2016 North American PRRS Symposium

Final Program

InterContinental Magnificent Mile Chicago
Chicago Marriott Downtown Magnificent Mile
Chicago, Illinois

December 3-4, 2016

The 2016 North American PRRS Symposium wishes to thank the following sponsors for their generous support:

Midwest Veterinary Services

Dr. David Benfield (Travel fellowships)
Dr. Joan K. Lunney (Travel fellowships)

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Luis Gimenez-Lirola, Iowa State University

Ben Hause, Cambridge Technologies

Sheela Ramamoorthy, North Dakota State University

Hiep Vu, University of Nebraska-Lincoln

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X.J. Meng elected to the National Academy of Sciences

By: Michael Sutphin, Public Relations Coordinator, Virginia-Maryland College of Veterinary Medicine, Virginia Tech

X.J. Meng, University Distinguished Professor of Molecular Virology at Virginia Tech, has been elected a member of the National Academy of Sciences. Membership in the academy is one of the highest honors given to a scientist in the United States.

Meng, a virologist in the Department of Biomedical Sciences and Pathobiology at the Virginia-Maryland College of Veterinary Medicine, is one of 84 new members and 21 foreign associates from 14 countries recognized for their distinguished and continuing achievements in original research.

Meng's research focuses on emerging and reemerging viral diseases that impact veterinary and human public health. Meng is widely considered one of the world's leading scientists in hepatitis E virus, porcine circovirus type 2, and porcine reproductive and respiratory syndrome virus. Meng's lab developed the first U.S. Department of Agriculture fully-licensed vaccine to protect against porcine circovirus type 2 infection and its associated diseases in pigs, a major threat to the global swine industry. In addition, Meng discovered the swine hepatitis E virus in pigs, which led to the recognition of hepatitis E as a zoonotic disease.

Meng has authored or co-authored more than 290 peer-reviewed articles and book chapters. These have been cited more than 18,380 times by other researchers. Meng has been awarded more than 40 research grants as a principal investigator with more than $15 million, and he is also a co-investigator or collaborator on more than 50 other awarded grants of more than $27 million.

A native of Qingdao, China, Meng earned a medical degree from Binzhau Medical College and master's degree in microbiology and immunology at Wuhan University College of Medicine. He then completed a Ph.D. in immunobiology from Iowa State University's College of Veterinary Medicine. Prior to joining the Virginia Tech faculty, he worked as the John E. Fogarty Visiting Scientist and a senior staff fellow at the National Institute of Allergy and Infectious Diseases, a part of the National Institutes of Health.

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. He is a member of the American Society for Virology, American Association of Veterinary Laboratory Diagnosticians, and Honorary Diplomat of the College of Veterinary Microbiologists, American Association of Swine Veterinarians and the American Association for the Advancement of Science. 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 PRRS Symposium to present their work on PRRS.

Joan Lunney, who is actively involved in mentoring younger scientists, is pleased to support the David A. Benfield Student Travel Fellowships for attendance at PRRS Symposia.

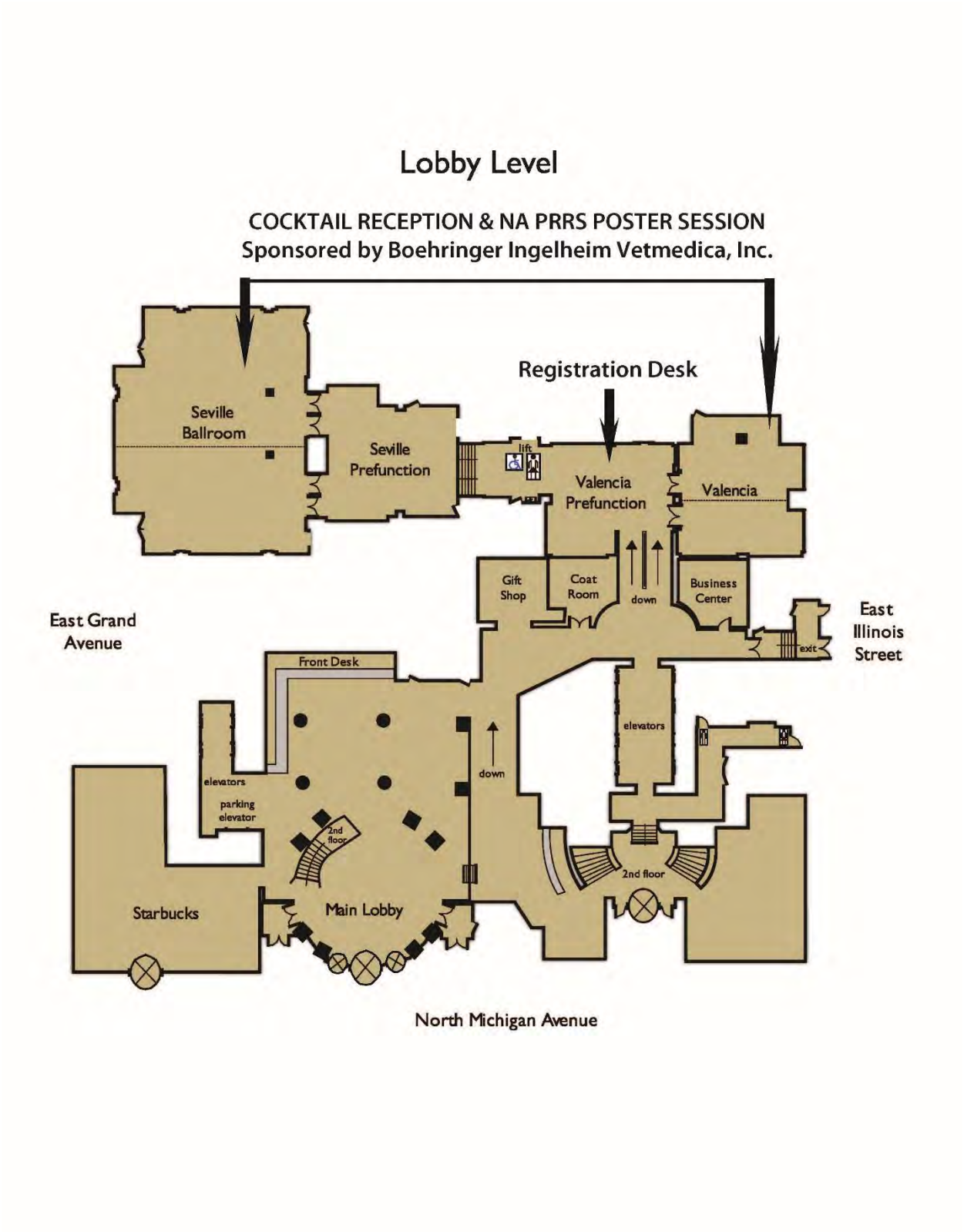
2016 Student Travel Fellowship Recipients

