



ABSTRACT BOOK

International PRRS Congress

JUNE 3-5, 2015 | Ghent, Belgium





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Tom Duinhof GD Animal Health, Deventer
Tanja Opriessnig The Roslin Institute and R(D)SVS, University of Edinburgh
Paolo Martelli Parma University

PRRS 2015 Invited Speakers

Frederick Chi-Ching Leung School of Biological Sciences, The University of Hong Kong,
Ying Fang Kansas State University, USA
Hanchun Yang China Agricultural University
Enric Mateu Autonomous University of Barcelona and CRESA
Tomasz Stadejek Warsaw University of Life Sciences, Poland
Raymond (Bob) Rowland Kansas State University, USA
Robert Morrison University of Minnesota, USA
Guangzhi Tong Shanghai Veterinary Research Institute
Poul Bækbo Danish Pig Research Centre



David A. Benfield Student Travel Fellowships

David A. Benfield received his BS and MS degrees from Purdue University and a PhD from the University of Missouri-Columbia. He has a distinguished 25 years in research related to virus diseases of food animals. In 1990, he was the co-discoverer of the cause of “mystery swine disease” or porcine reproductive and respiratory syndrome virus (PRRSV). He has remained a role model and mentor to many of those who are currently in the PRRS field. Currently, he is the Associate Director of the Ohio Agricultural Research and Development Center, The Ohio State University and a Professor in the Food Animal Health Research Program in the College of Veterinary Medicine. It is his generous donation that initiated this fellowship program. It is his hope that these fellowships provide students with the experience of attending the International PRRS Congress to present their work and collaborate on PRRS.

2015 David A. Benfield PRRS 2015 Travel Fellowship Recipients

Andreia Arruda	University of Guelph
Vanessa Max Kraus	Servicio Agrícola y Ganadero, Chile
Jin Cui	South China Agricultural University
Megan Niederwerder	Kansas State University
Benjamin Tribble	Kansas State University



SCIENTIFIC PROGRAM

WEDNESDAY, June 3rd 2015

- 17:00-20:00 **Arrival and Registration** (First Floor)
- 18:00-20:00 **Welcome reception** (Ground floor, Artevelde Forum)

THURSDAY, June 4th 2015

- 08:30-10:00 **PLENARY SESSION 1: PRRSV EVOLUTION**
Chairs: Kay Faaberg & Lars E. Larsen
- 08:30-09:00 **KEYNOTE LECTURE: AN OVERVIEW OF THE HISTORICAL EVOLUTION OF PRRSV**
Frederick Chi-Ching Leung
School of Biological Sciences, University of Hong Kong, China
- 09:00-09:15 **SELECTED PRESENTATION**
- O.01- EMERGENCE OF NOVEL NADC30-LIKE STRAIN OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN CHINA**
L Zhou^{*}, ZC Wang, YP Ding, XN Ge, X Guo and HC Yang
Key Laboratory of Animal Epidemiology and Zoonosis of Ministry of Agriculture, College of Veterinary Medicine and State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, People's Republic of China
- 09:15-09:30 **O.02- RECONSTRUCTION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS EVOLUTIONARY DYNAMICS IN ITALY**
G. Franzo^{1*}, G. Dotto¹, M. Cecchinato¹, D. Pasotto¹, M. Martini¹ and M. Drigo¹
¹Department of Animal Medicine, Production and Health (MAPS), Viale dell'Università 16, 35020 Legnaro (PD), Italy
- 09:30-09:45 **O.03- GENETIC AND PHENOTYPIC IN VITRO AND IN VIVO CHARACTERIZATION OF RECENT PRRSV ISOLATES FROM AUSTRIA REVEAL SIMILARITIES TO EAST ASIAN STRAINS**
L.J. Sinn^{1*}, L. Ziegłowski¹, H. Koinig², G. Mößlacher³, B. Lamp¹, I. Hennig-Pauka² and T. Rümenapf¹
¹Institute of Virology, University of Veterinary Medicine Vienna, Vienna, Austria;
²Clinic for Swine, University of Veterinary Medicine Vienna, Vienna, Austria;
³Oberösterreichischer Tiergesundheitsdienst (TGD), Linz, Austria



- 09:45-10:00 **O.04- GENOME ANALYSIS OF A NON-MLV-RELATED TYPE 2 HUNGARIAN PRRSV ISOLATE**
Gy. Balka¹, X. Wang², F. Olasz³, Á. Bálint⁴, I. Kiss⁵, K. Bányai³, M. Rusvai¹, T. Stadejek⁶, D. Marthaler⁷, M.P. Murtaugh², Z. Zádori^{3*}
¹Department of Pathology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary; ²Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA; ³Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; ⁴National Food Chain Safety Office Veterinary Diagnostic Directorate, Budapest, Hungary; ⁵Ceva-Phylaxia Veterinary Biologicals Co. Ltd., Budapest, Hungary; ⁶Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland; ⁷Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN 55108, USA
- 10:00-10:30 **Coffee break (Ground floor, Artevelde Forum)**
- 10:30-12:00 **PLENARY SESSION 2: PRRSV REPLICATION CYCLE**
Chairs: Lei Zhou & Tahar Ait-Ali
- 10:30-11:00 **KEYNOTE LECTURE: THE MOLECULAR BIOLOGY OF PRRSV: REPLICATION CHARACTERISTICS AND INTERPLAY WITH HOST CELL INFRASTRUCTURE**
Ying Fang
Department of Diagnostic Medicine/Pathobiology, Kansas State University, USA
- 11:00-11:15 **SELECTED PRESENTATION**
O.05- PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS NONSTRUCTURAL PROTEIN 2 MEMBRANE TOPOLOGY
Kay S. Faaberg^{1*}, Matthew A. Kappes^{1,2}, and Cathy L. Miller²
¹Virus and Prion Research Unit, USDA-ARS-National Animal Disease Center, Ames, Iowa, USA; ²Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA
- 11:15-11:30 **O.06- A COMPLEX INTERPLAY BETWEEN THE SIGNAL PEPTIDE, GLYCOSYLATION SITES AND INTERACTION WITH M DETERMINES SIGNAL PEPTIDE CLEAVAGE FROM GP5**
B. Thaa and M. Veit^{*}
Institute of Virology, Veterinary Faculty, Free University Berlin, Germany
- 11:30-11:45 **O.07- CHARACTERISING THE PRRSV DOUBLE MEMBRANE VESICLE**
A. Brown^{1*}, C. Burkard¹, T. Ait-Ali¹, S. Lilloco¹, A. Archibald¹, B. Whitelaw¹, A. Mileham²
¹The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Campus, Midlothian, Scotland; ²Genus PLC, Belvedere House, Basing View, Basingstoke, Hampshire, RG21 4HG



- 11:45-12:00 **O.08- INHIBITION OF PRRSV REPLICATION BY TARGETING THE FORMATION OF DOUBLE-MEMBRANE VESICLE**
J. Rappe^{1,2,3*}, V. Thiel^{1,3} and N. Ruggli^{1,3}
¹The Institute of Virology and Immunology IVI, Middelhäusern and Bern, Switzerland; ²Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland; ³Department of Infectious Diseases and Pathobiology, University of Bern, Switzerland
- 12:00-13:00 **Lunch (Ground floor, Artevelde Forum)**
- 13:00-14:45 **PLENARY SESSION 3: PRRS PATHOGENESIS**
Chairs: Johanna M.J. Rebel & Kristien Van Reeth
- 13:00-13:30 **KEYNOTE LECTURE: THE ROLE OF NON-STRUCTURAL PROTEINS IN VIRULENCE AND PATHOGENICITY OF PRRSV**
Hanchun Yang
College of Veterinary Medicine, China Agricultural University, Beijing, China
- 13:30-13:45 **SELECTED PRESENTATION**
O.09- DYNAMIC CHANGE IN LUNG MACROPHAGES AND CYTOKINES ENVIRONMENT DURING INFECTION OF PIGS WITH A HIGH OR LOW VIRULENT GENOTYPE 1 PRRSV STRAIN
P. Renson¹, N. Rose¹, M. Le Dimna¹, S. Mahé¹, A. Keranflech¹, F. Paboeuf¹, C. Belloc², M.F. Le Potier¹, O. Bourry^{1*}
¹ Anses Laboratoire de Ploufragan, BP53, 22400 Ploufragan, France; ² UMR BioEpAR, Oniris, INRA, LUNMA, BP4006, 44307 Nantes Cedex 03, France
- 13:45-14:00 **O.10- PATHOGENESIS OF EUROPEAN SUBTYPE 3 AND SUBTYPE 1 PRRSV STRAINS IN PIGS**
Eefke Weesendorp¹, Norbert Stockhofe-Zurwieden¹, Johanna Rebel^{1*}
¹Central Veterinary Institute, Wageningen University&Research
- 14:00-14:15 **O.11- MATERNAL AND FETAL PREDICTORS OF FETAL VIRAL LOAD AND DEATH IN PRRSV INFECTED PREGNANT GILTS**
A. Ladinig^{1*}, C. Ashley², S.E. Detmer³, J.K. Lunney⁴, G. Plastow⁵, J.C.S. Harding²
¹University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria; ²Department of Large Animal Clinical Sciences, University of Saskatchewan, Canada; ³Department of Veterinary Pathology, University of Saskatchewan, Canada; ⁴Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, U.S. Department of Agriculture, USA; ⁵Department of Agricultural, Food, and Nutritional Science, University of Alberta, Canada



- 14:15-14:30 **O.12- APOPTOSIS AT THE MATERNAL-FETAL INTERFACE OF TYPE 2 PRRSV INFECTED PREGNANT GILTS**
P. Novakovic¹, A. N. Al-Dissi¹, J.C.S. Harding², S. E. Detmer^{1*}
¹Dept. of Veterinary Pathology, ²Dept. of Large Animal Clinical Sciences Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada
- 14:30-14:45 **O.13- PATHOGENICITY OF HIGHLY PATHOGENIC PRRSV IN PREGNANT SOWS**
M. Takagi^{1*}, A. Bayanzul³, N. Hattori¹, K. Kuga¹, M. Ikezawa², T. Shibahara², K. Kawashima²
¹Viral Disease and Epidemiology Research Division, ²Pathology and Pathophysiology Research Division, National Institute of Animal Health, NARO, Tsukuba, Japan, ³Mongolian State University of Agriculture, Mongolia
- 14:45-15:15 **Coffee break (Ground floor, Artevelde Forum)**
- 15:15-17:00 **PLENARY SESSION 4: PRRS IMMUNITY/IMMUNE EVASION**
Chairs: Isabelle Schwartz-Cornil & Artur Summerfield
- 15:15-15:45 **KEYNOTE LECTURE: IMMUNITY IN PRRS VIRUS INFECTION**
Enric Mateu
Centre de Recerca en Sanitat Animal (CRESA), UAB-IRTA, Bellaterra, Spain
- 15:45-16:00 **SELECTED PRESENTATION**
O.14- POTENT INTERFERON-ALPHA PRODUCTION BY PLASMACYTOID DENDRITIC CELLS CO-CULTURED WITH MACROPHAGES PRE-INFECTED WITH GENOTYPE 1 OR GENOTYPE 2 HIGHLY PATHOGENIC PORCINE REPRODUCTIVE AND RESPIRATORY
O. García-Nicolás¹, G. Auray¹, J. Rappe¹, N. Ruggli¹, A. Summerfield^{*1,2}
⁽¹⁾Institute of Virology and Immunology, Mittelhäusern, Switzerland; ⁽²⁾ University of Bern, Bern, Switzerland
- 16:00-16:15 **O.15- THE IMMUNITY RAISED BY RECENT EUROPEAN SUBTYPE 1 PRRSV STRAINS ALLOWS A BETTER REPLICATION OF EAST EUROPEAN SUBTYPE 3 PRRSV STRAIN LENA THAN THE IMMUNITY RAISED BY AN OLDER STRAIN - INCREASED RISK FOR SPATIAL EXPANSION OF PRRSV LENA-LIKE STRAINS**
I. Trus^{1*}, I. S. Frydas¹, V. R.A.P. Reddy¹, C. Bonckaert¹, Y. Li¹, L. K. Kvisgaard², L. E. Larsen², H. J. Nauwynck¹
¹Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; ²National Veterinary Institute, Technical University of Denmark, Frederiksberg C, Denmark



- 16:15-16:30 **O.16- IMMUNOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF A SHORT PROTEIN, 7AP, TRANSLATED FROM AN ALTERNATIVE FRAME OF ORF7 OF PRRSV**
F. Olasz¹, A. Vizovszki¹, B. Dénes², Á. Bálint², T. Magyar¹, Z. Zádori^{1*}
¹Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; ²National Food Chain Safety Office Veterinary Diagnostic Directorate, Budapest, Hungary
- 16:30-16:45 **O.17- SPECIFIC DISRUPTION OF THE DEUBIQUITINATING ACTIVITY OF NIDOVIRUS PAPAINE-LIKE PROTEASES REVEALS THEIR ROLE IN SUPPRESSION OF THE INNATE IMMUNE RESPONSE DURING INFECTION**
R.C.M. Knaap^{1*}, B.A. Bailey-Elkin², P.B. van Kasteren¹, T.J. Dalebout¹, I. Sola³, L. Enjuanes³, P.J. Bredenbeek¹, E.J. Snijder¹, B.L. Mark² and M. Kikkert¹
¹Molecular Virology Laboratory, Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands; ² Department of Microbiology, University of Manitoba, Winnipeg, Canada; ³ Department of Molecular and Cell Biology, Universidad Autónoma de Madrid, Madrid, Spain
- 16:45-17:00 **O.18- SUBVERSION OF HOST PROTEIN SYNTHESIS BY PRRS VIRUS NONSTRUCTURAL PROTEIN (NSP) 1 β**
D. Yoo^{*}, M. Han, and H. Ke
Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
- 17:00-18:00 **SPONSORED SEMINAR: THE ECONOMICS OF PRRSV: PROGRESS MADE AND WORK YET TO BE DONE**
Derald Holtkamp
Dept. Veterinary Diagnostics and Production Animal Medicine, College of Veterinary Medicine at Iowa State University (Ames, Iowa US)
Sponsored by MSD Animal Health
- 18:00-20:30 **Congress Dinner (Room Pedro De Gante, ground floor)**
- 20:30-22:00 **Poster session with Open Bar and Dessert Buffet (Minneplein, first floor)**

FRIDAY, June 5th 2015

- 08:30-10:00 **PLENARY SESSION 5: PRRSV DIAGNOSTICS**
Chairs: Cinta Prieto & Ann Brigitte Cay
- 08:30-09:00 **KEYNOTE LECTURE: METHODS AND APPROACHES TO DIAGNOSE PRRS**
Tomasz Stadejek
Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life, Poland



- 09:00-09:15 **SELECTED PRESENTATION**
- O.19- PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) SHEDDING IN ORAL FLUIDS OF NAÏVE AND VACCINATED PIGS EXPOSED TO THE WILD-TYPE VIRUS**
E. Gibert^{1*}, E. Pileri^{1,2}, G.E. Martín-Valls¹, E. Cano¹ and E. Mateu^{1,2}
¹ Centre de Recerca en Sanitat Animal (CReSA), IRTA, Edifici CReSA, campus UAB, 08193 Cerdanyola del Vallès, Spain; ²Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain
- 09:15-09:30 **O.20- COMPARISON OF PROCESSING, RNA EXTRACTION METHODS AND QRT-PCR MIXES FOR THE DIAGNOSIS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) INFECTION USING ORAL FLUIDS**
E. Gibert¹, G.E. Martín-Valls¹, E. Cano¹ and E. Mateu^{1,2}
¹ Centre de Recerca en Sanitat Animal (CReSA), IRTA, Edifici CReSA, campus UAB, 08193 Cerdanyola del Vallès, Spain; ²Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Bellaterra, Spain
- 09:30-09:45 **O.21- APPLICATION OF THE ION TORRENT™ PGM™ INSTRUMENT FOR WHOLE GENOME SEQUENCING OF EUROPEAN PRRSV STRAINS**
S. Daly¹, P. Siddavatam^{1*}, S. Moine¹, and C. O'Connell¹.
¹ Thermo Fisher Scientific
- 09:45-10:00 **O.22- HERD MANAGEMENT STRATEGIES TO CONTROL PRRSV INFECTIONS USING THE PRIOCHECK® PRRSV VIA ELISA**
F. Kuhn^{1*}, R. Lausterer¹, B. Schröder¹, T. Kühn², S. Pesch³ and A.J. Räber¹
¹Thermo Fisher Legacy Prionics, Wagistrasse 27a, CH-8952 Schlieren-Switzerland;
²vaxxinova GmbH, diagnostics, Deutscher Platz 5d, D-04103 Leipzig, Germany;
³vaxxinova GmbH, diagnostics & Vertrieb, Johann-Krane-Weg 42, Münster
- 10:00-10:30 **Coffee break (Ground floor, Artevelde Forum)**
- 10:30-12:00 **PLENARY SESSION 6: PRRS VACCINES**
Chairs: Falko Steinbach & Nicolas Ruggli
- 10:30-11:00 **KEYNOTE LECTURE: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) VACCINES - LESSONS FROM THE PAST AND CHALLENGES FOR THE FUTURE**
Hans J. Nauwynck
Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium



- 11:00-11:15 **SELECTED PRESENTATION**
O.23- BIOINFORMATICS PREDICTION OF SWINE MHC CLASS I EPITOPES FROM PRRSV
S. Welner^{1*}, M. Nielsen², O. Lund², G. Jungersen³, L.E. Larsen¹
Departments of ¹Virology, ²Systems Biology, ³Immunology and vaccinology, Technical University of Denmark, Copenhagen, Denmark
- 11:15-11:30 **O.24- THE DESIGN AND EVALUATION OF A PARTICULATE FORMULATION OF VACCINE CANDIDATE T CELL ANTIGENS FROM THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS**
H. Mokhtar¹, L. Biffar¹, M. Pedrera¹, S. Somaravarapu², M.G. Duran³, M.J. Rodriguez³, J.P. Frossard¹, S. McGowan¹, R. Strong¹, J.C. Edwards¹, F. Steinbach¹, S.P. Graham^{1,*}
¹Virology Department, Animal and Plant Health Agency, Addlestone, United Kingdom; ²School of Pharmacy, University College London, United Kingdom
³Ingenasa, Madrid, Spain
- 11:30-11:45 **O.25- VACCINATION WITH A PRRS MODIFIED LIVE VIRUS VACCINE FOLLOWED BY CHALLENGE WITH PRRSV AND PCV2 PROTECTS AGAINST PRRS BUT ENHANCES PCV2 REPLICATION AND PATHOGENESIS**
M. Niederwerder^{1*}, B. Bawa¹, N. Serão², B. Triple¹, M. Kerrigan¹, J. Lunney³, J. Dekkers², R. Rowland¹
¹Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506, USA; ²Department of Animal Science, Iowa State University, Ames, Iowa 50011, USA; ³United State Department of Agriculture, Agricultural Research Services, Beltsville Agricultural Research Center, Beltsville, Maryland 20705, USA
- 11:45-12:00 **O.26- VACCINATION WITH UNISTRAIN® PRRS IN PIGLETS GIVES A PARTIAL CLINICAL AND VIROLOGICAL PROTECTION AFTER CHALLENGE WITH AN EAST EUROPEAN SUBTYPE 3 ISOLATE (LENA STRAIN)**
J. Miranda¹, D. Torrents¹, R. Pedrazuela¹, I. Rodriguez¹, M. Busquet¹, D. Llopart¹, H.J. Nauwynck², C. Bonckaert^{2*}.
¹HIPRA, Amer (Girona), Spain; ²Faculty of Veterinary Medicine, Ghent
- 12:00-13:00 **Lunch (Ground floor, Artevelde Forum)**
- 13:00-14:45 **PLENARY SESSION 7&8: PRRS CONTROL**
Chairs: Tom Duinhof & Tanja Opriessnig



- 13:00-13:30 **KEYNOTE LECTURE: PRRS CONTROL AND ELIMINATION: THE ROLE OF VIRUS AND HOST GENETICS**
Raymond R. Rowland
Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, USA
- 13:30-13:45 **SELECTED PRESENTATION**
O.27- ENVIRONMENTAL ENRICHMENT IN EARLY LIFE INFLUENCES SUSCEPTIBILITY TO PRRSV AND CO-INFECTIONS
I. Dixhoorn², L. Bolhuis³, I. Reimert³, J. Middelkoop³, A. Rebel¹, N. Stockhofe-Zurwieden^{1*}
¹Central Veterinary Institute, Wageningen University&Research; ²Wageningen Livestock Research, Wageningen University&Research; ³Department Animal Adaptation Physiology, Wageningen University
- 13:45-14:00 **O.28- EVALUATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME CONTROL METHODS USING AGENT-BASED MODELLING**
A.G. Arruda^{1*}, Z. Poljak¹, A. Greer¹, R. Friendship¹, J. Carpenter²
¹Department of Population Medicine, University of Guelph, Guelph, Canada; ²Ontario Swine Health Advisory Board, Stratford, Canada
- 14:00-14:15 **O.29- INACTIVATION OF PRRSV BY A NOVEL METHOD OF ON BOARD HEATING OF TRUCKS IS WITHIN RANGE FOR THE DAILY ROUTINE IN PIG TRANSPORT**
T. Duinhof^{1*}, A. van Nes², M. Houben³
¹GD Animal Health, Deventer, the Netherlands; ²Utrecht University, Veterinary Faculty, Utrecht, The Netherlands; ³PorQ, Son, The Netherlands
- 14:15-14:30 **O.30- PRELIMINARY ASSESSMENT OF RISK FACTORS RELATED TO PRRSV STABILITY IN FARMS WITHIN A CONTROL PROGRAM IN SPAIN**
Martín-Valls GE^{1*}, Alarcón LV¹, Allepuz A^{1,2}, Cano E², Armengol R³, Casanovas A³, Rosell C⁴, Jordi Casal^{1,2}, Mateu E^{1,2}
¹ Centre de Recerca en Sanitat Animal (CRESA), IRTA, Edifici CReSA, Spain; ² Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain; ³ Federació de Cooperatives Agràries de Catalunya, Casa de l'Agricultura Barcelona, Spain; ⁴ Parc Científic i Tecnològic Agroalimentari de Lleida (PCiTAL), IRTA, Edifici Fruitcentre, Lleida, Spain
- 14:30-14:45 **O.31- NATIONAL CONTROL AND ERADICATION PRRS VIRUS CHILE, SOUTH AMERICA**
J.I., Gómez¹, P. Pérez¹, J. Herrera¹, C. Mathieu¹, M. Johow¹, V. Max^{1*}
¹ Servicio Agrícola y Ganadero – SAG – Agricultural and Livestock Service - Ministerio de Agricultura Chile
- 14:45-15:15 **Coffee break (Ground floor, Artevelde Forum)**



- 15:15-16:50 **ROUND TABLE SESSION: PRRS CONTROL/ERADICATION PLANS**
Moderator: Paolo Martelli
- 15:15-15:40 **KEYNOTE LECTURE: PRRS CONTROL AND ERADICATION PLANS IN EUROPE**
Poul Baekbo
Danish Pig Research Centre, Danish Agriculture and Food Council, Denmark
- 15:40-16:05 **KEYNOTE LECTURE: CURRENT INCIDENCE, PREVALENCE AND STATE OF CONTROL OF PRRS VIRUS IN NORTH AMERICA**
Robert Morrison
Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA
- 16:05-16:30 **KEYNOTE LECTURE: PRRS CONTROL IN CHINA: VACCINATION AND VIRUS EVOLUTION**
Guangzhi Tong
Shanghai Veterinary Research Institute, CAAS, China
- 16:30-16:40 **Questions & answers**
- 16:40-16:50 **Round table discussion**
- 16:50-17:00 **BEST POSTERS/ORAL COMMUNICATION AWARDS CEREMONY**
- 17:00-18:00 **SPONSORED SEMINAR (Jan Van Eyck room, first floor)**
PRRS CONTROL: APPLICATION OF SCIENCE IN THE FIELD
Sponsored by Boehringer Ingelheim
- 20:00 **FAREWELL BANQUET AT THE GHENT OPERA HOUSE**



Invited Speaker

Keynote Lecture

An overview of the historical evolution of PRRSV

M.S. Brar¹ and F.C.C. Leung^{1,2*}

¹School of Biological Sciences, University of Hong Kong, HKSAR

²Bioinformatics Center, Nanjing Agricultural University, Nanjing, China

Porcine reproductive and respiratory syndrome virus (PRRSV) is the viral aetiological agent responsible for PRRS, a disease causing substantial economic loss to global swine husbandry annually. True to the nature of RNA viruses, PRRSV, with a single-stranded positive sense RNA genome with an approximate size of 15 kb, too, is a rapidly evolving virus and already having undergone extensive genetic diversification as evidenced from sequencing of field samples spanning a temporal scale of over two decades. Although there is less certainty surrounding the exact pre-recognition evolutionary events leading to the "initial" outbreaks of the 1980's, several hypotheses have been posited. After initial isolates from North America and Europe were characterized genetically and antigenically, the existence of two genotypes was established which still holds true today with no further genotypes discovered. The voluminous nature of sample collection and sequencing over the years has led to a greater appreciation of the significant genetic diversity within each genotype which in led to the formation of several intra-genotype classification schemes (for example subtypes in type 1 PRRSV and the (sub-)lineage system for type 2 PRRSV). The genetic heterogeneity of PRRSV is, at least in part, responsible for the inability to develop an efficacious vaccine offering good protection / immunity against heterologous infection. Although the direct impact of genetic diversity on antigenic diversity is understandable and established, the presumed genetic links underlying phenotypic diversity of PRRS is not as clear cut. Over the years, many examples of the emergence of PRRS in particularly virulent forms has been documented (for example HP-PRRSV in China and MN184 in the USA) with isolates from some of these outbreaks possessing genomic alterations (insertions / deletions) raising speculation on viral genetics as a possible cause. Aside from innate viral factors driving PRRSV evolution, viral diversity has also been shaped by extrinsic factors like transmission dynamics, swine management practices, vaccination practices and, possibly, pig genetic variation. Local variation in these factors is widespread, and has influenced regional characteristics of genetic diversity.



Invited Speaker

Keynote Lecture

The molecular biology of PRRSV: replication characteristics and interplay with host cell infrastructure

Y. Fang^{1*} and E.J. Snijder²

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive-sense single-stranded RNA virus, which replicates in the cytoplasm of infected cells. The PRRSV genome is about 15 kb in length, containing 11 known ORFs. The 3'-proximal part of the genome has a compact organization and contains eight relatively small genes, most of which overlap with neighboring genes. These ORFs encode structural proteins and are expressed from a 3'-co-terminal nested set of subgenomic mRNAs. The large replicase ORFs 1a and 1b occupy the 5'-proximal three-quarters of the genome, with the size of ORF1a being much more variable than that of ORF1b. ORF1a translation yields replicase polyprotein (pp) 1a, whereas ORF1b is expressed by -1 programmed ribosomal frameshifting (PRF) to generate pp1ab. Recently, a short transframe (TF) ORF was found. It overlaps the nsp2-coding region of ORF1a in the +1 frame and to be expressed by a novel -2 ribosomal frameshift mechanism that is controlled by a viral protein factor (nsp1beta) rather than an RNA signal. The replicase pp1a and pp1ab are posttranslational processed by viral proteases to generate at least 14 nonstructural proteins (nsps) that direct viral replication and transcription. Based on bioinformatics and experimental studies, a complete proteolytic processing map for the PRRSV pp1a and pp1ab has been developed recently. Following pp1a and pp1ab cleavage, mature nsps assemble into a membrane-associated replication and transcription complex, located in the perinuclear region of infected cells, where viral RNA synthesis takes place. Besides their roles in the proteolytic processing, the replicase subunits, nsp1alpha and nsp1beta, may be involved in transcriptional/translational regulation. Several PRRSV proteases also appear to be involved in evading host defense systems and modulating host cell functions to promote virus reproduction. The interplay of viral proteins and RNA elements with cellular molecules allows PRRSV to hijack the host cell's infrastructure for successful replication.



Invited Speaker

Keynote Lecture

The role of non-structural proteins in virulence and pathogenicity of PRRSV

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen severely affecting the swine industry worldwide. The syndrome caused by this virus is still prevalent in many countries, particularly in China. Meanwhile, the continuing appearance of novel variant strains of PRRSV with increased virulence and pathogenicity recurrently causes emerging and re-emerging of clinical outbreaks. Actually, our understandings of PRRSV pathogenesis remain insufficient although great efforts on the area of viral pathogenesis have been done. A growing number of researches have shown potential roles of certain structural and non-structural genes in virulence and pathogenicity of PRRSV. In recent years, much attention has been paid to the pathogenesis involved by the non-structural proteins (Nsps) of PRRSV. Several non-structural proteins (Nsps) have been shown to down-regulate the innate immune response via inducing the inhibition of INF- α , IFN- β and other cytokines production in host, which contribute to the pathogenesis of PRRSV. Among the Nsps of PRRSV, Nsp9, a RNA-dependent RNA polymerase (RdRp), is regarded as a crucial motor for viral RNA replication, and Nsp10, a RNA helicase, is considered another key enzyme that directly participates in RNA synthesis. By using reverse genetics, our recent studies have revealed that the Nsp9- and Nsp10-coding regions together are closely related to the replication efficiency of the Chinese highly pathogenic PRRSV (HP-PRRSV) *in vitro* and *in vivo*, and are related to its increased pathogenicity and fatal virulence for piglets, indicating the important roles of Nsp9 and Nsp10 in virulence and pathogenicity of PRRSV via driving viral replication. Further molecular mechanisms affecting PRRSV replication by Nsp9 and Nsp10 are required to be addressed to better understand the pathogenesis of PRRSV. Our latest work has discovered some pathway that regulates PRRSV replication by the interaction of Nsp9 with host cellular proteins. These findings will be helpful to design new strategies of controlling PRRSV, such as the development of novel anti-viral drugs and vaccines against PRRSV infection.



Invited Speaker

Keynote Lecture

Immunity in PRRS virus infection.

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Immunity against PRRS virus (PRRSV) is one of the more challenging areas of viral immunology in pigs. The virological course of PRRSV infection is characterized by a long viremia (weeks to months, depending on the age of the pig) and a relative persistence of the virus in lymphoid tissues. This picture reflects that the infection is able to alter the normal development of efficacious immunity. However this is not a classical persistent infection since the virus is finally cleared from the body and pigs develop immunity that can range from sterilizing (mostly against the homologous strain) to very partial (to other isolates). The examination of the classical parameters of the immune response has shown that upon infection pigs rapidly develop antibodies but these antibodies are devoid of neutralizing capabilities. Indeed, neutralizing antibodies (NA) rarely appear before the third or fourth week of infection and then only moderate titers occur; even NA may not develop at all in some animals. To add complexity, NA may be highly specific for the isolate that induced them or may be almost pan-neutralizing. Specific T-cell cytotoxic responses have not been clearly demonstrated and the development of interferon-gamma producing cells –a usual correlate of protection in other viral infections- is relatively delayed and shows ups and downs for many weeks before stabilizing. Similarly to NA, the cell-mediated response depends on the viral isolate. Finally, there is a debate about the participation of regulatory T cells in PRRSV infection and, if present, if those Tregs are natural or induced. The adaptive response depends on the events happening in the early encounter with the pathogen. This has been the focus of many studies in PRRSV immunology. Most published papers point towards two critical points: the interplay of the virus with the innate immune system and the genetic and immunobiological diversity of PRRSV. Regarding the innate response, it is widely accepted that PRRSV inhibits type I interferon responses in many cell types but it seems that this inhibition is not that clear when it comes to plasmacytoid dendritic cells, the specialized interferon-I-producing antiviral cells. Besides, PRRSV may also interfere with the production other cytokines such as IL-10 and TNF-alpha, altering thus the milieu in which the initial recognition of PRRSV occurs. Many of these cytokines (e.g. TNF-alpha) are released as a result of the activation of Toll-like receptors. Although the information is scarce, it seems that PRRSV may also interfere with the expression of TLR3 and TLR7 in infected cells. In addition, several studies have shown that the infection of dendritic cells may lead to the downregulation of immunologically relevant molecules in the cell surface. Interestingly, different isolates may have different properties with regards to the interaction with the immune system. The host component is also important in the response to PRRSV. The genetic background of the host may also influence the course of the infection and the immune response. In this presentation we will review these different elements involved in the immunity to PRRSV.



