-specific immune response in sows after a PEDV outbreak.

### Material & Methods

In a 250 sow herd located in Northern Germany a PEDV outbreak occurred. Subsequently, sows were fed with naturally infectious material to achieve protective levels of PEDV-antibodies in colostrum and milk. Fourteen sows were randomly selected and serum samples were obtained at 2, 8 and 16 weeks (wk) post-infection. PEDV-antibodies were measured using two different enzyme linked immunosorbent assays (ELISAs; IDvet and Biovet). Results of both ELISAs were used to evaluate PEDV-antibodies using repeated measures ANOVA and conduct correlation analysis. Cohen's  $\kappa$  was used to compare both ELISAs based on the cut-off for a positive test result (IDvet:  $\geq 60$  S/P % and Biovet:  $\geq 0.4$  S/P ratio).

### Results

PEDV antibody concentration of the sows measured by IDvet and Biovet ELISAs showed a significant decrease during the study period ( $p=0.0031$  and  $p<0.0001$ , respectively). A positive correlation was observed between both ELISAs ( $\rho: 0.69$  [95% CI: 0.49 to 0.83];  $p>0.0001$ ). PEDV-antibody positive sows were observed at wk 2 [6/14], wk 8 [4/14], but not at wk 16 [0/14] using IDvet, and at wk 2 [11/14], wk 8 [11/14] and wk 16 [6/14] using Biovet. A moderate agreement between both ELISAs was found (Cohen's  $\kappa=0.439$ ; 95% CI: 0.236 to 0.621).

### Discussion & Conclusion

Biovet ELISA results reveal an ongoing PEDV-specific immune response in sows to the next pig cycle based on a PEDV outbreak. However, discrepancy of different ELISAs warrants further investigation.



## VVD-036 - SEROLOGICAL RESPONSES AFTER VACCINATION WITH THE FIRST INTRADERMAL PORCINE CIRCOVIRUS VACCINE

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### Background & Objectives

Porcilis® PCV ID is the first porcine circovirus (PCV) vaccine for intradermal administration with the IDAL injector. PCV protection is based on both cellular and humoral immunity. The aim of this study was to evaluate serological antibody response after vaccination of piglets with Porcilis® PCV ID.

### Material and methods

The trial was conducted in 6 conventional farms. In each farm, piglets were vaccinated with Porcilis® PCV ID at three weeks of age. Depending on the herd size, 10 to 20 piglets were randomly selected and ear-tagged for blood sample collection at 3, 5, 7 and 9 weeks of age.

Samples were tested for antibodies against PCV2 (IgM/IgG - INGENASA and PCV2 - AlphaLisa MSD AH), M. hyo (IDEXX ELISA), PRRS (IDEXX ELISA) and APP OMP (ELISA MSD AH). PCV2 viraemia was tested with qPCR and results were reported as percentage of viremic pigs and average viral load (Log<sub>10</sub> DNA copies/).

### Results

The PCV2 IgM/IgG serological profile was very similar across the six farms. At three weeks of age, IgM seroconversion was absent while more than 80% of the piglets seroconverted by 5 weeks of age. At 7 weeks of age, the IgM response was still high followed by a drop by 9 weeks of age. The IgG response was delayed until 7 weeks of age and remained positive at 9 weeks of age. With the AlphaLisa-test, a quantitative test, the average antibodies remained above 6log<sub>2</sub> until 9 weeks of age. All study piglets remained negative for PCV2 in the qPCR-test.

### Discussion and Conclusion

In all farms, vaccination with Porcilis® PCV ID induced an IgM/IgG response which corresponded to high titers in the quantitative AlphaLisa until at least 9 weeks of age.



## VVD-037 - ASSESSMENT OF VERTICAL TRANSMISSION BY DETECTION OF PCV2 IMMEDIATELY AFTER FARROWING IN SPAIN

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### Introduction

Blood sampling after farrowing has been commonly used to assess virus vertical transmission. Several sample types have been described to determine sow herd PCV2 stability.

The objective of this study was to assess PCV2 qPCR information from placental umbilical cord serum (PUCS) samples in several commercial farms in Spain.

### Materials and Methods

Overall 591 individual serum samples were collected from different sow herds all over Spain. These sources had suboptimal growing parameters in the downstream flow. All of these farms were PRRSv positive and had implemented piglet vaccination for PCV2 before the study.

In all herds, two PUCS from each placenta were milked out into a single blood tube to create two samples per placenta.

Three groups were defined: 1-2 parities, 3-4 parities and 5 or more parities. Samples were taken across all these parity groups in each herd.

Individual PCV2 qPCR was run for each sample.

The prevalence was calculated using the PUCS sample as the experimental unit. The blood viral load was evaluated among positive samples expressed in exponential value but transformed to  $\log_{10}$  for comparison purposes.

An ANOVA was run to analyze the effect of the parities on prevalence and viral load.

### Results

The virus was detected in 188 from a total of 591 samples. This means that the prevalence of PCV2 per pig was 32% among all the farms.

The mean  $\log_{10}$  viral load was 6.54 ( $\pm 0.06$ ).

The qPCR distribution for the viral load in the younger sows (1-2 parities) was significantly higher compared older sows (5 +parities).

The prevalence was significantly higher in sows with 5 or more parities.

### Conclusions

This study, demonstrates that vertical transmission of PCV2 is highly prevalent in Spain.

PUCS samples are a good tool to detect PCV2 vertical transmission which is a consequence of virus circulation in the sow herd. This has been repeatedly shown in studies in other countries.



## VVD-038 - CONTROL AND ELIMINATION OF PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) INFECTION: A CANADIAN CASE STUDY

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### Introduction

Since January 2014, porcine epidemic diarrhea virus (PEDV) and porcine delta coronavirus (PDCV) have been present in Canada, causing severe clinical signs and mortality in over 100 farms. The incidence of cases declined before prevalence reached a high level, and now farm-based eradication projects have reduced prevalence to almost zero.

### Aim

We aim to describe and summarize a series of cases of PEDV from farms within a single veterinary practice in Ontario, Canada. We summarize sources of infection and eradication techniques—including pig movement, hygiene, surveillance and biosecurity.

### Methods

56 cases of PEDV involving 108 sites have occurred in an Ontario veterinary practice. Case definition and characteristics are summarized; including presumed source of infection Eradication plans are described. Follow up testing protocols and results are reviewed.

### Results

Of the 108 sites affected, 72 were sites with sows, and the remaining 36 were sites with weaned pigs or grower/finisher pigs. The annual incidence within the veterinary practice (total 1750 sites) was 3.7% (2014), 1.6% (2015), 0.8% (2016). The putative source of infection is summarized as follows: infective feed (13%); livestock trucks (21%); downstream site from known positive source (50%); unknown/other (16%). Of the 108 infected sites, 96 have now been declared negative. Key attributes of PEDV eradication include: rapid and thorough exposure of entire pig inventory; creating a farrowing gap; cleaning and disinfection techniques, aggressive internal biosecurity measures between positive and negative pigs; thorough testing of affected and downstream sites.

### Conclusion

In the past three years there has been very high success eradicating PEDV from different types of pig rearing sites in Ontario. Some of the remaining positive sites have plans to complete eradication but are not yet complete at the submission of this abstract. As there is no regulatory oversight from national or provincial governments for PEDV eradication, farm response to PEDV infection is voluntary.





## VVD-039 - PREVALENCE OF TAIL LESIONS IN FATTENING PIGS SLAUGHTERED IN SWITZERLAND

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### Presentation of the farm

This case report took place in a post-weaning (2500 weaners) to finishing herd (6000 finishers) in Brittany, France. The weaning piglets of 21 days of age (doa) came from a farrow-to-wean farm of 1000 sows from a long time. The farmers increased their finishing facilities in the first semester of 2015 (from 4000 to 6000 heads). In relation with this modification of the pig flow, despite the absence of clinical signs of porcine circovirus associated disease (PCVD), a PCV2 vaccination was implemented the day of piglet's delivery, in order to avoid a clinical destabilization.

### Clinical case report

In September 2015, clinical signs typical of PCVD (anorexia, wasting) were observed within the 3 first weeks of finishing period. The necropsy, histopathology and PCR on lymph nodes from 2 euthanized affected pigs of 90 and 105 doa revealed macroscopic and histopathologic lesions in agreement with PCV2 infection. The PCR results were positive for PCV2 detection with respectively  $10^9$  and  $10^{13}$  copies/g of tissue.

From November to February we implemented different actions: modification of the timeline of PCV2 vaccination, checking of the quality of injections, management of co-infections but endly without success on the mortality.

In February 2016, qPCR, histopathology and immunohistochemistry realized on lymph nodes from 3 euthanized pigs confirmed PCVD. The PCV2 genome was amplified and sequenced. The PCV2 isolate belonged to the PCV2b-1C genogroup also called PCV2b mutant and sometimes isolated in case of PCV2 vaccine failure.

### Conclusion

After changing the vaccine to another PCV2 vaccine, clinical situation was improved: the mortality rate which rose from 3.5% from weaning to slaughter to 6.5% during the outbreak returned to 3%. It is the first time that a PCV2 isolate of the PCV2b-1c genogroup is characterized in France although such isolates become predominant in China and in USA.



## VVD-040 - MATERNAL ANTIBODIES IMPAIR IMMUNE RESPONSES TOWARDS INFLUENZA INFECTION BUT DO NOT PREVENT PIGS FROM BEING PROTECTED AGAINST A SECOND INFECTION

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### Background & Objectives

Whereas maternally-derived antibodies (MDA) reduce piglets' susceptibility to influenza virus, they extend duration of virus dissemination in batches (Cador et al. (2016) Vet.Res.47:95). MDA interfere also with post-infectious immune responses in piglets, raising questions about protection once passive immunity has waned. Here, we investigated MDA impact on SPF pigs' immune responses following H1N1 infection and subsequent efficiency towards a second homologous infection.