Aleksandra Wozniak	Warsaw University of Life Sciences, Poland
Alexandria Van Noort	South Dakota State University
Ana Stoian	Kansas State University
Anton Yuzhakov	Scientific Research Institute of Epidemiology and Microbiology, Russia
Camila Valle Tejada	Pontifical Xaverian University, Canada
Carolina Malgarin	University of Saskatchewan, Canada
Cassandra Lynn Ferring	North Carolina State University
Elena Canelli	University of Parma, Italy
Emma Howson	The Pirbright Institute, United Kingdom
Hanzhong Ke	University of Illinois at Urbana-Champaign
Ignacio Correas	University of Nebraska-Lincoln
Jitka Frölichová	University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
Junru Cui	University of Connecticut
Kathryn Kimpston-Burkgren	University of Nebraska-Lincoln
Kristen Berg	North Carolina State University
Laura Constance	Kansas State University
Luca Popescu	Kansas State University
Mofazzal Hossain	Kansas State University
Nanhua Chen	College of Veterinary Medicine, Yangzhou University, China
Pablo Valdes-Donoso	University of Minnesota
Qian (Jessie) Dong	Iowa State University
Qingzhan Zhang	University of Illinois at Urbana-Champaign
Rachel Palinski	Kansas State University
Sergey Raev	Institute for Human and Animal Diseases, Russia
Vlad Petrovan	Kansas State University
Yanli Li	Universitat Autònoma de Barcelona, Spain

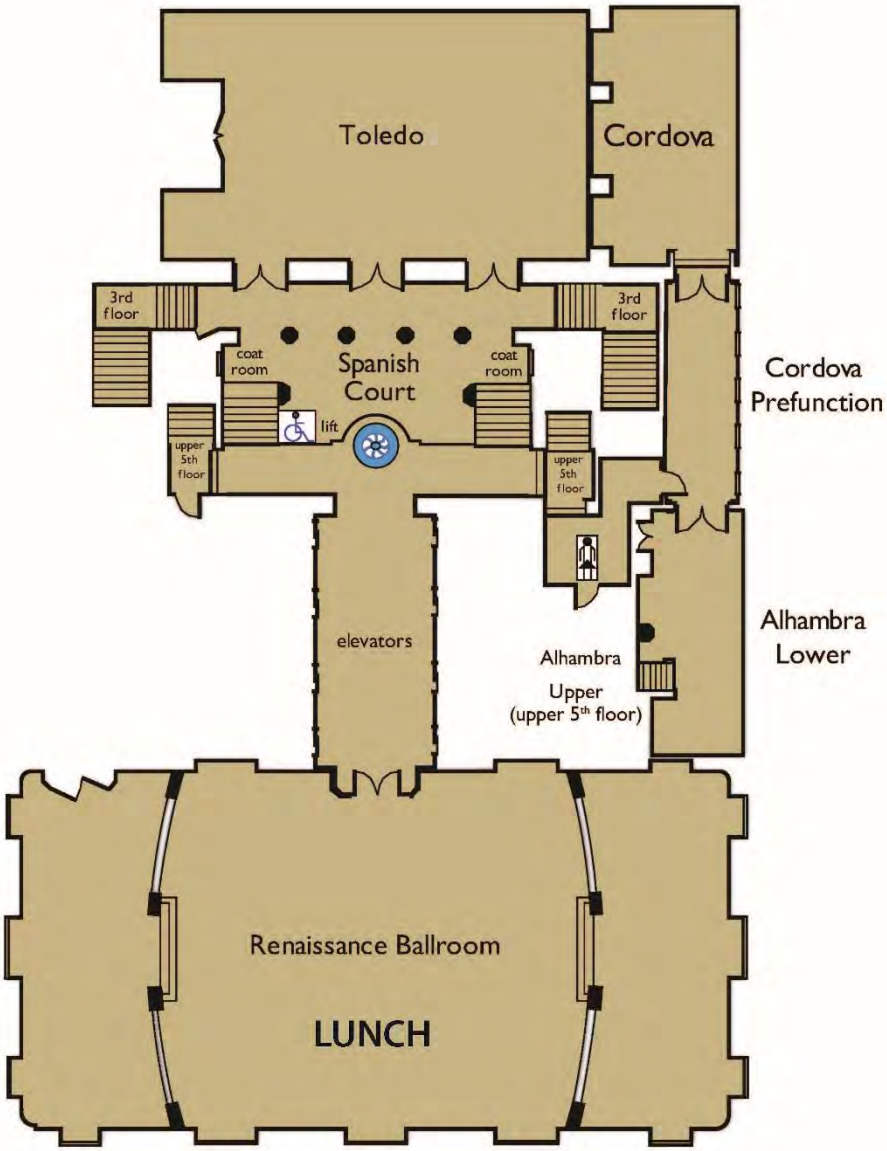
CE Credit Information

The 2016 North American PRRS Symposium Program has been approved for 13.5 hours of continuing education (CE) credit as approved by the Kansas Board of Veterinary Examiners. Kansas Board of Veterinary Examiners approves continuing education hours based on the guidelines set forth by the AAVSB RACE approval program for veterinarians and veterinary technicians. Signed CE confirmation forms are included in the attendee registration packet.