Invited Speaker

Keynote Lecture

Methods and approaches to diagnose PRRS

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Porcine reproductive and respiratory syndrome (PRRS) is a globally distributed viral disease clinically characterized by reproductive failure in breeding animals and respiratory disease in pigs of all ages. At present there are two distinct genotypes among PRRS viruses: Type 1 (European type) and Type 2 (North American type). Both genotypes are distributed globally.

Clinical signs of PRRSV infection are influenced by virulence of the strain, pre-existing immunity, co-infections and farm management practices, so any clinical diagnosis must be confirmed by detection of PRRSV, or its specific antibodies.

The most widely used methods for PRRS diagnosis are ELISA and PCR. The best clinical specimens for virus detection by PCR in individual pigs are serum, lung, lymph node, tonsil or spleen sections. Recently oral fluid became widely used sample for detection of PRRSV in pig populations. In case of reproductive problem, samples from aborted or stillborn piglets can be used.

The PCR assays should be able to detect and discriminate between the two genotypes of PRRSV, as they can co-infect individual pigs and pig populations. PRRSV strains of both genotypes are highly diverse genetically and several reports indicated that no single PCR assay is able to detect all current viruses with optimal sensitivity. This is particularly true for East European subtypes of Type 1 PRRSV, of which relatively few sequences are available for PCR primer design.

At present there are many commercially available ELISA kits for detection of serum antibodies specific for PRRSV. Recently, ELISA platform has also been adapted to testing oral fluid samples. Generally, any ELISA kit is useful for the detection of antibodies against either genotype of PRRSV. There are also kits available to discriminate seroconversion against the two genotypes but due to some cross reaction the results should be interpreted with care, and always discriminatory PCR is recommended to confirm the diagnosis. If available, IPMA and IFA tests can also be recommended for serological discrimination of infection with the genotypes of PRRSV. ELISA kits differ in specificity and sensitivity. Low sensitivity of a given test can be overcome by increasing the number of tested samples. On the other hand, low specificity can make serological monitoring of PRRSV free herds particularly challenging, especially in regard to adult pigs populations.

Sampling protocol in a pig farm has to be always designed individually, depending of the diagnostic goal, and a farm organization. The simplest question, whether a farm is infected with PRRSV, can be answered by ELISA testing of 10-20 finishers. Detailed analysis of PRRSV circulation in a farrow to finish farm, or a multi-site production system, requires testing of large number of samples representing several age groups and all locations of a system.

Interpretation of seroconversion or virus detection in farms where modified live vaccines are being used is challenging as there are no marker vaccines on the market, vaccine viruses can persist in vaccinated farms, and at least for some time they can co-exist with wild type strains. In such conditions DNA sequence analysis of amplicons obtained in different age groups of a farm is required for full understanding of PRRSV circulation.

In PRRS control program planning and execution, the best approach is to use ELISA in conjunction with PCR and DNA sequence analysis.



Invited Speaker

Keynote Lecture

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) vaccines - lessons from the past and challenges for the future

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One of the hallmarks of PRRSV is its genetic instability leading to the emergence of new strains. The drive behind this evolution is the viral adaptation to replicate in more subsets of its target cell, the macrophage, and to higher levels per cell allowing the virus to spread more efficiently. Especially the increase of susceptible macrophages in the upper and lower respiratory tract has increased the viral shedding via nasal secretions and improved the airborne spread. In addition, the virus became very skilled in escaping from immunity. Neutralizing antibodies appear late and at very low titers and are not very cross-reactive. A few reports on the cell-mediated immunity are indicative for a failure of cytotoxic T-lymphocytes and NK cells. All these aspects make it difficult to develop an ideal vaccine. In the past, attenuated vaccines gave a very good protection (homologous situation), however the efficacy dropped considerably when new divergent strains emerged (heterologous situation). Therefore, a regular adaptation of the vaccine virus strain should be advised. Inactivated vaccines should contain a high viral antigenic mass and a strong adjuvant to induce a protective immunity. In pigs vaccinated with an inactivated vaccine, a shorter virus replication is noticed upon a homologous challenge, however no effect is seen on the virus replication during the first week. Therefore, this vaccine can only be advised to boost an existing immunity, especially when the vaccine virus is closely related to the circulating field virus. In this context, an autogenous vaccine may be the ideal inactivated vaccine. The technology is available to start this approach. An interesting strategy for controlling field virus circulation in a pig population may be to vaccinate gilts and fattening pigs with an attenuated PRRSV vaccine that is genetically closely related to the prevalent field strain and sows with an adjuvanted, autogenous inactivated PRRSV vaccine. By performing cross-sectional serological analyses in fattening pigs, the impact of the maternal immunity on the induction of an active immunity and on the circulation of wild type virus may be assessed. In the future, efforts should be made to develop marker vaccines. This will ease the follow up of the prevalence of PRRSV in large pig populations.



Invited Speaker

Keynote Lecture

PRRS Control and Elimination: The Role of Virus and Host Genetics

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The eventual elimination of PRRS will be dependent on understanding its complex biology/ecology, the genetics of the virus-host interaction, and the development of new immunological tools for detection and control. This process begins with elucidating the molecular mechanisms of replication at the cellular and host levels, continues through the herd and ultimately ends in the analysis of metapopulation dynamics. As a principal control tool, modified live virus (MLV) PRRS vaccines have been available for 20 years and have yet to fully meet expectations. One important limitation is that vaccines show the greatest level of protection against homologous virus. New findings demonstrate that pigs develop different types of neutralizing antibody, including broadly neutralizing antibody (bnAb) capable of neutralizing multiple isolates. The existence of PRRSV-specific bnAb raises two important questions: what are the epitopes recognized by bnAb and what are the genetics of the host that enable the bnAb response? Therefore, in addition to understanding immunological mechanisms related to protection, pig genetics is likely to play an important role in developing the next generation of vaccines; the so-called “vaccine-ready” pig. Other promising control measures are linked to understanding how the virus interacts with the macrophage host cell. Previous work using transgenic pigs suggests that at least one receptor-associated molecule, CD169, may not be essential for infection. However, the modification of other macrophage surface proteins may prove more promising in blocking PRRSV infection.



Invited Speaker

Keynote Lecture

PRRS control and eradications plans in Europe

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Porcine reproductive and respiratory syndrome virus (PRRSV) is widespread and endemic in most European countries and only 4 countries has a free status: Norway, Sweden, Finland and Switzerland. Based on a short questionnaire mailed to 20 EU countries (response rate 50%), type 1 (EU strain) is the most prevalent and only serotype in most countries, whereas type 2 (US strain) have some prevalence in e.g. Denmark, Germany, Austria and Poland. Due to a general lack of systematic surveillance in most countries, the true prevalence of infected herds is unknown, but based on estimations expected to be 25-50% in e.g. Denmark and Romania, 50-75% in e.g. Germany, Greece and Austria and 80-95% in e.g. Italy and Spain.

The cost of the PRRSV infection in Europe seems not to be very well estimated in most countries. Studies in sow herds that experience *acute* PRRSV problems have shown a loss of 59 to 379 EURO/sow during outbreak in Holland (median 75 EURO) and of 4 to 95 EURO/sow in Denmark (median 44 EURO). When comparing a large number of *chronically* infected herds to non-infected herds, Danish studies show only a marginally reduction in productivity. The piglet mortality was 0.8-0.9 %-point higher and the nursery mortality was 0.4%-point higher in infected herds. No difference was seen in mortality among finishers.

Only the four PRRSV free countries have a national control strategy. For these countries stamping out (total depop/repop) of infected herds are mostly used. In Sweden and in Switzerland PRRSV was diagnosed for the first time in 2007 and 2012, respectively, in Switzerland due to import of infected semen. After a relevant screening all infected herds were culled and both countries regained PRRSV free status in less than a year.

In most EU countries the main measures of controlling PRRSV is by vaccination, change of management and by herd closure in combination with vaccination. Based on a high level of non-infected nucleus & multiplying herds in Denmark (90% PRRSV free) that ensure a reliable and safe source for PRRS free replacement stock, PRRSV is eliminated by total depop/repop or by partial depop/repop to some larger extend in Denmark. Thus the prevalence of infected herds has been declining for more than 10 years.

Apparently only a few EU countries are discussing national eradication as a possibility. In Holland a pilot program has been running for some years in a local area with group of motivated farmers. All farms are tested 3 times a year and regular meetings are held to promote a voluntary eradication approach. In Denmark the most likely national cost of PRRSV has been calculated to be around 15 million EURO per year, whereas cost of a national eradication program running over a 5 year period, has been estimated to 120 million EURO. This gives a pay-back-time of around 15 years. Based on this the most probable strategy in Denmark (under discussion) will be a voluntary monitoring program for all herds, followed by an individual control plan for all herds, which eventually might lower the prevalence of infected farms.



Invited Speaker

Keynote Lecture

Current incidence, prevalence and state of control of PRRS virus in North America

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Porcine reproductive and respiratory syndrome virus (PRRSV) has been reported to be the most costly pathogen causing reproductive disease and decreased growth performance. The virus continues to evolve and considerable variability exists among isolates in virulence, apparent protection from vaccination and our time required to eliminate the virus from sow herds.

Lacking any data on prevalence or incidence in the industry, we started a Swine Health Monitoring Program in 2012 (Tousignant et al). A convenience sample of volunteer herds were invited to participate and today, 538 sow herds are enrolled accounting for approximately 2 million sows, or about a third of the total sows in United States. Veterinarians provide weekly diagnostic data on PRRS status for these herds including new infections and progress made in controlling the virus. The data are analyzed to calculate incidence and prevalence. Location is also shared thereby facilitating tempero-spatial analysis. These data indicate a decrease in incidence in 2013 and 2014 to approximately 25% compared to approximately 35% in previous years. This decrease may reflect increased knowledge and implementation of effective biosecurity measures, however, it remains to be seen if this decrease is sustainable.

Substantial progress has been made in how we control PRRSV in sow herds. After a herd is infected, veterinarians commonly employ a program referred to as Load / Close / Expose (LCE). The expectation is that protective immunity is built at the population level resulting in the eventual elimination of PRRSV from the breeding herd. Linhares et al (2014) followed a cohort of 61 infected sow farms and reported the median time to eliminate virus from piglets as 26.6 weeks (25th to 75th percentile, 21.6–33.0 weeks). The average production loss was 2.2 pigs / sow and it took an average of 16.5 weeks for herds to recover back to baseline. Herds that received modified live virus as the exposure program recovered production sooner and had fewer piglets lost but took longer to eliminate field virus than herds that received field virus as the exposure program.

Recognizing that a herd's risk of infection is partially related to PRRSV status of neighboring herds, has led to voluntary efforts to control the virus within regions of the country (Corzo et al). Health status is shared among participants as well as successes and failures with regards to control efforts.

There is an overall sense of progress and this has led American Association of Swine Veterinarians to propose a national PRRS control program. The intention is to build on the existing regional control programs in an attempt to reduce regional incidence of the virus, while encouraging PRRSV elimination programs to decrease the prevalence of infected herds. The program will be producer led, producer funded and voluntary, at least at its initiation.



Invited Speaker

Keynote Lecture

PRRS Control in China: Vaccination and Virus Evolution

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Porcine reproductive and respiratory syndrome (PRRS), was first introduced into China in late 1995, and the first outbreak of a PRRS-like disease was reported on a pig farm near Beijing. Within a few years following the first outbreak, PRRS virus spread to most of the provinces of China, many pig farms have since become endemically infected. In 2006, a highly pathogenic PRRS(HP-PRRS), characterized by high fever, high morbidity and mortality to all age pigs, emerged in Jiangxi province and rapidly spread to most of the provinces in next a few months. Since then, HP-PRRS virus became the dominant epidemic strain circulating in swine farms and HP-PRRS is now one of the severest threats to the swine industry.

Since 2004, killed vaccine and live attenuated vaccine against classical PRRS were officially approved to use in China. As the time was limited before the HP-PRRS outbreak, we were not sure about effectiveness of vaccination on PRRS control, but for sure that those classical PRRS vaccine could not stop HP-PRRS. By 2011, three attenuated live vaccines developed from three HP-PRRSV isolates were released to use for control of HP-PRRS in China. With wide application of HP-PRRS vaccines, the disease was no longer pandemic, but more likely endemic or sporadic.

Since 2012, a number of PRRSVs have been isolated from clinically ill piglets, all these viruses contain a discontinued 30 aa deletion in nsp2 region indicating that they were derived from earlier HP-PRRSV. These are summarized as four situations: 1. There were large number of PRRSVs isolated from piglets distributing in most of provinces in China, these viruses showed a wide range of genetic diversity (some contain different additional deletion in either nsp2 region or ORF2 or ORF3). 2. In a period of five months, 14 wild type PRRSVs were isolated from an all-in, all-out farrow-finish farm of 8,100 sows, with a production of approximately 165,000 slaughter pigs per year. These isolates share 96.8%-99.2% nucleotide identity with earlier HP-PRRSV. 3. Importation and recombination are responsible for the latest emergence of high pathogenic PRRSV in China. A NACD30-like PRRSV recently introduced from North America and has undergone genetic exchange with the classic HP-PRRSVs in China, the recombinant virus was indicated highly pathogenic to piglets in experimental infection test. 4. Three PRRSV, NT1, NT2, and NT3, were isolated from three dying piglets from a single pig farm in Jiangsu Province, China. Whole genome sequencing revealed that the three isolates share the highest homology with JXA1-P80, an attenuated vaccine strain developed by serial passage of highly pathogenic PRRSV JXA1 in MARC-145 cells. More than ten amino acids residues in ORF1a, ORF1b, GP4, and GP5 that were thought to be unique to JXA1-P80 were each found in the corresponding locations of NT1, NT2, and NT3. Experimental infection of piglets with NT1, NT2, and NT3 indicated that they are highly pathogenic PRRSVs and they are likely to be revertants of the vaccine strain JXA1-P80.

Taking all together, we could see that evolution of PRRSV is active and PRRSVs are diverse, which is possibly the new challenge to current vaccination strategy and future prevention of PRRS.



O.01

Emergence of novel NADC30-like Strain of Porcine Reproductive and Respiratory Syndrome Virus in China

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Porcine reproductive and respiratory syndrome (PRRS) is one of most economically important viral diseases of swine for the global pork industry. The emerging novel strain of PRRSV with higher pathogenicity and virulence can cause clinical outbreaks of severe forms of PRRS. During August-December of 2014, severe clinical outbreaks of PRRS were observed on 7 intensive pig farms in 5 pig-producing areas of China. To genetically identify the PRRSV strains in recent emergence, the ORF5 genes of 7 novel isolates together with the whole genome of one strain, designated as CHsx1401, were sequenced. Comparative analyses of the deduced amino acid sequences showed that these amplified ORF5s (GenBank accession nos. KP861625-31) shared 88.6%-98.0% similarity with each other, and higher similarity (91.5%-96.5%) with the NADC30 isolated in the United States, lower similarity (84.1%-88.6%) with the Chinese representative PRRSV strains including CH1a, HB-1(sh)/2002, HB-2(sh)/2002 and JXwn06, and only 82.1%-86.1% similarity with VR-2332. The genome of CHsx1401 was 15,020 nucleotides in length, excluding its ploy A tail (GenBank accession no.KP861625). The amino acid alignment of its nonstructural protein 2 (Nsp2) highly variable region with other strains' showed that CHsx1401 had a amino acid deletion model which is identical to the NADC30, and MN184 isolated in the United States, namely 111-aa deletion at position 323-433, 1-aa deletion at position 481 and 19-aa deletion at position 533-551, compared with the prototype VR-2332. Phylogenetic analysis based on whole genome of PRRSV indicated that CHsx1401 was shown to be genetically more closely related to the NADC30, clustering into a branch. Our clinical observation and ORF5 gene variation analyses confirmed that novel NADC30-like PRRSV has been emerging and epidemic in China. Further whole genome analyses clearly showed that the virus shared an identical deletion model to the NADC30 of the United States origin in its Nsp2-coding region, and was genetically similar to the NADC30, and differed from previous Chinese PRRSV strains. Last, further investigation of its pathogenicity and cross protection between this virus and current commercial vaccines is needed to prevent and control this virus.



O.02

Reconstruction of Porcine Reproductive and Respiratory Syndrome Virus evolutionary dynamics in Italy.

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Porcine Reproductive and Respiratory Syndrome (PRRSV) is the most relevant and challenging infectious disease to affect swine breeding. Despite this, several aspects of virus evolution and virus-host interaction are still poorly understood and largely based on knowledge obtained through *in vitro* or *in vivo* experimental infections. Consequently, our understanding is often contradictory and difficult to infer toward actual field conditions due to peculiar experimental conditions. Our phylodynamic study, based on ORF5 sequences of 141 samples collected in Italy from 1993 to 2012, explores different aspects of PRRSV epidemiology, evolution and virus-host interaction. Two major clades, belonging to Type 1 subtype 1, were demonstrated to co-circulate while harboring a relevant intra- and inter-clade genetic diversity. Most Recent Common Ancestor (MRCA), evolution rates and population dynamics were estimated using a serial coalescent based approach and different demographic histories were reconstructed for the two clades. Analysis of selective pressure revealed that sites subjected to diversifying selection were mainly located in the region of the glycoprotein 5 (GP5) exposed to the host environment. Similarly vast majority of strains were highly glycosylated, confirming the proposed protective role of the glycan shield against humoral immune response. Overall, our study reports both interactions between viral population as well as virus-host to be relevant in shaping viral evolution: different population dynamics over time seemed to reflect a competition between clades. Some evidence arguments in favor of the role of immune pressure in affecting GP5 evolution, favoring frequent changes in region exposed to host immune response and preserving glycosylation profiles that can hamper humoral immunity.

O.03

Genetic and phenotypic in vitro and in vivo characterization of recent PRRSV isolates from Austria reveal similarities to East Asian strains

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Porcine reproductive and respiratory syndrome virus (PRRSV) outbreaks in Austria occur frequently in formerly free livestock without epidemiological links. Recently in two PRRS outbreaks in Upper Austria unusual type 1 subtype 1 strains were detected by the routine qPCR diagnostic procedures (ORF7; TGD Upper Austria). On one farm a mild yet long lasting PRRSV infection was noticed and eradication failed (883/13). The other remarkable case was a farm in the same region that encountered an abortion storm and also respiratory disease in piglets (440/14).

Virus isolation proved difficult for 883/13 that could be cultivated on porcine alveolar macrophages (PAMs). In contrast 440/14 readily grew on PAMs and MARC-145 cells without adaptation. A third PRRSV isolate from Lower Saxony 2009 (613) was included in the analyses. The genomes were fully sequenced and cloned. Phylogenetic analysis of nucleotide and amino acid (aa) sequences revealed that the two field isolates from Austria were not identical and clustered closely together with the German 613 and a South Korean isolate (KNU-07). While isolates 613 and 883/13 had small deletions in nsp2 (compared to the prototype strain Lelystad), isolate 440/14 had several deletions in nsp2 and a 12 aa deletion in GP3/4.

To confirm the pathogenic potential of the isolates 883/13, 440/14 and 613 an animal trial with five seven-week-old piglets per group was carried out. The piglets were infected oronasally with 1×10^5 TCID₅₀ of the respective strain. Along with daily clinical examination the piglets were weighed and blood and nasal swabs were collected regularly. At day 13 and 14 respectively the animals were euthanized and necropsied. Daily weight gain was lower in the two groups infected with the Austrian isolates compared to the control group. Significant respiratory symptoms were only observed for 440/14 but PRRSV specific lesions in lung tissue were found for all strains. Detection of PRRSV by qPCR or virus isolation revealed highest virus loads in serum of 440/14 infected animals, followed by 883/13 and 613.

The surprising results of our studies revealed that three different isolates from Austria and Germany cluster more closely to an East Asian strain than to European isolates. This is further supported by a deletion in ORF3/4 of 440/14, that is similar but not identical to one mainly found in Chinese sequences. Whether these molecular markers are indicative of the introduction of East Asian PRRSV isolates to central Europe or whether they result of a directed, immunevasive evolution is currently not to decide. Further in depth studies are necessary to functionally explain the changes in PRRSV.

O.04

Genome analysis of a non-MLV-related type 2 Hungarian PRRSV isolate

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The vast majority of the type 2 strains found in Europe are genetically related (>95% ORF5 nucleotide identity) to modified live virus (MLV) vaccine. However, recent studies revealed other groups of type 2 viruses that are less similar (86–94%) to the Ingelvac MLV, and their existence most probably cannot be attributed to the vaccine. The aim of our study was to characterize a member of the European type 2 viruses with 88% similarity to the Ingelvac strain on ORF5.

In 2012 lung, tissue and lymph node samples were obtained from the carcass of a young growing pig originating from an endemically PRRS positive herd. Only mild to moderate respiratory symptoms were observed among the young fatteners. No significant reproductive disorders were reported at the time of sampling. A closely related virus was first identified in 2005 in multiple sites of a swine breeding company with mild clinical signs of PRRS.

The genome of PRRSV-2/Hungary/102/2012 (Hu102) was amplified in five overlapping parts and sequenced with Ion Torrent semiconductor sequencing equipment. The phylogenetic analysis of the whole genome of Hu102 and 215 other full PRRSV genomes showed that it was a novel type 2 PRRSV isolate that was phylogenetically close to the progenitor type 2 PRRSV, and not related to VR2332 or Ingelvac PRRSV MLV. The ORF5 dendrogram that included a large dataset of other ORF5 sequences showed that Hu102 belongs to the ancient lineage 1 or lineage 2 of the type 2 viruses, which may have originated in Eastern Canada where the earliest isolates were found.

Hu102 has unique deletions of 10 amino acids (VR3223 aa 314–323) and 9 amino acids (VR2332 aa 792–800) in the nsp2 that were not present in prototype strain VR2332 or the prototype high pathogenicity Chinese strain JXA1. Beside that a 9 aa insertion could be found in the nsp2 of HU102 (aa 795–803) that was not present in VR2332 or JXA1.

Recombination analysis performed with the available full-length genome sequences showed no evidence of such event with other known PRRSV.

The antigenic regions (ARs) and glycosylation sites within GP2, GP3, GP4 and GP5 proteins of PRRSV-2/Hungary/102/2012 have been analyzed and compared to references VR2332, Ch-1a and NVSL 97-7895. Large amount of aa substitutions were found in the hydrophobic regions of the amino-terminal signal peptides of the GP proteins (GP2_{1–40}, GP3_{1–30}, GP4_{1–20} and GP5_{1–31}). A very rare glycosylation pattern (featured in less than 1% of the PRRSV strains found in Canada) with five glycosylation sites (N30, N34, N35, N44 and N51) was detected in the GP5.

Our data suggest that the ancestor of Hu102 was most probably imported directly from North America during the early stages of PRRSV diversification (likely from Canada or the North Central USA).



O.05

Porcine reproductive and respiratory syndrome virus nonstructural protein 2 membrane topology

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Nonstructural protein 2 (nsp2) of the porcine reproductive and respiratory syndrome virus (PRRSV) replicase polyprotein, implicated in formation of double membrane vesicles in infected cells as sites for viral replication and protein synthesis, has also been shown to be incorporated into ultrapurified virions as differently sized isoforms. PRRSV nsp2 has four to five predicted transmembrane helices located near the carboxyl terminus, a predicted prokaryotic signal sequence, and 1-6 potential N-glycosylation sites. In order to study membrane insertion and topology of this protein, nsp2 of Type 2 strain VR-2332 was cloned into pcDNA 3.0 without a FLAG tag, or with a FLAG tag located at the N- or C- terminus. The constructs were assessed using a rabbit reticulocyte lysate cell free translation system in the presence or absence of artificial canine pancreatic microsomal membranes. Expression of PRRSV nsp2 in the absence of all other viral factors resulted in the genesis of both full-length nsp2 as well as a number of nsp2 isoforms. Addition of microsomal membranes to the translation stabilized the translation reaction, resulting in predominantly full-length nsp2 as assessed by immunoprecipitation with α -FLAG antibodies. The microsomal membranes are also capable of core glycosylation at predicted N-glycan sites (NX[ST]), but no N-glycosylation was observed. Analysis further showed full-length nsp2 strongly associates with membranes, along with two additional large nsp2 isoforms. Membrane integration of full-length nsp2 was confirmed through high-speed density fractionation, protection from protease digestion, and immunoprecipitation. The results demonstrated that nsp2 integrated into the membranes with an unexpected topology, where the amino (N)-terminal (cytoplasmic) and C-terminal (luminal) domains were orientated on opposite sides of the membrane surface.

O.06

A complex interplay between the signal peptide, glycosylation sites and interaction with M determines signal peptide cleavage from GP5.

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Gp5 and M are the main components of the viral envelope, a driving force for virus budding and the target of antibodies. Their epitopes (mostly identified by pepsin analysis) are located near the (predicted) signal peptide cleavage site and it is thus not known whether they are actually present in the mature protein and hence in virus particles. Previously we have identified the signal peptide cleavage site of GP5 from American PRRSV strains. The results revealed that the signal peptide is cleaved at two sites. As a result a mixture of GP5 proteins exists in virus particles. All of them preserve a neutralizing epitope, but only a fraction contains the “decoy epitope”, a target of non-neutralizing antibodies that have been claimed to be one cause of viral persistence.

For GP5 of European PRRSV strains only one neutralizing epitope was identified for the strain Intervet-10 (I-10) using monoclonal antibodies from immunized mice. Surprisingly, the antibodies did not neutralize the infectivity of the closely related Lelystad strain. This is due to a single amino acid difference at position 24 (Pro → Cys) in the signal peptide of GP5 – yet, the epitope does not encompass this residue, but is located further downstream, right at the border between the predicted signal peptide and the ectodomain. It was speculated that the amino acid exchange affects the site of signal peptide cleavage, such that the epitope is present in the mature form of GP5 from I-10, but not from the Lelystad strain.

Here we report about signal peptide cleavage from GP5 of European PRRSV strains. Expression and western blotting revealed that the signal peptide is completely removed from GP5 of the Lelystad strain, but is partially preserved (~50%) in GP5 from a virus variant having a glycosylation site at position N35. Surprisingly, N35 is not glycosylated, but the glycosylation sequon is required to inhibit signal peptide processing. In contrast, glycosylation sites introduced at position N36, N37 and N38 are used, but do not inhibit signal peptide cleavage. Complete processing of the signal peptide of GP5(N35) was observed for the mutant Cys24Pro and also by co-expression of M with GP5. Thus, a complex interplay between glycosylation (sites), amino acids within the signal peptide and interaction with M affects processing of the signal peptide and thus the presence of antibody epitopes in virus particles.

Mass spectrometry of GP5(N35) purified from virus particles demonstrated that the signal peptide is completely removed by cleavage between G34 and N35. This result explains why a monoclonal antibody does not neutralize Lelystad as the epitope is not present in virions. The identification of the signal peptide cleavage site of the GP mutant C24P (corresponding to the II-10 strain) is currently under investigation.



O.07

Characterising the PRRSV Double Membrane Vesicle

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The replication cycle of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) depends upon the rearrangement of cellular membranes to form double-membrane vesicles (DMVs). Previously published data are consistent with a cellular origin of the DMV in the autophagy or ERAD tuning pathways. We aim to elucidate the nature of the DMV structures using the nsp2-GFP fusion protein of infectious clone SD01-08 as a DMV marker in Marc-145 cells. Using density separation of cellular organelles we identified and excluded cellular proteins associated with organelles of similar density to the DMV structures. The sub-cellular location of PRRSV in infected cells was analysed using SD01-08 nsp2-GFP and immunofluorescence staining of candidate co-localized host proteins. We are characterising DMVs in infected cells by proteomic and biochemical analysis of cellular organelles sorted by fluorescence-activated flow cytometry.



O.08

Inhibition of PRRSV replication by targeting the formation of double-membrane vesicles

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Recently, a novel antiviral compound (K22) that specifically interferes with membrane-bound RNA synthesis by impairing double-membrane vesicles (DMV) formation was described to inhibit replication of a broad range of animal and human coronaviruses (Lundin A. et al, 2014, PLoS Pathog. 10:e1004166). Both, coronavirus and porcine reproductive and respiratory syndrome virus (PRRSV) replication and transcription complexes were described to accumulate at virus-induced DMVs. Therefore, the present study was aimed at determining whether K22 does also inhibit replication of PRRSV by targeting DMVs, and if so, whether PRRSV escape mutants can be selected.

The effect of different doses of K22 on PRRSV replication was determined in MARC-145 cells with genotype 1 and genotype 2 PRRSV. DMSO treatment was used as control. Inhibition of PRRSV replication was detected with 30 uM K22 and complete inhibition was obtained with 50 uM K22, with no detectable cytotoxic effect. By successive virus passages in MARC-145 cells in the presence of increasing amounts of K22, drug-resistant PRRSV were selected. Nucleotide sequence analyses revealed potential adaptive mutations in the nonstructural proteins of the resistant viruses, which will be further investigated with functional full-length cDNA clones. Our data so far demonstrate that K22 has antiviral activity beyond the coronaviruses, supporting the involvement of DMV in PRRSV replication. Results from reverse genetics experiments are expected to provide insights into the PRRSV proteins involved in DMV-dependent RNA replication.

O.09

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

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Introduction

Lung macrophages play an important role in host defense at both innate and adaptive immune levels. During PRRSV infection, these key cells are the main target for virus replication and their functions are altered. In 2007, a new highly pathogenic PRRSV strain (Lena, genotype 1.3) was isolated in Eastern-Europe. To better understand the pathogenesis of Lena infection, we studied the evolution of macrophages and their cytokines environment in the lung of infected pigs and compared them to that of pigs infected by a low virulent genotype 1.1 PRRSV strain (Finistere).

Materials and Methods

Six-weeks-old SPF pigs were inoculated with either Lena (n=8; L group), Finistere strain (n=5; F group) or media (n=5, Control group). The animals were monitored daily for clinical signs. Bronchoalveolar Lavage Fluids (BALF) and blood were collected twice a week after infection to follow the macrophages (flow cytometry), the virus (qRT-PCR) and the cytokines (ELISA).

Results

In L group, pigs exhibited high fever and clinical score leading to death of 3 animals whereas in F group, pigs displayed only mild clinical signs. Compared with F group, PRRS viral load in L group was 10 to 100-fold higher in serum and BALF, but decreased faster from 22 dpi in BALF. After infection, BALF cells displayed a drop in viability and phagocytosis activity matching with a decrease of macrophages population for both groups. In F group, the loss of lung macrophages was delayed compared to L group. From 8 to 15 dpi, animals of L group showed a simultaneous increase of monocytes in BALF and blood whereas it was only observed in BALF of F group. Regarding cytokines, low levels of IFN α and TNF α were measured in BALF for both strains and with a delayed response for F group. In serum, high levels of IFN α and TNF α were detected in L group but not in F group. Conversely, IL1b and IL8 levels were higher in L group than in F group in BALF whereas low levels were measured in serum for both strains. High levels of IFN γ were also found in BALF in L group from 8 to 15 dpi. In contrast IFN γ level was low (6 times lower compared to L group at 8 dpi) in BALF of F group and no IFN γ were detected in serum for both groups.