### Material & Methods

In a first trial, 6 MDA- and 6 MDA+ piglets were H1N1-inoculated at 5 and 9 weeks of age. Three other pigs of each status were mock-inoculated. In a second trial, 14 MDA- and 14 MDA+ pigs were H1N1-inoculated at 7 weeks of age, while 14 other MDA- and MDA+ pigs were mock-inoculated. At 11 weeks of age, 7 MDA- and 7 MDA+ infected, or control, pigs were H1N1-inoculated. The last pigs were mock-inoculated. Virus excretion was quantified in nasal swabs by RT-qPCR. Antibody titers were measured in sera using indirect ELISA (anti-NP/M), competition ELISA (anti-NP), hemagglutination inhibition test and virus neutralization. Lympho-proliferation assays were performed on heparinized blood samples.

### Results

Primo-infected MDA- pigs showed symptoms, shed the virus and developed immune responses despite some age-dependent differences. In MDA+ animals, clinical signs increased with MDA level decay. Virus shedding was not prevented. No increase in anti-H1 and neutralizing antibodies was observed whatever the MDA level, but anti-NP and anti-M responses were less affected. Proliferative T cell responses were delayed by high MDA levels. Nevertheless, MDA+ animals were protected from the second infection, as MDA- piglets.

### Conclusion

The impact of MDA on post-infectious responses decreases gradually with their decay and MDA+ animals would not play a role individually in virus persistence at the farm level, at least during 4 weeks post-infection. Advice is provided on serological tests for influenza diagnosis in piglets born to vaccinated sows.



## VVD-041 - SUBTYPING OF SWINE INFLUENZA VIRUSES USING A HIGH-THROUGHPUT REAL TIME PCR PLATFORM

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### Introduction

Swine influenza is a respiratory disease caused by multiple subtypes of influenza A virus (IAV). The genome of IAV consists of 8 segments and subtype classification is based on the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). In Denmark, the influenza screening test and subsequent subtyping is performed by real time RT-PCR (RT-qPCR) but several assays are needed to cover the wide range of circulating subtypes which is expensive, resource and time demanding. To mitigate these restrictions the high-throughput qPCR platform BioMark (Fluidigm) has been explored. The BioMark platform uses less sample and reagent volume compared to standard qPCR platforms and allows for up to 9,216 parallel reactions on one chip.

### Materials and Methods

A total of 14 PCR assays specific for the different subtypes of HA and NA genes relevant for swine influenza and 6 assays specific for the internal genes of IAV were validated and optimised to run under identical reaction conditions and assembled on a dynamic array chip (Fluidigm).

### Results

The sensitivity and specificity of the chip was assessed by testing cell culture isolates and field samples with known subtypes (based on sequencing). The results revealed that the performance of the dynamic chip was similar to conventional real time analysis.

### Discussion and Conclusion

Application of the chip for subtyping of swine influenza has resulted in a significant reduction in time, cost and working hours. Thereby, it is possible to offer diagnostic services with reduced price and turnover time which will facilitate choice of vaccines and by that lead to reduction of antibiotic used.



## VVD-042 - EVALUATION OF NOSE-TO-NOSE TRANSMISSION OF BOVINE VIRAL DIARRHEA VIRUS IN EXPERIMENTALLY INFECTED PIGLETS

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### Background & objectives

Swine can be infected by BVDV under natural conditions. Direct contact with cattle in the same property is the main cause of the appearance of infections by this agent in pigs. Thus, the objective of this study was to verify the nose-to-nose transmission of BVDV in experimentally infected piglets.

### Material & Methods

Six piglets with 20 days of age were randomly selected, separated into three groups by isolators (control, infected and sentinel). Animals from the sentinel and infected group were connected to have direct contact. The animals of the infected group were inoculated orally and nasally (BVDV-1, Singer strain), and the experimental period was performed in 25 days (daily collection of nasal swabs and blood collection every 7 days); euthanasia and necropsy performed at the 25<sup>th</sup> day. Seroconversion was evaluated by means of Virusneutralization (VN). Detection of virus presence in nasal secretions was performed by RT-PCR.

### Results

The results showed that seroconversion of the infected piglets (I1 and I2) occurred on the 25<sup>th</sup> day after infection, presenting antibody titers of 80. Control and sentinel piglets did not present seroconversion. Although the presence of antibodies was detected only on the last day, viral excretion was detected in the nasal swab samples in one of the infected animals (I2) by RT-PCR on 17 and 21 dpi.

### Discussion & conclusion

The study was successful in inducing the experimental infection of pigs using BVDV, in addition to the detection of neutralizing antibodies against the virus. The inoculated piglets excreted the virus before the detection of seroconversion, however the transmission to the other animals was not observed. The possibility of transmission of BVDV by the nasal route in piglets was not proved within the evaluated period.

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## VVD-043 - INVESTIGATION OF PCV2 ANTIBODY PREVALENCE IN SOWS IN AUSTRIA

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### Background & Objectives

Prevalence of PCV2 antibodies (Ab) in sows has been rarely described and most reports describe data from times before piglet vaccination has been introduced. After several years of piglet vaccination and a concomitant reduction of viral loads, we hypothesised that natural booster of sows might have become unreliable. Therefore, we aimed to investigate PCV2 Ab prevalence in sows.

### Material & Methods

The study included 12 farms, representative for Austrian pig production, in the three main pig producing regions. On farms piglets have been vaccinated against PCV2 for at least 3 years. Sows used for sample collection were healthy, in early-/mid-gestation and have not been re-vaccinated against PCV2. No gilts were included. Cross-sectional blood samples were collected once per farm. The number of samples was calculated based on an expected prevalence of  $\geq 70\%$ , an accuracy of  $\pm 10\%$  and a confidence level of 90%. Samples were analysed using the INGEZIM CIRCO IgG ELISA by Ingenasa.

### Results

Forty out of 489 sows were PCV2 Ab negative (8.2%). The majority of negative sows were parity 1 (15/40). All sows of parity 6 and higher were Ab positive. Prevalence was above 90% in 9/12 farms. Three farms had a proportion of Ab negative sows of 22.0 – 31.4%. Parity reached from 1 to 15 and distribution was highly variable between farms, but the majority of the sows were parity 2-5.

### Discussion & Conclusion

Ab prevalence in 9/12 farms investigated was comparable to numbers reported before implementation of piglet vaccination. However, 3/12 farms had more than 20% of sows negative for PCV2 Abs, which may put those herds at risk for PCV-RD or increased in-utero infection of piglets. In a follow-up project the impact of sow vaccination will be investigated in one of these farms.



## VVD-044 - FIELD STUDY COMPARING TWO VACCINES USED IN THE CONTROL OF THE PORCINE CIRCOVIRUS TYPE 2 (PCV2)

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### **Background & Objectives**

Many PCV2 vaccines are available on the market. The selection of a vaccine is based on its efficacy but also on its impact on growth performances. The aim of this study was to compare two PCV2 vaccines by assessing the weight gain and the mortality rate.

### **Material & Methods**

This trial was conducted in a farrow to finish, 1400 sows farm in France. Overall 1187 piglets, from 2 consecutive batches were included in the study. The day before weaning piglets were weighed, identified individually and randomly allocated to one of the 2 vaccination group. Group C was vaccinated with 1 ml of Ingelvac CircoFLEX<sup>®</sup>. Group S was vaccinated with 2 ml of Suvaxyn PCV<sup>®</sup>. Ten non-vaccinated sentinel piglets per batch were included to assess the PCV2 infection status. Average Daily Gain (ADG) from weaning to Day 39 was calculated by weighing all piglets individually 14 days after vaccination. ADG from weaning to slaughter was calculated considering the slaughter data. Mortality was recorded during the whole study period. ADGs and mortality rates were compared between groups using a t-test and a Chi-square test respectively.

### **Results**

The PCV2 circulation was confirmed on the sentinel animals by PCR. Fourteen days after vaccination, ADG in Group C (184.4 g/day) was significantly higher than in group S (176.2 g/day) ( $p=0.026$ ). Also from inclusion to slaughter, ADG in group C was higher than in Group S however the difference was not significant. The mortality rate in Group C (3.3%) was significantly lower than in Group S (6.0%) ( $p=0.037$ ).

### **Conclusion**

The difference in ADG shortly after vaccination suggests that different PCV2 vaccines do not induce the same level of local and systemic reactions. The differences in performance parameters over the whole trial period especially in mortality rate indicate differences in efficacy between the two vaccines.



## VVD-045 - ROLE OF IFN- $\alpha$ IN MULTIFACTORIAL RESPIRATORY DISEASE OF SWINE

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Porcine reproductive and respiratory syndrome virus (PRRSV) can predispose pigs to the secondary respiratory infection with bacteria such as *Haemophilus parasuis*. Animals infected with both pathogens develop more severe clinical disease. Macrophages serve as the target of replication of PRRSV. There are two types of macrophages – porcine alveolar macrophages (PAMs) as resident cells provide one of the first lines of defence against microbes invading the lung tissue. On the other hand, monocyte-derived macrophages (MDMs) as inflammatory macrophages are naive cells accumulating in site of inflammation. Concurrent infection of PAMs or MDMs with PRRSV and/or *H. parasuis* was analysed *in vitro* and difference dependent on macrophage type were observed. MDMs were more sensitive to PRRSV infection resulting in higher mortality of cells and higher production of IFN- $\alpha$  compared to fully differentiated PAMs. Elevated level of IFN- $\alpha$  decreased expression of pro-inflammatory cytokines (IL-1 $\beta$  and IL-8) what was confirmed also by experimental addition of IFN- $\alpha$  to MDMs followed by infection with *H. parasuis*. In addition, MDMs infected with *H. parasuis* alone, but not in co-infection, formed multinucleated giant cells (MGCs). Infection with PRRSV could therefore facilitate the development of a secondary bacterial infection by avoiding the formation of MGCs by macrophages. Higher sensitivity of undifferentiated macrophages accumulating in site of inflammation could contribute to the development of multifactorial respiratory disease of swine. The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (project LO1218) and the Ministry of Agriculture of the Czech Republic (QJ1210120).