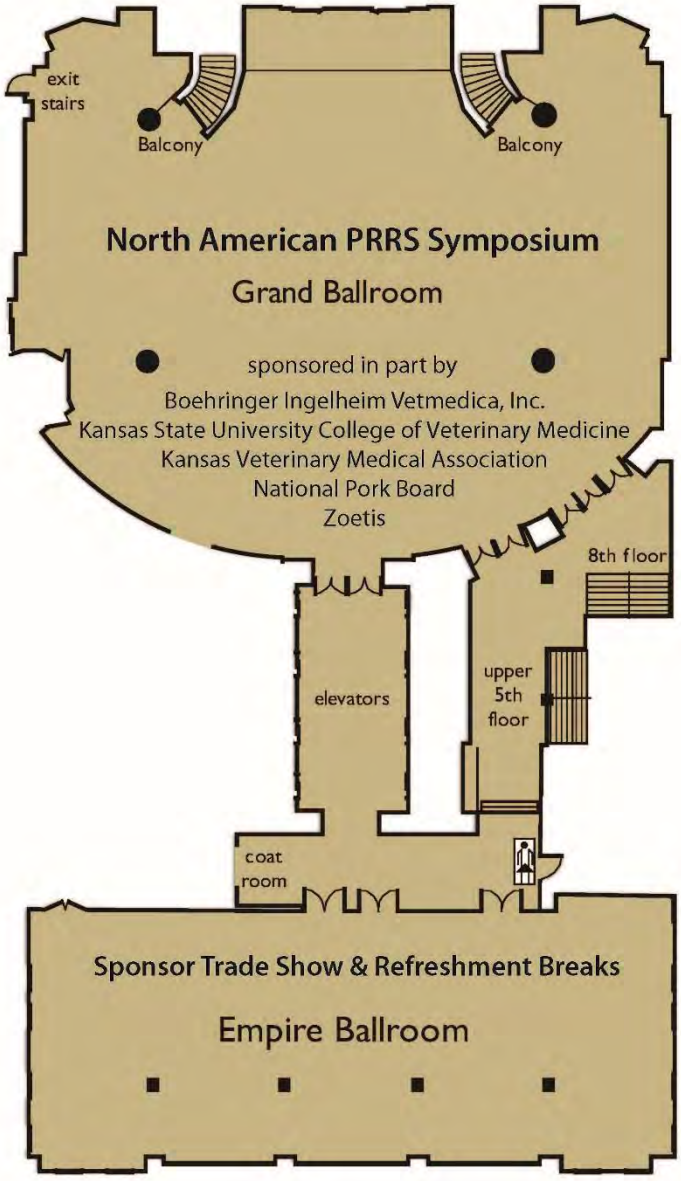
InterContinental Hotel Maps



5TH Floor


Toledo
Cordova
3rd floor
3rd floor
coat room
Spanish Court
coat room
Cordova Prefunction
lift
upper 5th floor
upper 5th floor
elevators
Alhambra Lower
Alhambra Upper (upper 5th floor)
Renaissance Ballroom
LUNCH

7TH Floor


exit
stairs
Balcony
Balcony
North American PRRS Symposium
Grand Ballroom
sponsored in part by
Boehringer Ingelheim Vetmedica, Inc.
Kansas State University College of Veterinary Medicine
Kansas Veterinary Medical Association
National Pork Board
Zoetis
8th floor
elevators
upper
5th
floor
coat
room
Sponsor Trade Show & Refreshment Breaks
Empire Ballroom

Map - InterContinental and Marriott Locations

2016 North American PRRS Symposium Program

PRRS, emerging and foreign animal diseases

Friday, December 2nd

***4:00pm-6:00pm* Registration Desk Open**

Valencia Prefunction Room, 1st Floor, Intercontinental Hotel

Saturday, December 3rd

***6:30am-10:00am* Registration Desk Open**

Valencia Prefunction Room, 1st Floor, Intercontinental Hotel

8am-12pm Intercontinental Hotel, Grand Ballroom, 7th Floor

Building on the best practices of PRRS Control

8:00am	Welcome-Tom Fangman
8:05am	*Bob Morrison & Carles Vilalta*-Update from Swine Health Monitoring Project (SHMP)
8:35am	*Daniel Linhares*-Update on PRRS stability studies and flash updates on PRRS detection and biosecurity studies
9:05am	*Jim Lowe*-Big data and models: Are they really useful in disease management?
9:35am	Q & A
9:50am	**Break**
10:05am	*PH Rathkjen*-PRRS around the World – What's new regarding Global cross-protection against PRRS
10:50am	*Roger Main & Mike Roof*-Impact of PRRS vaccination on infectious load and implications for area control and eradication
11:50am	Panel Discussion

12pm Lunch- Renaissance Ballroom, 5th Floor

Saturday, December 3rd

1pm-4pm Intercontinental Hotel, Grand Ballroom, 7th Floor

Alternative strategies for PRRS control

Moderator: Joan K. Lunney, USDA ARS BARC

1:00pm *Hanchun Yang*-China Agricultural University, "**Pathogenesis and control of Chinese highly pathogenic PRRSV"**

1:30pm *Megan Niederwerder*- Diagnostic Medicine/Pathobiology, Kansas State University, **"The role of the microbiome in PRRS"**

2:00pm *Jeff Zimmerman*-Vet Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University, **"Developments in infectious disease surveillance"**

2:30pm *Tanja Opriessnig*- The Roslin Institute, University of Edinburgh and Iowa State University, **"Update on novel experimental pig vaccine approaches"**

3:00pm *John Harding*-Western College of Veterinary Medicine, University of Saskatchewan, **"A multi-'omics approach to understanding the pathophysiology of reproductive PRRS"**

3:30pm *Jack Dekkers*, Animal Breeding and Genetics, Department of Animal Science, Iowa State University, **"Update on the host genetics of resistance to porcine diseases"**

5:00pm- Poster Session and Reception

Valencia and Seville Rooms, 1st Floor

Sunday, December 4th

7am-8am: Intercontinental Hotel, Renaissance Ballroom, 5th Floor
Breakfast

Special breakfast speakers sponsored by Zoetis

Meggan Bandrick-Zoetis, **"The latest on PEDV"**
Alex Morrow- STAR-IDAZ, **"Global Coordination of Animal Disease Research"**

Meeting moves to Downtown Marriott Hotel
5th Floor, Salons A/B/C/D

Joint session on PRRS, emerging and foreign animal diseases

Selected talks from NA-PRRS meeting abstracts

Moderators: *Ben Hause (Cambridge Technologies) & Luis Gimenez-Lirola (Iowa State University)*

8:15am *Diego Diel, South Dakota State University,* Pathogenesis and infection dynamics of Senecavirus A in pigs, Abstract #42

8:30am *Alexandra Buckley, USDA,* Senecavirus A infection in sows, neonates, and market weight gilts with subsequent protective immunity, Abstract #35

8:45am *Steven Lawson, South Dakota State University,* Development of antibody reagents & assays for Senecavirus A serodiagnosis, Abstract #10

9:00am *Fangfeng Yuan, Kansas State University,* Construction and characterization of a full-length cDNA infectious clone of emerging porcine Senecavirus A, Abstract #24

9:15am *Rachel Palinski, Kansas State University,* A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure, Abstract #18

9:30am *Chantale Provost, University of Montreal,* Development of a new molecular method to discriminate Porcine Epidemic Diarrhea Virus infectious viral particles, from non-infectious ones, which are contaminating pig derived food additives, Abstract #21