Conclusion

We confirmed that Lena strain induces high fever, high systemic viral load and inflammatory response. In the lung, high macrophages alteration linked to high viral load was associated with a rise of monocytes population and high IL1b / IL8 levels. For Finistere strain, we showed a delayed and lower innate response as well as a prolonged viral replication possibly related to weak IFN γ response. Better understanding the immune response to highly virulent PRRSV strain is a key point for a better control of these strains.



O.10

Pathogenesis of European subtype 3 and subtype 1 PRRSV strains in pigs.

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Vaccination against porcine reproductive and respiratory syndrome virus (PRRSV) results often in limited protection. Understanding host immune responses and pathogenesis elicited by different PRRSV strains could help to develop more efficacious vaccines. Differences in host response between the European subtype 3 strain Lena and subtype 1 strains Belgium A and Lelystad-Ter Huurne (LV) were examined in several studies and “meta-analyses” results across studies will be presented. Characteristic of infections with strain Lena, compared to strains Belgium A and LV, are fever, clinical symptoms, a systemic inflammatory response, higher virus titres in serum, differences in leukocyte populations in blood, lower numbers of IFN- γ secreting cells in blood, and a delayed antibody response against a non-related immunization. In the lungs, infection with strain Lena induce more severe acute pathology with an proinflammatory response and an influx of neutrophils and monocytes in bronchoalveolar lavage fluid (BALF). However, after a few weeks, all strains induce an increased percentage of cytotoxic T cells and higher levels of IFN- γ producing cells in BALF. We hypothesized that the stronger early inflammatory response of the Lena strain contributed to the observed faster clearance of virus after infection and better protection against reinfection. In a comparative study, this hypothesis was tested. Pigs were infected with strains Lena or LV and homologous challenged after 46 days. Challenge infection resulted in both groups in complete protection, with no viraemia, clinical symptoms or viral RNA in tissues. After the challenge, a boost in antibody levels in pigs infected with homologous strains mounted rapidly and IFN- γ ELISPOT assays showed comparable responses between Lena and LV, when cells were homologous stimulated. However, clear differences between the strains in levels of neutralizing antibodies and IFN- γ SC were observed when the assays were performed with heterologous virus. In conclusion, although, there are clear differences in immunological, clinical and virological responses after infection, with stronger inflammatory response for strain Lena-infected pigs, there were no differences observed in protection against homologous challenge. Whether this is also true for heterologous challenges needs further research.

O.11

Maternal and fetal predictors of fetal viral load and death in PRRSV infected pregnant gilts

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Although mechanisms associated with reproductive failure and fetal death induced by porcine reproductive and respiratory syndrome virus (PRRSV) are poorly understood, a relatively small amount of research has focused on this aspect of PRRS pathophysiology. We recently completed a large-scale project investigating phenotypic and genotypic predictors of reproductive PRRS severity. For this purpose, 114 pregnant Landrace gilts were inoculated with a virulent PRRSV strain (NVSL 97-7895, 10⁵ TCID₅₀ total dose) at gestation day 85 (\pm 1) and were followed for 21 days. Numerous clinical, pathological, immunologic and viral responses were characterized in dams and fetuses including quantification of PRRSV RNA in gilt and fetal sera and tissue samples by RT-PCR, measurement of cytokine levels in gilt sera and supernatants of peripheral blood mononuclear cells (PBMC) stimulated with PRRSV or PMA/Ionomycin, changes in leukocyte subsets in gilt blood analysed by flow cytometry, gross and microscopic evaluation of lesions in gilt and fetal tissues, characterization of fetal preservation, mortality and location within the uterus. Dams were specifically selected from high and low birth weight (BW) litters in order to determine if the dam's BW influences PRRS severity. The WUR10000125 genotype, associated with PRRSV resistance/susceptibility in experimentally infected nursery pigs, was determined in dams, sires and non-autolysed fetuses. Multilevel, mixed-effects regression models were used to determine which phenotypic responses in gilts and fetuses were predictive of fetal viral load and death.

Our results demonstrate that PRRSV RNA concentration in dam sera and systemic tissues were not associated with fetal death. PRRSV RNA concentration in the maternal-fetal interface was the strongest predictor of fetal viral load and the probability of fetal death, emphasizing its importance in the transmission of the virus from the maternal to the fetal compartment. Importantly, the presence of PRRSV in fetuses, particularly at high levels in thymus, increased the likelihood of fetal death. Fetal infection and death clustered within the uterus indicating that the status of adjacent fetuses and inter-fetal transmission of PRRSV significantly influence fetal outcome. Several systemic immune responses measured in gilts were associated with fetal outcome in a positive or negative manner. Whereas serum interferon-alpha contributed to the probability of fetal death, absolute numbers of T-helper cells in early infection were protective. Absolute numbers of myeloid cells over time and interleukin 12 levels also helped to protect against fetal viral load. These results suggest specific immune responses may either contribute to, or protect against, transplacental virus transmission. Unexpectedly, the WUR10000125 SNP on SSC4, associated with lower PRRS viral load and higher average daily gain in experimentally infected nursery pigs, was not associated with reproductive outcome after PRRSV infection.

Whereas past research suggested that fetal death results from events occurring at the maternal-fetal interface and was independent of direct fetal involvement, we conclude that viral replication in fetal tissues and subsequent spread of PRRSV to adjacent fetuses are pivotal events in the pathogenesis of reproductive PRRS.

O.12

Apoptosis at the maternal-fetal interface of type 2 PRRSV infected pregnant gilts

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The pathogenesis of fetal death induced by porcine reproductive and respiratory syndrome virus (PRRSV) is poorly understood. It has been hypothesized that initiation of apoptosis in fetal implantation sites plays a key role in the mechanism of PRRSV-induced fetal death. The objective of this study was to assess the apoptosis as a mechanism of cell death in uterine tissue and fetal placenta 21 days after type 2 PRRSV infection in pregnant gilts using terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) technique.

On gestation day, 85±1, 114 PRRS virus-naïve pregnant gilts were inoculated with PRRSV (10^5 TCID₅₀ total dose) and 19 negative control gilts were sham inoculated. At 21 days post-inoculation, dams and their litters were humanely euthanized for necropsy examination. Samples of uterus with fully attached placenta, as well as fetal thymus were collected and analyzed by an in-house qPCR to quantify PRRS viral load. Based on the PRRSV RNA concentration in the uterine/placental tissue adjacent the umbilical stump of each fetus, 3 groups of samples: negative (not detected), low (quantifiable, below mean), and high (quantifiable, above mean) were formed (n=40/group; 120 total). The corresponding formalin fixed paraffin-embedded uterine/fetal placenta sections were subjected to TUNEL staining. The number of apoptotic (TUNEL positive) cells per 1 mm² of endometrium and the fetal placenta was determined by Image ProPlus software.

At the maternal-fetal interface, there was more apoptosis observed in the fetal trophoblasts than uterine epithelial cells. Within the endometrium, the majority of the cells that were undergoing apoptosis were lymphocytes. The average number of apoptotic cells in the endometrium was 0.39±0.33, 13.99±6.6, and 16±9.41, respectively for the negative, low and high groups, whereas in the placenta was 3.15±3.56, 9.51±7.08, 24.99±14.87 cells per 1 mm² of the tissue. Statistical analysis revealed that the numbers of apoptotic cells in the endometrium and fetal placenta were both positively associated with PRRS viral load groups ($P<0.001$, $P<0.05$, respectively) suggesting significant increase in the numbers of apoptotic cells in the endometrium and the fetal placenta during type 2 PRRSV infection in pregnant gilts. This study confirmed that presence of the PRRSV virus in the uterus and the fetal placenta, and not in the fetus, was significantly associated with the presence of apoptosis in the fetal placenta at maternal fetal interface.

O.13

Pathogenicity of highly pathogenic PRRSV in pregnant sows

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Highly pathogenic porcine respiratory and reproductive syndrome (HP-PRRS) emerged in China in 2006 and thereafter, has occurred in Southeast Asia. The disease was characterized by a high fever of above 41°C, red discoloration of the ears and body, abortion in pregnant sows in different stages, and high mortality. In this study, we tested the pathogenicity in pregnant sows inoculated with HP-PRRS virus isolated in Vietnam in 2010.

Six PRRS virus (PRRSV) and porcine circovirus type 2 free SPF pregnant sows (90 days of gestation) were intranasally inoculated with 10⁵ TCID₅₀/sow HP-PRRSV Vietnamese strain (strain 100186 614). Sows were monitored daily for body temperature and clinical signs. Serum was sequentially collected for PRRSV antibody and the amount of viral RNA measured by a quantitative real-time RT-PCR. One pregnant sow was euthanized at 5 day post-inoculation (dpi) to examine systemic viral distribution in gilt and fetuses. Others were euthanized when they aborted, and lungs, lymph nodes and other organs were collected from sows and mummified, stillbirth and live fetuses for histopathology and the measurement of viral load.

The inoculated sows had a fever (>40°C) and were observed depression, anorexia and mild respiratory distress. Four pregnant sows aborted at 11-17 dpi, and expelled mummified, stillborn and live fetuses (a total of one liveborn and 59 stillborn). One sow was euthanized at 20 dpi because it didn't abort and was dying, and its fetuses were decomposed. Antibodies specific for PRRSV were detected at 10 dpi. Viral RNA was detected in blood samples of pregnant sows from 1 dpi, and its peak was shown at 5 dpi. The amount of viral RNA was shown approximately 10⁵ to 10⁶ TCID₅₀/ml at 5-10 dpi. In lungs, lymph nodes, uterus and other organs of sows and fetal organs including lungs, liver and spleen, viral RNA was also detected. Furthermore, viral RNA was detected in the uterus and umbilical cord of one sow euthanized at 5 dpi and in lungs, liver and spleen of one of 14 fetuses.

In all sows, mild interstitial pneumonia and small necrotic foci in lymph nodes were observed. No abnormal findings were observed in other organs. Viral antigens were detected in lungs and lymph nodes lesions, and uterine macrophages. In fetuses, no abnormal findings were observed by gross examination. Microscopically, some fetuses showed small necrotic foci in lungs and lymph nodes. Viral antigens were detected in some lymphoid organs including lymph nodes, spleen and thymus.

In this study, severe clinical signs were not observed, however, a higher incidence of abortion was reproduced in pregnant sows inoculated with HP-PRRSV Vietnamese strain. Our results could demonstrate high capability of HP-PRRSV Vietnamese strain to induce reproductive disorder in pregnant sows. This experimental model might contribute to clarify the mechanisms of reproductive disorder caused by PRRSV.



O.14

Potent interferon-alpha production by plasmacytoid dendritic cells co-cultured with macrophages pre-infected with genotype 1 or genotype 2 highly pathogenic porcine reproductive and respiratory syndrome virus strains

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Porcine respiratory and reproductive syndrome virus (PRRSV) is classified into genotype 1 (PRRSV-1), and genotype 2 (PRRSV-2). Within both genotypes, strains varying in pathogenicity are found. Porcine macrophages are the target cells for PRRSV replication but they do not produce interferon alpha (IFN-alpha) after viral infection. On the other hand, plasmacytoid dendritic cells (pDC) although not infected, produce IFN-alpha in response to free PRRSV particles with strain-dependent differences. Interestingly, the highly pathogenic PRRSV-1 Lena strain was unique in not inducing IFN-alpha production by pDC. We also demonstrate efficient pDC stimulation by PRRSV-infected macrophages, resulting in a higher IFN-alpha production than direct stimulation of pDC by virions. Even co-culturing Lena strain-infected macrophages with pDC resulted in high levels of IFN-alpha. Stimulation of pDC by PRRSV infected macrophages required cell contact, and was abrogated by an inhibitor of neutral sphingomyelinase inhibitor (GW4869). Importantly, GW4869 did not suppress CpG-induced pDC activation or replication and release of PRRSV from infected macrophages. Our data indicate a role for macrophages -derived exosomes in pDC stimulation by infected cells. In conclusion, although PRRSV is able to suppress IFN type I in macrophages, pDC are highly responsive PRRSV-infected macrophages by secreting large quantities of IFN-alpha. Considering the potency of pDC in the innate response, our results are questioning the concept of PRRSV as a virus, which is not well sensed by the innate immune system.



O.15

The immunity raised by recent European subtype 1 PRRSV strains allows a better replication of East European subtype 3 PRRSV strain Lena than the immunity raised by an older strain - increased risk for spatial expansion of PRRSV Lena-like strains

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The spatial distribution of PRRSV-1 subtypes in Europe is quite stable, most probably due to a strong population immunity induced by the local PRRSV strains. In this study, we evaluated the potential of the immunity induced by several West European subtype 1 PRRSV strains (2007 isolate 07V063 and 2013 isolates 13V091 and 13V117) to provide a protection against the highly virulent East European subtype 3 PRRSV strain Lena. Eleven-week-old pigs were inoculated with subtype 1 PRRSV strains (07V063, 13V091 or 13V117). Seven weeks later, the pigs were challenged with PRRSV strain Lena. Clinical, virological and serological parameters were monitored upon challenge. Number of fever days was higher ($P < 0.05$) in the non-immune control group (7.6 ± 1.7 days) compared to animals from immune groups (07V063-immune: 4.0 ± 1.2 days, 13V091-immune: 4.6 ± 1.1 days, 13V117-immune: 4.0 ± 2.9 days). In all groups, protection was characterized by reduction ($P < 0.05$) of AUC values of nasal shedding (control: 14.6 ± 5.6 , 07V063-immune: 3.4 ± 3.4 , 13V091-immune: 8.9 ± 6.1 , 13V117-immune: 8.0 ± 6.1) and viremia (control: 28.1 ± 11.0 , 07V063-immune: 5.4 ± 4.4 , 13V091-immune: 9.0 ± 1.5 , 13V117-immune: 8.3 ± 4.8). Reduction of respiratory disease, nasal shedding (mean AUC and mean peak values) and viremia (mean AUC and mean peak values) was more pronounced in 07V063-immune ($P < 0.05$) than in 13V091-immune and 13V117-immune animals. Inoculation of animals with subtype 1 PRRSV strains caused a priming of Lena-specific VN antibody response. Upon challenge with PRRSV Lena a serological booster effect was observed for neutralizing antibodies against strains used for the first inoculation. Our results indicate that immunity elicited by inoculation with subtype 1 PRRSV strains can partially protect against antigenically divergent subtype 3 strains. We conclude that the lower protection level elicited by recently isolated subtype 1 PRRSV strains may facilitate spatial expansion of subtype 3 strains from East Europe to West Europe.

O.16

Immunological and biochemical characterization of a short protein, 7ap, translated from an alternative frame of ORF7 of PRRSV

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Sequence analysis revealed a short alternative ORF named ORF7a within the nucleocapsid gene of 46 sequentially divergent PRRSV genomes. ORF7a localized in the +2 frame contains a methionine codon in a conserved position and depending on genotypes, continues in a coding region stretching between 26 and 53 amino acids. Alignment of the translated protein sequences (named 7ap) revealed five highly conserved and ten conserved amino acids in the proteins. 7aps from divergent strains have different physicochemical properties though they are all predicted to be positively charged.

To demonstrate the existence of 7ap and to investigate the translation of ORF7a, eGFP and 3xFLAG fusion constructs were created by cloning the cDNA of the 5' half of the ORF7 mRNA of the HU-14432/2011 PRRSV strain in the three consecutive frames on the + strand. Strong fluorescent eGFP signal and FLAG expression can be detected in the nucleus and cytoplasm of cells transfected by the frame+1 and frame+2 constructs confirm that 7ap is in fact translated from the nucleocapsid mRNA.

7ap proved to be highly toxic to bacteria. To complete the serological investigation of HU-14432/2011 PRRSV infected animals for the verification of *in vivo* translation, 7ap of HU-14432/2011 (Hu7ap) was synthesized chemically and used for indirect ELISA tests. Surprisingly, ELISA experiments revealed that Hu7ap binds strongly to mammalian (pig, mouse, goat, rabbit) IgG horse radish peroxidase (HRP) conjugates and that the binding is independent of HRP.

Protein-protein gel retardation assays were performed with Hu7ap and the 7ap of a type II PRRSV strain, WuH4 (Wu7ap) to confirm the results of ELISA and to localize the binding site/s of 7ap on swine IgG. Both 7aps were able to bind IgGs and the mayor binding site was localized on the IgG (Fc) fragment. Hu7ap was able to inhibit hemolysis in standard complement fixation experiment suggesting that Hu7ap binds to CH2 domain of the Fc.

The fact that 7ap contains several arginines and has a net positive charge are the trademarks of nucleic acid binding proteins so the RNA and DNA binding characteristics of 7ap were also investigated in protein-nucleic acid gel retardation assays.

Both Wu7ap and Hu7ap were able to completely reduce the motility of nucleic acids at relatively high molar charge ratios ((NA)-/(protein)) (Hu7ap: 7,31>0,5>0,34, Wu7ap: 1,5>0,37>0,07 for dsDNA, ssRNA and ssDNA, respectively) indicating strong dsDNA and ssRNA binding capability.

Having high sequential divergence and upkeeping RNA and DNA binding properties make it highly probable that nucleic acid binding is functional and it is not just an arbitrary consequence of the net positive charge of 7aps.

To raise antibody against 7ap two mice were immunized with SDS-PAGE slices containing the Hu7ap. None of the immunized animals' sera reacted with Hu7ap or Hu7ap-GFP. However, antinuclear antibodies were detected in both sera suggesting an ability of Hu7ap to interact or mimic auto-antigenic macromolecules.

Localization and the remarkable biochemical characteristics of PRRSV 7ap may indicate multiple functions for the protein including nuclear and cytoplasmic over-tuning of normal cellular processes and immunosuppression.

O.17

Specific disruption of the deubiquitinating activity of nidovirus papain-like proteases reveals their role in suppression of the innate immune response during infection

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The first line of defense against viral infections, the innate immune response, leads to the expression of pro-inflammatory cytokines and type 1 interferons (IFN), which ultimately establish an antiviral state strongly hampering virus replication. A full-blown innate immune response also stimulates effective adaptive immunity. Ubiquitination, the post-translational conjugation of monomeric ubiquitin (Ub) or poly-Ub chains to target proteins, is an essential factor in the regulation of the innate immune response. The positive-sense single-stranded RNA genome of nidoviruses encodes two replicase polyproteins that are processed into mature non-structural proteins, in part by a papain-like protease (termed PLP or PLpro depending on the virus family). In addition to this essential function in virus replication, several nidovirus PLPs, including PLP2 of porcine reproductive and respiratory syndrome virus (PRRSV), were shown to be deubiquitinating enzymes (DUBs) that likely suppress host Ub-regulated innate immune signalling.

To understand the molecular basis for Ub recognition and deconjugation, we determined the crystal structures of equine arteritis virus (EAV) PLP2 and Middle East respiratory syndrome coronavirus (MERS-CoV) PLpro in complex with Ub. The structural information revealed that EAV PLP2 adopts a fold that is consistent with DUBs of the ovarian tumor domain family, while MERS-CoV PLpro belongs to the ubiquitin-specific protease family of DUBs. To study the importance of the DUB activities during infection, we used the crystal structures of both proteases to design mutations targeting a Ub-binding region distant from the active site, in order to specifically disrupt Ub-binding without affecting the polyprotein cleavage. In cell culture, EAV and MERS-CoV DUB knockout viruses showed similar replication kinetics compared to the respective wild-type viruses, confirming unaffected polyprotein processing. Strikingly, compared to cells infected with wild-type virus, markedly increased levels of mRNA encoding IFN-beta and IFN stimulated genes were measured upon infection with these DUB knockout mutants.

This showed for the first time that the DUB activities of EAV PLP2 and MERS-CoV PLpro, despite the structural differences between these enzymes, both play a role in suppressing the host innate immune response during infection. Sequence analysis and structural modelling indicated that, like its EAV homolog, PRRSV PLP2 belongs to the ovarian tumor domain family of DUBs. Therefore the strategy to selectively remove the innate immune suppressing DUB activity is currently being applied in the design of novel modified live virus vaccines targeting PRRSV. As a result, these DUB knockout viruses are expected to elicit improved innate immune responses that may also allow better-protecting adaptive immunity compared to current vaccines.



O.18

Subversion of host protein synthesis by PRRS virus nonstructural protein (nsp) 1 β

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Plus-sense RNA virus genome functions as an mRNA and their translation is entirely dependent on host cell translation machinery. Some of these viruses compete with host protein translation to ensure efficient production of viral proteins and in some cases may stifle innate host defense. Picornaviruses impair the cap-dependent translation of mRNA and utilize the internal ribosome entry sequence for competitive viral protein synthesis. PRRSV genome is a positive-strand RNA with the 5' cap and 3'-polyadenylated tail and its translation is cap-dependent. In the present study, we show that PRRSV is able to subvert the translation of host cellular mRNAs by blocking their export from the nucleus to the cytoplasm to cause a biased translation of PRRSV proteins. The inhibition of mRNA export was specific for PRRSV, and the PRRSV-nsp1 β protein was pivotal to play for mRNA nuclear retention and subversion of host protein synthesis. By sequence analysis, a motif for SAP (SAF-A/B, Acinus, and PIAS) was identified with the consensus sequence of 126-LQxxLxxxGL-135. Site-specific mutagenesis was conducted to substitute individual residues in the SAP motif to alanine, and a total of seven SAP mutants were constructed to examine their subcellular localization and suppressive activities on the host protein synthesis. Exclusively cytoplasmic staining was observed for L126A, R129A, L130A, and L135A, and these mutants did not to suppress the host protein synthesis. In situ hybridization studies further unveiled that the mutants L126A, R128A, R129A, L130A, and L135A were unable to block the nuclear export of host cellular mRNAs. PRRSV-nsp1 β has previously been shown to inhibit the type I interferon response, and when the SAP mutants were examined for IFN suppressive activities, the mutants L126A, R128A, R129A, L130A, and L135A were unable to suppress the IFN production, IFN signaling, and TNF- α production pathways. Using the PRRSV reverse genetics, SAP mutant PRRS viruses were generated. Infectious mutant viruses were recovered for PRRSV-K124A, PRRSV-L126A, PRRSV-G134A, and PRRSV-L135A, whereas PRRSV-R128A, PRRSV-R129A, and PRRSV-L130A were non-viable. Wild-type nsp1 β was retained in the cytoplasm in PRRSV-infected cells, and so nsp1 β -L126A and nsp1 β -L135A did. Accordingly, no mRNA nuclear retention occurred in these cells. Importantly, PRRSV-L126A and PRRSV-L135A did not suppress the IFN production. Taken together, our study demonstrates that nsp1 β blocks cellular mRNA nuclear export and as a consequence, suppresses host-protein synthesis. The SAP motif in nsp1 β is essential for its nuclear localization and mediates the host cell mRNA nuclear retention, resulting in the subversion of host protein synthesis and innate immune response.

O.19

Porcine reproductive and respiratory syndrome virus (PRRSV) shedding in oral fluids of naïve and vaccinated pigs exposed to the wild-type virus

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Detection of Porcine reproductive and respiratory syndrome virus (PRRSV) using oral fluids (OF) is becoming an important tool for control of the infection in the herd. However, dynamics of oral shedding is not completely known. The aim of the present study was to examine shedding of PRRSV in naïve and vaccinated pigs exposed to infected animals. Seventy four-week old piglets were randomly divided in two groups (V, n=36 and NV, n=34) housed separately. On D0, V pigs were vaccinated with PORCILIS PRRS[®] according to the directions of the manufacturer. On day 30, 20 NV animals were inoculated intranasally with a genotype 1 wild-type strain 3267 of PRRSV (then onwards designated “seeder pigs” (S)). Two days later, each S pig was allocated with one NV (n=6) or one V (n=14) in 1:1 pens. Blood and oral fluids of each animal were collected periodically for the next 4 weeks using an oral fluid collection device (Salivette[®]). Previously, in order to minimise handling of pigs and increasing the amount of OF collected, pigs had been trained twice a week for chewing Salivette[®] devices flavoured with apple juice. Collected blood and OF were analysed by a qRT-PCR aimed to the amplification of ORF7. For comparison, all data for NV and V are referred as “post-contact” days. S pigs rapidly developed viremia (<3 days) after inoculation and viral shedding in OF started shortly thereafter. As a matter of fact all pigs were positive in OF testing for the first 19 days after inoculation and by day 23 shedding still occurred in 12/20 pigs (60%; CI_{95%}:36.4%-80.0%). For contact NV pigs, the shedding pattern was quite similar and thus, 21 days after contacting with S pigs, 50% of them were still shedding virus in OF. During the observed period viral load in OF of S or NV was in the range of 4.2 log₁₀-2.6 log₁₀ genomic copies/ml, with a significant decrease over time (r²=0.59; p=0.006). In vaccinated animals the shedding behaviour was different. Although pigs may develop viremia caused by the wild-type virus at least for one sampling (2-3 days), shedding in OF was significantly reduced (average shedding period 8 days). Thus, the maximum proportion of OF shedders in vaccinated pigs in a given time was 11/14 (78.5%; CI_{95%}:48.8%-94.3%) with a low of 21% of shedders at 14 days post-contact with infected pigs. Regarding the viral load, the number of viral genomic copies/ml ranged on average from 3.5 log₁₀ and 2.3 log₁₀. This load was significantly lower (p<0.05) compared to that of S and NV pigs. The Kappa value for the comparison of blood sampling and individual OF was 0.86 in S, 0.88 in NV and 0.68 in V pigs. In conclusion, OF fluids was a reliable method for detecting PRRSV shedding. In vaccinated pigs shedding was significantly decreased both in terms of duration and load. This work was funded by Spanish INIA (project RTA2011-00119-00) and by MSD Animal Health.

O.20**Comparison of processing, RNA extraction methods and qRT-PCR mixes for the diagnosis of Porcine reproductive and respiratory syndrome virus (PRRSV) infection using oral fluids.**E. Gibert¹, G.E. Martín-Valls¹, E. Cano¹ and E. Mateu^{1,2}¹ Centre de Recerca en Sanitat Animal (CRESA), IRTA, Edifici CReSA, campus UAB, 08193 Cerdanyola del Vallès, Spain.²Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Bellaterra, Spain

Detection of Porcine reproductive and respiratory syndrome virus (PRRSV) in oral fluids (OF) by qRT-PCR is gaining increasing popularity but is subjected to several circumstances that may affect the performance of the assay. Environmental contamination of samples is almost impossible to prevent and this includes bacterial contamination as well as remnants of feedstuff and other materials. Moreover, RNA extraction methods are diverse and not many have been proven with OF. The aim of the present study was to examine the effect of storage time and centrifugation of samples and the RNA extraction method on the performance of the qRT-PCR. OF were collected in several pens of a weaning unit in a commercial PRRSV-negative farm. In a first assay, storage time and centrifugation conditions were examined. Collected OF were pooled and a sample of the OF pool was spiked with 10^4 TCID₅₀ of the genotype 1 isolate 3267. Decimal dilutions up to 10^{-4} were made. Samples were kept at 4°C either for 24h or 72h and freshly made spiked samples were used as controls. For each time, Centrifugation forces tested were 15000 x g and 1,000 x g (15 min) and non-centrifuged samples were included for comparison. RNA extracted by MagMAX™ Pathogen RNA/DNA Kit and the real-time qRT-PCR reaction was performed with a commercial mix AgPath-ID™ One-Step RT-PCR Kit (Applied Biosystems). In a second experiment, freshly spiked samples made as above were centrifuged at 15000 x g 15 min and RNA was extracted using 6 different extraction systems: TRIzol LS Reagent (Ambion-Life technologies) used as a standard, BioSprint96 One-For-All Vet Kit (Qiagen), Nucleospin RNA Virus (Macherey-Nagel), MagMAX Pathogen RNA/DNA Kit (Applied Biosystems), High Pure Viral RNA Kit (Roche) and PureLink Viral RNA/DNA MiniKit (Invitrogen-Life technologies). The samples were subsequently analyzed by real-time qRT-PCR using un paparellel two commercial mixes, AgPath-ID™ One-Step RT-PCR Kit (Applied Biosystems) and LSI VetMAX® PRRSV EU/NA (Life technologies). Centrifugation at 15,000 x g resulted in a slight increase of sensitivity (1-2 cycles) at 0 and 24h. Regarding the extraction methods, Trizol and MagMax resulted in the lowest Cq at equivalent virus concentrations using a given mix. LSI VetMAX® was in general more sensitive than AgPath-ID™ (up to 5 cycles less for equivalent samples) although significant differences were noticed depending on the extraction system used. The present results may be useful to standardize procedures for PRRSV detection in OF by real time RT-PCR.



O.21

Application of the Ion Torrent™ PGM™ Instrument for Whole Genome Sequencing of European PRRSV Strains

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Thermo Fisher Scientific

Porcine Reproductive and Respiratory Syndrome (PRRS) is a highly infectious disease, endemic in pigs throughout the world. PRRSV strains are divided into two genotypes designated as 1 (formerly European) and 2 (formerly North American). Highly pathogenic strains that first emerged in Asia-belong to genotype 2 as well. PRRS is caused by a single stranded positive-sense RNA enveloped virus with a high mutation rate leading to greater heterogeneity of the nucleotide sequence between the individual strains. The increasing genetic diversity of the virus increases the risk of reduced sensitivity for existing diagnostic nucleotide detection methods. In order to reduce this risk, it is important to frequently assess the sensitivity of the diagnostic test methods, updating them when necessary.

The aim of this study was to optimize the next generation sequencing protocol using the Ion Torrent™ PGM™ instrument to sequence whole PRRSV genomes from European isolates representing different geographical regions. For accessing field samples, Thermo Fisher Scientific established several collaborations with laboratories and research institutes. Low titer PRRSV field samples were cultivated on macrophages in order to obtain higher viral load. After virus precipitation and RNA extraction using commercially available kits, we used different techniques to enhance PRRSV RNA recovery and to remove endogenous porcine rRNA. The PGM™ workflow was then followed to sequence the whole genome. Bioinformatics analysis is performed using an in-house developed tool, Viral Genome Assembly Pipeline (VGAP) for genome assembly and identification of a viral consensus sequence.

Sequencing data is of high quality with a mean coverage of ~4000x, a mean nucleotide read length of 114, and limited host genome interference (~12%). One sample was excluded from the assembly process due to high host genome interference (>75%). For nine samples, mapping reads against 405 PRRSV genomes we found three European reference strains (KF203132, GQ461593, and GU067771). Contigs generated from VGAP cover greater than 99% of the reference genomes; and sequence length, contig number, N50 and N95 statistics indicate that VGAP produced high quality consensus genomes. BLASTing consensus genomes against the NCBI database showed approximately 90% sequence identity with the reference genomes of European type 1 strains. In depth sequence and phylogenetic analyses are in progress to study the genetic diversity between the samples and against the reference strains.

We thank the group of Enric Mateu at Centre de Recerca en Sanitat Animal (CRESA-IRTA) Barcelona/Spain for providing PRRSV strains from Spain cultivated on macrophages.

O.22

Herd management strategies to control PRRSV infections using the PrioCHECK® PRRSV VIA ELISA

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PRRSV outbreaks are feared in the swine industries because of the devastating economic impact. The complex nature of the PRRSV disease indicates that single diagnostic tests may not be enough to successfully manage PRRS virus but rather comprehensive solution strategies are needed for the effective control of the disease. Here we present data for PRRSV disease management using the ELISA PrioCHECK® PRRSV VIA. The PrioCHECK® PRRSV VIA is an indirect ELISA being able to discriminate Type I (EU) from Type II (US) PRRSV infection by measuring the presence of neutralizing antibodies in serum of pigs.

In total 182 serum samples relating to 19 different pig herds were investigated with the PrioCHECK® PRRSV VIA. On herd level a sensitivity of 89.4 % was achieved, corresponding to 17 correctly identified herds. The specificity was calculated on individual samples derived from pigs with confirmed negative PRRSV status (240 samples) and resulted in 99.4% for Type I coated plates and 98% for Type II (US) coated plates.