## VVD-046 - ROTAVIRUS TYPE A ASSOCIATED DIARRHOEA IN NEONATAL PIGLETS: IMPORTANCE AND BIODYNAMICS

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### Background

Rotavirus A (RVA) is a well-known cause of diarrhoea in piglets, but the infection dynamics and clinical impact are not fully elucidated. The aim was to determine the significance of infection with RVA in relation to neonatal diarrhoea.

### Material/Methods

Two commercial swine herds with neonatal diarrhoea and a positive RVA diagnosis were included. Five litters from each of two herds and a total of 132 piglets were sampled. The animals were subjected to a daily clinical examination and faeces were collected daily from all piglets. The piglets were weighed at beginning and at the end of study. The outbreak day was defined as the day where >25 % of the litter showed clinical diarrhoea, and for all litters this was either the 4<sup>th</sup> or 5<sup>th</sup> day of life. On outbreak day, fecal samples from all piglets in the litter were analysed for RVA together with samples collected two days prior to outbreak day from 54 of the 132 piglets. The analyses were made by a RVA specific RT-qPCR. Virulent *E. coli* was ruled out by PCR as differential diagnosis in all litters by testing a pooled sample on the outbreak day.

### Results/Discussion

In total, 43%(57/132) of the piglets had clinical diarrhoea and 66%(87/132) were positive for RVA on the outbreak day. For comparison, 89%(51/57) of the diarrheic piglets and 48%(36/75) of the non-diarrheic piglets were positive for RVA which was significantly different ( $P<0.001$ ). Piglets that tested negative for RVA had a higher weight gain over the 4-day period (mean 363g vs. 278g,  $P<0.05$ ) despite that the positive piglets had a significantly higher birthweight (mean 1,45kg vs. 1,27kg,  $P<0.05$ ). Furthermore, 63%(34/54) of the piglets developed diarrhoea within 24 hours after a positive RVA diagnosis. The results confirmed that RVA has a significant impact on incidence of diarrhoea and weight gain also in *E. coli* negative litters.





## VVD-047 - THE USE OF BIOPORTAL TO ANALYSE ORF 5 PRRS SEQUENCES FROM COMMERCIAL FARMS IN SPAIN

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### Introduction

During the last 6 years, Boehringer Ingelheim Vetmedica, Inc. (BIVI) in collaboration with UC Davis have adapted the program Disease BioPortal to the particularities of PRRSV.

The aim of this work is monitoring the PRRS isolates at country level for Spanish production sites by using Disease BioPortal.

### Materials and Methods

Seven hundred and thirty six ORF5 sequences were obtained from 39 different companies corresponding to 74 different farms across Spain in 2015-2016. The Disease BioPortal software (<http://bioportal.ucdavis.edu/>) was used to generate the phylogenetic trees and evaluated the genomic distances between the sequences. To determine if a sequence is different to another, we assumed a threshold of 2 % even though there is no consensus in that point.

### Results

Overall, 44% of the sequences came from due to wean piglets, 47% from the piglets at the end of the nursery and only 4.9% and 3.2% came from finishers and gilts respectively.

We were able to detect all the relevant PRRSV modified live vaccine strains in Spanish market even through the vaccination program implemented was based on BI vaccines. Rerouting the phylogenetic tree to the first isolate detected in Europe Lelystad (1991) we found that the nearest and the most heterologous wild-type isolate found in our data base (excluding vaccine like strains) range from (7.23% to 17.42%). This result suggests an annual evolution of the virus between 0.29 and 0.69% similar (0.5% annual evolution) to that described in other studies.

### Conclusions and Discussion

Farms selected were representative for Spanish conditions. This study confirms that PRRS MLV strains can be found in the study farms despite the use of a different vaccine. The study confirms the large heterogeneity of PRRS type 1 ORF5 in Spain. BioPortal will be able to help monitor the strains within a farm, company, region even at a country level as this study has demonstrated.



## VVD-048 - STUDY OF THE EFFICIENCY OF ORF5 PCR DEPENDING ON THE CT VALUES OF ORF7 PCR IN PRRS VIRUS

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### Introduction

PRRS virus is one of the most important pathogens in the swine industry. Sequencing of ORF 5 can help you to monitor your biosecurity. The main problem of this technique is the lower sensibility usually that ORF5 PCR has compared to ORF7 PCR.

This work, analyze two methods of real time RT-PCR for ORF7 and ORF5 from field serum samples belonging to potentially infected animals.

### Materials and Methods

2309 pools of 5 serum samples were obtained from 74 different PRRS positive farms in Spain during a year. Commercial primers were used for ORF7 PCR. For ORF5 primers more than 68 divergent isolates from Genbank sequences were used.

The positive ORF 7 PCR samples were divided in 5 groups regarding to their threshold Cycles (CT) values. Group 1: <24 CT, 2: 24-29, 3: 30-32 CT, 4: 33-35 CT, 5: >36 CT. Each of these groups was correlated with the % of positive in ORF5 PCR.

### Results

In average 81% of ORF7 positive samples gave a positive result for ORF5 but these results are quite variable depending of the Cts values results of the ORF7 PCR. Group 1 and 2 reached 100% and 95.1% of positive results coming from a positive ORF 7 PCR sample. Group 3 and 4 obtained 74.9% and 51.5% of positive results respectively, been 0% the result in case of group 6.

### Conclusions and Discussion

This study shows that the preferred ORF to diagnose is ORF7 otherwise according to our results 19% of positive samples been positive they would have been considered negative using ORF5 PCR. The ability to have a positive result in ORF 5 PCR from a positive ORF7 PCR sample is inversely proportional to the CT value of the ORF7 PCR result. This probably is related with the size of the amplicon, the efficiency of the primers or both.



## VVD-049 - DETECTION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) DNA IN PIGS VACCINATED AGAINST PCV2

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### Background & Objectives

The aim of the study was to compare the presence of PCV2 in serum, feces and oral fluid of pigs from 12 Polish farms vaccinated against PCV2.

### Materials & Methods

Serum, feces and oral fluid samples from piglets, weaners and fatteners were obtained in 12 farms using CircoFlex (Boehringer Ingelheim), Porcilis PCV (MSD) or Suvaxyn PCV (Zoetis). Vaccination protocols were implemented from 4 months to 8 years. Samples were analyzed with in house Real Time PCR for PCV2. Ct>37 was considered negative.

### Results

PCV2 was detected in serum from 6 out of 12 farms. In farms 6 and 8 viremia was detected in fatteners and in farms 2 and 5 in weaners and fatteners. In farm 3 it was detected only in 13-week-old pigs and in farm 12 only in 17-week-old pigs. On average PCV2 was detected in only 15.6% of serum samples. PCV2 was detected in 37.8% of fecal samples and the prevalence ranged from 0% in farms 1, 3 and 7 to 78.3% in farm 2. On average 50% of oral fluid samples were positive for PCV2, but the prevalence ranged from 0% in farms 3 and 7 to 100% in farms 2, 4 and 5. PCV2 was detected in oral fluid of weaners and fatteners from most of the farms. In farm 1 it was detected only in 12-week-old pigs, in farm 9 only in 4-week-old pigs and in farm 10 only in 3 and 18-week-old pigs.

### Discussion & Conclusion

Vaccination against PCV2 is known to significantly limit viremia and shedding but this effect can be very different between the farms. At present it is unclear whether the observed differences in PCV2 circulating patterns are related to different efficacy of PCV2 vaccination. Oral fluid can be a recommended sample for PCV2 monitoring in negative farms.



## VVD-050 - DOES EXTENDED STORAGE OF ORAL FLUID IMPACT PCR AND ELISA RESULTS OF DETECTION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS?

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### Background & Objectives

Oral fluid and serum are commonly used samples for PRRSV diagnosis and monitoring by ELISA and PCR. The aim of the study was to evaluate the impact of oral fluid sample handling and preservatives addition on sensitivity of Real-time PCR and ELISA after storage at room temperature.

### Material & Methods

Serum and oral fluid samples were obtained from PRRSV positive farms and were confirmed to contain PRRSV or specific antibodies. The study was performed to assess the impact of PBS and chlorhexidine addition on Real Time PCR and ELISA results from samples stored at room temperature for up to 10 days. Additionally the impact of oral fluid centrifugation (3min/8000 rpm) on PCR and ELISA performance was assessed. The PRRSV antibodies in oral fluid and serum were detected with IDEXX PRRS OF Ab Test, ELISA IDEXX X3 PRRS, respectively. PRRSV RNA was detected with EZ-PRRSV MPX 4.0 Real Time PCR reagents (Tetracore Inc.), following the extraction with QIAamp Viral RNA Mini Kit (Qiagen). The Ct>37 was considered as negative.

### Results

The Ct values increased and S/P values decreased gradually over time suggesting degradation of the analytes. This degradation occurred faster in oral fluid than in serum samples. Oral fluid centrifugation decreased sensitivity of Real Time PCR and ELISA. The impact of PBS and chlorhexidine addition on PCR was variable while it was non existing for ELISA.

### Discussion & Conclusion

As expected serum samples were more stable at room temperature than oral fluid. However, our results indicate that the native oral fluid samples, with Ct values of about 31 at day 0, without stabilizing additives, remained positive at day 4 at room temperature. Oral fluid samples remained positive in ELISA after 10 days in room temperature.

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## VVD-051 - DETECTION OF PORCINE PARVOVIRUSES 1, 2, 3, 4, 5 AND 6 IN SERUM AND ORAL FLUID OF PIGS IN POLAND

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### Background & Objectives

In the last two decades novel parvoviruses were detected in pigs worldwide. The aim of the study was to compare the presence of parvovirus DNA in serum and oral fluid samples from Polish pigs.

### Material & Methods

Samples were obtained from six farms from pigs of 5 to 19 weeks of age. From each sampled age group 4 to 12 serum samples were obtained and pooled. One oral fluid sample was collected per pen. Samples were tested with Real Time PCR.