9:45am *Rolf Rauh, Tetracore, Inc.,* Evaluation of a portable real-time PCR platform (T-COR 8™) for ASF during outbreaks in an endemically infected population in Uganda., Abstract #11

Break

Moderators: *Ying Fang (Kansas State University) & Gustavo Delhon (University of Nebraska-Lincoln)*

10:15am *Ignacio Correas, University of Nebraska-Lincoln,* Cross-reactivity of immune responses against Porcine Reproductive and Respiratory Syndrome virus, Abstract #38

10:30am *Joseph Darbellay, VIDO-InterVac,* The progression to T cell immunity after infection with porcine reproductive and respiratory syndrome virus, Abstract #40

10:45am *Junru Cui, University of Connecticut,* A GP5 Mosaic T-cell Vaccine for Porcine Reproductive and Respiratory Syndrome Virus Is Immunogenic, Induces Broad Cellular Responses to Diverse Strains and Confers Partial Protection to Pigs, Abstract #64

11:00am *Kay Kimpston-Burkgren, University of Nebraska-Lincoln,* Contribution of PRRSV minor glycoproteins to a protective immune response in swine, Abstract #51

11:15am *Tanja Opriessnig, University of Edinburgh, Iowa State University,* An interferon inducing PRRSV vaccine candidate protects against challenge with a heterologous virulent type 2 strain in a conventional pig model , Abstract #71

11:30am *Volker Gerdts, Vaccine and Infectious Disease Organization-InterVac,* Development of a Novel Vaccine for Porcine Epidemic Diarrhea Virus, Abstract #7

11:45am *Jishu Shi, Kansas State University,* Pigs immunized with a novel E2 subunit vaccine are protected from subgenotype heterologous Classical Swine Fever Virus challenge, Abstract #76

Lunch **On your own**

Moderators: *Diego Diel (South Dakota State University) & Sheila Ramamoorthy (North Dakota State University)*

1:30pm *Marti Cortey, IRTA-CReSA,* Changes in the genetic composition of PRRSV quasispecies and its relationship with long and short viral infections, Abstract #39

1:45pm *Rui Guo, Kansas State University,* Porcine reproductive and respiratory syndrome virus takes advantage of host intercellular

mitochondria transferring pathway for cell to cell spreading of the infection, Abstract #48

2:00pm *Ana Stoian, Kansas State University,* Identification of CD163 domain involved in the infection with Type II Porcine Reproductive and Respiratory viruses, Abstract #86

2:15pm *Hanzhong Ke, University of Illinois at Urbana-Champaign,* SAP domain in nsp1-beta of porcine reproductive and respiratory syndrome virus (PRRSV) correlates with interferon suppression in cells and pathogenesis in pigs, Abstract #50

2:30pm *Federico Zuckermann, University of Illinois,* The unfolded protein response induced by porcine reproductive and respiratory syndrome virus infection of alveolar macrophages is involved in immune dysregulation, Abstract #62

2:45pm *Pengcheng Shang, Kansas State University,* A novel mechanism of protein-stimulated trans-activation of ribosomal frameshifting in porcine reproductive and respiratory syndrome virus: implication in improved vaccine development, Abstract #85

Break

Moderators: *Dan Rock (University of Illinois) & KJ Yoon (Iowa State University)*

3:15pm *Susan Detmer, University of Saskatchewan,* Pathological findings at the maternal-fetal interface during the early type 2 PRRS virus infection of late gestation pregnant gilts, Abstract #41

3:30pm *Yanli Li, UAB,* Resolution of genotype 1 PRRSV attachment on bone marrow-derived dendritic cells, Abstract #53

3:45pm *Lei Zhou, China Agricultural University,* Attenuate Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus by incorporating target site of hematopoietic-specific MicroRNA into viral genome, Abstract #82

4:00pm *Jenelle Dunkelberger, Iowa State University,* A major gene for host response under PRRS challenge is not negatively associated with overall performance in commercial pig lines under non-challenged conditions, Abstract #44

4:15pm *Natasha Gaudreault, Kansas State University,* Genetically edited pigs lacking CD163 show no resistance following infection with the African Swine Fever Virus isolate, Georgia 2007/1, Abstract #47

5:30pm **CRWAD Keynote: Randy Prather, University of Missouri, "Genetic Engineering for Host Resistance to PRRSV and other swine diseases"**

6:30pm CRWAD poster session, 7th Floor

ABSTRACTS

Number	Last Name	First Name	Abstract Title & Authors	Submitting Institution
Speakers				
S1	Dekkers	Jack	Update on the host genetics of resistance to porcine diseases. *Jack Dekkers, Iowa State University, USA.*	Iowa State University
S2	Harding	John	A multi'omics approach to understanding the reproductive pathophysiology of type 2 porcine reproductive and respiratory syndrome (PRRS). *J.C.S. Harding1*, A. Ladinig2, P. Novakovic1, S.E. Detmer1, J.M. Wilkinson3, T. Yang3, J.K. Lunney4, G.S. Plastow3. 1University of Saskatchewan, Saskatoon, SK. 2University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria. 3Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB. 4Animal Parasitic Diseases Laboratory, USDA ARS, Beltsville, MD.*	University of Saskatchewan
S3	Niederwerder	Megan	The role of the microbiome in PRRS. *M.C. Niederwerder1,2*, C.J. Jaing3, R.A. Ober1, J.B. Thissen3, A.G. Cino-Ozuna1,2, K.S. McLoughlin4, R.R.R. Rowland1. 1Department of Diagnostic Medicine/Pathobiology. 2Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS. 3Physical & Life Sciences Directorate, 4Computations Directorate, Lawrence Livermore National Laboratory, Livermore, CA.*	Kansas State University
S4	Opriessnig	Tanja	Update on novel experimental pig vaccine approaches. *T. Opriessnig1,2,* P.G. Halbur2, P. Gauger2, J.Q. Zhang2, Q. Chen2, D. Tian3, Y.Y. Ni3, X.J. Meng3, M. Tan4. 1The Roslin Institute, University of Edinburgh, Midlothian, UK. 2Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA. 3Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. 4Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA and Department of Pediatrics, University of Cincinnati, Cincinnati, Ohio, USA.*	University of Edinburgh, Iowa State University
S5	Prather	Randall	Genome editing for PRRSV resistance and beyond. *R.S. Prather. Division of Animal Science, National Swine Resource and Research Center, University of Missouri, Columbia, MO.*	University of Missouri
S6	Yang	Hanchun	The latest on pathogenesis and control of highly pathogenic PRRSV in China. *Hanchun Yang. College of Veterinary Medicine, China Agricultural University, Beijing, China.*	China Agricultural University
S7	Zimmerman	Jeff	Developments in infectious disease surveillance. *M. Rotolo,1 Y. Sun,2 C. Wang,1,2 R. Main,1 J. Zimmerman1*. 1Department of Veterinary Diagnostic and Production Animal Medicine. 2Department of Statistics, Iowa State University.*	Iowa State University