In one particular case study pigs showed clinical signs for PRRSV despite regular vaccination with a Type I (EU) vaccines. PRRSV Screening ELISA data revealed that all samples were positive. PrioCHECK® PRRSV VIA results detected very high antibody titers to Type II (US) in more than 90% of the animals. In contrast low antibody titers to Type I (EU) were seen in 2 of the 17 animals. These results indicated that protection to heterologous PRRSV strain failed. This finding could be confirmed by positive Type I and Type II PRRSV PCR. As a consequence the veterinarian decided to change the vaccine in this farm.

In a second investigation serum samples of 10 boars known to be continuously infected with Type I (EU) PRRSV were tested with the PrioCHECK® PRRSV VIA. All samples were negative to Type I (EU) PRRSV indicating that no neutralizing antibodies against Type I were present. In contrast 50% of the samples revealed high antibody titers to Type II (US) PRRSV. The same samples were also positive in the screening ELISA, PrioCHECK® PRRSV Ab porcine. Type II (US) positive results could be confirmed by PCR indicating that the herd was affected by a fresh infection of Type II (US).

In summary we show that the PrioCHECK® PRRSV VIA is a reliable tool to discriminate Type I (EU) from Type II (US) PRRSV infections on herd level, detect single or mixed infections of different PRRSV types on herd level and help to decide over the optimal type and schedule for PRRSV vaccination.



O.23

Bioinformatics prediction of swine MHC class I epitopes from PRRSV

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PRRSV possesses multiple immunoevasive strategies, from suppression of the host cell antiviral machinery, to the deceptive induction of a non-neutralizing antibody response through decoy antigen presentation.

This, combined with a very high mutation rate, is continuously hampering the development of safe and effective vaccines.

With the overall aim to design a vaccine that induces an effective CTL response against PRRSV, we have taken a bioinformatics approach to identify concerned PRRSV epitopes predicted to react broadly with predominant swine MHC (SLA) class I alleles. First, the genomic integrity and sequencing method was examined for 334 available complete PRRSV type 2 genomes leaving 104 strains of high quality. For each strain, a library of all possible 9- and 10-mer peptides was generated considering the known ribosomal frame shift sites and sites for post translational cleavage.

All peptides were in silico analyzed for binding affinity to either of five common SLA class I alleles. A quantitative rank score was generated for each peptide by combining two algorithms based on the NetMHCpan neural network and lab determined SLA binding affinity of each amino acid at any position in the peptide, respectively.

Peptides with a rank score above a predefined threshold were further analyzed by the PopCover algorithm, providing a final list of 53 epitopes prioritized according to maximum coverage of PRRSV strains and SLA alleles.

The peptide-SLA binding affinities and stabilities were subsequently measured in the lab with the convincing outcome that about 40 % of the peptides bound stably with SLA molecules with a half-life longer than 30 minutes.

This bioinformatics approach provides a rational strategy for selecting peptides for a CTL-activating vaccine with broad coverage of both virus and swine diversity. The immunogenicity of the selected peptides is in the process of being verified further in vivo and ex vivo.

O.24

The design and evaluation of a particulate formulation of vaccine candidate T cell antigens from the porcine reproductive and respiratory syndrome virus

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CD8 T cells are crucial to the control of many viruses through cytolysis of infected cells and secretion of IFN-gamma and TNF-alpha. Since clearance of porcine reproductive and respiratory syndrome virus (PRRSV) infection is not dependent on the development of neutralising antibodies, it has been proposed that T cell mediated immunity plays a key role. We therefore hypothesised that conserved CD8 T cell antigens represent potential candidates for development of a novel PRRS vaccine.

To identify suitable antigens, a PRRSV-1 proteome-wide synthetic peptide library was screened *in vitro* to determine the specificity of T cell IFN-gamma responses from cohorts of immune pigs. Both the matrix (M) protein and non-structural protein 5 (NSP5) were identified as well conserved targets of, in many instances, dominant T cell responses. IFN-gamma responses to NSP5 peptides were exclusively from CD8 T cells, whereas both CD8 and CD4 T cells responded to the M protein peptides. The majority of responding CD8 T cells expressed an effector memory-like phenotype with co-expression of TNF-alpha and mobilisation of the cytotoxic degranulation marker CD107a.

To assess their vaccine potential, peptides from M and NSP5 were encapsulated in hydrophobically modified chitosan particles adjuvanted by incorporation of the synthetic multi-TLR2/TLR7 agonist Adilipoline™. For comparison, empty particles and adjuvanted particles encapsulating beta-propiolactone inactivated PRRSV-1 were prepared. *In vitro* characterisation of the formulations showed highly efficient antigen encapsulation. Particles were found to be in the low micron rather than nanometre size range and differed significantly in their surface charges dependant on composition. Particles bearing Adilipoline™ induced IL-12 and type I IFN responses from dendritic cells and IL-8 responses from both dendritic cells and monocytes. Immunisation of pigs with the M/NSP5 peptide loaded particles primed a measurable antigen-specific CD4 but not CD8 T cell response. Upon PRRSV-1 challenge, CD4 T cell responses were boosted and CD8 T cell responses detected but with limited evidence of enhanced control of viraemia. Analysis of the lungs during the resolution of infection showed high levels of M/NSP5 specific IFN-gamma responses from CD8 rather than CD4 T cells. These data suggest that vaccine priming of a CD8 T cell response is required for protection from PRRSV infection, and alternative delivery systems should be evaluated in the context of M and NSP5 antigens.



O.25

Vaccination with a PRRS modified live virus vaccine followed by challenge with PRRSV and PCV2 protects against PRRS but enhances PCV2 replication and pathogenesis

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Co-infections involving PRRSV and PCV2 contribute to a group of disease syndromes collectively termed porcine circovirus-associated disease (PCVAD). The complex etiology of PCVAD, including the role of PRRSV infection, has yet to be fully understood. Presumably, PRRSV infection enhances PCV2 replication as a result of modulating host immunity and increasing PCV2-permissive cells. The purpose of this study was to evaluate PCV2 replication and pathogenesis in pigs vaccinated with a PRRS modified live virus (MLV) vaccine and subsequently challenged with a combination of PRRSV and PCV2. Three week-old barrows ($n = 226$) were randomly allocated into one of two groups. One group was vaccinated with a heterologous PRRS MLV vaccine and both groups were co-challenged 28 days after vaccination. During the early post-challenge period, PRRS-associated clinical signs were decreased and average daily gain (ADG) was increased in the vaccinated group. Overall, 38% of non-vaccinated pigs and 19% of vaccinated pigs were documented with aural cyanosis, a clinical sign associated with acute PRRSV infection. The vaccinated group also had significantly less PRRSV viremia on days 4, 7, 11, 14, 21, 28, and 35 post-challenge ($p < 0.015$). These results demonstrate the protective effects of PRRS vaccination. However, during the later post-challenge period, the vaccinated group showed increased PCVAD, increased mortality, and decreased ADG. Overall, 12% of non-vaccinated pigs and 26% of vaccinated pigs were documented with clinical signs associated with PCVAD, such as dyspnea, muscle wasting, weight loss, rhinorrhea, lethargy, and pallor or jaundice. In addition, the vaccinated group had significantly greater PCV2 viremia on days 11 and 14 post-challenge ($p < 0.0001$). These results demonstrate the negative effects of PRRS vaccination. In this disease model, the early benefits of PRRSV vaccination were outweighed by the later amplification of PCVAD.



O.26

Vaccination with UNISTRRAIN[®] PRRS in piglets gives a partial clinical and virological protection after challenge with an East European subtype 3 isolate (Lena strain).

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Introduction: Lena strain is a highly pathogenic PRRSV subtype 3 strain isolated from a Belarusian farm with severe reproductive failure in sows and respiratory disorders in young piglets. The aim of the present study was to evaluate the cross-protection of a commercially available live attenuated PRRSV vaccine (UNISTRRAIN[®] PRRS) assessing the evolution of viremia and secretion upon challenge with Lena strain.

Materials and Methods: Twelve 4-weeks-old piglets, clinically healthy and free from virus and antibodies against PRRS were randomly assigned in two groups: in vaccinated group animals were intramuscularly vaccinated with UNISTRRAIN[®] PRRS (VP-046 BIS) and control group was left unvaccinated. Four weeks after vaccination, all pigs were intranasally inoculated with the highly pathogenic Lena strain (subtype 1.3; 82.3 % ORF5 homology to the vaccine strain; 10⁵ TCID₅₀/ml). Blood samples and nasal swabs were collected from all the animals at 0, 3, 5, 7, 10, 14, 21 and 28 days post challenge (dpc) to assess viremia (measuring virus titration of sera) and viral shedding (measuring virus titration of nasal swabs), respectively. Viremia and secretion were analysed using non-parametric Mann-Whitney (p<0.05).

Results: Virus was present in serum of all animals as of 3 dpc. A peak was observed at 10 dpc in the control group (4.2 ± 0.2 log 10 TCID₅₀ /ml) and at 5 dpc in vaccinated group (4.7 ± 0.6 log 10 TCID₅₀ /ml). In the control group, viremia lasted at least until 28 dpc with 2 out of 6 animals (2.1 and 1.6 log 10 TCID₅₀ /ml). In the vaccinated group, viremia lasted at least until 21 dpc. Viremia was reduced 7 days in the vaccinated group although no significant differences were observed between groups. Virus secretion was observed as of 3 dpc in both groups. Titres peaked at 3 and 7 dpc in the control group (5.6 ± 0.8 and 5.4 ± 0.6 log 10 TCID₅₀/100mg) group and at 5 dpc in vaccinated group (5.0 ± 0.3 log 10 TCID₅₀/100mg). In the control group, viral shedding was observed until at least 28 dpc and in the vaccinated group up till 21 dpc. Significant differences in secretion titers were found at 3, 7 and 10 dpc between groups. Moreover, the AUC value of virus secretion was significantly lower in the vaccinated pigs (11.6 ± 3.5) than in the non-vaccinated pigs (18.4 ± 1.9).

Conclusion: The results of the study demonstrate that vaccination with UNISTRRAIN[®] PRRS provides a partial virological protection against challenge with the East European subtype 3 PRRSV strain Lena.

O.27**Environmental enrichment in early life influences susceptibility to PRRSV and co-infections**I. Dixhoorn², L. Bolhuis³, I. Reimert³, J. Middelkoop³, A. Rebel¹, N. Stockhofe-Zurwieden^{1*}¹Central Veterinary Institute, Wageningen University&Research²Wageningen Livestock Research, Wageningen University&Research³Department Animal Adaptation Physiology, Wageningen University

Modern pig production is characterized by highly efficient use of animal rooms with high stocking density and often barren and stimulus poor housing conditions. The effect of rearing under barren conditions on behaviour, fear and stress are well known. However, few information is available on the influence of these conditions on infectious disease susceptibility. In this study the effects of environmental enrichment already in early life of piglets on the susceptibility for a PRRSV infection and disease development after co-infection with *Actinobacillus pleuropneumiae* (*A. pp*) were examined. Two groups of 4 sows with their litters were either allocated to Treatment A in common housing conditions (barren conditions) or to Treatment B in enriched conditions, i.e. twice as much space for the piglets with solid floor with regularly replenished bedding material and playing material (branches, jute bags, peat) to stimulate and accommodate exploratory behaviour. All piglets were weaned at 31 days of age and randomly regrouped in new groups, but each piglet was kept in the same environmental conditions as before. From each treatment one half was allocated to a non-infected control group, the other to the PRRSV/*A.pp* infected group ((1)barren-control, (2) enriched-control, (3) barren PRRSV/*A.pp*, (4) enriched PRRSV/*A.pp*, n = 14 per group). At about 6 weeks of age piglets from groups 3 and 4 were inoculated intranasally with PRRSV serotype 1 strain LV and eight days later exposed to an aerosol infection with *A.pp* serotype 2. This model was used, because both pathogens individually applied induce no overt clinical signs and in combination the co-infection can lead to mild clinical signs and pathological findings in a limited number of animals and can therefore be used to study conditions which decrease or enhance disease susceptibility. Three days after inoculation with *A.pp* all piglets were euthanized and pathological and bacteriological examinations performed. After inoculation with PRRSV no difference in virus titer was found at 4 days post inoculation (dpi), however at 8 dpi a significant reduction in virus titer in serum was observed in group 4 (enriched housing). Pathological examinations revealed gross pathological changes in the lungs, characteristic for an *A.pp* infection in 43% of pigs in group 3 and 7% in group 4 ($p < 0.05$) and *A.pp* bacteria were re-isolated from the respiratory tract or the adjacent lymph nodes in 36% (group3) and 7% (group 4) of piglets, respectively. Also, histological findings in the lungs in regard to PRRSV specific interstitial pneumonia were significantly less expressed in group 4 than in group 3. During the infection period fever after *A.pp* infection was more pronounced in group 3. No significant differences in systemic T cell phenotypes were found between the groups at certain time points after infection, nor in the bronchoalveolar lavage cell fraction at the end of the study.



O.28

Evaluation of porcine reproductive and respiratory syndrome control methods using agent-based modelling.

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Porcine reproductive and respiratory syndrome (PRRS) is an endemic swine disease in North America, and even though elimination of the virus is possible, virus re-introduction and recurrent outbreaks in previously positive sites are common. Gilt acclimation is a procedure commonly used to minimize the likelihood of new virus introductions and re-emergence of previously existing viruses occurring in the breeding herd. Acclimation can be achieved using vaccination with a commercial modified live vaccine (heterologous virus), or immunization based on exposure to the PRRS virus resident to the herd (homologous virus).

The objective of this project was to investigate which of the control measures mentioned above would best minimize the likelihood of an outbreak in a typical Ontario farrow-to-wean swine site compared to a baseline of no control measures implemented. Secondary objectives were to investigate how the duration of infection and number of infectious animals introduced would affect the overall size of such an outbreak.

A stochastic, agent-based model was developed in order to capture different animal characteristics such as disease state and location in the farm for female pigs and produced offspring, as well as heterogeneity of contacts within a herd. The model was created using Anylogic[®]7.1.2, and the outcome of interest was the maximum number of infected animals resulting from re-introduction of the virus into herds of different compositions. One hundred model iterations were generated for each scenario and sensitivity analysis was conducted to examine the impact of variation in the expected outcome when duration of infection and initial conditions related to virus introductions were modified.

Model results demonstrated that both PRRS control strategies produced a higher frequency of simulations resulting in no outbreak after the introduction of the virus, compared to the baseline scenario. A decrease in the duration of infectiousness resulted in an overall reduction in the maximum number of pigs infected. Finally, the frequency of no outbreaks occurring decreased as the number of infected animals introduced in the herd increased.

In conclusion, our findings suggest that homologous virus exposure would decrease the likelihood of occurrence of large PRRS outbreaks the most; and attempts to reduce the introduction of infected animals/ viral loads are valuable in decreasing the likelihood of major outbreaks.

O.29

Inactivation of PRRSv by a novel method of on board heating of trucks is within range for the daily routine in pig transport

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Introduction

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

Materials and Methods

The used truck was equipped with an on board heating device, which can be used for heating the truck while traveling between farms. Experiments included application of type 1 vaccine virus in a dose of 5×10^5 TCID₅₀ in a full size truck (4 sites, duplicate testing). The experiments included a time period of 30 and 60 minutes and an air temperature of 70° C. The inactivation of the virus was tested by using virus infectivity assay on MA 104 cell cultures. The temperature and relative humidity in the truck were recorded, the drying of wet floors was checked visually. Samples were taken by using swabs on the sites where the virus was applied.

Results and Conclusions

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

Keywords: PRRSv, biosecurity, transport trucks

O.30

Preliminary assessment of risk factors related to PRRSV stability in farms within a control program in Spain

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In 2014, the Catalan Federation of Agrarian Cooperatives (FCAC) with the support of the Spanish Ministry of Agriculture (MAGRAMA) and together with the Centre de Recerca en Sanitat Animal (CRESA-IRTA) started a 3-year project directed to the voluntary control of PRRSV in associated cooperatives in Catalonia and Aragon regions. Six cooperatives (approximately 90,000 sows) accepted to participate and 78 farms initiated the project. In a first step a 139-question survey, including questions related to the apparent health status and internal and external biosecurity measures was filled in an on-line application for each farm. A biosecurity improvement plan was done for each of the participating farms. Also, 61 out of these 78 farms were selected for a categorization scheme based on the determination of the PRRS-status of sows and piglets. Briefly, serum samples from sows (n=20) and 3, 6 and 9-week-old piglets (n=10 each) were analyzed by ELISA and up to 20 weak-born piglets in farrowing units were analyzed by RT-PCR. According to the results farms were categorized as unstable or stable with or without vaccination. Sequencing (ORF5) was performed with positive RT-PCR samples were sequenced when possible. Of the known biosecurity risk factors associated with the entrance of PRRSV into a farm, 65% of the farms did not analyze replacement gilts before entering them to the stock, 21% use semen from positive or unknown sources and the 20% of the farms included in the survey receive pigs in trucks that have visited other farms in the same day and have not been properly cleaned. Regarding the internal biosecurity risk factors associated with PRRSV dissemination, 65% of the farms did not have a policy for eliminating little viable suckling piglets, 96% of the farms are not using complete clean protocols in the pig rooms, and in the 72% of the farms there is not an appropriate workflow. According to the perception of the veterinarians, 12/61 (19.7%) herds were unstable to PRRS because of noticeable clinical problems although based on the analytical results 44/61 (72.1%) of the herds had active PRRSV as indicated by positive RT-PCR in suckling piglets and/or seroconversion of weaners, indicating an important discrepancy between perception and reality, probably because in many cases circulation of PRRSV did not cause overt clinical problems. Moreover, sequencing showed a high genetic diversity of PRRSV and probably multiple introduction sources. preliminary results suggest that there is a considerable way for improvement in biosecurity and monitoring and, also, that there is a high proportion of apparently subclinical infections with unknown impact in health and welfare of the piglets. Also, the methodology used may serve to the immediate development of regional control plans. In a next step, data obtained in the questionnaire will be analyzed considering the PRRSV status of the farm in order to screen risk factors related to the instability of the farms.



O.31

Title: National control and eradication PRRS virus Chile, South America

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Chile is one of the six leaders of pork meat production, producing more than 583,673 carcass tons in 2012 and 49% of that production is exported, mainly to Asian countries. This accomplishment is related to the excellent sanitary status; being free of Foot and Mouth Diseases, Classical Swine Fever among others diseases.

Chile is one of the few countries that were able to eradicate PRRS virus after a national control and eradication program. That achievement was done with a solid work between the public and the private sectors involved in the pig industry and from 2003 and 2007. In 2012 SAG auto declared as a free PRRS virus country and that was recognized by OIE in 2013, unfortunately in October of 2013 a new outbreak was detected with another PRRS virus being genetically similar to one isolated in Sonora Mexico.

An eradication program was implemented for a second time; a National Committee was created between SAG and stakeholders. A case definition was done as well as a diagnosis protocol and a procedure for affected farms and closure in the affected s1. A national surveillance system was put in practice in order to verify that the virus was contained in only two regions and just in few sites. A communicational campaign was done to compromise workers in their jobs with the pig industry and with backyards owners so they notify suspicions of the disease to SAG. Surveillance was done using a risk based method; in the industry doing a quantification of their previous status, closeness to an outbreak, biosecurity standard among others and the same was done with backyards owners, if they were sellers, had more than 50 mothers or had had positive serological results.

Since the index case to March 8th of 2015 more than 2,830 sites were visited and investigated and more than 7,300 checkups were performed, getting more than 64,395 samples analyzed in private as well as SAG laboratories. A total of 101 sites have been PCR reactors; 45 in the industry and 6 in the backyard farms, until today only 17 sites have virus circulation and they are under quarantine or sanitary official plan to eradicate the disease. To March 8th of 2015 we have 151,710 animals affected in the premises positive to PRRS being all of them in the industry farms.



Poster

1. PRRSV EVOLUTION

P.01

Characterisations of three novel porcine reproductive and respiratory syndrome virus isolates from one swine farm with highly homologous to a vaccine strain

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Introduction

In 2006, a highly pathogenic PRRSV (HP-PRRSV) with a 30-amino acid deletion in the Nsp2 gene, and characterized by high fever, high morbidity and high mortality in pigs emerged in China, and soon after the HP-PRRSV became the dominant strain circulating in swine herds. Since 2010, three attenuated vaccines developed by in vitro passing HP-PRRSV strains (JXA1, HuN4, TJ) for 80, 120, or 90 passages, respectively, were widely used. In 2012, we isolated three PRRSVs from three dying piglets in a farm suspect of PRRS outbreak. Genomic sequence analysis and animal infection indicated that the three viruses were highly pathogenic to piglets and they share high homology with a vaccine strain derived from HP-PRRSV JXA1.

Materials and Methods

PRRSV NT1, NT2 and NT3 were isolated from sera of three dying piglets in a pig farm in Jiangsu province, China, in 2012. The complete genome sequence of the three isolates were determined and viral growth kinetics were tested on PAMs and MARC-145 cells. Homology and phylogenetic were analyzed with other PRRSV strains including LV, VR2332, Ch-1a, HP-PRRSV isolates, and some vaccine strains. Finally, 26 healthy piglets were used to test pathogenicity of the three isolates.

Results

1. All the three isolates NT1, NT2 and NT3 had a unique discontinuous deletion of 30 amino acids in Nsp2 coding region, which is the same as HP-PRRSVs isolated in 2006.
2. Viral growth properties were tested on PAM cells and MARC-145 cells, NT2 showed faster and higher replication rate than that of NT1 and NT3 on PAM cells, and had lower replication rate than that of NT1 and NT3 on MARC-145 cells.
3. Analysis of full-length genomic sequence showed that the three isolates shared the highest nucleotide identity with JXA1-derived vaccine strains.
4. Phylogenetic analysis based on the complete genomic sequences showed that the NT isolates were clustered in a minor branch with the JXA1-derived vaccine strains belonging to HP-PRRSV subgroup3. NT1, NT2 and NT3 share 13, 12 and 12 amino acids unique/identical with JXA1-derived vaccine strains, respectively.
5. The results of pathogenicity test showed that piglets infected with NT1, NT2 or NT3 appeared clinical manifestations including high fever, anorexia, respiratory signs and 4/7 (NT1), 5/7 (NT2) and 4/7 (NT3) piglets died in two weeks, indicating that NT1, NT2 and NT3 are highly pathogenic PRRSV.

Conclusions

Our study found that the PRRSV isolates NT1, NT2, and NT3 were highly pathogenic to piglets, and shared the highest homology with JXA1-derived vaccine strains. The three novel isolates are very possibly the back reverse mutants of JXA1-derived vaccine strain.

P.02

H-Index as a measure of the impact of PRRSV in porcine viruses' research outcome

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The bibliometric indicator H-index (or Hirsch index) has been proposed as a factual and accurate parameter to evaluate research impact for a given subject. H-index measures the number of published papers (N) that have been cited N or more times. It combines the quantity and the quality –number of citations– to evaluate a large number of search terms. In order to measure the importance of Porcine reproductive and respiratory syndrome virus (PRRSV) research in the scientific publications, the present study aimed to calculate the H-Index of PRRSV publications and to compare it with other swine viruses.

The H-index for 54 swine virus was calculated using the bibliographic software package Web of Science (WOS) v.5.16.1. Searches (March 2015) were done using: “porcine”, “pig” or “swine”, each virus complete name, acronym, common names, their synonymous and the associated disease/s. Selected papers were reviewed one by one to ensure database accuracy. Viruses showing the ten highest H-indices were selected, classified by their zoonotic potential and analysed for journal quartile and the country of origin of each paper.

H-index for non-zoonotic viruses was higher than that of zoonotic ones (70.33 and 58.00, respectively). In decreasing order, the ten highest H-indices were: 1-PRRSV (95), 2-Porcine circovirus type 2 (PCV2) (85), 3-Swine influenza virus (SIV) (79), 4-Classical swine fever virus (72), 5-Aujeszky's disease virus (58), 6-Foot-and-mouth disease virus (56), 7-African swine fever virus (56), 8-Pig endogenous retrovirus (55), 9-Porcine rotavirus (49) and 10-Hepatitis E Virus (49). Focusing on the three highest H-Indices, the number of total citations in the dataset were: 16398 (PRRSV), 14068 (PCV2) and 16008 (SIV); the mean number of citations was 172 (CI_{95%}:148.95-195.05) for PRRSV, 163 (CI_{95%}:144.1-181.9) for PCV2 and 202 (CI_{95%}:136.81-267.19) for SIV and the mean quartile 1.65 (CI_{95%}:1.48-1.82) for PRRSV, 1.77 (CI_{95%}:1.59-1.95) for PCV2 and 1.28 (CI_{95%}:1.13-1.43) for SIV. Of 95 PRRS publications, 62.1% were from North America (52 from USA and 7 from Canada), 31.6% from Europe (8 The Netherlands, 6 Denmark, 5 Belgium, 4 Spain, 3 France, 2 UK, 1 Germany and 1 Poland), and 6.3% from Asia (5 China and 1 Singapore); none were from South-America, Oceania or Africa. When these highly cited papers were distributed into three periods (1991-1995; 1996-2000 and 2001-onwards), one third of them were presented in each period (33, 32 and 30, respectively). However, as expected, papers published in the first period describing the virus, its isolation and the general characteristics of the disease, had the highest impact in terms of number of references. Finally, in the top-ten there were 7 papers from 1991-1995, 1 from 1995-2000 (describing the divergent evolution of the virus on two continents) and 2 from 2001-onwards (evaluating the PRRSV economic impact in USA and the fatal cases of HP-PRRSV in China). Despite being a non-zoonotic and a relatively new virus, PRRSV has an extremely high impact and research interest among porcine viruses as H-index demonstrates.



Poster

1. PRRSV EVOLUTION

P.03

Hypervariable regions of the porcine reproductive and respiratory syndrome virus (PRRSV) genome evolve under diversifying selection from acute to persistent infection

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Genetic variation in both structural and nonstructural genes is a key factor in the capacity of porcine reproductive and respiratory syndrome virus (PRRSV) to maintain persistence within animals, farms and metapopulations. However, the exact mechanisms of how genetic variation contributes to persistence remain unclear. As a part of a study to understand the role of host genetics in disease resistance, a subpopulation of pigs showed virus rebound at 42 days post infection (dpi) regardless of the existence of homologous neutralizing antibodies. To assess ongoing changes in viral quasispecies from acute to persistent infection, mutations in hypervariable regions within nsp1, nsp2, ORF3 and ORF5 genes were analyzed by 454 sequencing in four selected pigs. Samples for sequencing included the parental virus NVSL 97-7895 and sera collected at 4, 28, 42dpi, as well as tonsils collected at 42dpi. When compared to the NVSL sequence, virus collected at 28dpi showed the highest genetic diversity. Assessment of mutations in sera and tonsils collected at 42dpi from the same pigs revealed the existence of distinct quasispecies. The dN/dS rates were < 1 for all sera except one collected at 42dpi indicating that hypervariable regions evolved under negative selection, whereas eight substitutions were under significant levels of positive selection. Positively selected A₂₇V and N₃₂S substitutions in GP5 resided at the decoy epitope cleavage sites and N₃₂S created a new N-glycosylation site, which might contribute to viral persistence. This study suggests that the introduction of new viruses under diversifying selection serves as a mechanism of PRRSV persistence.

P.04

Development of PRRSv prevalence and ORF-5 homology in The Netherlands and its possible influence on reproductive disorders in sows.

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Introduction: Porcilis PRRS vaccination effectively reduces PRRSv vertical transmission and the risk of piglets born weak¹. Van Groenland controlled PRRSv in infected SPF herds by optimizing biosecurity and vaccination of sows and piglets resulting in a negative outflow². These measures are implemented more frequently to control or even eradicate the virus from particular groups or farms. MSD-AH's ResPig offers the opportunity to investigate the status of PRRS via cross-sectional blood sampling of gilts, sows, weaners and nursery pigs, including saliva testing (PCR) of newly weaned and 10-week old piglets. The aim of this study was to investigate the development of PRRSv prevalence in Dutch farm-, farrowing unit and nursery level and determine its possible influence on reproductive disorders, and to compare the ORF-5 sequences of the 2014 ResPig results with previous years.

Material and methods:- PRRS vaccination rates are calculated from ResPig anamneses.

- 2014: 99 farms sampled weaners and 10week old piglets for serum investigations including saliva PRRS PCR of both groups.

- The PRRSv- and genotype prevalence are compared with previous data (Table 1).

- If PRRSv circulation in nurseries is a risk factor for reproductive disorders was estimated by comparing farms with or without reproductive disorders (weak born piglets and/or early farrowing and/or abortion) and the PRRSv positivity of the nurseries and calculation of odds ratio.(Table 2).

-Molecular epidemiology of PRRSv was evaluated by comparing the ORF-5 sequences of sequenced strains in 2014 with published data^{2,3,4,5} (Table 3).

Results

2014: 89% of ResPig sow herds were vaccinated (95% EU DV mlv), and 29% of piglets (100% EU DV mlv): both rates are increasing (sows 80% (2004-2007) and 87% in 2012 / piglets: 15% in 2012)⁵

Table 1. PRRSv prevalence

PRRSv prevalence	2014	EU / US (%)	2012(5) (pig dense region)	2010(3)	EU / US (%)
at weaning	14% (14/99)	86 / 14	29%(12/42)		
at 10 weeks	36%(36/99)	92 / 08	67%(28/42)	41%(14/34)	86 / 14
Farm (in piglet population)	43%(43/99)	90 / 10	74%(31/42)		

Table2:PRRSv prev. nursery and reproductive disorders

	farms with PRRSv+ nursery	farms with PRRSv- nursery
farms with repro probl.	12 (80%)	3 (20%)
farms with no repro. probl.	35 (36%)	64 (64%)

Odds ratio:7.3 (95%CI: 1.9-27.7) P=0.0034

Tabl. 3: % ORF-5 homology of PRRSv strains compared with LV

2005	18	28	41	12	1	
2006-2007	5	50	45			
2008-2009	21	65	14			
2012 peelh	11	41	26	11		11
2014	5	26	0	16	11	42
category % hom.:	80-82	83-85	86-88	89-91	92-94	95-97 98-100

2014: 21strains sequenced

Strains with 98-100% homology originated out of recent DV PRRS-vaccinated animals (except one in direct contact).

2 genotypes 2 strains in non-vaccinated animals

Conclusion and discussion: PRRS sow vaccination rate in the Netherlands is high and increasing. The PRRSv prevalence tends to decrease: the incidence of PRRS virus in saliva of piglets at weaning decreased (29% to 14%) as well as at the end of the nursery (41% to 36%). Since sows cannot protect their offspring long after birth, piglets can easily be infected in multiplying farms. PRRSv infections in the nurseries seem to be a big risk factor for having reproductive disorders. This is probably due to the fact that in The Netherlands almost all multiplying farms have a single-site system. Comparing ORF-5 sequences over time, there is no evidence of further genetic drift towards more heterologous strains.

Ref. ¹Cruijssen T. et al. (2011) ISERPD Proc. P.239; ²v Groenland G.J.et.al.(2010) IPVS Oral.Proc.P272; ³Wellenberg G. et.al. (2010) IPVS Proc.P.554; ⁴Geurts V. et.al.(2012) IPVS Proc.P.1036; ⁵Vercammen Th. et.al (2014) IPVS Oral Proc.P.218



Poster

1. PRRSV EVOLUTION

P.05

Molecular epidemiology of PRRSV in South China during 2011-2014

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV), which is the etiologic agent of porcine reproductive respiratory syndrome, was well known as widely disseminated virus with reproductive disorders in sows and respiratory symptoms in pigs of all ages. In June 2006, a highly pathogenic PRRSV (HP-PRRSV) was reported as the main cause of porcine high-fever disease in China and then HP-PRRSV became dominant strain in China. To elucidating the genetic relationships between Chinese isolates in recent years, the ORF5 gene of 59 PRRSV strains from 2011 to 2014 were sequenced and compared with representative strains obtained from GenBank. Phylogenetic tree based on ORF5 gene was constructed using MEGA 5.