### Results

PPV1, PPV2, PPV3, PPV4, PPV5 and PPV6 were detected in 12.50%, 46.67%, 23.33%, 5.83%, 25% and 16.67% of oral fluid samples and in 3.08%, 33.87%, 26.15%, 7.69%, 15.38% and 9.23% of serum samples, respectively. PPV2 occurred in 5/6 farms, PPV1, PPV5 and PPV6 in 4/6 farms, PPV3 and PPV4 in 3/6 farms. In 5/6 farms from 3 to 6 parvovirus species were co-circulating. In one farm only PPV2 occurred. PPV6, PPV1 and PPV3 were detected only in oral fluid samples in 3/6, 2/6 and 1/6 farms respectively. Overall, 16.84% of the weaner samples were positive and 20.10% of fatteners. In 5-12-weeks-old pigs mainly PPV2 (57.58% of positives) was detected. In 13-14-weeks-old pigs mainly PPV2, PPV3 and PPV5 occurred (23.4% each). In 15-16 and 17-19-weeks-old pigs the most prevalent was PPV5 (35% of positives) and PPV3 (21.57% of positives) respectively.

### Discussion & Conclusion

Novel PPVs are commonly present in Polish farms. They were detected more often in oral fluid samples than in serum (except PPV4). This shows that oral fluid is better sample for monitoring of PPVs circulation in swine herds. High PPV2 prevalence in young pigs and PPV3 and PPV5 in older pigs suggest potential impact of pig health.



## VVD-052 - COMPARISON OF SIX COMMERCIAL ELISA KITS FOR THE DETECTION OF ANTIBODIES AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY VIRUS

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### Background & Objectives

ELISA is the most popular method of PRRS diagnosis and monitoring in swine herds. The aim of the study was to compare the performance of six commercially available PRRSV ELISA kits using clinical samples from 10 Polish farms.

### Material & Methods

The following kits were used: CIVTEST SUIS PRRS E/S (HIPRA) (Kit 1), IDEXX PRRS X3 Ab Test (IDEXX) (Kit 2), used as a reference kit, INgezim PRRS 2.0 (Ingenasa) (Kit 3), VetExpert PRRS Ab ELISA 4.0 (Bionote) (Kit 4), Pigtype PRRSV Ab (Qiagen) (Kit 5) and PrioCHECK PRRSV Antibody ELISA (ThermoFisher) (Kit 6). For the specificity assessment, sera from sows (n=139) from 5 PRRSV-naïve herds, and for the sensitivity assessment sera from growing pigs (n=253) from 5 PRRSV- positive farms were tested.

### Results

The highest specificity was obtained with Kit 1 and 2 (100%). Kit 3 had 96%, Kit 4 and 5 had 98% and Kit 6 had 94% specificity. The highest number of positive samples was detected with Kit 6. Its sensitivity as compared to Kit 2 was 104.5%. Sensitivity of other investigated kits was 81%, 92%, 88.5%, 99% for kit 1, 3, 4 and 5 respectively, as compared to our reference Kit 2.

### Discussion & Conclusion

All tests are suitable for general evaluation of PRRS status in positive, unstable farms. The overall best performance had Kits 2 and 5. The highest sensitivity of the Kit 6 is likely to originate from relatively low specificity, as it was shown on samples from negative sow farms.



## VVD-053 - DIFFERING REMOVAL (MORTALITY AND CULLS) RATES IN COMMINGLED PIGS FROM PRRS POSITIVE AND NEGATIVE SOURCES

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Differing Removal (Mortality and Culls) Rates in Commingled Pigs from PRRS Positive and Negative Sources

### Background

PRRS continues to be a challenge for the USA swine industry especially in the grow finish phase of production. Unfortunately, sow herds continue to break, this is particularly a problem when pigs are commingled from multiple sow farm sources. The health of the flow of pigs is only as good as its weakest link. The objective of this study was to evaluate the mortality percentage based on source farm (wean to finish). To determine the impact of mixing sources when one source is positive.

### Materials and Methods

Identified a commingled flow of pigs from 3 different source farms. One source had just turned positive at the time of stocking the wean to finish site. The other sources continued to stay negative during the filling of site. These pigs were all tagged to identify the pigs individually and recorded to the sow farm. Pigs were individually weighed and allocated to pens by weight to ensure standard weight distribution in all of the pens. Mortality was recorded for all dead pigs

### Results

The mortality was higher in the pigs sourced from the positive source, even when mixed at weaning. Removal rate for positive source was 32.3% in, for negative first negative source 6.0% and second negative source 9.2%. Other production parameters were also affected such as ADG, ADFI, F/G and number of culls from the group.

### Discussion and Conclusion

It is ideal to split flows of positive and negative pigs once a herd turns positive however this not always possible or may take some time to accomplish. Fortunately, the mortality is biggest problem in the pigs from the positive site despite the other negative production impacts of PRRS on the group.



## VVD-054 - IDENTIFICATION OF THE NOVEL ATYPICAL PORCINE PESTIVIRUS CIRCULATING IN SPAIN FROM 1997 TO 2016

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### Background and objectives

Atypical porcine pestivirus (APPV) has been recently described in USA, The Netherlands and Germany associated to Congenital Tremor (CT). This study aimed to determine the presence of APPV in Spain to provide information regarding pathogenesis and the molecular epidemiological link with recently reported strains.

### Material and Methods

Samples from 2-day-old piglets with moderate-severe CT were evaluated by qRT-PCR. Subsequently, a retrospective study to detect APPV RNA was performed in a serum collection (Veterinary School of Barcelona) from necropsied pigs (1997-2016). Sanger method for sequencing partial NS2-3 fragment was conducted. Partial NS2-3 sequence from 11 Spanish APPV strains from 2001-2016 were obtained; a phylogenetic tree was constructed using the Maximum Likelihood method.

### Results

A high APPV RNA load was detected in lymphoid organs of CT affected piglets, mainly in the tonsil; virus was also detected in cerebrospinal fluid, brain, and nasal/rectal swabs. Additionally, positive samples were detected from 1997 to 2016 in 89/642 (13.9%) serum samples analyzed. In the first week of age, 54 out of 161 (33.5%) piglets were viraemic for APPV, but viraemic pigs at older ages were also detected. The highest RNA load was found in animals <1 week of age. Phylogenetic analysis from 1615 NS2-3 nucleotides showed three well defined APPV clusters.

### Discussion and conclusion

APPV has been circulating in Spain at least since 1997. A close phylogenetic relationship of APPV circulating in Spain and Germany was found. Results suggest that lymphoid tissues might constitute a target for APPV, thus the potential immunosuppressive capacity of APPV cannot be underrated. APPV presence in Spain during last two decades strongly suggests it is probably a rather widespread virus

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## VVD-055 - PNEUMONIC MANIFESTATIONS OF PORCINE RESPIRATORY DISEASE COMPLEX IN FATTENING PIGS AND ROLE OF PRRSV AND PCV2 INFECTION

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### Background & Objectives

Porcine respiratory disease complex (PRDC) is a major health concern in the pig industry worldwide. PRDC is associated with different pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), *Pasteurella multocida* and porcine mycoplasmas (i.e.: *M. hyopneumoniae* and *M. hyorhinis*). Taking into account condemned lungs from fattening pigs at slaughterhouse, we aimed to characterize the different morphologic types of pneumonia and the role of PRRSV and PCV2 in PRDC affected pigs.

### Material & Methods

A total number of 127 condemned lungs from 12 farms were referred to the Diagnostic Services of the Veterinary Medicine Faculties of Córdoba and Murcia. Lung samples from each pulmonary lobe were collected and fixed in 10% formalin for both histopathological and immunohistochemical examinations, making use of specific antibodies against PRRSV and PCV2 antigens.

### Results

Single pneumonic processes were rarely diagnosed; in contrast, coexistence of 2 and 3 pneumonic patterns dominated. Thus, the triple concurrent association of suppurative bronchopneumonia, interstitial pneumonia and bronchiointerstitial pneumonia was the most frequent diagnosed process (52 out of 127 cases, 40.95%). Interestingly, 97 lungs showed a marked bronchial-associated lymphoid tissue hyperplasia, lesion related to *M. hyopneumoniae* infections. The immunohistochemical analysis against PRRSV and PCV2 antigens revealed 31 lungs positive for PRRSV, 7 lungs positive for PCV2 and 18 lungs positive for both viral antigens.

### Discussion & Conclusion

These findings demonstrate the multiethiological origin of PRDC in all studied farms, showing a significant role for PRRSV, and likely for *M. hyopneumoniae*, as infectious agents. Although PCV2 antigen was also detected, either alone or in combination with PRRSV, its contribution appears to be less determining in these farms, probably due to immunization against this agent.



## VVD-056 - AN ATTEMPT TO ERADICATE PRRS IN 11 WALLOON PIG FARMS (BELGIUM)

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### Background & Objectives

From February 2009 until January 2016, 11 pig farms located in the South part of Belgium (Wallonia) were included in a project of PRRS eradication. These farms were farrowing-to-finish units with on average 176 sows [12 to 600].

### Material & Methods

In a way to demonstrate the possibility to become PRRS-free or to maintain a free status, continuous education and communication meetings with producers, technicians and veterinarians were organised. Serological tests (Elisa and PCR) were performed every 4-6 months. Biosecurity scoring was performed using Biocheck (UGent). When necessary, vaccinations of sows and/or piglets were realized with modified live virus in a way to reduce virus circulation.

### Results

Three farms were and stayed PRRSv-free until the end of the project. One farm became PRRSv-free on Augustus 2011. Four farms began to produce negative finishing pigs while sows continued to be vaccinated. In two farms, active viral circulation was demonstrated in sows and/or growing pigs. Finally, one farmer decided to stop pig production. The present poster presents and details the results obtained.

### Discussion & Conclusion

In some farms, some mistakes (non respect of vaccination protocol and/or biosecurity measures such as introduction of PRRS-positive semen) were identified that could explain the active viral circulation. Otherwise, to become and remain PRRS-free is possible in Wallonia, a low density area of swine production.