Detection, diagnostics and surveillance				
1	Chen	Nanhua	Differential detection, genomic characterization and phylogenetic analysis of Porcine Epidemic Diarrhea Viruses from vaccinated pig herds in Fujian, China 2016. *N. Chen*, J. Zhu. College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, 225009, P.R. China.*	Yangzhou University
2	Clement	Travis	A cost effective method for surveillance of PRRSV and IAV-S in oral fluids using a newly developed multiplex rtRT-PCR. *T. Clement1*, R. Rauh2, W. Nelson2, J. Kelly2, E. Nelson1, J. Christopher-Hennings1.* *1Veterinary and Biomedical Sciences Department, South Dakota State University, Brookings, South Dakota.* *2Tetracore, Inc., Rockville, Maryland.*	South Dakota State University
3	Diaz	Ivan	ELISAs based on recombinant nsp7 proteins from PRRSV type 1 and 2 as DIVA tests. *M. García Duran1, I. Díaz2*, J. Sarraseca1, N. de la Roja1, I. Hernández-Caravaca3, H. Nauwynck4, E. Mateu5, M.J. Rodríguez1. 1INGENASA. Madrid, Spain. 2IRTA (CReSA, IRTA-UAB), Bellaterra, Barcelona, Spain. 3Boehringer-Ingelheim España, Sant Cugat del Vallés, Barcelona, Spain. 4Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium. 5Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, UAB, Bellaterra, Spain.*	IRTA-CReSA (Centre de Recerca en Sanitat Animal)
4	Diel	Diego	Genetic characterization and phylogenetic analysis of Senecavirus A. *L.R. Joshi1*, K.A. Mohr1, D. Gava2, G. Kutish3, P. Piñeyro4, J. Zhang4, L. Caron2, R. Schaefer2, D.G. Diel1. 1Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD. 2EMBRAPA Swine and Poultry, Concordia, SC, Brazil. 3Department of Pathobiology, University of Connecticut, Storrs, Connecticut, USA. 4Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA.*	South Dakota State University
5	Frolichova	Jitka	Epitope mapping of M protein of PRRS virus. *J. Frölichová1*, V. Celer1,2. 1Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic. 2CEITEC, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.*	University of Veterinary and Pharmaceutical Sciences Brno
6	Frossard	Jean-Pierre	Assessing the value of full genome sequence analyses for genotype 1 PRRS viruses. *J.P. Frossard*, S. McGowan, D. Dorey-Robinson, B. Choudhury. Animal and Plant Health Agency, Weybridge, Surrey, United Kingdom.*	Animal and Plant Health Agency
7	Gerdts	Volker	Development of a novel vaccine for Porcine Epidemic Diarrhea Virus. *N. Berube, S. Hauta, Y. Popowych, W. Connor, J. Erickson, S. Tetland, K. Bock, B. Allan, J. van den Hurk, N. Makadiya, E. van Moorlehem, R. Brownlie, A. Zakhartchouk, N. Rawlyk, S. Walker, C. Wheler, D. Wilson, S.K. Tikoo, Y. Zhou, A.A. Potter, V. Gerdts. Vaccine and Infectious Disease Organization-International Vaccine Centre, 120 Veterinary Rd., Saskatoon, SK, S7N 5E3 Canada.*	Vaccine and Infectious Disease Organization-InterVac

8	Hossain	Mofazzal	Development of a new strategy for detection of classical swine fever virus antibodies in alphavirus based replicon particle derived E2 and Erns vaccinated swine. *M.M. Hossain*, R.R.R. Rowland. Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506 USA.*	Kansas State University
9	Howson	Emma	Progress in the development of real-time PCR assays for detection of foot-and-mouth disease. *E. Howson1*, V. Fowler1, D. King1, V. Mioulet1, W.M. Nelson2, R. Rauh2. 1The Pirbright Institute, Pirbright, Surrey, UK. 2Tetracore, Rockville, MD.*	The Pirbright Institute
10	Lawson	Steven	Development of antibody reagents & assays for Senecavirus A serodiagnosis. *S. Lawson*, A. Singrey, L. Joshi, J. Leat, J. Nelson, D.G. Diel, J. Christopher-Hennings, E.A. Nelson. Veterinary and Biomedical Sciences Department, South Dakota State University, Brookings, SD.*	South Dakota State University
11	LeBlanc	Neil	Evaluation of a portable real-time PCR platform (T-COR 8™) for ASF during outbreaks in an endemically infected population in Uganda. *N. LeBlanc1*, L. Liu2, S. Atim3, M. Esau3, E. Chenais2, R. Mwebe3, N. Nantima3, R.A. Okurut3, C. Ayebazibwe3, K. Ståhl2. 1Consultant molecular diagnostics, Uppsala, Sweden. 2National Veterinary Institute, Uppsala, Sweden. 3National Animal Disease Diagnostics and Epidemiology Center, Entebbe, Uganda.*	NGL Consulting
12	Leung	Frederick	Molecular phylogenetic diversity of PRRSV Type 2 in Mexico, 2005-2013. *M. Shi1, M.S. Brar2, J.H. Lara3, R. Cortes3, F. Quezada3, B. Lozano3, E. Soto3, D. Sarfati3, F.C. Leung2,4. 1Sydney Emerging Infections & Biosecurity Institute, School of Biological Sciences and Sydney Medical School, The University of Sydney, NSW 2006, Australia. 2School of Biological Science, The University of Hong Kong, Hong Kong SAR, China. 3Laboratorio Avi-Mex, S. A. de C. V.; Mexico. 4FCL Bioscience, Hong Kong Science and Technology Park, Shatin, New Territories, Hong Kong SAR, China.*	The University of Hong Kong
13	Maamar	Achacha	Comparison of five different strategies for high level expression of soluble recombinant PRRSV-nucleocapsid protein for its use in diagnostic. *M. Achacha1, A. Kheyar1, A. Bensari1, M. Arbour2 L. Masson2. 1Arivac Inc, St-Hyacinthe, Quebec, Canada. 2National Research Council, Montreal, Canada.*	ARIVAC INC
14	Martin-Valls	Gerard	Alternative sampling methods for assessing vertical transmission of PRRSV. *G.E. Martin-Valls*, M. Hidalgo, E. Cano. IRTA-CReSA, Campus de la UAB, 08193, Bellaterra (Barcelona).*	IRTA-CReSA (Centre de Recerca en Sanitat Animal)
15	Mhamdi	Zeineb	Type of influenza A virus reassortants present in Quebec swine herds from 2011 to 2015 and their antiviral drugs resistance. *Z. Mhamdi, C. Savard, C.A. Gagnon. Swine and Poultry Infectious Diseases Research Centre (CRIPA) and the Research Group on Infectious Diseases in Animal Production (GREMIP), Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Qc, Canada.*	University of Montreal