Materials and Methods

From January 2011 to May 2014, 59 PRRSV positive samples were collected from pig farms in south China, including 8 samples in 2011, 10 samples in 2012, 19 samples in 2013 and 22 samples in 2014. ORF5 gene was amplified by reverse transcription-PCR (RT-PCR) as reported previously. Then the PCR products were cloned into pMD19-T vector, and sequenced in both directions using special primers. Phylogenetic tree was constructed using the MEGA 5 with the neighbor-joining method.

Results

Phylogenetic analysis of the nucleotide sequences of ORF5 gene revealed that all PRRSV strains used in this study were clustered into four subgenotypes which were represented by strain JXA1, CH-1a, VR2332 and GM2, respectively. Most of strains belonged to subgenotype 1 and 4, whereas only three strains shared great similarity with CH-1a. Furthermore, 17 PRRSV strains formed a novel branch with representative strains GM2 which was recombined between Chinese prototype field strain QYYZ and vaccine strain MLV.

Conclusions and Discussion

Phylogenetic relationship presented in this study demonstrated that most of PRRSV strains obtained in south China during 2011-2014 were clustered into subgenotype 1, 3 and 4, therefore we can conclude that Chinese classical strains, highly pathogenic strains and recombinant coexist in the swine herds. Moreover, the appearance of natural recombination reminded us of the extensive use of MLV vaccines for controlling PRRS. So we should learn more about molecular evolution and recombination mechanism of PRRSV to control variants of PRRSV circulated in swine herds.



Poster

2. PRRSV REPLICATION CYCLE

P.06

Cellular miR-130 inhibits replication of type 2 PRRSV by directly targeting its RNA

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Introduction: Approximately 22 nucleotides in length, microRNAs (miRNAs) have a great impact on viral infections, especially by binding to sequences with partial complementarity on viral RNA transcripts, usually resulting in the repression of virus replication. In recent years, growing evidence indicates that the cellular miRNA-mediated RNAi also reveal a big role in porcine reproductive and respiratory syndrome virus (PRRSV) replication. In the present study, we investigated whether more potent cellular miRNAs could play an important role in controlling PRRSV replication.

Methods: We performed computational prediction analysis of the HP-PRRSV strain vJX143 and the classic PRRSV strain vAPRRS using ViTa and miRanda database to predict miRNA target sites in order to find more potent PRRSV inhibitors. The next task was defining whether miR-130 could inhibit PRRSV replication by transfected chemically modified miRNA mimics into PAMs or MARC-145 cells before PRRSV infection and then examined virus production, viral gene and protein expression by virus titer assays, IFA, Western blotting and qRT-PCR assays. Then, twenty cDNA fragments encompassing the PRRSV genome were amplified by PCR from PRRSV pJX143 and subcloned into the pGL3-Control vector downstream of the luciferase ORF. Relative luciferase activities between cells transfected with miR-130 mimic and negative control (NC) mimic for different vectors containing various PRRSV cDNA segments were used to determine the target sites of miR-130b.

Results: Prediction results indicated that miR-130 could target the sites (bp 155 to 162) in viral genomic RNA through seed base pairing. It was pleasantly surprising that the target region was highly conserved in type 2 PRRSV, which now circulates in most commercial swine industries through out the world, but absent in type 1 PRRSV. As expected, delivered miR-130 family mimics, especially miR-130b inhibited PRRSV replication in vitro according to the results of virus titer assays, IFA, Western blotting and qRT-PCR assays. We also found that the anti-PRRSV effect of miR-130 is confirmed in multiple type 2 PRRSV strains but not in vSHE, a classical type 1 strain. Moreover, over-expression of miR-130 did not induce IFN-alpha or TNF-alpha expression in either mock- or PRRSV-infected PAMs. Luciferase reporter assays showed that relative luciferase activity produced a 60% decrease in pGL3-5UTR while no decrease in relative luciferase activity was observed in any other vectors. The above results indicated that miR-130 directly targets PRRSV RNA.

Conclusions: Taken together, these results demonstrated that miR-130 family, especially miR-130b inhibits replication of type 2 PRRSV in vitro by directly targeting its RNA. Our study reveals an example of a miRNA that affects viral propagation and highlights a host factor that may be important for future control measures against PRRS.



Poster

2. PRRSV REPLICATION CYCLE

P.07

A peek into the transcriptional mechanism of Type 1 PRRSV

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Porcine reproductive and respiratory syndrome virus (PRRSV) expresses a nested set of subgenomic RNAs (sgRNA1–7) flanked by a common terminal 5'-untranslated region (UTR) leader (L), a 3'UTR and a polyA tail. To gain further insights into the yet to be characterised mechanism of PRRSV sgRNA transcription, we evaluated the transcriptome of two Type 1 PRRSV strains, LV and Su1-Bel (subtype 3). RNA-seqs of pig lymph nodes and lungs three days post infection were performed. A total of 26 and 20 different sgRNA transcripts, inclusive of those that encode the known ORFs and the full-length genome, were detected in tissues infected by LV and SU1-Bel respectively. Inspection of the junction sequences - splitting translation regulatory sequence (S-TRS) - of the sgRNAs reveal that these short sequences are variable and they are derived from the regions located in the ORFs, i.e. body-TRS (B-TRS). This finding has therefore ruled out the 'leader-primed transcription model' for PRRSV 1 sgRNA transcription and lent, instead, further support for the proposed discontinuous minus strand RNA synthesis model (DMSSM). However, the lack of absolute conservation in the B-TRS suggests that transcription switching and template swapping may proceed through modified mechanism from that of DMSSM. We propose that in addition to the B-TRS, L-TRS is also recruited in the replication/transcription complex to provide the transcription switching and template swapping signals. We have tentatively called this model the "quasi-continuous minus strand synthesis model" (QMSM). With QMSM, complimentary matching between L-TRS and B-TRS over at least three bases, even with some mismatches, is sufficient to guide sgRNA template swapping which then allows the switching of transcription onto the the 3' terminal of nascent minus RNA body. In addition, differences in nucleotide usage on the TRS are also found between LV and Su1-Bel. While TRSs in LV mostly end with the CC dinucleotides, SU1-Bel seems to rely on the UU at the 5'end. Interestingly, we have also identified a specific trinucleotides alteration of "CCT" (LV) to "TTC" (Su1-Bel) immediately upstream of the L-TRS. This alteration was found only in subtype 3 strains including Lena, suggesting a potential difference in the transcriptional characteristics.



Poster

3. PRRS PATHOGENESIS

P.08

PRRSV envelope (E) protein interacts with mitochondrial proteins, inhibits ATP synthesis and induces apoptosis.

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In order to detect cellular proteins that potentially associate with the PRRSV E protein inside the cell, we employed an immunoprecipitation (IP)-coupled mass spectrometry (MS) approach. A plasmid encoding EYFP-tagged E or an empty vector encoding only the EYFP sequence was transfected into human embryo kidney (HEK) 293 cells. Subsequently, E and its associated cellular proteins were affinity purified using GFP-Trap beads from the cell lysates. The proteins were resolved by SDS-PAGE and visualized by Coomassie blue staining. In addition to detecting several common bands in both vector and E lanes, at least five specific bands were detected in the E lane. These protein bands were subjected to MS analysis, and several cellular proteins were identified in the analysis of these bands. Interestingly, 25% of these proteins were mitochondrial proteins. Interaction between E and two mitochondrial proteins (ATP synthase and ADP/ATP translocase) was further confirmed by co-IP and Western blot. Since these proteins are involved in ATP synthesis and ADP/ATP transport between mitochondria and cytoplasm, we checked ATP production in the transfected or infected MARC-145 cells. In cells either expressing E or infected with PRRSV, we observed significant reduction of ATP production. To check if E induces apoptosis, we assessed PARP and caspase-3 cleavage by Western blot after the E protein expression in HEK 293 cells. Our data revealed a pro-apoptotic role of E. Taken together, our results suggest that PRRSV E interacts with mitochondrial proteins and induces apoptosis by inhibiting ATP production.



Poster

3. PRRS PATHOGENESIS

P.09

Replication characteristics of eight virulent and two attenuated genotype 1 and 2 porcine reproductive and respiratory syndrome virus (PRRSV) strains in nasal mucosa explants

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Porcine reproductive and respiratory syndrome virus (PRRSV) can spread in between pigs via contact and airborne route. It was shown before that the highly pathogenic PRRSV strain Lena was able to replicate 10 to 100 times more in the nasal mucosa compared to the low pathogenic PRRSV strain LV. In this work, the replication characteristics of four type 1 (LV, 07V063, 08VA, 13V091), three type 2 (VR2332, MN-184, VN) and two attenuated (MLV-DV, MLV-VR2332) PRRSV strains were studied. After 72 hpi, mean virus titers reached $10^{4.5 \text{ to } 4.8}$ TCID₅₀/ml for LV and 08VA, $10^{5.2 \text{ to } 5.4}$ TCID₅₀/ml for VR2332 and Lena, and $10^{5.8 \text{ to } 6.3}$ TCID₅₀/ml for 07V063, 13V091, MN-184 and VN strains, whereas attenuated strains remained below detection limit. The mean number of PRRSV-positive cells/mm² at 72 hpi was 1.1 and 1.3 for the attenuated strains and LV, 13.3 for 08VA, 23.5 and 29.3 for VR2332 and 07V063, 31.1 and 33.8 for 13V091 and Lena, and, 39.1 and 59.2 for MN-184 and VN respectively. All the LV and MLV-DV infected cells were Sn⁺, whereas all other strains also infected Sn⁻ macrophages. In conclusion, (i) based on the virus shedding in the respiratory explants, PRRSV strains can be categorized as poor (MLV-DV, MLV-VR2332, LV, 08VA), moderate (Lena, VR2332) and strong (07V063, 13V091, MN-184, VN) secretors, and (ii) based on the number of infected cells isolates can be categorized as low (MLV-DV, MLV-VR2332, LV), moderately (08VA, VR2332), highly (07V063, Lena, 13V091) and hyper (MN-184, VN) virulent in the nasal mucosa.



Poster

3. PRRS PATHOGENESIS

P.10

CD163 and CD169 markers at the maternal-fetal interface during type 2 PRRSV infection

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Fetal deaths due to porcine reproductive and respiratory syndrome virus (PRRSV) infection during late gestation are hypothesized to be the consequence of transplacental migration of virus. While it still remains unclear how the virus crosses the pig epitheliochorial placenta and productively infects the fetus, macrophages containing CD163 and CD169 (sialoadhesin) receptors have been implicated in the process. In this study, our objectives were to identify and quantify CD163 and CD169 positive macrophages within the PRRSV infected uterus and fully attached fetal placenta, and to evaluate their potential use in predicting PRRSV infection outcome in the fetus.

On gestation day, 85 ± 1 , 114 PRRS virus-naïve pregnant gilts were inoculated with PRRSV (10^5 TCID₅₀ total dose) and 19 negative control gilts were sham inoculated. At 21 days post-inoculation, dams and their litters were humanely euthanized for necropsy examination. Samples of uterus with fully attached placenta, as well as fetal thymus were collected and analyzed by an in-house qPCR to quantify PRRS viral load. Based on the PRRSV RNA concentration in the uterine/placental tissue adjacent the umbilical stump of each fetus, 3 groups of samples: negative (not detected), low (quantifiable, below mean), and high (quantifiable, above mean) were formed (n=40/group; 120 total). The corresponding formalin fixed paraffin-embedded uterine/fetal placenta sections were subjected to immunohistochemistry for monoclonal antibodies directed against CD163, CD169 and PRRSV (SDOW17) antigens. Numbers of PRRSV positive cells and CD163 and CD169 positive macrophages per 1 mm² area of the endometrium and fetal placenta were determined by Image ProPlus software.

Results of immunohistochemistry revealed that PRRSV antigen was clearly present in macrophages, uterine epithelial cells and fetal trophoblast cells and there was a statistically significant difference in numbers of cells present in the endometrium and placenta between the low and high viral load groups ($P < 0.001$). Additionally, the numbers of CD163 positive macrophages in the fetal placenta and endometrium were significantly different between negative, low and high groups ($P < 0.05$). The numbers of CD163 positive macrophages in the fetal placenta ($P < 0.001$) and endometrium ($P < 0.001$) demonstrated statistically significant association with PRRS viral load in the fetal thymus. There were no significant relationship between numbers of CD169 positive macrophages and PRRS viral loads in the fetus placenta ($P = 0.159$) and endometrium ($P = 0.1$). While the CD169 results contradict previously reported results for type 1 PRRSV, they are consistent with recent studies in the transgenic pigs that demonstrated the CD169 receptor was not required for productive type 2 PRRSV infection.

In summary, these results confirm that numbers of CD163 positive macrophages in the endometrium and fetal placenta are significant predictors of the outcome of PRRS infection in the fetus and suggest an important role in the transplacental spread of type 2 PRRSV infection.



Poster

3. PRRS PATHOGENESIS

P.11

A reverse genetics system for the highly pathogenic European subtype 3 PRRSV strain Lena

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The recent appearance of highly virulent strains of porcine reproductive and respiratory syndrome virus (PRRSV) in Asia and Eastern Europe represents a major threat for pig industry that requires the identification of virulence determinants and possible atypical cell tropism of these new strains. To investigate these points, we have constructed an efficient reverse genetics system for the European subtype 3 field strain « Lena », isolated in Belarus. This strain was recently characterized as highly pathogenic in experimentally infected pigs (Karniychuk et al., 2010). Using a « Synthetic Virology » approach, we assembled the complete cDNA of the Lena strain genome from cloned synthetic DNA fragments designed from the published sequence (Genbank # JF802085, Van Doorselaere et al., 2012). A plasmid-based infectious clone was constructed by inserting the full-length cDNA downstream of the CMV promoter and placing appropriate signals (polyA and ribozyme sequences) at the 3' end of the cDNA. We obtained an efficient viral rescue by transfecting the BHK-21 cell line with this plasmid. We performed direct amplification of the rescued virus on primary cultures of porcine alveolar macrophages (PAMs). The recombinant Lena (rLena) strain was efficiently propagated on PAMs to reach titers above 10⁶ TCID₅₀ / ml after 2-3 passages. The growth properties of rLena on these cells appear similar to those of the low passaged, PAM propagated, natural Lena isolate. Experiments are currently planned to compare the pathogenicity of the rLena strain with its natural counterpart in pigs. We also modified our PRRSV Lena infectious clone to construct two recombinant reporter strains efficiently expressing the fluorescent proteins GFP and RFP. These recombinant strains will be useful to characterize more precisely the *in vivo* targets of the PRRSV Lena strain in the tissues of experimentally infected pigs.

P.12

Transcriptional profiles of host-pathogen responses in PBMCs in pigs infected with subtype 1 and 2 PRRSV strains of Type 1.

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The purpose of this study was to conduct a transcriptomic comparison between pigs experimentally infected with classical subtype 1 (DAN) PRRS strain and highly diverse East European strains belonging to subtype 2 (Russian strain ILI and Belarusian strain BOR). For this purpose transcriptome of peripheral blood mononuclear cells lymphocytes (PBMC) of infected and control piglets were analyzed using oligonucleotide microarrays specific for *Sus Scrofa* from Agilent. The experiment was performed using pigs of highly uniform genetic and environmental background, therefore RNA from each control and experimental group (DAN, ILI and BOR) were pooled and analyzed separately 7 days post infection. Each pool was processed in 4 repeats using Two-Color Microarray-Based Gene Expression Analysis, Low Input Quick Amp Labeling. The genes were determined to be differentially expressed if the fold change (FC) was greater than 1.5 in up or down-regulation. Statistical differences were determined with a Student's t-test at $p \leq 0.05$. All differentially expressed genes were imported into the Ingenuity Systems Pathway Analysis program (IPA; <http://www.ingenuity.com>) to identify most significant immunological pathways. Statistical analysis showed that at 7 pid the number of differentially expressed genes was 12518, 7335 and 4253 in groups infected with DAN, ILI and BOR respectively. Interestingly, the number of up and down-regulated genes engaged in immunology pathways was comparable in DAN group but in case of BOR and ILI groups significantly higher amount of up-regulated genes predominated over down-regulated. Within top 10 canonical immunology pathways "Production of Nitric Oxide and Reactive Oxygen Species in Macrophages" was identified as the only one common for all groups. Further pathways common for DAN and ILI groups were "Acute Phase Response Signaling", "iNOS Signaling" and "Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses". One common process for DAN and BOR groups was identified as "Clathrin-mediated Endocytosis Signaling". Four immunological pathways were shared between BOR and ILI groups: "Leukocyte Extravasation Signaling", "Agranulocyte Adhesion and Diapedesis", "Granulocyte Adhesion and Diapedesis" and "Caveolar-mediated Endocytosis Signaling". To validate the microarray results six genes were selected for RT-qPCR. First two genes CXCL10 (chemokine (C-X-C motif) ligand 10) and OAS1 (2'-5'-oligoadenylate synthetase 1) were identified among top up-regulated molecules in all three infected groups of animals. Two other genes selected for qRT-PCR analysis were CXCL2 (chemokine (C-X-C motif) ligand 2) and IL-8. Interestingly, both those genes were up-regulated in ILI (CXCL2 FC=2.931, IL-8 FC=2.240) and BOR groups (CXCL2 FC=5.455, IL-8 FC=5.331) but down-regulated in DAN group (CXCL2 FC= -2.3, IL-8 FC= -2.759). The expression of last two genes coding for FOS and IL-4 were down regulated in all three groups of infected animals and FC revealed values as follows: DAN =-2.90; ILI=-2.1; BOR FC=-3.49 and DAN =-3.89; ILI =-3.22; BOR FC=-2.04, respectively. The expression levels found by RT-qPCR were highly reliable for FC values determined by microarrays.

The most severe clinical lesions were observed in pigs inoculated with Bor strain, while Ili strain from the same subtype 2 resulted in mild clinical picture. However, no clear correlation between clinical picture and obtained results could be indicated.

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Poster

4. PRRS IMMUNITY / IMMUNE EVASION

P.13

The vOTU domain of highly-pathogenic porcine reproductive and respiratory syndrome virus displays a differential substrate preference.

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Arterivirus genus member Porcine reproductive and respiratory syndrome virus (PRRSV) causes an economically devastating disease, recently exacerbated by the emergence of highly pathogenic strains (HP-PRRSV). Within the nonstructural protein 2 of PRRSV is a deubiquitinating enzyme domain belonging to the viral ovarian tumor (vOTU) protease superfamily. vOTUs, which can greatly vary in their preference for their host ubiquitin (Ub) and Ub-like substrates such as interferon stimulated gene 15 (ISG15), have been implicated as a potential virulence factor. Since various strains of PRRSV have large variations in virulence, the specificity of vOTUs from two PRRSV strains of varying virulence were determined. While both vOTUs showed de-ubiquitinating activity and markedly low deISGylating activity, HP-PRRSV demonstrated a strong preference for lysine 63-linked poly-Ubiquitin, tied to innate immune response regulation. This represents the first report of biochemical activity unique to HP-PRRSV that has implications for a potential increase in immunosuppression and virulence.

P.14**Nuclear retention of host cell transcripts by nonstructural protein (nsp) 1 of arteriviruses and suppression of host innate immunity**

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The genome of arteriviruses is a single-stranded positive-sense RNA, and its coding strategy is relatively conserved with their genome length between 12.7 and 15.7 kb. Arteriviruses infect immune cells and may cause persistence in infected hosts. Inefficient induction of proinflammatory cytokines and type I interferons (IFN) are observed during infection of this group of viruses, suggesting that they may have evolved to escape the host immune surveillance for efficient survival. Recent studies have identified viral proteins regulating the innate immune signaling, and among these, nonstructural protein (nsp) 1 is the most potent IFN antagonist. For PRRSV, two subunits (nsp1 α and nsp1 β) of nsp1 suppress type I IFN production. In particular, PRRSV-nsp1 α degrades CREB (cyclic AMP responsive element binding)-binding protein (CBP), a key component of the IFN enhanceosome, whereas PRRSV-nsp1 β degrades karyopherin-1 which is known to mediate the nuclear import of ISGF3 (interferon-stimulated gene factor 3). Our studies on PRRSV were expanded to EAV, LDV, and SHFV. EAV-nsp1 remained uncleaved, whereas LDV-nsp1 was cleaved to LDV-nsp1 α and LDV-nsp1 β . SHFV-nsp1 was initially predicted to be cleaved to generate three subunits (nsp1 α , nsp1 β , and nsp1 γ), but only two subunits were generated as SHFV-nsp1 $\alpha\beta$ and SHFV-nsp1 γ . The papain-like cysteine protease (PLP) 1 α motif in SHFV-nsp1 α remained inactive, and only the PLP1 β motif of SHFV-nsp1 β was functional to generate the SHFV-nsp1 γ subunit. All subunits of arterivirus nsp1 showed the IFN suppressive activity and inhibited IRF3- and NF- κ B-mediated IFN production. Similar to PRRSV-nsp1 α , CBP degradation was evident in cells expressing LDV-nsp1 α and SHFV-nsp1 γ , but no such degradation was observed for EAV-nsp1. Regardless of CBP degradation, all subunits of arterivirus nsp1 also suppressed the IFN-sensitive response element (ISRE)-dependent antiviral protein expression. Since cellular mRNA nuclear export was found to be blocked by PRRSV, this study was expanded to other member viruses in the family Arteriviridae using individual subunits of nsp1. The nuclear accumulation of host mRNAs was evident for LDV-nsp1 β and SHFV-nsp1 β , whereas such mRNA retention was not observed for EAV-nsp1. Our data show that the nsp1-mediated IFN modulation is a common strategy for all arteriviruses, and the blocking of mRNA export to the cytoplasm is associated with subversion of host-protein synthesis, resulting in the enhanced viral protein translation and the suppression of IFN production, IFN signaling, and TNF- α production.



Poster

4. PRRS IMMUNITY / IMMUNE EVASION

P.15

PRRSV isolated from a pig with rebound in viremia following initial clearance is resistant to homologous neutralizing antibody but susceptible to broadly neutralizing antibody

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During a 42 day experimental PRRSV infection trial, approximately 20% of pigs presented with virus rebound, which we describe as a biphasic viremia profile. Previously, we analyzed over 1,600 serum samples for neutralizing activity to the respective homologous isolate, as well as three to 10 genetically diverse PRRSV isolates. Based on the results, pigs were placed into distinct neutralizing activity (NA) categories, including pigs with no NA, homologous NA (NA against only the challenge isolate), heterologous NA (NA against the homologous isolates as well as one or more genetically diverse Type II isolates), and broad NA (neutralizing activity against all Type I and Type II isolates tested). We predict that rebound virus will possess a distinct NA phenotype compared to the parental (challenge) isolate. The approach was to compare the NA of serum samples possessing homologous or broad NA against parent and rebound isolates. Briefly, virus was isolated from serum of a pig presenting with rebound in viremia at 56 days after challenge with the Type II isolate KS62. The challenge isolate (KS62) and rebound isolate (KS62-Reb) were then reacted with 4 samples with homologous NA and 3 samples with broad NA in a PRRSV neutralization assay. The samples with broad NA had similar neutralizing titers against KS62 and KS62-Reb. In contrast, samples with homologous NA neutralized KS62, but showed no or very low neutralizing activity towards KS62-Reb. These results provide further support the notion that homologous and broad neutralizing antibodies recognize distinct epitopes in the PRRSV proteome. These results have implications for the design of future vaccines. For example, vaccines that elicit high levels of homologous neutralizing antibody are not only unable to protect during challenge from genetically diverse isolates, but they also may not be sufficient to protect from homologous virus challenge. Future vaccine designs should take into account the category of neutralizing antibodies that are elicited.

P.16

Mutations in a highly conserved motif of nsp1beta protein impairs the innate immune suppression function of porcine reproductive and respiratory syndrome virus

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PRRSV nonstructural protein 1beta (nsp1beta) was identified as a multifunctional viral protein involving in suppressing host innate immune response and activating a unique -1/-2 programmed ribosomal frameshifting (PRF) for the expression of PRRSV nsp2TF and nsp2N. In this study, site-directed mutagenesis analysis showed that R128A or R129A mutation impaired programmed ribosomal frameshifting (PRF) transactivation function of nsp1beta, as well as reduced ability of nsp1beta to suppress IFN-beta reporter gene expression. However, only R128A mutation affects the ability of nsp1beta to suppress “self-expression” *in vitro*. In a previous study, we generated a recombinant virus vSD95-21-K124A/R128A (v1BKO) carrying double mutations in nsp1beta. In the current study, we further created three viable recombinant viruses, vSD95-21-R128A (vR128A), vSD95-21-R129A (vR129A) and vSD95-21-R128A/R129A (vRR128AA), carrying single or double mutations in the GKYLQRRLQ motif. In comparison to the wild-type virus, vR128A and vR129A showed similar growth ability, while vRR128AA and v1BKO mutants had reduced growth ability with approximately 0.5-1 log₁₀ decrease in viral titer in MARC-145 cell. With the stimulation of IFN-alpha, a 10-150 fold higher level of ISG15 expression were detected in mutant-infected Marc-145 cells in comparison to that of wild type virus-infected cells. In pigs infected with vR128A and vR128AA (but not vR129A) viruses, an increased level of IFN-alpha expression was observed, which correlated with an increased NK cell cytotoxicity. Further, augmented innate response was supported by a significant increase in the production of an important adaptive immune cytokine IFN-gamma in those mutants-infected pigs. These data demonstrate that R128 and R129 residues are critical for nsp1beta function, and modifying these key residues in the GKYLQRRLQ motif attenuated virus growth and improved the cellular innate and adaptive immune responses.



Poster

4. PRRS IMMUNITY / IMMUNE EVASION

P.17

Preliminary *in vitro* characterization of some immunobiological properties of HP-PRRSV isolates in Italy

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most important pig diseases worldwide because of its high incidence and economic impact on swine industry. Since the late 90's highly pathogenic (HP) isolates have emerged causing overt and severe clinical signs either in sows (Sow Abortion and Mortality Syndrome – SAMS) or in weaners-growers. The clinical, virological and serological outcomes of HP-PRRSV-1, i.e. Lena (PRRSV-1 subtype 3) from Belarus and, more recently, 13V091 (PRRSV-1, subtype 1) from Belgium, have been studied and experimentally reproduced (Karniychuk et al., 2010; Frydas et al., 2015).

This study aims at *in vitro* characterizing some immunobiological properties of two Italian PRRSV-1, subtype 1 strains (PR-392014 and PR-402014) isolated from distinct outbreaks of SAMS and severe respiratory and systemic disease with high mortality in weaners (up to 50%), referable to as HP-PRRSV. The nucleotide sequence of the ORF5 showed 83% and 85.9% homology to Lelystad virus, respectively for PR-392014 and PR-402014. Two other PRRSV strains isolated from outbreaks with a more conventional clinical course in weaners (PR-112014 and PR-252014) were used as non-HP/controls. Porcine alveolar macrophages (PAM) obtained from PRRSV naïve pigs were infected with the four different strains at a MOI of 0.01. The expression of TLR 3, TLR 9, SWC3, CD163, CD16 and CD14 was assessed in PAM by flow cytometry (FCM) at 0, 24 and 48 hours of infection.

HP-PRRSV strains caused the most intense reduction of CD16 cell percentage (6-15%) and MFI (Mean Fluorescence Intensity) (45-50%). All PRRSV strains, with the exception of the strain 392014, showed an increase of CD163+ cells associated with the highest MFI change. Only in PAM infected by the PR-392014 strain the TLR3 expression drastically increased upon infection from 0.7 to 52%. After 48 hours of infection the proportion of all the investigated subsets was reduced probably as a consequence of the cytopathic effect of the virus.

This study confirms that strains showing different clinical outcomes have also dissimilar immunomodulatory features on susceptible target cells, namely PAM. The effect of the HP-PRRSV strains on the target cells needs further investigations, by evaluating the effect of the viruses at early infection time points and by associating these functional markers with the production/secretion of antagonist cytokines.



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4. PRRS IMMUNITY / IMMUNE EVASION

P.18

Towards a better understanding of the within-host dynamics to PRRSv: insights from modelling approach.

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PRRSv is responsible for significant worldwide production losses and its control is a major challenge for the swine industry. Vaccination, the main control measure, does not allow to eradicate the infection and only confers a partial protection to the host. This lack of efficiency is mainly due to the strong variability in PRRSv strain virulence, which induces highly variable within-host dynamics. Consequently, there is a real need to better understand the interactions between the virus and the immune response in order to improve PRRSv control.

To tackle this issue, a dynamic and deterministic modelling approach was chosen. We developed an original immunological model consisting in an integrative representation of the within-host dynamics. It describes the immune mechanisms at the between-cell scale, including the innate response, the activation and orientation of the adaptive response and their complex regulations by the major cytokines. By the definition of parameter sets based on published experimental and modelling studies, this approach allowed us to explore various scenarios in terms of host susceptibility, strain virulence, as well as dose and duration of viral exposure.

Our first results show that similar infection durations associated with contrasted immune dynamics can be explained by the consideration of the immune mechanisms affected by the strain virulence. They provide new insights to explain apparent inconsistencies between experimental data. We then showed that the exposure, whose effect is often neglected, has an impact on the within-host dynamics, which varies depending on the virulence level. Finally, the within-host dynamics induced by the infection of a vaccinated pig is currently under exploration, opening up promising leads to improve vaccine efficiency.

All these results provide new insights to guide further experimental and modelling approaches and they offer promising prospects to improve PRRSv control at the herd level by an immuno-epidemiological modelling approach.



Poster

4. PRRS IMMUNITY / IMMUNE EVASION

P.19

Local and systemic antibody and cell-mediated immune response after vaccination and infection with porcine respiratory and reproduction system (PRRS) virus

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In spite of many different data about the immune response after PRRS vaccination or infections only few of them compare the systemic and local antibody and cell mediated immune response in various compartments. In our experiment we compared shedding of PRRS virus in blood, saliva and faeces and systemic and local antibody and cell mediated immune response of piglets in blood, saliva and bronchoalveolar lavage (BALF) after immunization with four different (2 inactivated and 2 modified live - MLV) vaccines. We also monitored the changes in leukocyte population and lymphocyte subpopulation in blood and BALF after immunization and infection. In piglets vaccinated with inactivated vaccines the first specific antibodies were detected (in ELISA) in the serum 7 days after the second dose of. Antibodies were also detected in BALF (14 days after the second dose) but not in saliva. Vaccination with inactivated vaccine with oil adjuvant cause strong cellular reaction *in vivo*, therefore it was difficult to estimate the specific cell mediated immune response *in vitro*. The virus in piglets vaccinated with killed vaccine was detected in all samples including faeces 3 days after infection and the shedding was massive at the end of the experiment (21 days post infection). In piglets vaccinated with MLV, the first specific antibodies appeared 14 days after the administration of vaccine and were detected both in serum and in low concentration in saliva and BALF. The cell mediated immunity tested *in vitro* with blood lymphocytes (lymphocyte transformation test and ELISPOT) appeared 7 days after immunization but a strong response was detected only after experimental infection. The antigen cell mediated immunity was also detected by ELISPOT in leukocytes derived from bronchoalveolar lavages. The virus in piglets vaccinated with MLV was detected in blood and saliva 3 days, and in faeces 14 days after immunization. The virus was also detected after infection but the shedding decreased with time and the virus was not detected in some of the animals 14 – 21 days post infection.

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P.20

Next-generation sequencing for multiple gene genotyping of PRRSV from field samples.