## VVD-057 - DETECTING PORCINE CORONAVIRUSES PEDV, PDCOV AND TGEV BY REVERSE TRANSCRIPTASE REAL-TIME PCR

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Porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine delta coronavirus (PDCoV) cause gastrointestinal diseases in pigs. Clinical signs can include diarrhea, vomiting, dehydration and anorexia. Histologically, the diseases are often associated with villous atrophy. In Europe PEDV is an endemic disease. Our knowledge about the spread of porcine coronaviruses and the importance of early detection has led to the need for regular environmental testing that includes checking trucks, barn floors, walls and incoming feed components to prevent new outbreaks in naive herds.

We designed a reverse transcriptase real-time PCR (RT-rtPCR) assay to detect all three coronaviruses in a single reaction. This test can be performed with environmental samples taken off the floors and walls of barns, swabs taken from trucks, or swabs from farm personnel. The multiplex RT-rtPCR assay uses a different fluorescent dye for each target for pathogen ID, and includes an internal positive control. Bioinformatic tests show that the assay will detect all known strains of PEDV, TGEV, and PDCoV based on sequences from around the world that were entered into GenBank. We have found that the multiplex coronavirus assay worked with all environmental samples tested, including samples collected from pigs at the individual and herd level. The sensitivity and specificity of the multiplex coronavirus assay was determined on field samples consisting of oral fluids, feces, and diverse types of environmental samples. Over 400 field samples were tested. The workflow for testing field samples includes RNA isolation, RT-rtPCR and data analysis. Results from testing field samples showed a sensitivity of  $\geq 97\%$  and a specificity of 100% for all three targets.

In conclusion, a multiplex RT-rtPCR assay was designed for detecting three enteric coronaviruses. Here we show a workflow that is simple, sensitive, and accurate for detecting PEDV, PDCoV, and TGEV in diverse biological and environmental samples.



## VVD-058 - MOLECULAR IDENTIFICATION OF PORCINE EPIDEMIC DIARRHEA VIRUS IN SPANISH SWINE HERDS

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### Introduction

PEDV causes porcine epidemic diarrhea, a disease reported in several countries from Europe, Asia and recently USA, where a highly-virulent strain caused severe economic losses. In Spain, it was described in 1990's, in fattening pigs with low-mortality rates. However, there is sparse information about its current status in the country. This study describes PEDV identification in Spain in the last years

### Material and Methods

A qPCR assay targeting the Nucleoprotein gene was developed for identification of PEDV. A total of 232 clinical cases of enteric disease received in our lab during 2013-2014 were evaluated using this qPCR; samples included intestine, feces and rectal swabs. Partial nucleotide sequences for S gene from four Spanish PEDV strains were obtained to study their genetic characterization.

### Results

The qPCR showed specific detection of PEDV with LOD of 50 copies/reaction. PEDV was identified in 29% of clinical cases, in 9 from 17 Spanish provinces and in intestine (15%), feces (45%) and rectal swabs (32%) samples. Partial sequence of S gene showed 99% homology to recent PEDV sequences reported in Europe, but only 91% to highly-virulent PEDV emerged in USA.

### Discussion and Conclusion

This study describes molecular identification of PEDV in herds from several provinces in recent years, suggesting disease is still endemic in Spain. None clinical cases were compatible with those recorded for highly-virulent PEDV variant. Furthermore, partial sequences of spike gene also support that Spanish PEDV strains are different from variants that caused highly severe outbreaks recently in Asia and USA.



## VVD-059 - EVALUATION OF A NEW REAL-TIME PCR TEST FOR THE DETECTION OF AFRICAN SWINE FEVER VIRUS

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### Introduction

African-swine-fever-virus (ASFV) is a highly contagious, enveloped DNA virus. Isolates can vary in virulence, with up to 100% mortality. AFS is one of the most important swine diseases. IDEXX has developed the RealPCR ASFV DNA Test, multiplex-real-time PCR-test, to detect ASFV-DNA. The test uses an internal-sample-control approach for detection of endogenous swine-DNA. Summary of the evaluation of the test at the European reference laboratory for ASF, INIA-CISA in Spain will be presented here.

### Materials and Methods

Reaction mixes contained equal parts of RealPCR-DNA Master-Mix and target-specific IDEXX detection-mix for ASFV 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. These 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 analytical sensitivity was determined as < 10 copies/reaction with an efficiency of 101.7 % over an 8-log range. A high copy number of synthetic-swine-ISC target did not outcompete a low copy number for ASFV. In experimental-evaluations at the European reference laboratory for ASFV (INIA-CISA), up to 93% of the infected-animals could be detected, versus 91% with the in house PCR and 67% with the OIE real-time-PCR-test. Additionally, it showed highest-sensitivity for all 162-tested-samples versus the OIE real-time PCR-test and detected all 22 reference ASFV genotypes tested. The RealPCR-ASFV-DNA-Test is fully compatible with the modular IDEXX-RealPCR-platform, with an internal-sample-control specific to a swine DNA target.

### Conclusions

These results demonstrate that the RealPCR ASFV DNA Test is a useful new tool for the early detection of ASFV DNA. The test can be run on the IDEXX RealPCR platform using the shared reagents and standardized RealPCR cycling program for all RNA and DNA targets.



## VVD-060 - PIGS ARE SUSCEPTIBLE TO MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS INFECTION

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### Background and Objectives

Middle East respiratory syndrome (MERS) cases in humans continue to rise in Saudi Arabia and, occasionally, in other countries. Dromedaries are the main virus reservoir, but other animal (domestic or wild) species might be susceptible to MERS coronavirus (MERS-CoV) and act as reservoir species. The present study aimed to understand the potential role that pigs could play in MERS-CoV transmission.

### Material and Methods

Five pigs were intranasally inoculated with  $10^7$  TCID<sub>50</sub>/animal. Ten animals were used as direct contact (n=5) or indirect contact (n=5) to assess transmission, and they were exposed to their inoculated counterparts on day 2 post-inoculation (PI). RT-PCR and viral titration to detect MERS-CoV was performed on nasal swabs from several days PI (1, 2, 3, 5, 7, 10, 15 and 25). Sera were obtained before challenge and at 14 and 24 days PI to detect specific MERS-CoV antibodies. Pigs were euthanized at 25 days PI, and a complete necropsy was performed. Histopathological and immunohistochemistry assessment was done on the respiratory tract.

### Results

Viral RNA was detected in all inoculated animals and in 3 of 5 in direct-contact animals, but infectious virus was only detected in inoculated pigs. Positive RT-PCR results were detected in nasal swabs from days 1 to 10 PI, and in direct contact animals from day 1 to 4 after contact. No viral RNA was detected in indirect contact pigs. All inoculated animals sero-converted, but not the contact ones.

### Discussion and Conclusion

Pigs are susceptible to MERS-CoV infection, although limited animal-to-animal transmission was demonstrated. Thus, wild *Suidae* (i.e. common warthogs and bushpigs), present in geographical areas where MERS-CoV circulation has been evidenced, could also be susceptible to the virus.