16	Monger	Vijay	Detection of Porcine Reproductive and Respiratory Syndrome (PRRS) in Bhutan. *V.R. Monger1, T. Stadejek2, K. Rinzin1, K. Chabros2. 1National Center for Animal Health, Serbithang, Thimphu, Bhutan. 2National Veterinary Research Institute, Department of Swine Diseases, OIE Reference Laboratory, Pulawy, Poland.*	National Centre for Animal Health
17	Murtaugh	Michael	An indirect enzyme-linked immunosorbent assay for the identification of antibodies to Senecavirus A in swine. *C.M.T. Dvorak1, Z. Akkutay-Yoldar 1,2, S.R. Stone1, S.J. Tousignant3, F.A. Vannucci4, M.P. Murtaugh1*. 1Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Ave, St. Paul, MN 55108, USA. 2Ankara University, Department of Virology, Diskapi 06110, Ankara, Turkey. 3Swine Vet Center P.A., 1608 S. Minnesota Ave, St. Peter, MN 56082, USA. 4Department of Veterinary Population Medicine, University of Minnesota, 1365 Gortner Ave, St. Paul, MN 55108, USA.*	University of Minnesota
18	Palinski	Rachel	A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. *R. Palinski1, P. Pineyro2, P. Shang1, F. Yuan1, R. Guo1, Y. Fang1, E. Byers3, B.M. Hause1,4*. 1Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, Kansas, USA. 2College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA. 3Smithfield Hog Production, Warsaw, North Carolina, USA. 4Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas, USA.*	Kansas State University
19	Petrovan	Vlad	Development, characterization and diagnostic application of monoclonal antibodies against ASFV p30. *V. Petrovan*, P. Shang, F. Yuan, M.V. Murgia, Y. Fang, R.R.R. Rowland. Department of Diagnostic Medicine & Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.*	Kansas State University
20	Popescu	Luca	Oral fluids diagnostic methods for chronic Classical swine fever virus. *L.N. Popescu, N.N. Gaudreault, R.R.R. Rowland. Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University.*	Kansas State University
21	Provost	Chantale	Development of a new molecular method to discriminate Porcine Epidemic Diarrhea Virus infectious viral particles, from non-infectious ones, which are contaminating pig derived food additives. *C. Provost1*, P. Garneau1, D. Ojkic2, J. Harel1, C.A. Gagnon1. 1Centre de recherche en infectiologie porcine et avicole (CRIPA) et Groupe de recherche sur les maladies infectieuses en production animale (GREMIP), Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada. 2Animal Health Laboratory (AHL), University of Guelph, Guelph, Ontario, Canada.*	University of Montreal

22	Rotolo	Marisa	Proof of concept: PRRSV IgM/IgA ELISA detects infection in the face of circulating maternal IgG antibody. *M. Rotolo1*, J. Ji2, L. Gimenez-Lirola3, R. Magtoto3, C. Wang2, D. Baum3, M. Hoogland4, R. Main3, J. Zimmerman1. 1Veterinary Diagnostic & Production Animal Medicine Department, College of Veterinary Medicine, Iowa State University, Ames, IA. 2Statistics Department, Iowa State University, Ames, IA. 3Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA. 4Murphy-BrownLLC, Algona, IA.*	Iowa State University
23	Rotolo	Marisa	Spatial autocorrelation and implications for oral fluid-based PRRSV surveillance. *M. Rotolo*, M. Haddad, Y. Sun, L. Gimenez-Lirola, S. Bade, C. Wang, D. Baum, P. Gauger, M. Hoogland, R. Main, J. Zimmerman. Iowa State University, Ames, IA.*	Iowa State University
24	Shang	Pengcheng	Construction and characterization of a full-length cDNA infectious clone of emerging porcine Senecavirus A. *Z. Chen1, F. Yuan1,2, Y. Li1, P. Shang1, R. Schroeder3, K. Lechtenberg3, J. Henningson1,2, B. Hause1,2, J. Bai1,2, R.R.R. Rowland1, A. Clavijo1,2, Y. Fang1,2.* 1Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States. 2Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States. 3Midwest Veterinary Services, Inc., Oakland, NE 68045, United States.*	Kansas State university
25	Valdes-Donoso	Pablo	Assessment of Porcine Reproductive and Respiratory Syndrome (PRRS) impact in US sow farms. *P. Valdes-Donoso1,2*, J. Alvarez1, L.S. Jarvis2, R. Morrison1, A. Perez1. 1Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA. 2Department of Agricultural and Resource Economics, University of California Davis, Davis, CA, USA.*	University of Minnesota
26	Wozniak	Aleksandra	Detection of PCV2 DNA in serum, feces and in oral fluid of pigs vaccinated against PCV2. *A. Woźniak1*, D. Miłek1, P. Matyba2, P. Karbowiak3, P. Biernacki3, K. Biernacka1, T. Stadejek1. 1Department of Pathology and Veterinary Diagnostics. 2Department of Large Animals Diseases, Faculty of Veterinary Medicine, WULS, Warsaw, Poland. 3Vet-Com Sp. z o.o., Olsztyn, Poland.*	Warsaw University of Life Sciences
27	Wu	Shaoqin	The duration period of PRRSV Ab under serum therapy and herd closure. *S. Wu, * Z. Wu, Y. Xiao, Lujiang, L. Xie. Guangxi State Farms Yongxin Husbandry Company Nanning Hengxian 530317.*	Guangxi State Farms Yongxin Husbandry Company
28	Yu	Ji Eun	Development of immunochromatographic strip tests for on-site rapid and early detection of specific antibodies against porcine respiratory reproductive syndrome virus. *J.E. Yu1, I.O. Ouh1, H.Y. Lee2, E.S. Jeon2, K.M. Cheong2, I.S. Cho1, S.H. Cha1,*. 1Animal and Plant Quarantine Agency, Gimcheon, Gyeongsangbukdo, Republic of Korea. 2Median Diagnostics, Chuncheon, Gangwon, Republic of Korea.*	Animal and Plant Quarantine Agency