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a small, enveloped, single-stranded positive-sense RNA virus of Arteriviridae family. The 15kb PRRSV genome contains eight open reading frames (ORFs). ORF1a and ORF1b located at the 5'end represent nearly 75% of the viral genome and encode for proteins involved in replicase and polymerase activities. ORFs 2-7 encode for structural proteins associated with the virion: glycoproteins (GP) 2,3,4 and 5, membrane protein (M) and nucleocapsid protein (N), respectively.

The virus is a major cause of reproductive disorders, neonatal loss, or affliction of the respiratory system in swine. PRRSV infection leads to significant economic losses and sanitary problems, and so far no effective therapeutic measure exist. The disease was first reported towards the end of the 1980s simultaneously in the United States and in Europe, and since then extended all over the world.

PRRSV can be split into two genotypes: type 1 (European type: EU) and type 2 (North American type: NA). These two types are sharing only 50-60% genome sequence homology, due to a high degree of genetic variability. Within the EU genotype, four subtypes have been found: 1.1, 1.2, 1.3 and 1.4. PRRSV genetic diversity has a direct impact on immunobiology, epidemiology, diagnosis, and vaccine efficacy.

PRRSV genetic typing is currently based on ORF5 nucleotide sequence, which encodes for the main surface glycoprotein of the virus and has a critical role in inducing virus neutralizing (VN). This Sanger based strategy allows discrimination of each genotype and subgenotype, however it is worth pointing out that no correlation can be established between ORF5 based phylogenetic groupings and virulence or cross-protection. Moreover, existence of mosaic viruses resulting from recombination events may explain why some strains cannot be clustered using ORF5 phylogenetic analysis. Therefore, a single-gene sequence strategy is useful but not sufficient to genotype PRRSV.

The purpose of this study was to sequence several PRRSV genes from field samples. Besides to the classical ORF5 and ORF7 sequences, genes with the greatest genetic variability and/or encoding for proteins that potentially produce VN antibodies were selected, namely: Nsp2, ORF2, ORF3 and ORF4. Amplification of these six genes was performed in separate polymerase chain reactions (PCR) using primers and PCR conditions optimized to detect samples with low viral load. Amplicons generated from each individual sample were then pooled, subjected to enzymatic library generation and then tagged with different barcodes. This method enabled the six genes of 96 individuals to be sequenced in a single run using the Ion Torrent PGM™ Sequencer. Low failure rate and good amplification were obtained for samples with low viral load (eg samples with qPCR Ct values comprised between 18-36).

To conclude, we designed and set up a fast and robust method, based on RT-PCR amplification and subsequent next generation sequencing. This method enables to simultaneously sequence at once 6 independent PRRSV genes representing about 50% of the PRRSV genome from field samples and enables the identification of recombinant viruses. Besides the fact this approach avoids the need of cell culture isolation and amplification steps, it allows to generate more accurate phylogenetic trees and thus a more precise genotyping of the virus.

P.21

Detection of PRRSV in air sampled inside and outside PRRSV-positive herds in Denmark

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PRRS is one of the most important diseases in Danish swine. Approximately 40 % of Danish herds are positive for PRRSV type 1 or/and type 2. Air borne transmission via aerosols has previously been described for PRRS type 2 viruses in the US, but there have been no reports of aerosol transmission of PRRSV under field conditions in Europe. Information on aerosol transmission is important for the control of the disease and methods to detect PRRSV in the air could be an effective tool in control and eradication programs. The aim of the study was to validate methods to be used for sampling, storage and analysis of air samples for PRRSV.

The stability of PRRSV stored under a range of practical feasible conditions were first tested and revealed that the samples were stable for at least 10 days at 4°C. A validation of viral precipitation of liquid air samples under different conditions established the optimal concentration of the Polyethylene Glycol 8000 (PEG8000) used for precipitation and the optimal incubation- and centrifugation time. Finally, the ability of the cyclone to collect aerosolized PRRSV were confirmed in the laboratory using artificial aerosols containing different amounts of PRRSV confirming a decrease in Ct-values for PRRSV after precipitation when tested by real time RT-PCR.

The liquid cyclonic collector was placed inside a PRRSV-positive wean-to-finisher herd following mass vaccination of all pigs. Air was collected in the cyclone for 30 minutes in the middle of each of three rooms and blood samples were collected from selected animals.

PRRSV was detected by real time RT-PCR in the majority of samples and no significantly difference was found between the detection of PRRSV in air and blood samples in a given room, indicating that the cyclone was an effective tool for detection of PRRSV within a section.

In addition, the cyclonic collector was placed approximately 30 meters downwind from 4 PRRSV-positive swine herds. Air was sampled in the cyclone for 30 minutes. The samples were subsequently tested for PRRSV by real-time RT-PCR.

In total, 4 out of 20 samples were PRRSV-positive and all were PRRSV type 1. From herds 1, 2 and 3, 2/4, 1/4 and 1/6 of the samples were positive, respectively. All air samples collected from herd 4 were PRRSV negative. The four positive air samples had a Ct-value of 35.01-38.8 indicating, that the levels of virus were relatively low in all samples.

In conclusion, to our knowledge, this is the first report on detection of PRRSV genotype 1 in air samples collected outside PRRSV-positive swine herds in Europe. Furthermore, test of air samples collected inside herds were as sensitive as blood samples for detection of PRRSV providing an alternative methods for the monitoring of PRRSV status of herds/sections.



Poster

5. PRRSV DIAGNOSTICS

P.22

Development of a real-time RT-PCR assay that detects a broad range of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 subtypes

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Porcine Reproductive and Respiratory Syndrome is a very important pig disease worldwide. The causative agent for the syndrome is a small enveloped positive-sense RNA virus (PRRSV) of approx. 15 kb. There is a high genetic and antigenic diversity among PRRSV isolates and isolates are divided into two genotypes designated Type 1 and Type 2. The Type 1 viruses can further be divided into subtypes, where subtypes 1, 2, and 3 have been acknowledged. Type 1 subtype 1 is found globally, whereas subtypes 2 and 3, so far, have only been detected in Eastern Europe and Russia. Clinical and experimental data suggest that infection with Type 1 subtype 2 and 3 cause more severe disease in pigs than Type 1 subtype 1. The movement of pigs between countries increases the risk of spread of subtypes 2 and 3 outside Eastern Europe and therefore it is very important that the diagnostic tools are able to detect all subtypes in order to provide the correct treatment and control. Based on in silico analyses of published primer sequences, most of the existing real-time RT-PCR assays for detection of PRRSV do not recognize all PRRSV Type 1 subtypes.

The aim of the study was to develop a real-time RT-PCR assay capable of detecting a broad range of PRRSV Type 1 subtype 1, 2, 3 and isolates not allocated to subtypes (atypical Type 1 strains) with a high PCR efficiency and good range of quantification.

For the design of the real-time RT-PCR assay five (5) complete genomes from viruses representing all Type 1 subtypes 1, 2, and 3 were used. The five complete genomes were aligned and a dual labeled probe chemistry assay was designed at a conserved region of the genome (ORF2 coding region). The assay was validated on RNA extracted from cell culture supernatant or serum from samples representing all subtypes and one of the atypical Type 1 strains.

The assay recognized all tested strains with PCR efficiencies in the range 90-99% and a range of quantification of four-five 10-fold dilution steps.

Surveillance programs of PRRSV are in most countries based on serology, but most of the applied serological tests cannot discriminate between genotypes or subtypes. Thus, to be effective, PRRSV monitoring programs must be based on the use of sensitive real-time RT-PCR tests that recognize a broad range of PRRSV isolates. The presented assay provides laboratories with a new assay.



Poster

5. PRRSV DIAGNOSTICS

P.23

Characterization and identification of the polyclonal antibodies against the Nsp9 protein encoded by PRRSV

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Porcine reproductive and respiratory syndrome (PRRS) is considered to be one of the most important infectious diseases to affect the swine industry and characterized by reproductive failure in late term gestation in sows and respiratory disease in pigs of all ages. The Nsp9 gene, encoding the RNA dependent RNA polymerase, generally regarded fairly conserved when compared to other viral proteins. Currently, antibodies have not yet been developed against this specific protein even though such entities could have potential applications regarding the diagnosis and characterization of PRRSV. Therefore, the current study was undertaken to generate polyclonal antibodies against the immunodominant Nsp9 protein of this virus. For this purpose, the Nsp9 protein was expressed in *Escherichia coli* and subsequently used as an antigen to immune New Zealand rabbit. Antiserum was identified via an indirect ELISA. And then verified based on the ability to react with both naturally and artificially expressed Nsp9 protein in western blots. Antiserum was found to successfully recognize the Nsp9 protein in a variety of immunological assay. Thus, the widespread utility of this antiserum as a diagnostic core reagent should prove invaluable for further investigations regarding the mechanism of PRRS pathogenesis and the control of this disease. In this regard, it should be noted that the proteins were highly expressed in the supernatant from the Nsp9-producing cells that were incubated with a binding buffer containing the following compounds: beta -mercaptoethanol, urea, Tween 20, glycerol, and SDS, while they were rarely expressed in the supernatant from the Nsp9-producing cells that were incubated with binding buffer without the compounds. The result of virus neutralization test showed that antiserum can not neutralize the PRRSV. We firstly expressed and purified the Nsp9 gene of PRRSV. These provide a good way for the purification of proteins expressed in inclusion bodies and the preparation of the corresponding antibodies.

P.24

Earlier and easier diagnostic tools for PRRSV herd management: Comparison of sampling and prevalence under field conditions

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Real time RT-PCR (rtRT-PCR) and ELISA tests are often used to assess the PRRSV infection status of pig herds. rtRT-PCR tests on blood/serum and tissue samples are the most used technique to detect earlier PRRSV. Recently, the detection of many pig pathogens in oral fluids was reported as an alternative technique being more economical, easier and less invasive. The main goal of several studies was to validate oral fluid against blood/serum and tissue and also establish a sampling recommendation for oral fluids under field conditions for an earlier diagnostic of PRRSV with an easier sampling method.

Thermo Fisher Scientific requested several laboratories and research institutes throughout the world to evaluate the rtRT-PCR tools on over 800 field samples from different genotypes. A field study in Spain allowed evaluation of the performance of the kit on oral fluids samples. Results per pen are compared to individual results (blood/serum). Based on these results, a biostatistical/epidemiological study was launched to calculate the probability of genotype 1 and 2 virus detection in a pen using oral fluid samples taking into account the prevalence of PRRSV in serum as an independent variable. In all these studies, sample extraction was carried out with the MagMAX™ Pathogen RNA/DNA Kit (5X) or the MagVet™ Universal Isolation Kit. Purified RNAs were analyzed by rtRT-PCR with the LSI VetMAX™ PRRSV EU/NA kit or VetMAX™ NA and EU PRRSV Reagents.

The LSI VetMAX™ PRRSV EU/NA kit has shown a sensitivity of 98.2% on more than 400 positive field samples and a specificity of 100% on more than 400 negative field samples. In the second study, the kit showed an excellent correlation at pen level (oral fluid sample) compared to animal level (blood/serum sample) with a difference of $\pm 1C_T$. The PRRSV RNA was identified in early infectious stages compared to antibody detection: from 7 days after infection up to 7 weeks on oral fluids and 8 weeks on blood/serum compared to 28 days after infection with ELISA test. The number of oral fluids that need to be sampled on herd level in order to find at least 1 positive PRRSV oral fluid was for example 3 for a serum prevalence of 50%.

Ready-to-use rtRT-PCR tools allow detecting the virus in early stage of infectious compared to antibody detection. rtRT-PCR results obtained with blood/serum and oral fluids demonstrate an excellent correlation. Based on epidemiology and prevalence of the virus in the herd, oral fluids sample are able to provide the same if not better information due to ease of use compared to randomly taken blood samples on herd level. Oral fluids allow the swine industry to generate a more accurate and reliable monitoring system where early detection can result in faster response time. It is an opportunity to increase the number of pigs tested while decreasing the cost of analysis and to estimate the circulation of pathogens (PRRSV, PCV2 and SIV) in swine population for effective herd health monitoring.

P.25**Investigating the clinical significance of porcine reproductive and respiratory syndrome virus detected in vaccinated pigs – a diagnostic dilemma?**J-P. Frossard^{1*}, S.M. Williamson², C. Bidewell² and S. Scholes³¹Animal and Plant Health Agency (APHA, formerly AHVLA) Weybridge, New Haw, Addlestone, Surrey, UK²APHA, Rougham Hill, Bury St Edmunds, Suffolk, UK³APHA, Lasswade, Easter Bush, Scotland, UK

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important viral infections diagnosed in disease outbreaks in pig herds in Great Britain. In the last three months of 2014, the diagnostic rate of PRRS incidents was the highest of any quarter since 2004 when the diagnostic PCR was introduced, with 12.4% of submissions tested diagnosed compared to 7.0% for the same period in 2013. In 2014, 48 outbreaks of PRRS were diagnosed by Animal and Plant Health Agency and Scottish Agricultural College Consulting Veterinary Services diagnostic laboratories in Great Britain. Forty five of these were systemic and/or respiratory in nature and in post-weaned pigs, while three were of reproductive disease. Disease was diagnosed at all stages from weaning to slaughter age. The criteria which must be fulfilled to confirm a diagnosis of PRRS at these diagnostic laboratories are a) suspicious clinical history and gross pathology with virus detection by PCR, immunohistochemistry (IHC) or virus isolation or b) in live pigs, suspicious clinical history and detection of virus or seroconversion in unvaccinated pigs. In herds with known PRRSV infection or where a challenge is considered likely during rear, there has been increasing use of modified-live PRRS vaccination at or around weaning to mitigate disease during the growing period. This raises a potential issue when using PCR to diagnose disease due to PRRS, especially when there are other pathogens identified, as commonly occurs due to the immunosuppressive nature of PRRSV. It is recognized that, following challenge by field virus, pigs may become viraemic even when protected against clinical disease by vaccination. Thus detection of PRRSV by PCR alone in live-vaccinated pigs could result in overstating the significance of the virus in the disease outbreak. An approach taken in our diagnostic laboratories when pig carcasses or lungs are submitted, is to follow-up detection of PRRSV by PCR in vaccinated pigs with histopathology and IHC on lung. Where pigs are PCR-positive and PRRSV antigen is detected by IHC in association with pneumonic lesions, there is good evidence that PRRSV is involved in disease. As PCR-testing pigs within the first few weeks after vaccination can detect vaccine-derived virus, sequencing can be used to differentiate field and vaccine-like strains. However, as only a short segment of the PRRS viral genome (ORF5) is sequenced, viruses which appear vaccine-like could be different in other genetic regions through recombination or mutation. Here we present a breakdown of PRRS cases where combinations of PCR, sequencing, histopathology and IHC have been applied to samples from diseased pigs of known vaccination status. These illustrate the value of additional testing to fully investigate disease attributed to PRRSV and demonstrate an area of potential diagnostic uncertainty in PRRSV PCR-positive vaccinated pigs which do not have evidence of PRRSV antigen in the lung using IHC. It would be useful to compare this approach to evaluations by other diagnostic laboratories for such cases.



Poster

5. PRRSV DIAGNOSTICS

P.26

Identification and characterization of phage-displayed peptides recognizing Porcine Reproductive and Respiratory Syndrome Virus GP4 protein

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Phage display is a powerful technology to identify specific ligands of a target protein by a biopanning process. It has been successfully applied in various aspects, including the drug discovery, diagnostic analysis. PRRSV GP4 protein a critical viral envelope protein that not only mediates interactions with other viral envelope glycoproteins but also mediates interactions with scavenger receptor CD163 for virus entry. Although both attenuated live and inactivated PRRSV vaccines have been developed, the effectiveness and safety of these commercial vaccines are controversial. Moreover, until now there are no effective antiviral agents against PRRSV available to control PRRSV infection. Therefore, it is of great importance to develop effective antiviral approaches. In this study, a recombinant GP4 protein was expressed and used as a target, after four rounds of biopanning by using a random 12-mer peptide library, ten phage clones were selected. These phage clones can interact with both GP4 protein and PRRSV, and distinguish with seven other porcine viruses by ELISA assays. The DNA sequencing results showed that three identical peptides were identified, and designated as peptide-YHM, WHN or WHE. MARC-145 cells were treated with three chemically synthesized peptides at various stages of PRRSV life cycle. Virus titration assay was performed to determine the virus infectivity. The expression of viral mRNA and protein was measured by Realtime PCR and indirect immunofluorescence assay, respectively. These results indicated that all the three peptides were unable to block PRRSV binding nor repress viral replication, however, the peptide-WHE significantly impaired virus entry in a dose-dependent fashion through competitively binding to GP4 against CD163. In conclusion, our findings imply that PRRSV GP4 is an attractive target for diagnostics and drug development. The selected phages were able to be used as a novel diagnostic tool to distinguish PRRSV from other viruses, and the peptide-WHE has the potential to be developed as a therapeutic agent for control and treatment of PRRSV infection.



Poster

5. PRRSV DIAGNOSTICS

P.27

Differences in performance of commercial real-time RT-PCR kits in detection of diverse PRRSV strains

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Several authors suggested that high genetic diversity of PRRSV may affect diagnostic methods, especially RT-PCR assays based on amplification of a specific fragment of viral nucleic acid.

The aim of the present study was to compare the performance of commercially available real-time RT-PCR kits based on collection of strains representing known diversity of PRRSV.

The panel consisting of 40 samples employed in this study included RNA extracted from 20 different PRRSV strains (isolates and sera samples from naturally and experimentally infected pigs). Sixteen strains belonged to Type 1 and represented subtype 1 (4 strains), subtype 2 (3 strain), subtype 3 (4 strains) and tentative subtype 4 (2 strains). Two strains switched between subtypes 1 and 2 depending on the region analysed (ORF7 or ORF5) and in one case subtype was not determined. Remaining four strains were of Type 2, including high pathogenic (HP) strain from Vietnam. Additionally, serial dilutions of strains belonging to Type 1 subtypes 2, 3 and 4 as well as HP-PRRSV were included into the panel of samples.

The comparison included seven commercial real-time RT-PCR assays (I-VII) designed to allow detection and differentiation of two PRRSV genotypes. All samples were tested in duplicates according to the instructions provided by manufacturers.

Overall variable sensitivity, especially regarding diverse Eastern European strains from Type 1 subtypes 2-4 was observed. The percentage of samples classified as positive in at least one of duplicates varied between analysed kits and reached 95% (Kit I), 87.5% (VII), 80% (Kit VI), 72.5% (Kits IV and V), 57.5% (Kit III) and 50% (Kit II). Only three assays (Kit I, V and VII) detected at least one sample per every PRRSV strain. Among remaining kits from one to seven strains, mostly from Type 1 subtypes 2-4 were classified as false negative. Kit III failed to detect any sample from subtype 3, but other assays detected at least one strain from every subtype. Additionally one field strain of Type 2 was not detected by one of the kits. All tests detected HP-PRRSV strain up to at least 10^{-5} dilution.

Obtained results indicated that genetic variability may strongly affect diagnostic results. In the present study commercial kits failed to detect up to 7 different PRRSV strains. Abovementioned findings emphasize that continuous monitoring of PRRSV genetic diversity, evaluation of diagnostic tests and updating primers and probes sequences are necessary to ensure proper diagnostics. Close cooperation between diagnostic laboratories and the manufacturers of diagnostic kits would be beneficial for both sides.

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P.28

Differentiation of PRRSV Type 1 and 2 serological responses by an immunofluorescence antibody assay

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Study objective

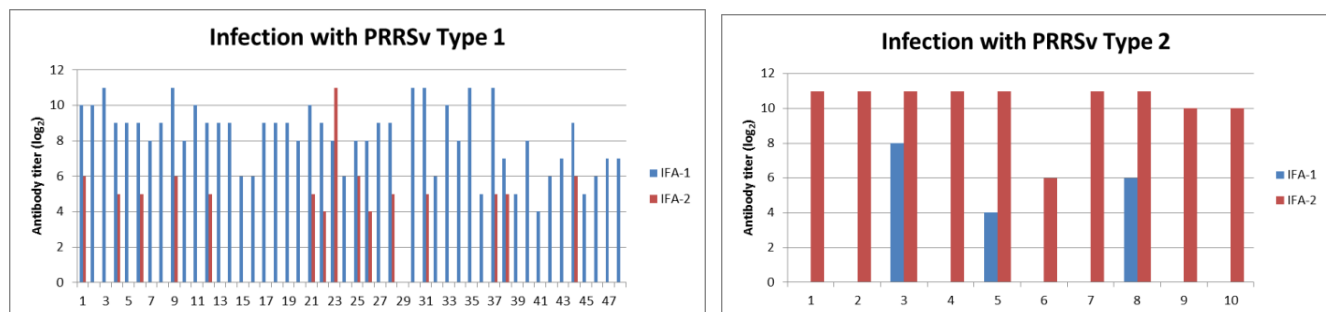
The study objective was to establish criterions for interpretation of test results from immunofluorescence antibody assays (IFA) in order to differentiate antibodies against PRRSV Type 1 and 2. Such criterions are useful when testing field samples of unknown PRRSV infection status.

Materials and methods

In a study by Nielsen et al. (2014), sensitivities and specificities for the IFA test specific for the two different types (IFA PRRSV Type 1 (IFA-1) and 2 (IFA-2)) were calculated for cut-offs at 4 log₂ and 6 log₂, respectively. A cut-off level of ≥ 4 log₂ provided the best test performance with sensitivities and specificities of 0.98/0.98 for PRRSV Type 1 and 1.00/0.90 for PRRSV Type 2, respectively.

Results and conclusion

The specific titers from the IFA-1 and IFA-2, 21 days after infection with PRRSV, are summarized in the figures below (48 pigs infected with Type 1 and 10 pigs infected with Type 2).



Infection with PRRSV Type 1 induced IFA-1 antibody titers ≥ 4 in 47 (98%) and IFA-2 antibody titers ≥ 4 in 15 (31%) of samples. IFA-2 reactions were ≥ 2 log values lower than IFA-1 in 14 of 15 samples. Infection with PRRSV Type 2 induced IFA-2 antibody titers ≥ 4 in 10 (100 %) and IFA-1 antibody titers ≥ 4 in 3 (30%) of samples. IFA-1 reactions were ≥ 2 log values lower than IFA-2 in 3 of 3 samples.

Based on these results it is suggested to classify serum samples with unknown PRRSV infection status accordingly:

Positive for PRRSV Type 1: IFA-1 ≥ 4 and (IFA-1 minus IFA-2) ≥ 2

Positive for PRRSV Type 2: IFA-2 ≥ 4 and (IFA-2 minus IFA-1) ≥ 2

By using these criterions, a correct classification was obtained for all except two of the 58 tested sera (97%). Only animal no. 29 in figure 1, which was negative in both IFA-tests, and animal no. 23, in which Type 1 infection resulted in higher heterologous titers (IFA-2), did not comply with this classification.

Reference:

G.B. Nielsen, C. Drexler, B. Kroezen-Maas, R. Jolie (2014): Evaluation of an immunofluorescence antibody assay for detection of type 1 and type 2 PRRSV infection. Proceedings, PRRS Seminar 2014, Chicago, USA.



Poster

5. PRRSV DIAGNOSTICS

P.29

Validation of PRRS serological diagnostic test using sow colostrum.

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Introduction

Although several PRRS serological methods exist, collecting blood or oral fluid samples from sows for such testing is difficult. The aim of this study was to investigate the possibility of using individual or pooled sow colostrum samples for PRRS antibody detection, which is an easy sample in the field and pooling samples lowers the cost of individual analysis.

Materials and Methods

The study included 18 PRRS free herds with various vaccination and feeding protocols to control the specificity of the diagnosis. In each herd, colostrum of 10 young sows (First or Second parity) and 10 older ones (i.e Third parity or more) were sampled. In addition, 6 known PRRS infected herds were selected, in which a total of 72 paired sera and colostrum were collected to test the sensitivity of this method. All samples were tested with the IDEXX 3XR PRRS Antibody Elisa kit at Labocea diagnostic laboratory.

Results

The specificity of the diagnosis on individual colostrum was calculated as 98.8 % (confidence interval 96,7 – 99,6), and the specificity on pooled colostrum was 100 % (CI pools: 71,5 – 99,8). At the herd level, the specificity, was 82,4% on individual samples (taking into account 3 false positive results among 260 individual colostrum samples, originating from 3 out of 17 herds), while the herd's specificity on pooled colostrum was 100 %. The sensitivity of the diagnosis on individual and pooled samples was optimal (100%) (CI individuals: 95-100; CI pools: 71,5-100).

Conclusion

Based on the results from this study, PRRS serological diagnosis on sow colostrum appears to be a new and simple way to monitor the status of PRRS negative herds, and the rate of positive animals in infected sow herds.

P.30

Comparative evaluation of the immune response to field Porcine Respiratory and Reproductive Syndrome virus (PRRSV) infection in terms of serum and mucosal antibody, and cell-mediated immunity

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Porcine reproductive and respiratory syndrome (PRRS) remains a cause of considerable economic losses and welfare problems in pig farms throughout Europe as an important component of the porcine respiratory disease complex (PRDC), and as a cause of reproductive failure in gilts and sows. The need for extensive surveillance programmes on farm makes the case for new and refined diagnostic approaches, aimed at obtaining crucial information about PRRSV infection and relevant control programs, while optimizing the allocation of manpower and resources. In this respect, we set out to work on oral fluids as a most promising, robust and cost-effective matrix for a wide array of diagnostic assays. In this respect, oral fluids hold promise for use in large-scale sampling and disease monitoring programs, as well as for use in the assessment of PRRSV-specific immunization procedures. The aim of this study was to define the profile of Ab response to PRRSV in oral fluids in a PRRS-stable breeding herd.

The aim of this work was to compare the time-course of humoral and cell-mediated immunity in 3 groups of PRRS-free replacement gilts introduced into a PRRSV-infected breeding herd. In particular, we investigated serum IgG antibody, PRRSV-specific IgA and IgG in oral fluids and the cell-mediated response (PRRSV-specific release of interferon-gamma). These parameters were measured in order to identify possible discrepancies in the development and kinetics of the immune response against PRRS virus.

Gilts got regularly infected by PRRSV around 7-9 weeks after entering the farm. 4 results must be highlighted: A) the precocity of the Ab response in oral fluids group was similar to that seen in sera; B) good conditions of animal health, welfare and farm management were associated with an early humoral immune response and cell-mediated immunity, as well (gamma-IFN test), in contrast to what is observed in PRRS "problem" herds; C) the cell-mediated response may be considerably different among subjects of the same group, but each group tends to clearly distinguish itself with respect to this parameter; D) Ab-positive oral fluid samples can derive from a minority of seropositive pigs out of the 8-10 individuals that deposit the oral fluids.

Lastly, some problems were reported regarding the use of the cotton rope for collecting oral fluids of pigs aged > 12 weeks. These problems should be dealt with by proper modifications of the applied protocol.

P.31

Disrupting the deubiquitinase function of PRRSV papain-like protease 2 to improve vaccine efficacy.

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Several virus species encode deubiquitinating enzymes (DUBs), suggesting that they remove ubiquitin (Ub) to evade Ub-dependent antiviral responses. Arteriviruses like Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) encode an OTU-domain DUB known as papain-like protease 2 (PLP2) in nonstructural protein 2 (nsp2), which removes Ub from cellular proteins, and is also essential to virus replication by cleaving a site within the viral replicase polyproteins.

To dissect this dual specificity, which relies on a single catalytic site, we determined the crystal structure of equine arteritis virus (EAV) PLP2 in complex with Ub (1.45 Å). PLP2 binds Ub using a zinc finger that is uniquely integrated into an exceptionally compact OTU-fold, which we believe represents a new subclass of zinc-dependent OTU DUBs. Notably, the Ub-binding surface is distant from the catalytic site, which allowed us to mutate this surface to significantly reduce DUB activity without affecting polyprotein cleavage. Viruses harboring such mutations exhibited wild-type replication kinetics in cell culture, confirming that PLP2-mediated polyprotein cleavage was intact. However, the loss of DUB activity strikingly enhanced innate immune signaling, resulting in nearly an order of magnitude increase in beta-interferon mRNA expression by primary equine lung cells infected with the mutant.

Our findings not only establish PLP2 DUB activity as a critical factor in arteriviral innate immune evasion, the selective inactivation of DUB activity also opens new possibilities for developing improved live attenuated vaccines against arteriviruses like PRRSV and other viruses encoding similar dual-specificity proteases. The focus of our current research is to develop PRRSV vaccine strains that display reduced DUB activity with the aim to improve vaccine efficacy.

P.32**Generation of PRRS VLPS by multiple protein co-expression in the baculovirus system**

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In the last years, many efforts have been done in the generation of Virus Like Particles (VLPs) for vaccine development to control human or animal diseases (Martinez-Torrecuadrada 2003; Latham and Galarza, 2001; Brun, Barcena et al., 2011; Rueda, Hurtado et al., 1999) because they combine some of the advantages of attenuated and subunit vaccines. Recently, the generation of basic structures of PRRS virions through the baculovirus system has been reported by the co-expression of Gp5 and M proteins (Nam, Chae et al. 2013). Gp5 and M have been the focus of many studies during years because its implication in virus internalization and in neutralization. Protective immune response in PRRS is based on neutralizing antibodies and cellular response but, until now, there is not a clear epitope or protein considered to be the only responsible of the protective mechanisms. Moreover, the results seem to point out that other structural proteins are contributing to the global immune response against the virus. As a result, this work aimed at the construction of PRRS VLPs that incorporate Gp5, M, Gp2, Gp3, Gp4 and E proteins.

The cDNA sequences corresponding to the six structural proteins were amplified from Olot virus strain. The sequences were cloned into the multiple expression baculovirus vector pBAC4x-1 (Novagen) in two separate plasmids: Gp5 and M in one plasmid (pBAC4x-1A) and Gp2, Gp3, Gp4 and E in another one (pBAC4X-1B). The expression of the different proteins was assessed by Western blot with specific antibodies or pig sera. The culture supernatants after single infection with pBAC4x-1A or by co-infection with pBAC4x-1A plus pBAC4x-1B were semi purified with a sucrose cushion and, subsequently, loaded on the top of a 20-60% sucrose layer gradient. The formation of VLPs was confirmed, both in single infections and in co-infections, by electron micrograph of the gradient fractions corresponding to PRRSV density (1,15-1,16 g/cc).

Gp5 and M proteins were easily detected in the two types of VLPs with the specific mAbs, showing an apparent molecular weight similar to the one observed in the virus. The minor structural proteins are, in general, more difficult to detect both in the VLPs and in the virus. This fact could be reflecting the stoichiometry of the virus, whose envelope is mainly composed of Gp5/M complexes (Wissink, Kroese et al. 2005; Mardassi, Massie et al. 1996; Wissink, Kroese et al. 2005) whereas the Gp2-Gp3-Gp4 complexes and E protein are nestled (Dokland 2010). In this work, Gp3 protein had an apparent size smaller than the one in the wild type virus, which can be indicating a lower glycosylation level.

The system presented here has the flexibility to easily add or remove complete structural proteins to the basic Gp5/M VLPs, providing a useful approach to study the implication of particular epitopes or proteins. Besides, the possibility to exchange individual proteins to generate PRRS VLPs from different strains would be a helpful tool for the development of specific vaccines. Nevertheless, further work is needed to clarify important aspects of the VLPs generated as their immunological abilities.

Acknowledgements

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P.33

Construction and in vivo Analysis of Recombinant PRRSV Expressing E2 Gene of CSFV

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) and classical swine fever (CSF) are important infectious diseases for swine industry. Currently live vaccines against both diseases are widely used in China. Previous reports indicated that PRRSV infection could interfere immune efficacy of CSF vaccine. In this study, based on the full-length cDNA clone of highly-pathogenic PRRSV cell attenuated vaccine strain HuN4-F112, CSFV E2 gene was inserted between ORF1b and ORF2. An extra transcription regulatory sequence 6 (TRS6) was inserted behind the exogenous gene in order to make E2 gene as a unique sg mRNA expression.