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Faldyna, M.	KL08, IMM-OP-03, REP-010, VAC-013, VAC-015, VAC-049, VPH-008, VVD-045	Gallardo Frontaura, C.	VVD-059	Górmez Laguna, J.	BBD-073
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Falkenau, A.	VAC-021	Gallissot, M.	HME-028, WEL-021	Gotter, V.	BBD-002, BBD-027, BBD-043
Fang, L.	WEL-023	Gambade, P.	VPH-009	Gow, L.	VVD-059
Fang, L.H.	WEL-019	Ganges, L.	VVD-054, VVD-OP-05	Gracia, M.I.	WEL-026
Fano, E.	BBD-OP-06, HME-054, BBD-004, BBD-006	Gantelet, H.	BBD-046	Græsbøl, K.	VPH-OP-02
Farneti, S.	BBD-039	Garbes, N.	VVD-027	Grahofer, A.	REP-OP-05, RES-005, REP-012, VAC-024
Farzan, V.	BBD-OP-07	Garbo, A.	WEL-030	Gresland, B.	MIS-004, VVD-033, VVD-016
Felföldi, B.	BBD-001, BBD-072	García-Bocanegra, I.	BBD-078	Gréau, P.	HME-028
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<b>grosse Beilage, E.</b>	BBD-047, BBD-081, WEL-032	<b>Hidalgo, Á.</b>	VAC-004, VAC-016, IMM-OP-05	<b>Jolie, R.</b>	VAC-008, HME-017, VAC-007, VAC-017, VAC-033, VAC-034, VVD-018, VVD-021
<b>grosse Kamphake, M.</b>	MIS-007	<b>Hidalgo, D.</b>	VVD-017	<b>Jordà Casadevall, R.</b>	BBD-045, VAC-028
<b>Grütznér, N.</b>	BBD-047, BBD-081, VVD-035, WEL-027, BBD-044	<b>Hidalgo, F.</b>	BBD-078	<b>Jordà, R.</b>	BBD-064, BBD-065
<b>Guadagnini, G.</b>	BBD-061	<b>Hillen, S.</b>	HME-021	<b>Josselin, M.</b>	VVD-039
<b>Guazzetti, S.</b>	VVD-OP-02	<b>Hjulsager, C.</b>	VVD-041, VVD-046	<b>Jung, S.W.</b>	WEL-019
<b>Gugler, C.</b>	HME-027	<b>Hlavová, K.</b>	KL08, REP-010, VPH-008	<b>Jung, Y.</b>	HME-003
<b>Guibe, L.</b>	IMM-OP-02	<b>Hoang, Q.</b>	VVD-057	<b>Junker, K.</b>	HHM-OP-06
<b>Gurtner, C.</b>	REP-OP-05	<b>Holbert, S.</b>	VAC-014	<b>Junnikkala, S.</b>	RES-001, WEL-035
<b>Gutzwiller, A.</b>	WEL-039	<b>Holstege, M.</b>	HME-023	<b>Kaalberg, L.</b>	VAC-055
<b>Gwenaël, B.</b>	VVD-039	<b>Holtkamp- DVM, D.</b>	HME-055	<b>Kádas-Tóth, E.</b>	WEL-022
<b>Haagmans, B.L.</b>	VVD-060	<b>Honegger, J.</b>	REP-OP-06	<b>Kahila, M.</b>	VVD-059
<b>Haesaert, G.</b>	BBD-058	<b>Hong, J.S.</b>	WEL-019, WEL-023	<b>Kaiser, M.</b>	VVD-005
<b>Haesebrouck, F.</b>	BBD-057, BBD-058, BBD-066, BBD-OP-05	<b>Hou, F.</b>	VVD-006	<b>Kálmán, A.</b>	HME-040
<b>Haimi-Hakala, M.</b>	WEL-007	<b>Houben, M.</b>	HME-023, MIS-014, HHM-OP-06, VPH-002	<b>Kamphues, J.</b>	WEL-027
<b>Halas, M.</b>	VAC-018	<b>Houe, H.</b>	VAC-037, VVD-007	<b>Kang, S.</b>	HME-003
<b>Han, Y.G.</b>	WEL-023, WEL-025	<b>Hu, D.</b>	WEL-039	<b>Kanora, A.</b>	BBD-028, BBD-030, BBD-032, BBD-034, PPD-001
<b>Hänninen, M.L.</b>	HHM-OP-03	<b>Huber, L.</b>	VVD-043	<b>Kaptur, R.J.</b>	BBD-017
<b>Hansen, C.</b>	VPH-001, VVD-046	<b>Huerta Lorenzo, B.</b>	BBD-075	<b>Karabasil, N.</b>	VPH-OP-04
<b>Hansen, T.S.</b>	VAC-001, VAC-051	<b>Huerta, B.</b>	BBD-083	<b>Karbowiak, P.</b>	VVD-049
<b>Hansen, U.</b>	WEL-036	<b>Huerta, E.</b>	HHM-OP-05	<b>Karembe, H.</b>	BBD-060
<b>Hanson, D.</b>	BBD-076, VVD-OP-03	<b>Huerto Lorenzo, B.</b>	BBD-063	<b>Karién, K.</b>	BBD-043
<b>Harakova, B.</b>	MIS-003	<b>Hug, C.</b>	MIS-OP-01	<b>Karlsson Lindsjö, O.</b>	BBD-025
<b>Harder, T.</b>	RES-002, VVD-041, VVD-OP-06	<b>Hug, P.J.</b>	REP-OP-06, MIS-OP-01, WEL-006	<b>Kartner, T.</b>	VVD-010
<b>Hasan, S.</b>	RES-001, WEL-035	<b>Hummelrose Diness, L.</b>	HME-022	<b>Kaschubek, T.</b>	WEL-008
<b>Haugegaard, J.</b>	MIS-002, VAC-003, VAC-037, VVD-007	<b>Imre, A.</b>	VAC-018	<b>Kateman, L.</b>	VVD-OP-06
<b>Haugegaard, S.</b>	HME-022	<b>Isera</b>	REP-OP-04	<b>Kauffold, J.</b>	REP-014, REP-015, VVD-030
<b>Hautekiet, V.</b>	WEL-009, WEL-010	<b>Jacob, C.</b>	HHM-OP-04	<b>Kavanová, L.</b>	VVD-031, VVD-045, VPH-008
<b>Hayden, J.</b>	HME-018	<b>Jacobsen, S.</b>	VVD-005	<b>Keith, M.</b>	VAC-050
<b>Heenemann, K.</b>	VVD-030	<b>Jacobson, M.</b>	BBD-025, VVD-005	<b>Kelderman, K.</b>	HME-014, IMM-OP-06
<b>Heggelund, M.</b>	MIS-013	<b>Jagu, R.</b>	VAC-040	<b>Kerényi, K.</b>	HME-039
<b>Heinonen, M.</b>	HHM-OP-03, WEL-007	<b>Jakubowski, T.</b>	VVD-050, VVD-052	<b>Kerloc'h-Dagorn, D.</b>	RES-006
<b>Hellmann, K.</b>	VAC-016	<b>Jang, J.C.</b>	WEL-018	<b>Kern, P.</b>	BBD-059
<b>Helmer, C.</b>	MIS-007	<b>Jansen, R.</b>	RES-OP-03, WEL-012	<b>Kerros, S.</b>	WEL-005
<b>Hemonic, A.</b>	HHM-OP-04	<b>Janssen, C.</b>	VAC-011	<b>Kieckens, E.</b>	BBD-050, BBD-052
<b>Hennig-Pauka, I.</b>	HME-027, MIS-OP-02	<b>Janssens, B.</b>	HME-019, VAC-009	<b>Kielland, C.</b>	MIS-013
<b>Henritzi, D.</b>	RES-002, VVD-OP-06	<b>Janssens, G.</b>	MIS-OP-03	<b>Kim, B.O.</b>	WEL-015
<b>Hermanns, W.</b>	VAC-021, VVD-021	<b>Jardin, A.</b>	BBD-002, BBD-027	<b>Kim, D.</b>	HME-003
<b>Hernández Caravaca, I.</b>	VVD-013, VVD-014, VVD-047, VVD-048, BBD-004, VVD-004, VVD-037, BBD-006, BBD-054, HME-006	<b>Jędryczko, R.</b>	BBD-059	<b>Kim, H.S.</b>	WEL-025
<b>Hernandez, J.</b>	REP-OP-04, VAC-025	<b>Jenkins, T.</b>	WEL-016	<b>Kim, Y.Y.</b>	WEL-015, WEL-018, WEL-019, WEL-023, WEL-025
<b>Hernández, M.</b>	VPH-007	<b>Jennes, M.</b>	RES-OP-03	<b>Kiss, I.</b>	BBD-001, BBD-072
<b>Herrera-León, S.</b>	VPH-006	<b>Jenny, B.</b>	HME-032	<b>Klein Koerkamp, M.J.A.</b>	VPH-OP-06
<b>Herrero-Fresno, A.</b>	VPH-OP-02	<b>Jensen, P.M.</b>	VAC-001, VAC-051	<b>Klein, J.</b>	HME-001
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<b>Heyndrickx, M.</b>	VPH-005	<b>Jiménez, M.</b>	REP-003, REP-004, REP-005, IMM-OP-04, VAC-030, VAC-031, VAC-036	<b>Klinkenberg, M.</b>	HME-043, HME-044
		<b>Jinghui, F.</b>	VVD-OP-01	<b>Klit, K.J.M.</b>	WEL-011
		<b>Joisel, F.</b>	BBD-022, BBD-029, HME-038, RES-OP-05	<b>Kloepfer, C.</b>	HME-005



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Lamrani, Á.	IMM-OP-05	Lopez, S.	VPH-009	Maris, P.	HHM-OP-04
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<b>Martinelli, N.</b>	VAC-041	<b>Miguel, J.</b>	REP-008, REP-009	<b>Nauwynck, H.</b>	VVD-001, VVD-002, VVD-019, VVD-020
<b>Martinez Gilaberte, A.</b>	BBD-004	<b>Milani, A.</b>	VVD-032	<b>Navarro, F.</b>	VVD-059
<b>Martinez-Abarca, A.</b>	VAC-052	<b>Milanov, D.</b>	HME-002	<b>Nechvatalova, K.</b>	BBD-059, KL08, VAC-015
<b>Martinez-Pulgarin, S.</b>	IMM-OP-01	<b>Mitek, D.</b>	VVD-051, VVD-049	<b>Nedbalcová, K.</b>	VAC-049, VVD-045
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<b>Mateu, E.</b>	VAC-036, WEL-041	<b>Moine, S.</b>	VVD-026, VVD-057	<b>Nielsen, E.O.</b>	VPH-OP-01
<b>Matiašková, K.</b>	VAC-049, VVD-045	<b>Mølgaard Sommer, H.</b>	HME-022	<b>Nielsen, G.B.</b>	VAC-037, VAC-003
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<b>Matyba, P.</b>	VVD-049	<b>Montali, A.</b>	VAC-041	<b>Nienhoff, H.</b>	HME-031
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<b>Mayerhofer, S.</b>	MIS-OP-02	<b>Moon, S.H.</b>	BBD-070, HME-046	<b>Nitzel, G.P.</b>	VAC-042
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<b>Mechler, M.</b>	VVD-022, VVD-024, VVD-042	<b>Moronato, M.L.</b>	BBD-023, BBD-036	<b>Ogno, G.</b>	VVD-OP-02
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<b>Mendoza, N.</b>	REP-008, REP-009	<b>Moser, L.</b>	BBD-011, VVD-038	<b>Oliva, J.E.</b>	IMM-OP-05
<b>Menjón, R.</b>	REP-003, REP-004, REP-005, VAC-031, VAC-036, IMM-OP-04, VAC-030	<b>Mouchet, H.</b>	RES-006	<b>Oliveira, L.G.</b>	VVD-022, VVD-024, VVD-042
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<b>Merdy, O.</b>	BBD-022, BBD-029, HME-038, RES-OP-05, VAC-053	<b>Mozo, R.</b>	REP-002	<b>Olivier, M.</b>	VAC-014
<b>Merlot, E.</b>	WEL-034	<b>Mößler, A.</b>	WEL-027	<b>Oliviero, C.</b>	WEL-007, WEL-035, HHM-OP-03, REP-007, REP-OP-01, RES-001
<b>Mertens, N.</b>	HME-021	<b>Muentener, C.</b>	VPH-003	<b>Olkkola, S.</b>	HHM-OP-03
<b>Mesonero Escuredo, J.A.</b>	RES-OP-02	<b>Mul, M.F.</b>	VPH-OP-06	<b>Ollila, A.</b>	WEL-042
<b>Mesonero, S.</b>	RES-002	<b>Mulberry, L.</b>	HME-055	<b>Olsen, J.E.</b>	VPH-OP-02
<b>Messenger, I.</b>	MIS-004, VAC-040, VVD-023, VVD-044	<b>Müller, A.</b>	MIS-001	<b>Olteanu, M.</b>	WEL-017
<b>Mesu, A.P.</b>	VAC-005, VAC-006	<b>Muñoz-González, S.</b>	VVD-054, VVD-OP-05	<b>Ondrackova, P.</b>	KL08
<b>Metais, J.</b>	BBD-013, BBD-046, BBD-048, HME-029, HME-030, VAC-057, VVD-023	<b>Muñoz, M.</b>	VVD-060	<b>Oorborg, D.</b>	VPH-OP-06
<b>Metzler-Zebeli, B.U.</b>	MIS-OP-02	<b>Munsterhjelm, C.</b>	AWN-OP-01		
<b>Mevius, D.</b>	KL01	<b>Nadeau, É.</b>	VAC-004, VAC-016, IMM-OP-05		
		<b>Nagy, Z.</b>	VAC-018		
		<b>Nalbert, T.</b>	VVD-052		
		<b>Nam, S.O.</b>	WEL-018		
		<b>Napoleoni, M.</b>	BBD-039		