29	Yuzhakov	Anton	Complete genome sequencing of a genotype 1 subtype 2 PRRSV isolate obtained in Western Siberia. *A.G. Yuzhakov1,2*, S.A. Raev1,2, A.A. Sviridova2, T.V. Grebennikova1,2, O.A. Verkhovskii1, A.D. Zaberezhny2, T.I. Aliper1,2 , H. Nauwynck3. 1Diagnostics and Prevention Research Institute for Human and Animal Diseases, Moscow, Russia. 2All-Russian Research Institute of Experimental Veterinary Medicine named Ya.R. Kovalenko, Moscow, Russia. 3Ghent University, Ghent, Belgium.*	Diagnostics and Prevention Research Institute for Human and Animal Diseases
30	Zakhartchouk	Alexander	Identification of immunodominant B-cell linear epitopes present in the nucleocapsid protein of Porcine Epidemic Diarrhea Virus. *N. Makadiya, V. Gerdts, A. Zakhartchouk*. Vaccine and Infectious Disease Organization-International Vaccine Center (VIDO-InterVac), University of Saskatchewan, Saskatoon, SK, Canada.*	Vaccine and Infectious Disease Organization (VIDO-InterVac), University of Saskatchewan
31	Zhao	Mengmeng	Establishment of a SYBR Green I based real time PCR for rapid detection of PRRSV Nsp9 gene and its expression in PRRSV infected cells. *M.M. Zhao1,2*, G.H. Zhang2. 1College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China. 2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, National Engineering Research Center for Breeing Swine Industry, College of Veterinary, South China Agricultural University, Guangzhou 510642, China.*	Henan Agricultural University
32	Zhao	Mengmeng	Influence of antiserum of PRRSV to replication in Marc-145 cells. *M.M. Zhao 1,2*, G.H. Zhang 2. 1College of Animal Science and Veterinary Medicine,Henan Agricultural University, Zhengzhou 450002,China.2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province,National Engineering Research Center for Breeing Swine Industry,College of Veterinary,South China Agricultural University,Guangzhou 510642, China.*	Henan Agricultural University
33	Zhao	Mengmeng	Preparation and identification of the monoclonal antibodies against Nsp9 protein of Porcine Reproductive and Respiratory Syndrome Virus. *M.M. Zhao1,2*, G.H. Zhang2. 1College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China. 2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, National Engineering Research Center for Breeing Swine Industry, College of Veterinary, South China Agricultural University, Guangzhou 510642, China.*	Henan Agricultural University
99	Zhang	Yongning	Epitope mapping of monoclonal antibodies against emerging porcine circovirus subtype 3. *Y. Zhang1,3, P. Shang1, F. Yuan1,2, R.Guo1, B.M. Hause1,2, Y. Fang1,2. 1Department of Diagnostic Medicine and Pathobiology; 2Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas; 3Institute of Animal Quarantine, Chinese Academy of Inspection and Quarantine, Beijing, China.*	Kansas State University

Host response to infection, including host genetics and innate/adaptive immunity				
34	Berg	Kristen	Effect of PEDV and PRRSV outbreaks on reproductive performance of commercial sows. *K.E. Berg1*, C.L. Ferring1, K.A. Gray2, N.V.L. Serão1. 1Department of Animal Science, North Carolina State University, Raleigh, NC. 2Smithfield Premium Genetics, Rose Hill, NC.*	North Carolina State University
35	Buckley	Alexandra	Senecavirus A infection in sows, neonates, and market weight gilts with subsequent protective immunity. *A. Buckley1*, B. Guo2, N. Montiel1, V. Kulshreshtha1, A. van Geelen1, K.J. Yoon2, K. Lager3. 1Oak Ridge Institute for Science and Education and National Animal Disease Center and U.S. Department of Agriculture, Ames, IA, USA. 2College of Veterinary Medicine, Iowa State University, Ames, IA, USA. 3National Animal Disease Center, U.S. Department of Agriculture, Ames, IA, USA.*	USDA
36	Canelli	Elena	Phenotypic characterization of a novel HP Italian PRRSV-1 isolate in experimentally infected pigs. *E. Canelli1*, A. Catella1, A. Corradi1, B. Passeri1, E. De Angelis1, G. Sandri2, F.C. Leung3, G. Ogno1, L. Ferrari1, P. Borghetti1, P. Martelli1. 1Department of Veterinary Science – University of Parma – Italy. 2Gruppo Veronesi – Italy. 3School of Biological Science, The University of Hong Kong, Hong Kong SAR, China, and FCL Bioscience, Hong Kong Science and Technology Park, Shatin, New Territories, Hong Kong SAR, China.*	University of Parma
37	Constance	Laura	Comparison of morbidity and mortality after challenge with two North American PRRS virus isolates shows marked variation in time course and prevalence of clinical disease between isolates. *L.A. Constance1*, B. Bloomberg3, J.K. Lunney4, J.C.M. Dekkers5, R.R.R. Rowland1, M.C. Niederwerder1,2. 1Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS. 2Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS. 3Comparative Medicine Group, Kansas State University, Manhattan, KS. 4United State Department of Agriculture, Agricultural Research Services, Beltsville Agricultural Research Center, Beltsville, MD; 5Department of Animal Science, Iowa State University, Ames, IA.*	Kansas State University
38	Correas	Ignacio	Cross-reactivity of immune responses against Porcine Reproductive and Respiratory Syndrome virus. *I. Correas*, H.L.X. Vu, A.K. Pattnaik, F.A. Osorio. Nebraska Center for Virology and School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE.*	University of Nebraska-Lincoln
39	Cortey	Marti	Changes in the genetic composition of PRRSV quasispecies and its relationship with long and short viral infections. *M. Cortey1*, G.M. Arocena1, E. Mateu1,2. 1IRTA-CReSA, UAB Campus, E-08193 Bellaterra, Barcelona (Catalonia-Spain). 2Departament de Sanitat i d'Anatomia Animals, UAB, E-08193 Bellaterra, Barcelona (Catalonia-Spain).*	IRTA-CReSA