Materials and Methods

HuN4-F112 is an attenuated vaccine derived from highly pathogenic PRRSV HuN4. rHuN4-F112 is an infectious molecular clone of HuN4-F112. The chimeric plasmid pA-SM-E2 was constructed using SOE PCR that could express CSFV E2 gene. The chimeric virus vA-SM-E2 was rescued by in vitro RNA transfection. 30-day-old piglets free of PRRSV and CSFV antigen and antibody were selected for evaluating immune efficacy of the recombinant virus vA-SM-E2 and sera were collected before and after immunization for antibody level evaluation and neutralization test.

Results

1. After the identification for viral characteristics and genetically stability, the results showed that the vA-SM-E2 could be stable for at least 20th cell passages, and their E2 expression could be detected in cell culture by IFA.
2. The animal inoculation test for this chimeric virus compared with vaccine strain HuN4-F112 in serological examination were performed, 15 30-day-old PRRSV and CSFV-free piglets were obtained and divided randomly into three groups, i.e., five piglets in each group. Piglets in group 1 (vA-SM-E2), group 2 (vHuN4-F112), group 3 (EMEM) were injected intramuscularly on day 0 with 2mL of diluted viruses (1×10^5 TCID₅₀) and EMEM, respectively. The piglets were monitored daily to assess their general health status and rectal temperature. PRRSV and CSFV E2 specific antibody response were analyzed in sera collected at 0, 7, 14, 21, 28, 35, 42, and 49 dpi using a commercial ELISA kit. The results showed that chimeric virus vA-SM-E2 inoculated group developed the similar PRRSV-specific immunizing effect with the vHuN4-F112 vaccinated group 2 while developed high level of CSFV E2 neutralization antibody. The E2 antibody level began to rise at 7 dpi and all group 1 piglets had sero-converted by 14 dpi. They all had the similar rise tendency with PRRSV seriological responses.

Conclusions

Recombinant PRRSV expressing E2 of CSFV was successfully constructed, inoculation of the recombinant virus could induce high level neutralization antibodies against CSFV. The further protective immunity test against CSFV and PRRSV challenge would be performed to detect the feasibility of the recombinant virus as the vaccine candidate.

P.34

Strain-specific serological response after simultaneous vaccination with PRRS MLV against PRRSV types 1 and 2: Impact on interpretation of surveillance data

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Both type 1 (subtype 1) and type 2 PRRSV are currently circulating in Denmark. In some double-infected herds, the pigs are simultaneously vaccinated with PRRSV modified live vaccines (MLV) against both genotypes. There is a lack of data on the impact on serological response following simultaneous administration of PRRSV MLVs against both type 1 and type 2 PRRSV. The objective of this experimental study was to compare the level of serological response of single-vaccinated pigs with responses of double-vaccinated pigs and to compare the serological response of the two vaccination strategies following challenge with homologous and heterologous virus strains.

Sixty-six four-week-old PRRSV-negative pigs were included in the study. The pigs were purchased from a specific pathogen-free herd and tested free of a range of pathogens including PRRSV by serology at the beginning and end of the study. The pigs were housed at the experimental animal facilities at the National Veterinary Institute under appropriate biosecurity conditions. On arrival (week 0), the pigs were randomly distributed into four groups (1-4). Each group was housed in a separate room. One week after arrival (week 1), the pigs in groups 1-3 were vaccinated with either Porcilis® PRRS VET or Ingelvac® PRRS VET, or both. The last group was kept as a non-vaccinated control. Nine weeks after vaccination, all pigs were divided into three new groups and were then challenged with PRRSV type 1 (DK strain 18794), PRRSV type 2 (DK strain 19407b) or PRRSV atypical strain (strain BOR59) by the intranasal route. Blood samples were collected daily from all pigs during the first week after vaccination and challenge. In the remaining periods, samples were taken once a week from all pigs. The level of antibodies against PRRS virus was measured by type-specific ELISA and IPMA.

All pigs developed antibodies against PRRSV two to three weeks after vaccination as measured by ELISA and IPMA. The onset and level of antibodies developing in response to single and double vaccination were equal. Similarly, the development of type-specific antibodies in pigs vaccinated with the type 2 vaccine was comparable to that in pigs vaccinated with the type 1 vaccine. The profile of antibody responses following challenge with the atypical PRRSV type 1 strain BOR59 was identical to the responses of the animals challenged with the subtype 1 strain. These results indicated that simultaneously vaccination with two different PRRSV vaccines is feasible, although they also revealed that standard serological assays cannot distinguish between infections with the different type 1 subtypes. This emphasises the fact that viral detection and genomic characterisation are required for adequate surveillance of circulating PRRSV type 1 strains in Europe.

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P.35**Characterization of ELISA negative sows in multiply MLV vaccination protocols.**I. Díaz^{1*}, B. Genís-Jorquera¹, G.E. Martín-Valls¹ and E. Mateu^{1,2}¹ Centre de Recerca en Sanitat Animal (CReSA), IRTA, Edifici CReSA, UAB, 08193 Bellaterra, Spain.² Departament de Sanitat i Anatomia Animals, Facultat Veterinària, UAB, 08193 Bellaterra, Spain.

In most breeding herds, sows are re-vaccinated periodically with the idea of maintaining immune memory against PRRS virus; e.g. vaccination schedules including blanket vaccination with modify live vaccines (MLV) every 3-4 months. Swine veterinarians sometimes report that a variable percentage of sows may become or remain negative by ELISA in spite of having received multiple vaccinations. At present, it is difficult to say if the presence of seronegative vaccinated sows could be related to the lack of compliance of vaccine administration, to factors related to the immunological response of some sows or, to a failure of the ELISAs for detecting those animals, among other causes. This study was aimed to preliminary assess the causes explaining the presence of seronegative sows in multiply vaccinated herds. The study was conducted in two farms (F1 and F2) with a history of seronegative vaccinated sows. In both farms, adaptation of gilts was done by deliberate exposure to wild-type PRRSV and afterwards, by recall immunization with a type 1 MLV administered in a blanket vaccination every third month (4 doses/year). In each farm, 60 females (sows and gilts distributed by parities) were sampled before a recall vaccination. Then, animals were vaccinated with the MLV vaccine as usually and were bled again 21 days later. Sera were analysed by three commercial ELISAs: ELISA PRRS X3 (E1) (Idexx Laboratories), CIVTEST suis PRRS E/S (E2) (Laboratorios Hipra) and, Ingezim PRRS Universal (E3) (Ingenasa). All samples yielding a negative result were re-tested. In the second sampling, animals testing negative in any of the ELISAs were bled again one week later to evaluate virus-specific IFN-gamma response in ELISPOT. In F1, the percentage of seronegative animals either before or after the vaccination was low and varied depending on the ELISA (3.3-10%; most of them from parity 6). One sow (parity 7) was negative in all the ELISAs both before and after vaccination, and that animal also tested negative in the IFN-gamma ELISPOT. In F2, depending on the ELISA 12 to 27/60 animals (20-45%) were seronegative before the vaccination of which 10 to 12/16 were gilts. After vaccination most gilts (75-93.8%) seroconverted, indicating that the initial exposure failed indeed. With E1, before vaccination 15 sows were negative and after vaccination 7 did not seroconvert; with E2 6 sows tested negative before vaccination and 9 were so after vaccination. With E3, all 13 negative sows before vaccination seroconverted after the recall dose. Regarding IFN-gamma ELISPOT results, specific responses were only demonstrated in 4 of the sows that tested simultaneously negative in E1 and E2. Taken together our results suggest that the existence of apparently seronegative vaccinated sows may be the result of the ELISA used but also in a small percentage of cases of the lack of response of the sow.

P.36

Vaccination of piglets at 2 weeks-of-age with Ingelvac PRRSFLEX® EU provides protection against heterologous PRRSV field challenge in the face of homologous maternally derived antibodies

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Due to the lack of efficient DIVA vaccines, successful control of porcine reproductive and respiratory syndrome (PRRS) requires strict management measures including vaccination. The marked genetic differences observed among various PRRSV isolates has a negative effect on MLV efficiency. MLV vaccination with the CP used in our study is reported to reduce clinical losses, but it did not prevent heterologous infection in a field infection trial.

The objective of the present work was to determine and compare the efficacy of a recently developed MLV vaccine (Ingelvac PRRSFLEX® EU) to a not vaccinated cohort (PBS) in 2 weeks old piglets born to sows mass-vaccinated with ReproCyc PRRS® EU and assess the possible interference with homologous maternally derived antibodies with vaccine efficacy.

The study was performed on a commercial Hungarian pig farm. A total of 475 piglets were either vaccinated at 2-weeks of age under the sow with Ingelvac PRRSFLEX® EU or vaccinated with a control product (PBS). The sow herd was previously vaccinated with ReproCyc PRRS® EU (homologous MLV strain with PRRSFLEX). All piglets were seropositive at time of vaccination due to maternally derived antibodies.

ORF5 sequence homology of the vaccine strain and the circulating field strain was determined to be 87.8%.

Serum samples were obtained from pre-selected and permanently marked animals (20%) throughout the study. PRRS specific real-time quantitative PCR was performed to measure both the viral load and the frequency of virus positive animals.

Low levels of viraemia and low proportion of viraemic animals (15%) have been observed among the vaccinated animals after vaccination.

No increase in viraemia was detected during naturally occurring field challenge in the Ingelvac PRRSFLEX® EU vaccinated group, and significant differences were found at peak of field challenge (2.23 for the PBS vs. 0.37 log/GE per ml for Ingelvac PRRSFLEX® EU; $p < 0.0001$). Consequently, no increase in the proportion of viraemic animals during peak of field challenge in case of the Ingelvac PRRSFLEX® EU group (46 [PBS] vs. 11%; $p = 0.0001$) was observed.

The present study aimed to assess the efficacy of a recently developed MLV vaccine (Ingelvac PRRSFLEX® EU) in piglets at 2-weeks of age in the presence of homologous maternally derived antibodies. At the time of the natural infection observed in the PBS group (10–12 WOA), surprisingly the number of viraemic animals did not increase in the vaccinated group. Our data indicate that large majority of piglets vaccinated at 2 WOA with Ingelvac PRRSFLEX® EU were protected both in terms of viraemia levels and proportion of viraemic animals. Our results are in contrast to previous studies where other MLV products were not able to prevent viraemia in a natural infection model. It has to be highlighted that results were achieved in piglets with high levels of homologous maternally derived antibodies at the time of vaccination.



P.37

REPROCYC PRRS® EU possessed an excellent safety profile in a breeding sow herd under field conditions

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Successful control of PRRS requires strict management measures including vaccination. The marked genetic differences observed among various PRRSV isolates has a negative effect on MLV efficiency, however the degree of similarity between the vaccine and the field virus in the herd cannot predict the degree of protection. MLV vaccination is reported to reduce clinical losses, but it cannot prevent heterologous infection. The objective of the present work was to determine the safety of a recently developed MLV vaccine (ReproCyc PRRS® EU) in a sow herd in comparison to a commercially available MLV vaccine.

The study was performed on a commercial Hungarian sow farm in a historical comparison design. All sows on the farm were vaccinated in a whole herd mass vaccination program with one vaccine, either with a commercially available PRRS vaccine or ReproCyc PRRS® EU (PRRS 94881).

Safety parameters were measured twice on the day of vaccination (one and four hours post vaccination) and daily for 14 days post vaccination. Injection site reactions were investigated for pain, redness, heat and swellings. Clinical observations were divided in four categories such as behavior, respiration, digestion and other.

Serology data were generated from pre-selected animals (10% of herd) throughout the study. Semi-quantitative analysis was based on IDEXX PRRS X3 ELISA results.

When comparing the local reactions at the sites of administration significantly higher percentage of injection site reactions were observed in case of the id. applied CP in terms of redness ($p \leq 0.0001$), heat ($p \leq 0.0059$) and swelling ($p \leq 0.0001$) when compared to the im. injected ReproCyc PRRS® EU.

Significantly higher percentages of systemic clinical signs were observed in terms of behavior (e.g. lethargy, ($p = 0.0011$)) after id. vaccination with the CP. No post vaccination fever was recorded in case of both vaccines.

Marked rise in the IDEXX ELISA-measured antibody levels was observed after vaccination with ReproCyc PRRS® EU. Antibody levels were $\sim 1.5 \times$ higher when compared to the CP.

The present study aimed to assess the safety of a recently developed MLV vaccine (ReproCyc PRRS® EU) in a breeding sow herd. Our data indicate that in case of both local and systemic reactions, the new vaccine candidate caused significantly less symptoms. It has to be noted however, that whatever reaction the id. administration might cause will always be easier to notice, especially under field conditions. On the other hand one might speculate that im. administration would result in more frequent systemic reactions, which was not the case in our experiment.

The rise in the antibody levels proved successful seroconversion of the animals, however the greater ELISA levels observed after vaccination with ReproCyc PRRS® EU does not necessarily refer to better protection.

P.38

Efficacy of intradermal vaccination with UNISTRRAIN® PRRS in piglets after a heterologous challenge at 24 weeks post-vaccination

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Objective

The aim of this study was to demonstrate that UNISTRRAIN® PRRS applied by intradermal route (ID) with a suitable device was as efficacious as applied with the conventional intramuscular injection (using needle and syringe) in piglets after a heterologous challenge at 24 weeks post-vaccination.

Materials and Methods

Thirty-seven 2-week-old piglets, clinically healthy and free from virus and antibodies against PRRS, were randomly assigned to three different groups: ID vaccinated group (n=11), intramuscularly (IM) vaccinated group (n=12) and control group (CTR; n=14). Animals in the ID group were intradermally immunised with UNISTRRAIN® PRRS (0.2 ml/dose; 103.5 CCID50/animal) applied with a suitable device. Animals in the IM group were intramuscularly immunised with UNISTRRAIN® PRRS (2 ml/dose; 103.5 CCID50/animal; applied with needle and syringe). Animals in the CTR group received 2 ml of PBS using the same strategy as the IM group. At 26 weeks of age, all piglets were challenged by intranasal route with a heterologous pathogenic strain of European genotype I of the PRRSV (89% ORF5 homology; 106.39 CCID50/animal). Animals were examined daily after challenge during the following 35 days. Virus detection was performed by real time RT-qPCR (at 2, 5, 8, 14, 21, 28 and 35 days post-challenge) and Area Under de Curve (AUC) of viraemia was calculated from the challenge to the end of the study. AUC and length of the viraemia were analysed using non-parametric Mann-Whitney U test ($p < 0.05$) and percentage of viraemic animals using two-tailed chi-square test/Fisher exact ($p < 0.05$).

Results

Vaccinated groups had a significantly lower vial load, as determined by AUC, when compared to non-vaccinated pigs (mean: ID: 5.1×10^1 and IM: 1.2×10^1 vs 3.6×10^4), the minimum (ID: 0.0×10^0 and IM: 0.0×10^0 vs 6.5×10^2 ; maximum: ID: 8.8×10^1 and IM: 1.8×10^2 vs. 1.2×10^5 ; median: ID: 0.0×10^0 and IM: 0.0×10^0 vs 3.1×10^4). In the vaccinated groups, it was also observed a significant reduction in the number of viraemic animals at: 5 days post challenge (dpc) (ID: 1/11; IM: 2/12 ; CTR: 10/14), 8 dpc (ID: 3/11; IM: 1/12; CTR: 13/14), 14 dpc (IM: 1/12; CTR: 7/14), at 21 dpc (ID: 0/11; IM: 0/12; CTR: 10/14), 28 dpc (ID: 0/11; IM: 1/12; CTR: 10/14) and 35 dpc (ID: 0/10; IM: 1/12; CTR: 9/14). Regarding the length of the viraemia after challenge, significant differences were observed in both vaccinated groups compared with control (ID: 4.7 ± 5.8 days; IM: 6.8 ± 12.3 days vs. CTR: 29.1 ± 9.3 days).

Conclusion: The results obtained allow to conclude that, vaccination with UNISTRRAIN® PRRS ID with a suitable device in piglets, had a comparable effect on the fast clearance of the virus to the IM traditional syringe and needle administration at 24 weeks post-vaccination. UNISTRRAIN® PRRS administered ID or IM seems to be a useful tool to decrease viraemia and thus a reduction of the infection pressure of PRRSV in an infected farm.

P.39

Efficacy of UNISTRAIN[®] PRRS administered intradermally in piglets in a multicentric field trial

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Objectives

The aim of this study was to evaluate the efficacy of UNISTRAIN[®] PRRS administered intradermally (ID) to piglets in front of a PRRS outbreak under field conditions.

Material and Methods

A multicentric, randomized and double blinded trial was carried out in 1532 animals (piglets) distributed in 3 commercial farms (farms n° 1, 2 and 3) in Spain with previous history of outbreaks of PRRS. The animals in each farm were randomly divided into two treatment groups. Group A (n=693) was vaccinated once at 3-4 weeks of age (day 0; d0) with 0.2 ml of UNISTRAIN[®] PRRS ($10^{3.5}$ CCID₅₀/animal) administered ID with a suitable device. Group B (n=839) was injected with 0.2 ml PBS also ID with the same device and left as the control group.

All animals included in the trial were individually identified by means of two ear tags. An outbreak was considered when at least 10% of pigs showed respiratory symptoms. To assess the efficacy of the vaccine when a PRRS virus outbreak occurred, viraemia and lung lesions among positive at PRRSV (detection of virus by RT-qPCR), clinical respiratory signs, mortality, body weight and antibiotics administered were registered from all the animals (from d0 up to slaughterhouse). Mann-Whitney U test and a chi-square test were used ($p < 0.05$).

Results and conclusions

A clinical PRRS outbreak was confirmed in two farms (outbreak beginning farm n° 1: day 76 with a PRRSV 95% ORF5 homology; outbreak beginning farm n°3: day 148 with a PRRSV 88% ORF5 homology) and thus, just the results of these two farms will be presented. Statistical differences were observed in farms n°1 and 3 between the vaccinated and the control group in terms of: **control of the viraemia** around and during the outbreak (farm n°1: d45 and d90; farm n°3: d90, d120 and d150); **clinical index assessment** during the outbreak (farm n°1: d76, d78, d82 and mean; farm n°3: d152 and mean); **mortality** at fattening period (farm n°1: 5.55% group A vs 10.10% group B; farm n°3: 1.92% group A vs 6.41% group B); percentage with lung lesions among RT-qPCR PRRSV positive animals (farm n°1: 33.3% group A vs 63.9% group B; farm n°3: 33.3% group A vs 90.0% group B) and **average daily weight gain** (kg) from weaning up to slaughterhouse (farm n°1: 0.61 ± 0.08 group A vs 0.57 ± 0.09 group B; farm n°3: 0.55 ± 0.09 group A vs 0.51 ± 0.11 group B). In farm n°3, it was also observed a significant decrease on the percentage of **animals treated (on the fattening period) with antibiotics** (3.2% group A vs 9.6% group B). The results obtained allow to conclude that, UNISTRAIN[®] PRRS administered ID with a suitable device, is efficacious when administered according to the recommended vaccination program and it is a useful tool to reduce viraemia and the negative clinical and productive consequences due to PRRSV infection in the field.

P.40

Safety of UNISTRRAIN[®] PRRS administered intradermally in piglets in a multicentric field trial

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Objectives

The aim of this study was to evaluate the safety of UNISTRRAIN[®] PRRS administered intradermally (ID) to piglets under field conditions.

Material and Methods

A multicentric, randomized, double blinded and controlled trial was carried out in 1532 animals (piglets from 3-4 weeks of age) distributed in 3 commercial farms (farms n° 1, 2 and 3) in Spain with previous history of PRRS outbreaks. The animals in each farm were randomly divided into two treatment groups. Group A (n=693) was vaccinated once at 3-4 weeks of age (day 0; d0) with 0.2 ml of UNISTRRAIN[®] PRRS ($10^{3.5}$ CCID₅₀/animal; strain) administered ID with a suitable device. Group B (n=839) was injected with 0.2 ml of PBS also ID with the same device and left as the control group. All animals included in the trial were individually identified by means of two ear tags. Rectal temperature (d0, d0+4h, d1, d2), general signs (d0, d0+4h, d1, d2, d3), local reactions at the inoculation site (d0, d0+4h, d1, d2, d3; inflammation: absence, slight, moderate, severe; redness: absence, presence; nodules: absence, presence) and adverse events (from d0 up to slaughterhouse) were registered from all the animals included in the group A compared with group B to assess the safety of the UNISTRRAIN[®] PRRS vaccine administered ID. Chi-square test and ANOVA test were used ($p < 0.05$).

Results

No serious or unexpected adverse events (death or anaphylactic shock) attributable to the vaccination with UNISTRRAIN[®] PRRS ID were observed. Likewise, no post-vaccination general clinical signs attributable to vaccination with UNISTRRAIN[®] PRRS ID were observed. No significant differences were observed on the increase on rectal temperature after vaccination (throughout the different monitoring days) between the UNISTRRAIN[®] PRRS vaccinated animals and control group (group A d0+4h: 0.20 ± 0.45 ; d1: -0.06 ± 0.46 ; d2: 0.04 ± 0.50 ; group B: d0+4h: 0.20 ± 0.44 ; d1: -0.01 ± 0.46 ; d2: -0.07 ± 0.44). Regarding local reactions observed after vaccination, a slight to moderate inflammation could be observed in group A which was resolved within 2 days post-vaccination (group A d0+4h: 50% absence, 27.7% slight, 22.2% moderate; d1: 75.5% absence, 22.2% slight, 2.2% moderate; d2: 87.7% absence, 12.2% slight; d3; 100% absence). Redness was also observed at the inflammation area of some animals (group A d0+4h: 68.9% absence, 31.3% presence; d1: 80% absence, 20% presence; d2: 94.4% absence, 5.6% presence; d3; 100% absence; group B: d0+4h: 95.5% absence, 4.4% presence; d1: 98.9% absence, 1.1% presence; d2: 100% absence; d3; 100% absence). No nodules were observed in any of the animals throughout the different monitoring days. In all cases, reactions spontaneously resolved three days post-vaccination without treatment.

Conclusions

The results obtained allow to conclude that, vaccination with UNISTRRAIN[®] PRRS, is safe when administered in piglets by intradermal route with a suitable device according to the recommended vaccination program.



P.41

Vaccination with UNISTRRAIN® PRRS in piglets reduces excretion of PRRSV after a heterologous challenge with a UK strain

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Introduction: Infected pigs with PRRSV can shed the virus through multiple routes and for a long time. The amount of virus shed, together with the duration of the shedding contributes to the spread of PRRSV within and between pig farms. Vaccines contribute to the control of PRRS infection by reducing the transmission of the virus. The aim of the present study was to demonstrate that vaccination of piglets with UNISTRRAIN® PRRS reduces virus shedding in saliva after a heterologous challenge.

Materials and Methods: Seventy 3-week-old piglets, clinically healthy and free from virus and antibodies against PRRS were randomly assigned to vaccinated group (n=35) and control group (n=35). Animals in vaccinated group were intramuscularly immunised with UNISTRRAIN® PRRS (2ml/dose; $10^{3.5}$ CCID₅₀/animal) and animals in control group received 2 ml of PBS using the same strategy as vaccinated group. At 8 weeks of age, all piglets were intranasally challenged with a heterologous pathogenic genotype I PRRSV strain (isolated in UK in 2011; 88 % ORF5 homology; $10^{7.7}$ CCID₅₀/animal). Saliva swabs were collected at 2, 5, 7, 14, 19, 21, 28 and 34 days after challenge. Virus detection and virus titration in saliva were analysed by real time RT-qPCR. Virus titre and length of the excretion were analysed using non-parametric Mann-Whitney U test ($p < 0.05$) and percentage of animals excreting virus using two-tailed chi-square test/Fisher exact ($p < 0.05$).

Results: The percentage of shedding piglets was statistically reduced in the vaccinated group at 5 dpc (18/35 vs. 34/34), at 7 dpc (19/35 vs. 28/34), at 14 dpc (11/35 vs. 31/34), at 19 dpc (2/35 vs. 9/34 and at 21 dpc (12/35 vs. 21/34). Vaccinated group had a significantly lower viral load in saliva (CCID₅₀/ml) when compared to non-vaccinated pigs at 5 days post challenge (dpc) (2.2×10^1 CCID₅₀/ml vs. 3.3×10^1), 7 dpc (1.2×10^0 vs. 1.8×10^0), 14 dpc (2.9×10^0 vs. 5×10^0), 19 dpc (5.7×10^{-2} vs. 2.6×10^{-1}) and 21 dpc (4.4×10^{-1} vs. 1.8×10^0). Furthermore, the length of virus excretion after challenge was also statistically lower in vaccinated group (12.9 ± 8.8 days vs. 20.1 ± 4.9).

Conclusion: Vaccination with UNISTRRAIN® PRRS significantly reduced the amount of virus excreted, the percentage of piglets excreting the virus and also the duration of viral excretion in saliva after a heterologous PRRS challenge with a pathogenic UK strain. Therefore, UNISTRRAIN® PRRS is a useful tool in order to reduce the transmission of PRRS virus within and between pig populations.

P.42

Vaccination with UNISTRRAIN® PRRS in piglets reduces viraemia of PRRSV after a heterologous challenge with a UK strain

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Introduction: Prolonged viraemia and persistent infection after a challenge increase the possibility of transmission of PRRS virus. Therefore, the control of the viraemia is essential to control PRRS disease. The aim of this study was to demonstrate that piglets vaccinated with UNISTRRAIN® PRRS control better viraemia than non-vaccinated piglets after a heterologous challenge.

Materials and Methods: Seventy 3-week-old piglets, clinically healthy and free from virus and antibodies against PRRS were randomly assigned to vaccinated group (n=35) and control group (n=35). Animals in vaccinated group were intramuscularly immunised with UNISTRRAIN® PRRS (2ml/dose; $10^{3.5}$ CCID₅₀/animal) and animals in control group received 2 ml of PBS using the same strategy as vaccinated group. At 8 weeks of age, all piglets were challenged by intranasal route with a heterologous pathogenic genotype I PRRSV strain (isolated in UK in 2011; 88 % ORF5 homology; $10^{7.7}$ CCID₅₀/animal). Animals were examined daily after challenge until 13 weeks of age. At 5, 7, 14, 19, 21, 28 and 34 days post challenge (dpc) virus detection and quantification from sera samples was performed by real time RT-qPCR. Area Under the Curve (AUC) of virus titre was calculated from all the post-challenge period. AUC and length of the viraemia were analysed using non-parametric Mann-Whitney U test ($p<0.05$) and percentage of viraemic animals using two-tailed chi-square test/Fisher exact ($p<0.05$).

Results: The percentage of viraemic animals was statistically reduced in the vaccinated group compared to control group at 14 days post challenge (dpc) (29/35 vs. 34/34), at 19 dpc (23/35 vs. 34/34), at 21 dpc (25/35 vs 31/34) and at 34 dpc (7/34 vs. 22/34). Vaccinated group had a significantly lower viral load (CCID₅₀/ml), as determined by AUC, when compared to non-vaccinated pigs (median: 2.3×10^3 vs. 1.5×10^4). Regarding the length of the viraemia after challenge significant differences were observed (26.2 ± 6.1 days in vaccinated group vs. 30.9 ± 4.8 in control group).

Conclusion: Vaccination with UNISTRRAIN® PRRS reduced significantly the viral load, the percentage of viraemic animals and the length of the viraemia after challenge with a heterologous pathogenic UK strain. Therefore, UNISTRRAIN® PRRS is useful to reduce the infection pressure of an infected farm.

P.43

Vaccination with UNISTRRAIN® PRRS in piglets reduces excretion of PRRSV after a heterologous challenge with a Spanish strain

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Introduction: Infected pigs with PRRSV can shed the virus through multiple routes and for a long time. The amount of virus shed; together with its duration are the first steps to the spread of the virus within and between pig farms. Vaccines contribute to the control of PRRS infection by reducing the transmission of the virus. The aim of the present study was to demonstrate that vaccinated piglets with UNISTRRAIN® PRRS reduce the excretion of PRRSV after a heterologous challenge.

Materials and Methods: Thirty two 4-week-old piglets, clinically healthy and free from virus and antibodies against PRRS were randomly assigned to vaccinated group (n=16) and control group (n=16). Animals in vaccinated group were intramuscularly immunised with UNISTRRAIN® PRRS (2ml/dose; $10^{3.5}$ CCID₅₀/animal) and animals in control group received 2 ml of PBS using the same strategy as vaccinated group. At 8 weeks of age, all piglets were challenged by intranasal route with a heterologous pathogenic genotype I PRRSV strain (Spanish isolated at 2005; 92 % ORF5 homology to the vaccine strain; 10^5 CCID₅₀/animal). Animals were examined daily after challenge until 14 weeks of life. Virus detection and virus titration in saliva were analysed by real time RT-qPCR. Virus titre and length of the excretion were analysed using non-parametric Mann-Whitney U test ($p<0.05$) and percentage of animals excreting virus using two-tailed chi-square test/Fisher exact ($p<0.05$).

Results: The percentage of animals excreting virus was statistically reduced at 2 days post challenge (dpc) in the vaccinated group (1/16 vs. 15/16 in control group). Also, vaccinated group had a significantly lower viral load (CCID₅₀/ml) in saliva when compared to non-vaccinated pigs at 2 dpc (6.56×10^{-2} vs. 2.49×10^0 in control group). Although there was not significant differences, virus saliva load was reduced at 7, 10, 14, 21 and 42 dpc among vaccinated animals... Regarding the length of the salivary excretion after challenge significant differences were observed (12.9 ± 8.8 days in vaccinated group vs. 20.1 ± 4.9 in control group).

Conclusion: Vaccination with UNISTRRAIN® PRRS significantly reduced the percentage of animals excreting, the amount of virus excreted, and the duration of the viral excretion in saliva after a heterologous PRRS challenge with a pathogenic Spanish strain. Therefore, UNISTRRAIN® PRRS is a useful tool in order to decrease the transmission of PRRS virus within and between pig populations.



P.44

Vaccination with UNISTRRAIN[®] PRRS in piglets reduces viraemia of PRRSV after a heterologous challenge with a Spanish strain

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Introduction: Prolonged viraemia and persistent infection after a challenge increase the possibility of transmission of PRRS virus. Therefore, the control of the viraemia is essential to control PRRS disease. The aim of this study was to demonstrate that piglets vaccinated with UNISTRRAIN[®] PRRS control better viraemia than non-vaccinated piglets after a heterologous challenge.

Materials and Methods: Thirty two 4-week-old piglets, clinically healthy and free from virus and antibodies against PRRS were randomly assigned to vaccinated group (n=16) and control group (n=16). Animals in vaccinated group were intramuscularly immunised with UNISTRRAIN[®] PRRS (2ml/dose; $10^{3.5}$ CCID₅₀/animal) and animals in control group received 2 ml of PBS using the same strategy as vaccinated group. At 8 weeks of age, all piglets were challenged by intranasal route with a heterologous pathogenic genotype I PRRSV strain (Spanish isolated at 2005; 92 % ORF5 homology; 10^5 CCID₅₀/animal). Animals were examined daily after challenge until 14 weeks of age. At 2, 5, 7, 10, 14, 21, 28, 35 and 42 days post challenge (dpc) virus detection and quantification from sera samples was performed by real time RT-qPCR. Area Under de Curve (AUC) of virus titre was calculated from all the post-challenge period. AUC and length of the viraemia were analysed using non-parametric Mann-Whitney U test ($p < 0.05$) and percentage of viraemic animals using two-tailed chi-square test/Fisher exact ($p < 0.05$).