Opriessnig, T.	VVD-OP-01	Pérez, L.	WEL-020	Raes, M.	VAC-007
Oreskovic, Z.	VAC-015	Perozo, E.	VAC-045	Raffi, V.	VVD-034
Oropeza-Moe, M.	WEL-040	Perrea, D.	VAC-035	Raj, V.S.	VVD-060
Orro, T.	WEL-007	Perreten, V.	BBD-044	Ramírez, H.	VVD-017
Orsini, S.	BBD-039, BBD-041	Persson, M.	MIS-008	Ramis, G.	HME-026, VVD-055
Ostanello, F.	BBD-OP-03, VAC-053	Petrov, D.	BBD-015	Rasmussen, M.	VVD-046
Österberg, J.	MIS-006	Petzoldt, D.	MIS-007	Rathkjen, P.H.	VAC-005, VAC-006
Ostović, M.	HME-034	Pezzotti, G.	BBD-041	Raunio-Saarnisto, M.	BBD-055
Otto, G.	HME-001	Philips, R.	HME-054, VVD-OP-03	Redant, V.	VVD-019, VVD-020
Oudshoorn, A.K.	RES-OP-02	Piel, Y.	VPH-009, HME-049, MIS-004	Reiner, G.	VVD-018
Ózsvári, L.	HME-007, HME-008, HME-009	Pieters, M.	BBD-004, BBD-OP-06, HME-035	Reiter, M.	MIS-001
Paboef, F.	VAC-032	Pikalo, J.	VAC-026	Relić, R.	HME-002
Paboouf, F.	VVD-033, VVD-040	Pina-Pedrero, S.	IMM-OP-01	Renken, C.	VAC-022
Padrell, M.	HME-037	Pinchbeck, G.	HMM-OP-02	Renson, P.	VAC-032
Pagot, E.	BBD-007	Piñeiro, C.	HME-020, HME-043	Revilla-Fernández, S.	VVD-OP-04, VAC-026
Pall Dalmau, A.	REP-OP-04	Plate, H.	HME-031	Richard, O.K.	VAC-024
Pallarés, F.J.	VAC-052, VVD-055	Playter, S.	BBD-OP-04	Rieck, W.	REP-014
Pallisera, J.	REP-OP-04	Pleguezuelos, P.	HMM-OP-05	Riedel, C.	VVD-010
Pálmai, N.	VAC-018	Plourde, L.	VVD-059	Rigamonti, S.	VPH-OP-05
Palomo Yague, A.	BBD-054	Pogácsás, I.	HME-008	Rigaut, M.	BBD-031
Palomo, A.	WEL-020	Pollard, C.	VVD-027	Risch, L.	BBD-047, BBD-081, WEL-027
Palya, V.	BBD-001, BBD-072	Polo, J.	BBD-005, VVD-015	Ritzmann, M.	VAC-017, VAC-022, VAC-023, VVD-018, VVD-021
Palzer, A.	HME-043	Pommelet, C.	IMM-OP-02	Rivera, F.	VVD-017
Panaite, T.D.	WEL-017	Pommellet, C.	BBD-046	Robben, N.	VVD-057, VVD-026
Pandolfi, F.	HME-044	Poppe, W.	MIS-014	Robert, N.	VVD-023
Papatsiros, V.	VAC-035	Pordán, M.	HME-007, HME-008, HME-009	Roca, M.	VAC-043, VAC-044, VAC-047, VAC-048
Pasmans, F.	BBD-057, BBD-066, VPH-005	Posthaus, H.	VAC-024	Rocadembosch, J.	HME-038
Passeri, B.	VAC-041	Pozo, M.	VVD-048	Ródenas, J.	BBD-005, VVD-015
Pastyria, A.	VVD-011	Pradervand, N.	WEL-039	Rodrigues da Costa, M.	MIS-016, MIS-017
Pauline, B.	VVD-039	Prati, P.	VPH-OP-05	Rodríguez Vega, V.	BBD-004, VVD-004, VVD-037
Pearce, D.	VAC-050	Prieto, C.	VVD-047	Rodríguez-Estévez, V.	BBD-069
Pearson, R.	BBD-014, HMM-OP-02	Pringle, M.	MIS-005	Rodríguez-Fernandez, J.C.	WEL-003, WEL-004
Pedersen, K.S.	BBD-067, BBD-068, BBD-071, BBD-OP-02	Prodanov-Radulović, J.	HME-002	Rodríguez-Gómez, I.M.	VVD-055, BBD-069, BBD-078
Pedrazuela, R.	VAC-020	Prodanović, R.	HME-002	Rodríguez-Guerra, M.Á.	BBD-033
Peeters, L.	VPH-005	Prodelałova, J.	VVD-031	Rodríguez-Ortega, M.J.	BBD-063
Peitzmeier, E.U.	VAC-005, VAC-006	Pruglo, V.	HME-051	Rodríguez-Vega, V.	BBD-006, BBD-054, HME-006
Pelliza, B.	REP-011	Puig, A.	VAC-045	Rodríguez, C.	BBD-005, VVD-015
Peltoniemi, O.	REP-OP-01, AWW-OP-01, REP-007, RES-001, WEL-035, WEL-042	Puig, R.	IMM-OP-04	Rodríguez, M.A.	WEL-021
Peña-Calzada, K.	WEL-003, WEL-004	Puigvert, E.	VAC-047	Rodríguez, V.	REP-OP-04, VVD-013, VVD-014, VVD-047, VVD-048
Pendl, W.	HME-032	Pujols, J.	VVD-015	Rogožarski, D.	HME-002
Penny, P.	REP-017	Pupin, P.	HMM-OP-04, HME-029, HME-030	Rohde, J.	HME-021
Pénzes, Z.	VAC-018	Putt, M.	HME-018	Romagosa, A.	HME-035
Perea, A.	BBD-033	Puyalto, M.	WEL-026	Romanov, O.	VVD-011
Pérez-Simó, M.	VVD-OP-05	Queguiner, S.	VVD-040	Romeiro, C.	BBD-074
Pérez-Simón, M.	VVD-054	Quijada, A.	VVD-026	Romeo, L.	REP-011
Pérez, D.	HMM-OP-05	Raber, A.	VVD-026	Romero, A.	REP-OP-04, VAC-025
Perez, J.	VVD-048	Rabie, A.	BBD-OP-03		
		Rach, S.	REP-014		