Results: The percentage of viraemic animals was statistically reduced in the vaccinated group compared to control group at 7 dpc (5/16 vs. 15/16), at 10 dpc (5/16 vs. 16/16), at 14 dpc (4/16 vs 16/16), at 21 dpc (1/16 vs. 16/16) and at 28 dpc (1/16 vs. 10/16). Vaccinated group had a significantly lower viral load (CCID₅₀/ml), as determined by AUC, when compared to non-vaccinated pigs (median: 5.3×10^0 vs. 3.33×10^3). Regarding the length of the viraemia after challenge significant differences were observed (8 ± 9 days in vaccinated group vs. 31 ± 8 days in control group).

Conclusion: Vaccination with UNISTRRAIN[®] PRRS reduced significantly the viral load, the percentage of viraemic animals and the length of the viraemia after challenge with a heterologous pathogenic Spanish strain. Therefore, UNISTRRAIN[®] PRRS is useful to reduce the infection pressure of an infected farm.

P.45

Efficacy of UNISTRRAIN[®] PRRS in front of a PRRS outbreak in a European farm

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Objectives: The aim of this field trial was to demonstrate under field conditions the efficacy of UNISTRRAIN[®] PRRS in the control of negative consequences due to PRRS outbreak.

Material and Methods: A randomized, double blinded and controlled field trial was carried out in a problematic PRRS farm with 711 piglets from 3-4 weeks of age at vaccination. The animals were randomly divided into two treatment groups: vaccinated group (n=357) received 2ml of UNISTRRAIN[®] PRRS by intramuscular route and control group (n=354) was injected with 2ml of PBS. An outbreak was considered when at least 10% of pigs showed respiratory symptoms. To assess the efficacy of the vaccine different parameters were registered: clinical respiratory signs, animals with lung lesions and positive to PRRSV (RT-PCR), average daily weight gain (ADWG) from weaning up to PRRS outbreak (kg) and percentage of animals treated with antibiotics were registered. Chi-square test and a Mann-Whitney U test were used for the statistical analysis.

Results: A clinical PRRS outbreak appeared in animals at 140-150 days of age. The outbreak was confirmed by PCR and serology. The strain isolated from the outbreak had a homology with the vaccine strain of 88 %. Signs observed were tachypnoea and dyspnoea at rest, with cough and nasal secretions in some animals and moderate to severe depression. The overall percentage of animals with clinical respiratory signs associated to PRRS during the outbreak was statistically lower in the vaccinated group (10.9 % vs. 18.2 %). Moreover, percentage with lung lesions among RT-PCR PRRSV positive animals were lower in the vaccinated group than in the control one (26.6 % vs. 31.2 %). The mean ADWG (kg) was statistically higher in the vaccinated group (0.57±0.08 vs. 0.54±0.08 in control group). The percentage of treated animals was statistically lower in the vaccinated group (9.5 % vs. 14.4 %).

Conclusions: Vaccination was demonstrated to be a useful tool to reduce clinical respiratory signs associated to PRRS, reduce animals with lung lesions and positive to PRRSV, and reduce the percentage of antibiotic treated animals. Also, vaccination improved ADWG of the piglets after a PRRS outbreak. So, UNISTRRAIN[®] PRRS is efficacious in piglets and it is a useful tool to reduce the negative clinical and productive consequences due to a PRRS outbreak.



Poster

6. PRRS VACCINES

P.46

Efficacy of UNISTRAIN® PRRS in farms with previous outbreaks of PRRS in a multicentric field trial

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Objectives: The aim of this study was to evaluate under field conditions the efficacy of UNISTRAIN® PRRS by IM route in piglets from farms with history of PRRS outbreaks and current virus circulation.

Material and Methods: A multicentric, randomized, double blinded and controlled trial was carried out in 2.037 piglets from 3-4 weeks of age at vaccination. The trial took place in 3 commercial farms (farms n° 1, 2 and 3) in Spain with previous PRRSV outbreaks and virus current virus circulation. The animals in each farm were randomly divided into two treatment groups: vaccinated group (n=1.057) received 2ml of UNISTRAIN® PRRS by intramuscular route and control group (n=980) was injected (IM) with 2ml of PBS. To assess the efficacy of the vaccine, different parameters were evaluated: viraemia (RT-PCR), percentage of mortality, percentage of animals treated with antibiotics and finally, animals with lung lesions positive to PRRSV (RT-PCR). Chi-square test and a Mann-Whitney U test were used (p<0.05).

Results: The percentage of viraemic animals in the vaccinated group was statistically lower than in the control group after entry into fattening unit at 45 days post vaccination (dpv) (in farms 2 and 3) and at 90 dpv (in farm 1, 2 and 3). In all three farms the percentage of mortality was lower in the vaccinated group compared to the control group being statistically different in farm n° 1 (1.02 % vs. 4.8 %). The percentage of animals treated with antibiotics was lower in the vaccinated groups (farm 1: 5.6 % vs. 9.2 %; farm 2: 9.3 % vs. 13.9 %; farm 3: non statistical differences). Moreover, percentage with lung lesions among RT-PCR PRRSV positive animals was statistically lower in the vaccinated group in two farms (farm 1: 0 % vs. 54.5 %; farm 2: 0 % vs. 28.6 %; farm 3: non statistical differences).

Conclusion: Vaccination with UNISTRAIN® PRRS was demonstrated as a useful tool to reduce mortality, animals with lung lesions positive to PRRSV, number of viraemic animals and the percentage of animals treated with antibiotics in farms with previous history of PRRS outbreaks. UNISTRAIN® PRRS is efficacious in piglets and it is a useful tool to reduce the negative clinical and productive consequences due to PRRSV infection in the field.

P.47

Assessment of safety of a modified live-virus PRRS genotype 1 vaccine in pregnant sows at various stages of gestation.

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes significant economic impact on the swine industry worldwide. PRRSV vaccination has been demonstrated as an effective tool to control clinical signs related to PRRSV infection. The aim of the present study was to evaluate field safety of a new PRRS genotype 1 modified live virus vaccine in sows and gilts at various stages of gestation.

Materials and methods

The study was conducted in a piglet producing farm with 660 sows. On study day -1 (D-1) a total of 505 sows were randomly allocated to group 1 (n=235) and group 2 (n=270). At the time of inclusion, 10% of sows and gilts from each study group were randomly assigned as sample animals. The study groups were housed in separated barns with separate air spaces. On study day 0 sows of group 1 were vaccinated with 2mL of the Control product (CP), a commercial modified live PRRSV vaccine. Sows of group 2 were administered intramuscularly with 2 mL of the Investigational Veterinary product (IVP), a modified live-virus PRRS genotype 1 vaccine (PRRS 94881 MLV). An individual examination for clinical signs was performed daily, starting the day before vaccination till D14. Clinical observation score included an assessment of behavior, respiratory signs and digestion. Additionally rectal temperatures were measured from sample animals on D-1, D0+1h, +4h to D14. Local reactions at the injection site were investigated from sample animals on study day 0, 1h and 4h post vaccination and subsequently daily till 14 days post vaccination. Injection sites were examined for redness, swelling, heat and pain during palpation. Blood samples were collected from sample animals on D-1, D14, D28, D84 and D119 to determine viremia by qRT-PCR.

Results

No differences were detected between groups for clinical signs behavior and respiration. In contrast, y-treatment ranged from 38.0 °C to 38.9°C and from 37.9°C to 38.7°C for the CP and IVP groups, respectively. No significant differences between groups were detected for rectal temperatures. Injection site reaction parameter pain (p= 0.039), redness (p= 0.030), heat (p= 0.016) and swelling (p= 0.002) were significantly less frequent in the IVP group compared to the CP group. The average duration of local reactions was 5.7 and 2.8 days in the CP and IVP groups, respectively. The average duration of local reactions was 5.7 days in the CP and 2.8 days in the IVP group. The average swelling size at the injection site was 4.4 cm and 2.5 cm for the CP and IVP groups, respectively. The maximum swelling size was reported with 20 cm in the CP group (severe score) and 8 cm in the IVP group (moderate score).). All serum samples from sample animals were negative for the detection of PRRSV by qPCR at all scheduled time points.

Discussion

Local or systemic reactions were less frequently observed in pigs receiving the IVP than in those receiving a registered PRRS vaccine, therefore supporting the field safety of PRRS 94881 MLV in sows and gilts at various stages of gestation.

P.48

Reproductive performance after vaccination with a modified live-virus PRRS genotype 1 vaccine in pregnant sows at various stages of gestation.

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is considered to be one of the major pathogens in pigs and causes significant economic impact on the swine industry worldwide. PRRSV vaccination has been demonstrated as an effective tool to control clinical signs related to PRRSV infection. The aim of the present study was to evaluate field efficacy of a new PRRS genotype 1 modified live virus vaccine in sows and gilts at various stages of gestation

Materials and methods

The study was conducted in a piglet producing farm with 660 sows. On study day -1 (D-1) a total of 505 sows and gilts were randomly allocated to group 1 (n=235) and group 2 (n=270). At the time of inclusion, 10% of sows and gilts from each study group were randomly assigned as sample animals. The study groups were housed in separated barns with separate air spaces. On study day 0 sows of group 1 were vaccinated with 2mL of the Control product (CP), a commercial modified live PRRSV vaccine. Sows of group 2 were administered intramuscularly with 2 mL of the Investigational Veterinary product (IVP), a modified live-virus PRRS Genotype 1 vaccine (PRRS 94881 MLV). To assess the efficacy of the IVP reproductive performance parameters were recorded from all sows/gilts till D119. The number of piglets alive per litter at weaning was selected as the primary criterion for the evaluation of vaccine efficacy. Farrowing performance (healthy live born piglets, weak born piglets, stillborns, mummies and crushed piglets per litter), abortion rate and returned to estrus were selected as secondary parameters. To evaluate piglet performance, piglets from sample animals were ear tagged and body weight was recorded at farrowing and at weaning. Piglets from sample animals were blood sampled at weaning to determine viremia by using an in-house quantitative real-time PCR assay (qRT-PCR).

Results

The primary parameter, mean total number of piglets alive at weaning did not differ significantly between group 1 and group 2, but the mean percentages of mortalities per litter till weaning was significantly higher in the CP group (14.1%) than in the IVP group (10.9%) respectively (p= 0.005). Examination of farrowing performance data for both groups resulted in no statistical differences between groups for percentage or number of alive, healthy live, weak live, stillborns, mummified, and crushed piglets per litter at farrowing. Abortion and return to estrus rate did also not differ significantly between both groups. No differences were detected between both groups for mean body weights on the day of farrowing, however, mean body weights at weaning (p= 0.026) and average daily gain (p= 0.037) were significantly higher in the IVP group compared to the CP group. The mean proportion of viremic piglets per litter at weaning did not differ significantly between both groups.

Discussion

Reproductive performance, survival rate, and piglet growth performance observed in the present study support the efficacy of PRRS 94881 MLV at various stages of gestation.

P.49**A porcine reproductive and respiratory syndrome virus vaccine based on the synthetic attenuated virus engineering approach is attenuated and effective in protecting pigs against macroscopic lung lesions associated with homologous virus challenge**D. Evenson¹, P.F. Gerber², C.T. Xiao¹, P.G. Halbur¹, D. Tian³, Y.Y. Ni⁴, X.J. Meng³, T. Opriessnig^{1,2*}¹Iowa State University, Ames, Iowa, USA²University of Edinburgh, Midlothian, UK³Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

Current porcine reproductive and respiratory syndrome virus (PRRSV) vaccines are often minimally protective. Rapid production of attenuated farm-specific vaccines is a feasible alternative to utilizing commercial vaccines. Recently the synthetic attenuated virus engineering (SAVE) approach was utilized to rapidly attenuate the wild-type PRRSV isolate VR2385. Specifically, the codon-pairs of the major envelope GP5 gene of PRRSV were rapidly deoptimized through a computer algorithm which resulted in a modified GP5 nucleotide sequence while at the same time retaining the original amino acid sequence. The resulting virus was designated SAVE5.¹ The objective of this study was to determine the efficacy of SAVE5 to reduce or prevent PRRSV-associated clinical signs, lesions and viremia following experimental challenge with the homologous virulent parental strain. Four groups of 9-10 three-week-old pigs were utilized. At day 0, two groups were vaccinated with the SAVE5 virus which is an attenuated version of PRRSV strain VR2385, while the two other groups were sham-vaccinated with saline. At day 42, the SAVE-5 and sham-vaccinated groups were challenged with PRRSV VR2385, and the experiment was terminated at day 54. Blood samples were collected on a regular basis for determining presence and amount of PRRSV RNA and PRRSV antibody levels. At necropsy tissues were collected and macroscopic and microscopic lesions were evaluated and compared across groups. The model to analyse continuous data collected over time was a repeated measures analysis of variance. A p-value of less than 0.05 was considered significant.

SAVE5 was effectively attenuated as evidenced by a low magnitude of viremia and lack of nasal shedding of SAVE5 vaccine virus in any of the pigs. However, by day 42 only 40% of the vaccinated pigs had detectable anti-PRRSV IgG. After challenge, clinical signs, microscopic lesions, virus shedding and PRRSV viremia were not different between vaccinated and unvaccinated groups; however, vaccinated pigs (14.9 ± 3.0) had significantly reduced macroscopic lung lesions compared to unvaccinated pigs (37.7 ± 5.6). Under the study conditions, the SAVE approach was successful in attenuating a PRRSV isolate and was also successful in reducing macroscopic lung lesions after homologous challenge compared to unvaccinated pigs. Although the results are encouraging, additional work needs to be done to further improve SAVE5 vaccine efficacy including testing other administration routes in growing pigs and testing the vaccine in pregnant sows.

P.50

The future of PRRSV vaccination: boosting with Progressis® to capitalize on pre-existing immunity, the innovation towards stronger protection.

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Introduction: PRRSv is endemic in swine producing countries worldwide and causes important losses due to reproductive and respiratory disorders. Specifically, the variability and the immune evading properties of the virus are leading to difficulties in its control. Inducing a strong immunity against PRRSv through vaccination remains a challenge, and therefore innovative approaches are required to further reduce clinical problems linked to PRRSv. MERIAL introduced an innovative vaccination strategy, capitalizing on an existing and widely applied technology, which creates new opportunities for the induction of protective immune responses in pigs with prior exposure to PRRSv.

Background: The origin of this vaccination strategy goes back to the successful track record of the technology in various fields of both human and veterinary vaccinology. The principle is based on the fact that different types of vaccines with the same virus or antigen show different antigenic epitopes and may induce a different immune response. It is suggested that prime-boost can be done with different types of vaccines containing the same antigens. In many cases such heterologous prime-boost can be more immunogenic than homologous prime-boost¹. The booster injection completed with a different vaccine may also limit the partial interference of neutralizing antibodies induced by the priming vaccine. This concept is well known and frequently used, e.g. in humans for influenza, where higher serological responses are obtained in persons primed with an experimental live vaccine before vaccination with an inactivated vaccine, compared to vaccination of naïve individuals². In animal vaccinology, the same principle is already described in cattle³. It is suggested that these prime/boost vaccination schedules generally result in a broader and more long lasting immunity, especially with increased cellular immunity and potentially against hidden epitopes difficult to immunize against with conventional vaccination procedures.

Results: In pigs, the additional value of the prime boost concept with inactivated vaccines has been described experimentally^{4,5} and was later on confirmed by field observations. In the first report⁶, the implementation of the immunity boost with Progressis at D90 of pregnancy in dams previously vaccinated with MLV, reduced the number of piglets that seroconverted from natural infection at 10 weeks of age from 10/15 to 1/20. In another report⁷, the PRRSv circulation in the herd was stabilized after one year of applying Progressis sow vaccination at D90 in combination with MLV vaccination at D60, and piglet mortality in the nursery room decreased from 4.5 to 2%. In two other reports^{8,9}, the production parameters in the nursery period significantly improved after introduction of Progressis vaccination in addition to sow MLV vaccination.

Conclusion: These results clearly show the benefit of the inactivated vaccine to broaden the immunity and to capitalize on a pre-existing immunity, either acquired by natural infection or by vaccination, both on immunological and on production parameters.

References: ¹Shan Lu (2004). *Curr Opin Immunol*; ²Talaat *et al.* (2014) *J Infect Dis*; ³Reber *et al.* 2006 *Comp Immunol Microbiol Infect Dis*; ⁴Diaz *et al.* (2013) *Vet Journal*; ⁵Nilubol *et al.* (2004) *Vet. Microbiol*; ⁶Dekens *et al.* (2013) *Proc. 5th ESPHM*; ⁷Defoort *et al.* (2014) *Proc. 23rd IPVS*; ⁸Knockaert *et al.* (2015) *Proc. 7th ESPHM*; ⁹Willems *et al.* (2015). *Proc. 7th ESPHM*

P.51

Case report: Controlling highly pathogenic PRRSv infection with attenuated classic PRRSv strain vaccine in a farrow to finish farm in China

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Highly pathogenic PRRS (hpPRRS) has caused enormous economic losses in China's pig industry since its first outbreak in 2006. Now, there are at least 6 strains of PRRS live vaccine for Chinese pig farmers to use, which initially played a positive effect on the prevention and control of PRRS but in recent years there still are many outbreaks in vaccinated pig farms. The objective of this paper is to report a case in a pig farm of China which controlled hpPRRS successfully with attenuated classical PRRSv strain vaccine (Ingelvac® PRRS MLV).

This case involved a 500 sow farm with farrow-to-finish production style. Breeding pigs were mass vaccinated with hpPRRS live vaccine (TJM-F92 strain) 4 times per year. Piglets were vaccinated once with same vaccine at 15 days. Production performance of this pig farm was good and stable for almost 2 years. However, nursery piglets (30-60 days) suffered from respiratory signs starting in April 2014 and lasting for 4 months. The clinical signs for piglets included anorexia and depression, periocular edema and conjunctivitis, back leg joint swelling. 55 day old pigs were in the peak incidence with mortality rate up to 15% (average of almost 10%).

Tissue samples collected from infected pigs were sent for laboratory diagnosis in July 2014. The results showed that those pigs were infected with hpPRRS. Based on the diagnostic results, in August 2014 the following measures were implemented to control PRRS.

- 1) Sows and boars were mass vaccinated twice with Ingelvac® PRRS MLV 30 days apart, and then vaccinated 4 times per year.
- 2) All piglets older than 14 (farrowing and nursery house) were vaccinated with Ingelvac® PRRS MLV, and newly born piglets were vaccinated at 14 days.
- 3) Medication and some herbs were added to the nursery feed for 7 days to reduce co-infection. Meanwhile biosecurity and production managements were strengthened, including temporarily stopping the introduction of replacement gilts, isolation and culling out of sick pigs, strict pig all in all out.

Three months after implementing those measures, pig production performance of the farm was stabilized. The numbers of weaned piglets per litter were improved by 2 piglets and the survival rate of nursery piglets by more than 4% in average.

In this case report, hpPRRS was successfully controlled by applying attenuated PRRS vaccine Ingelvac® PRRS MLV. Furthermore, the continuous flow single site production of this farm also was a disadvantage for the PRRS control.

Among the existing PRRS vaccines in China, Ingelvac® PRRS MLV vaccine provides protection against different strains, which was in line with other field observations and published papers. The key to controlling PRRS was an appropriate vaccine and good basic herd immunity, biosecurity and production management to complement it.

P.52**Preliminary results on the behavioural response of sows to intradermic vaccination**Déborah Temple¹, Marta Jimenez², Rika Jolie³, Marta Amat¹, Eva Mainau¹, Xavier Manteca¹¹ School of Veterinary Science, UAB, Spain ² MSD AH, Spain ³ MSD AH, NJ, USA³

In commercial pig production, sows are often vaccinated against PRRS several times per gestation period which can result in acute and chronic fear due to the painful procedure. This preliminary experiment investigated whether intradermal vaccination (IDAL) reduces sow's fear reaction to the vaccination procedure and has an effect on resting pattern and general activity compared to the traditional vaccination intramuscularly. Two treatments (IDAL and Traditional) were performed with 6 replicate pens of gestating sows (14 sows per pen), using the vaccine Porcilis PRRS® (MLV European strain). Behavioural indicators of fear or pain at the time of injection (high pitch vocalizations, retreat attempts, turning back, changes in activity) were recorded at individual level. Resting pattern and general activity were recorded the day before and after the vaccination by means of scan samplings and analyzed by means of non-parametric GEE models using the GENMOD procedure. The reactivity of each individual sow towards a person present in the pen was evaluated using the fear to human test validated by the Welfare Quality® for sows. The frequency of sows exhibiting an acute fear (or pain) response at the time of injection was significantly lower in the IDAL sows for the four indicators studied (high pitch vocalizations, IDAL=15.4% vs. Traditional=95.6%, $\chi^2=56$, $p<0.0001$; retreat attempts, IDAL=2.6% vs. Traditional=56.5%, $\chi^2=28$, $p<0.0001$; turning back, IDAL=5.1% vs. Traditional=69.6%, $\chi^2=36$, $p<0.0001$; change in behaviour, IDAL=18% vs. Traditional=95.6%, $\chi^2=53$, $p<0.001$). Sows from the traditional vaccination treatment decreased ($p=0.039$) activity the day after vaccination compared to IDAL sows. No significant difference was observed for the other resting patterns. Fearful reaction towards the assessor significantly ($\chi^2=12$, $p=0.0006$) increased in Traditional sows compared to IDAL sows the day after vaccination. Indeed, 33% of Traditional sows that did not show any sign of fear before the vaccination exhibited a total withdrawal from the observer during the fear to human test the day after vaccination, compared to 3% in the IDAL group. Those preliminary results show that intradermal vaccination can be a very promising strategy to reduce fear and pain reaction of gestating sows when vaccinated.

P.53**Age of PRRSv infection in commercial Belgian farrow-to-finish herds**E. de Jong^{1*}, W. Van Praet¹, S. Van Poucke² and T. Vandersmissen¹¹Animal Health Care Flanders (DGZ), Drongen, Belgium²Merial NV, Diegem, Belgium

Porcine Reproductive and Respiratory Syndrome virus (PRRSv) can cause severe economic losses through growth retardation, susceptibility to other infections and respiratory problems during the nursery and finishing periods. The object of the present study was to determine at what age pigs mainly become infected in commercial farrow-to-finish herds in Flanders, with a history of PRRSv related problems.

In 20 Belgian farrow-to-finish herds, blood and oral fluid samples were collected in 3 age categories: (1) 4 to 7 weeks, (2) 8 to 12 weeks and (3) 13 to 21 weeks. Per age category one pen was selected in which 10 to 13 pigs were housed. This pen was first sampled by means of one cotton rope/pen and afterwards individual blood samples of all pigs in the pen were taken by jugular venipuncture. Oral fluid was collected after 20 minutes and immediately chilled for transportation to the laboratory. All serum samples were analysed for PRRSv antibodies using a commercial indirect ELISA (PRRS X3 AB, Idexx). A positive result was defined as an s/p ratio ≥ 0.4 . Additionally, presence of viral RNA was detected on the oral fluid samples by RT-PCR (Virotype PRRS). Vaccination of the piglets against PRRSv was not allowed during the trial, whereas sow vaccination was optional and comprised several vaccination schemes.

The mean s/p ratio (\pm standard deviation) in the sera in the different age categories was 0.9 (± 0.7), 0.9 (± 0.9) and 2.0 (± 0.7) respectively and median values were 0.6, 0.4 and 2.1 respectively, with large herd differences. The analyses of the RT-PCR revealed 68%, 25% and 56% negative results for the three subsequent age categories. In the majority of the PCR positive samples, the European type was identified apart for one herd where both the European and North American strain was detected in the youngest age category.

In conclusion, most variation was seen in the antibody titres of the pigs between 8 and 12 weeks old. Most positive PCRs were also found in that age category, indicating that PRRSv infection mostly occurs near the end of the nursery and the beginning of the fattening unit. A subsequent trial investigates the effect of piglet vaccination in these herds on (1) the level of antibody titres during the fattening period and on (2) the stabilization of PRRSv infection pressure.

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A summary of four large scale systems-based PRRS control projects

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Introduction

Four collaborative large-scale, long term PRRS control projects applying a systems-based methodology were conducted. These projects occurred in four different geographical locations. The objective of these projects was to improve PRRS stability and overall growing pig performance through the strategic use of modified-live vaccine (MLV) compared to 18 months of prior production data.

Materials and Methods

Four breeding herd (BH) populations of 30,000 (system A), 70,000 (B), 24,000 (C) and 22,000 (D) sows and respective growing pig flows were involved in these PRRS control projects. All BH populations were infected with diverse heterologous PRRSv isolates; including PRRSv Type-1 and Type-2 isolates, and had experienced severe reductions in production. The primary interventions consisted of herd closures ranging from 147 to 210 days, mass vaccinations of BH populations with Ingelvac PRRS® MLV twice 30 days apart and re-vaccination quarterly. Replacement gilts were vaccinated with two doses of Ingelvac PRRS® MLV prior to introduction to BH's. All weaned pigs were vaccinated with Ingelvac PRRS® MLV. PRRSv circulation was monitored monthly in weaned pig and growing pig populations. The growing pig populations were monitored monthly using serum samples and oral fluids. Production data for ADG, and mortality by phase of production was analyzed using SPC technology.

Results

All four projects demonstrated significant improvements in ADG and mortality, following the interventions for PRRS control in the growing pig phase of production (Table 1).

Table 1. Summary of production changes 24* months post-implementation of protocol

	System				
	A	A-FP	B	C	D
ADG, % change					
Nursery	+23%	-	+7%	+0%	+9%
Finisher	+6%	+5%	+7%	+2%	+1%**
WTF	-	-	-	+6%	n/a
Mortality, % change					
Nursery	-63%	-	-23%	-38%	-11%
Finisher	-33%	-32%	-35%	-30%	+0%
WTF	-	-	-	-45%	n/a

FP= Feeder Pig Flow; * or until project completion; ** concurrent enteric disease outbreak

Discussion and Conclusions: Consistent implementation of a methodology that utilized herd closure and modified-live vaccine for the control of PRRSv infections in both the BH and weaning pigs was effective in improving pig performance and reducing mortality. Reductions in mortality in the nursery period (11-63%), finisher period (30-35%), and WTF period (45%) were realized. Improvements in ADG in the nursery phase (7-23%), finisher phase (1-7%), and WTF (6%) were consistent and repeatable across the four systems. These four systems-based PRRS control projects demonstrate that the implementation of a systematic methodology that utilizes modified-live vaccine for the control of wt-PRRSv infections can mitigate the consequences of infection on health and performance.

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Reduction of wild-type PRRS virus shedding in aerosol of growing pigs by modified-live virus vaccination at weaning

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Introduction: The risk of area-spread of porcine reproductive and respiratory syndrome virus (PRRSV) continues to be high in swine-dense regions potentially because of PRRSV shedding from large populations of growing pigs. The therapeutic use of modified-live virus (MLV) vaccine in infected pigs has been shown to reduce the duration of wild-type virus (WTV) shedding to sentinels and in aerosol. The objective of this study was to quantify the effect of MLV vaccine on performance and measure WTV shedding in pigs vaccinated at weaning and challenged 4 weeks later.

Materials and Methods: A total of 2100 PRRS-negative weaned pigs were randomly allocated to either a non-vaccinated control (NVC) or to a MLV vaccinated group, each housed in separated rooms. Biosecurity protocols were implemented to avoid PRRSV transmission between rooms. Pigs in the MLV group were IM vaccinated with Ingelvac PRRS[®] MLV (Boehringer Ingelheim Vetmedica, Inc.) at 4 weeks of age. Four weeks post-vaccination, 10% of the pigs in each group were IM inoculated with 1 mL of PRRS WTV RFLP pattern 1-18-2 at a concentration of 4.2x10⁷ RNAc/mL. Infection dynamics was monitored by PCR and ELISA tests on serum and oral fluid (OF) samples. Daily air samples were collected from each group at 8 AM using Liquid Cyclonic Collectors (Midwest MicroTek, Brookings, SD) placed in front of exhaust fans for 30 minutes and tested by PCR. Mortality, cull rate, ADG and feed conversion (FC) were recorded for wean to finish performance.

Results: Mild clinical signs developed in both groups following inoculation with PRRS WTV. No significant differences between groups were detected in PCR or ELISA results in serum or OF samples (P>0.10). MLV vaccine was not detected in the NVC group. The frequency of detection of PRRSV RNA in air was significantly higher in NVC than in the MLV group (P<0.0001) (Table 1). The duration of detection of PRRSV RNA in air samples was numerically shorter in the MLV group than in the NVC group (Table 1). Performance is summarized in Table 1.

Table 1. Detection of PRRSV in air by PCR and barn performance by treatment

Parameter (days)	NVC	MLV
Frequency post-vaccination	0/2 ^a	5/28 ^b
Duration post-vaccination	0	6
Frequency post-inoculation	21/118 ^b	4/118 ^a
Duration post-inoculation	36	6
Mortality, %	4.8	5.1
Cull rate, %	5.9	2.8
ADG, kg	0.712	0.739
Feed Conversion	2.38	2.40

Rows with different superscripts differ, P<0.05

Discussion and Conclusions: The prophylactic use of PRRS MLV vaccine in growing pigs at risk of infection represents a valuable tool to reduce the risk of transmission between herds in swine-dense-areas. The observed performance benefits as well as shedding reduction in MLV vaccinated pigs challenged with WTV support the recommendation of MLV vaccination of growing pigs at risk of infection.

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Porcine reproductive and respiratory syndrome virus serosurveillance in the wild boar (*Sus scrofa*) population of Southern Belgium, 2014

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A recent study has shown that, at herd and at animal level, the apparent seroprevalence of PRRSV in pig farms of Wallonia, the South part of Belgium, is respectively 48% (IC95%: 39 – 57%) and 32 % (IC 95%: 29 – 35%). In that region of low density of pig production (0.05 pig farms/km²), wild boars (*Sus scrofa*) are largely distributed, with an estimated population of about 25 000 animals over 16 844 km². As wild boars share a variety of pathogens with farm animals, they theoretically constitute a source of contamination. It is therefore important to evaluate the fraction of the wild boar population that eventually hosts the PRRS virus as they could (re)introduce it, especially where domestic pigs are raised outdoors. The present study had for objective to determine the global seroprevalence of PRRSV in the wild boar population of Southern Belgium. Results will be presented per age and sex and distributed per location of sampling. Finally, the results obtained in wild boars will be brought close to the proximity of outdoors pig farms.

In the frame of a targeted surveillance program, 434 wild boars were sampled during the hunting season 2014 (October to December). The study was conducted in 31 forest districts and a two-stage cluster sampling was realised. Firstly, some hunting areas were randomly chosen in each forest district and, secondly, some animals were randomly sampled in each hunting area. Wild boars populations being inconstant, a proportional allocation was realised per forest district. All animals were necropsied in the field, within 2 to 3 hours after shot. Individual postmortem examinations included determination of sex, age, body weight, and body condition. Age was determined on the basis of tooth eruption patterns and weight. Animals were classified as piglets (less than 6 months old), juveniles (between 6 and 12 months old), sub-adults (between 1 and 2 years old) and adults (over 2 years old). After examination of the intact whole body, the abdominal, thoracic, and naso-buccal cavities and corresponding organs were checked. Afterwards, blood was immediately collected in dry tubes exclusively by venipuncture from major vessels or by cardiac puncture. Sampling of blood in the thoracic and abdominal cavities was not carried out. Samples were transported to the lab within 12 hours. After centrifugation, sera were stored at –20°C until analysis. An ELISA (IDEXX PRRS X3) was used to screen the presence of IgG anti PRRSV. An individual result was considered negative if the s/p ratio was <0.4.

A total of 6/434 wild boars were found serologically positive for PRRS. The overall apparent prevalence was 1.38%. The present study is the first PRRS serological screening in wildlife in Southern Belgium. Our results provide indirect evidence of the presence of PRRS virus in wild boars but a screening at larger scale is needed in order to confirm this low seroprevalence in the region.