Rose, N.	MIS-004, VVD-029, VAC-032, VVD-033, VVD-040	Schötta, A.M.	MIS-001	Soica, C.	WEL-017
Rosell, R.	VVD-054, VVD-OP-05	Schüpbach-Regula, G.	REP-OP-06	Sol, C.	WEL-026
Roth, N.	VPH-OP-03	Schwanitz, S.	VAC-021	Solà-Oriol, D.	WEL-041
Roubos-van den Hil, P.	RES-OP-02, VPH-004	Schwarz, A.	REP-OP-06	Sola, X.	HME-016
Rouillier, J.	BBD-012	Schwarz, B.A.	VVD-035	Solanes, D.	VVD-060
Rozsnyay, Z.	VAC-018	Schwarz, L.	VVD-010, MIS-OP-02, RES-OP-06	Solarte, A.L.	BBD-073, BBD-083
Rubio, P.	BBD-040	Scimia, G.	MIS-010	Sønksen, U.W.	VPH-OP-01
Ruczizka, U.	MIS-OP-02	Scollo, A.	HME-053, VAC-054, WEL-030, BBD-037	Souza, A.	VVD-024, VVD-042
Rueckner, A.	VVD-030	Seate, J.	BBD-076, BBD-OP-04	Sparreboom, M.	RES-OP-01
Rümenapf, T.	VVD-010	Seehusen, F.	REP-016	Spergser, J.	RES-OP-06
Ryytty Sylván, K.	MIS-015	Segalès, J.	KL03, BBD-056, BBD-077, HHM-OP-05, HME-038, RES-002, RES-OP-04, RES-OP-05, VVD-015, VVD-054, VVD-060, VVD-OP-05, HME-035	Sperling, D.	BBD-060, HME-036, HME-051, BBD-061
Saborido, N.	BBD-005, VVD-015	Segarra, S.	WEL-020	Spiegel, F.	REP-016, REP-OP-03
Sala Ceballos, V.	VVD-037	Segers, H.	HME-011, HME-013, HME-019, VAC-009, VVD-008, VVD-009	Spiegel, S.	REP-016, REP-OP-03
Sala Echave, R.H.	HME-006, VVD-048	Segers, R.	VAC-002, VAC-007	Spindler, C.	HME-048
Sala, V.	VVD-014	Seltzhammer, S.	WEL-022	Spindler, D.	BBD-017
Salát, J.	VVD-045	Selves, J.	BBD-011	Spring, P.	HME-032
Salbu, B.	WEL-040	Serrano J.D.	BBD-079, VVD-058	Squire, J.	BBD-011
Saleri, R.	KL07	Serrano, E.	RES-OP-04	Stadejek, T.	KL04, HME-044, VVD-049, VVD-050, VVD-051, VVD-052
Sallaberry, S.	REP-008	Servais, V.	WEL-037	Staffolani, M.	BBD-039
Salvi, C.	MIS-009	Sevastyanova, M.	BBD-015	Stanek, G.	MIS-001
Sánchez Uribe, P.	IMM-OP-05	Shah, R.	HME-033	Stárvik, T.	MIS-013
Sánchez-Matamoros, A.	HME-037, VAC-039, VAC-052, VAC-019, VAC-038	Shaykhet, E.	VVD-011	Štáštý, K.	VPH-008
Sánchez, P.	HME-026	Sialelli, J.N.	RES-006	Steen Pedersen, K.	WEL-024
Sandri, G.	HME-041, BBD-036, VVD-OP-02	Sibila, M.	BBD-056, BBD-077, HHM-OP-05, HME-035, HME-038, RES-OP-04, RES-OP-05	Steens, R.	VAC-005, VAC-006
Sanjoaquin, L.	REP-003, REP-004	Sidler, X.	HME-032, VPH-003	Stege, H.	WEL-011
Santos, F.	VVD-024	Sierra, M.A.	REP-OP-04	Steiner, T.	WEL-031
Sanz C.	BBD-079	Simek, B.	BBD-059	Steinrigl, A.	VVD-OP-04, VAC-026
Saracila, M.	WEL-017	Simeunović, P.	HME-002	Štěpánová, H.	KL08, VPH-008
Sattler, T.	VAC-026	Simon-Grifé, M.	VAC-044	Stockinger, H.	MIS-001
Saun, X.	VAC-043	Simon, G.	VVD-040	Stoeckl, S.	VVD-021
Saunders, G.	VAC-042	Simon, M.	VAC-043, VAC-048	Stoiber, J.	VAC-017, VVD-018
Savić, S.	HME-002	Sipos, S.	REP-001, VAC-046	Streckel, E.	WEL-028
Sayyari, A.	WEL-036	Sipos, W.	MIS-001, VAC-046, REP-001	Struik, D.	VVD-OP-06
Scandurra, S.	BBD-037	Sisteré-Oró, M.	IMM-OP-01	Strutzberg-Minder, K.	BBD-019
Schafzahl, W.	KL06	Sitjá, M.	VAC-043, VAC-044, VAC-047, VAC-048	Stueger, H.P.	HME-042
Schatzmayr, D.	WEL-033	Sivertsen, T.	WEL-036	Svilovich, V.	HME-051
Scheer, P.	VPH-003	Sjölund, M.	MIS-005, MIS-008, MIS-012	Swan, K.M.	AWN-OP-01
Scheidt, A.	BBD-OP-04	Skoeries, O.	VAC-022	Swanenburg, M.	VPH-OP-06
Schiavon, E.	VVD-032	Skovgaard, K.	VVD-041	Swimley, M.	VVD-057
Schipper, D.	VVD-060	Smith, R.	HHM-OP-02	Szórádi, M.A.	VAC-018
Schivo, A.	VVD-032	Smith, R.P.	BBD-OP-03	Tabeling, R.	BBD-042
Schmaling, E.	BBD-076, HME-054, VVD-OP-03	Smola, J.	BBD-060	Tarradas Iglesias, C.	BBD-063, BBD-073, BBD-075
Schmidt, U.	MIS-006	Sno, M.	VAC-017, VVD-018, VVD-021	Tarradas, C.	VPH-006
Schmidtová, A.	VPH-010	Sobko, I.	VVD-011	Tassis, P.	VAC-035
Schmoll, F.	VAC-026, VVD-OP-04			Taylor, L.	VAC-027, VAC-029, VAC-050
Schneider, C.	VAC-016			Taylor, L.P.	VAC-042
Scholz, A.M.	VAC-021			Tebbs, R.	VVD-057



Teichmann, K.	WEL-008	Van Hagen, G.	WEL-012	Vuković, V.	HME-034
Telkänranta, H.	AWN-OP-01	Van Hamme, V.	WEL-002	Wacheck, S.	VVD-OP-06
Temple, D.	VAC-031, VAC-036	Van Hees, H.	RES-OP-01	Waddilove, J.	HME-017
Tenk, M.	VAC-018	van Kilsdonk, E.	VAC-002	Waehner, C.	VAC-022
Teodorović, V.	VPH-OP-04	van Limbergen, T.	HME-043, HME-044	Wähner, C.	HME-045, REP-013, REP-015
Tesařík, R.	IMM-OP-03, VAC-013, VAC-049	van Lochem, S.	VVD-012	Wähner, M.	REP-015
Thilmant, P.	VVD-056	van Maanen, K.	VVD-003	Wallenbeck, A.	MIS-006
Tignon, M.	VVD-019, VVD-020	van Nes, A.	VAC-011, HME-004, MIS-014, VVD-003	Wallgren, P.	MIS-008, MIS-012, MIS-005, MIS-006
Tind Sørensen, J.	AWN-OP-02	Van Ooyen, J.	VVD-059	Wallgren, T.	MIS-005
Tobias, T.	HME-004, HME-023, MIS-014, VVD-003	Van Poucke, A.	MIS-OP-03	Wang, C.	VVD-OP-01
Tobias, T.J.	RES-OP-01	van Staaveren, N.	MIS-016	Waret-Szkuta, A.	BBD-031
Tobin, F.	HME-016	Van Wagenberg, C.P.A.	VPH-OP-06	Wavreille, J.	WEL-037
Toman, M.	VAC-049, VVD-031	Vande Maele, L.	BBD-057, BBD-066	Waxenecker, F.	VPH-OP-03
Tonon, F.	VVD-028	Vandersmissen, T.	VPH-005	Weber, N.	BBD-067, BBD-068, BBD-071, BBD-OP-02
Torgerson, P.	HME-032	Vangroenweghe, F.	BBD-009, BBD-018, BBD-080, HME-010, HME-012, HME-014, IMM-OP-06	Weiss, C.	VAC-017
Tosser, H.	BBD-016	Vanhara, J.	HME-036	Weissenbacher-Lang, C.	REP-001
Tóth, I.	VAC-018	Vanhooydonck, K.	HME-012, IMM-OP-06	Weissenboeck, H.	MIS-001, REP-001
Touzain, F.	VAC-032	Vanrompay, D.	BBD-050, BBD-051, BBD-052	Wendt, M.	REP-016, REP-OP-03
Tremblay, D.	VAC-004, VAC-016	Vansteenkiste, K.	BBD-057	Wetzell, T.	VVD-OP-03, BBD-076
Trombani, C.	HME-025	Varela, E.	WEL-041	Widagdo, W.	VVD-060
Trus, I.	VVD-001, VVD-002	Vasilev, D.	VPH-OP-04	Wideman, G.	BBD-011, VVD-038
Tubbs, R.	BBD-OP-04	Vela, A.	REP-003, REP-004	Wijnveld, M.	MIS-001
Tyszka, A.	VVD-052	Velarde, A.	REP-OP-04	Willem, L.	VVD-029
Tzika, E.	VAC-035	Veldhuis, H.	VVD-OP-06	Willems, E.	HME-010
Úbeda, J.L.	HME-026	VELOCI, M.	BBD-037	Willems, H.	VVD-018
Ueffing, B.	HME-001, MIS-007	Velu, S.	VAC-040	Williams, N.	HMM-OP-02
Uhlig, S.	WEL-036	Venck, E.	MIS-009	Wim, V.D.B.	BBD-OP-01
Unterweger, C.	RES-OP-06, MIS-OP-02	Verbrugge, T.	WEL-012	Wisselink, H.J.	VPH-OP-06
Urlings, H.A.P.	VPH-OP-06	Vergara-Alert, J.	VVD-060, IMM-OP-01	Witvliet, M.	VAC-007
Urniza, A.	VAC-027, VAC-029	Verspohl, J.	REP-016	Wodak, E.	VAC-026
Ustulin, M.	BBD-023, BBD-036	Vetró, P.	VAC-018	Wolf, F.	HME-042
Vágó, L.	HME-009	Vicari, N.	VPH-OP-05	Wolf, R.	VAC-016
Vahlenkamp, T.W.	VVD-030	Vidondo, B.	VAC-024	Wolswinkel, T.	VVD-003
Valls, L.	BBD-062	Viehmann, M.	BBD-042	Woźniak, A.	VVD-049, VVD-051
Valros, A.	AWN-OP-01, WEL-042	Vio, D.	BBD-023, BBD-036, VVD-028, VVD-032	Wuyls, N.	HME-020
Van Asseldonk, M.A.P.M.	VPH-OP-06	Virginia, R.	VAC-025	Yeske, P.	VVD-053
van den Born, E.	VAC-002	Vismarra, A.	VPH-OP-05	You, D.H.	WEL-015
van den Brandt, J.M.A.	VVD-060	Vlaicu, P.A.	WEL-017	Yun, J.	WEL-042, HMM-OP-03
van den Elzen, P.	VAC-002	Voglmayr, T.	RES-OP-06	Zamperin, G.	VVD-032
Van den Oever, B.	WEL-012	Voigts, B.	VVD-012	Zeeh, F.	BBD-044, WEL-029
Van der Giessen, J.W.P.	VPH-OP-06	Voisin, F.	BBD-016	Zeineldin, M.	BBD-038
van der Hallen, E.	VVD-001	Von Ah, S.	WEL-006	Zeineldina, M.	BBD-035
van der Wolf, P.	BBD-043, VVD-OP-06	von Altrock, A.	REP-OP-03, REP-016	Zentek, J.	KL05, VAC-016, WEL-031
van Doorn, D.	VPH-002	Von dem Busche, I.	VAC-005, VAC-006	Ziron, M.	WEL-028
van Esch, E.	BBD-049	von Gunten, C.	WEL-029	Zizlavsky, M.	MIS-003
Van Gansbeke, S.	MIS-OP-04	Vos, J.	HMM-OP-06	Zoels, S.	VAC-023
Van Garderen, E.	HMM-OP-06	Vrielinck, J.	MIS-OP-03	Zoric, M.	MIS-006
Van Gorp, S.	HME-011, HME-013, HME-019, VAC-009, VVD-008, VVD-009				