40	Darbellay	Joseph	The progression to T cell immunity after infection with porcine reproductive and respiratory syndrome virus. *J. Darbellay1*, J. Van Kessel1, T. Käser2, V. Gerdts1. 1Vaccine and Infectious Disease Organization-International Vaccine Centre, Saskatoon, Sk. Canada. 2College of Veterinary Medicine, NC State University, Raleigh, North Carolina, United States.*	VIDO-InterVac
41	Detmer	Susan	Pathological findings at the maternal-fetal interface during the early type 2 PRRS virus infection of late gestation pregnant gilts. *P. Novakovic1, S.E. Detmer1*, S. Muhammad1, C.M. Malgarin1, A. Ladinig2, D.J. MacPhee1, J.C.S. Harding1. 1Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada. 2University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria.*	University of Saskatchewan
42	Diel	Diego	Pathogenesis and infection dynamics of Senecavirus A in pigs. *L.R. Joshi1, M.V.H. Fernandes1, T. Clement1, S. Lawson1, T.P. Resende, F.A. Vannucci2, E.A. Nelson1, D.G. Diel1*. 1Animal Disease Research and Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD. 2Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN.*	South Dakota State University
43	Dong	Qian	The effect of PRRS viral level and isolate on tonsil gene expression 42 days after infection. *Q. Dong*1, J. Lunney2, E. Fritz-Waters1, Y. Nguyen3, R.R.R. Rowland4, A. Hess1, J. Reecy1, J. Dekkers1. 1Department of Animal Science, Iowa State University, Ames, Iowa. 2USDA, ARS, BARC, APDL, Beltsville, Maryland; 3Department of Statistics, Iowa State University, Ames, Iowa. 4College of Veterinary Medicine, Kansas State University, Manhattan, Kansas.*	Iowa State University
44	Dunkelberger	Jenelle	A major gene for host response under PRRS challenge is not negatively associated with overall performance in commercial pig lines under non-challenged conditions. *J.R. Dunkelberger1*, P.K. Mathur2, M.S. Lopes2, E.F. Knol2, J.C.M. Dekkers1. 1Department of Animal Science, Iowa State University, Ames, IA, USA. 2Topigs Norsvin Research Center, Beuningen, Gelderland, the Netherlands.*	Iowa State University
45	Feng	Wen-hai	Porcine reproductive and respiratory syndrome virus (PRRSV) up-regulates IL-8 expression through TAK-1/JNK/AP-1 pathway. *Y.H. Liu1,2, W.H. Feng1,2*. 1State Key Laboratory of Agrobiotechnology. 2Department of Microbiology and Immunology, College of Biological Sciences, China Agricultural University, Beijing 100193, China.*	China Agricultural University
46	Ferring	Cassandra	Genetic evaluation of reproductive performance during PRRS/PED outbreaks. *C.L. Ferring1*, K.A. Gray2, N.V.L. Serão1. 1Department of Animal Science, North Carolina State University, Raleigh, NC. 2Genetic Research and Development, Smithfield Premium Genetics, Rose Hill, NC.*	North Carolina State University

47	Gaudreault	Natasha	Genetically edited pigs lacking CD163 show no resistance following infection with the African Swine Fever Virus isolate, Georgia 2007/1. *L.N. Popescu1, N.N. Gaudreault1*, K.M. Whitworth2, M.V. Murgia1, J.C. Nietfeld1, A. Mileham3, M. Samuel2, K.D. Wells2, R.S. Prather2, R.R.R. Rowland1. 1Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA. 2Division of Animal Science, College of Food Agriculture and Natural Resources, University of Missouri, Columbia, Missouri, USA. 3Genus, plc, DeForest, Wisconsin, USA.*	Kansas State University
48	Guo	Rui	Porcine reproductive and respiratory syndrome virus takes advantage of host intercellular mitochondria transferring pathway for cell to cell spreading of the infection. *R. Guo, Y. Fang. Department of Diagnostic Medicine and Pathobiology, Kansas state University, Manhattan, KS, 66506.*	Kansas State University
49	Hong	Linjun	Evaluation of fetal and maternal gene expression responses to reproductive porcine reproductive and respiratory syndrome virus infection. *L. Hong*1, A. Ladinig2, J.C.S. Harding3, J.K. Lunney1. 1Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, ARS, USDA, Beltsville, MD, USA. 2University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria. 3Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada.*	Beltsville Agricultural Research Center, ARS, USDA
50	Ke	Hanzhong	SAP domain in nsp1-beta of porcine reproductive and respiratory syndrome virus (PRRSV) correlates with interferon suppression in cells and pathogenesis in pigs. *H. Ke1*, M. Kerrigan2, M. Han1, R. Rowland2, D. Yoo1. 1Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL. 2Department of Diagnostic Medicine & Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.*	University of Illinois at Urbana-Champaign
51	Kimpston-Burkgren	Kay	Contribution of PRRSV minor glycoproteins to a protective immune response in swine. *K. Kimpston-Burkgren1,3*, H. Vu2,3, I. Correas2,3, D. Steffen2, A. Pattnaik2,3, Y. Fang4, F. Osorio2,3. 1School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE. 2School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE. 3Nebraska Center for Virology, Lincoln, NE. 4Department of Diagnostic Medicine/Pathology, Kansas State University, Manhattan, KS.*	University of Nebraska-Lincoln
52	Lee	Yoo Jin	Porcine deltacoronavirus induces caspase-dependent programmed cell death. *Y.J. Lee*, C. Lee. Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea.*	Kyungpook National University

53	Li	Yanli	Resolution of genotype 1 PRRSV attachment on bone marrow-derived dendritic cells. *Y.L. Li1,2*, E. Cano2, L. Darwich1,2, E. Mateu1,2. 1Departament de Sanitat i Anatomia Animals, UAB, Cerdanyola del Vallès Barcelona, Spain. 2Centre de Recerca en Sanitat Animal (CReSA)-IRTA, Cerdanyola del Vallès Barcelona, Spain.*	Universitat Autònoma de Barcelona
54	Liu	Pinghuang	IFN-lambda preferably inhibits PEDV infection of porcine intestinal epithelial cells compared to IFN-alpha. *L. Li1, F. Fu1, M. Xue1, W. Chen1, J. Liu2, H. Shi1, J. Chen1, Z. Bu1, L. Feng1, P. Liu1*. 1State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China. 2Jiangxi Province Key Laboratory of Bioprocess Engineering, Jiangxi Science & Technology Normal University, Nanchang, China.*	Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences
55	Ma	Jinyou	PRRSV nsp5 downregulates expression of antiviral genes by suppressing phosphorylation of STAT2. *J. Ma*, Q. Zhang, H. Ke, W. Liang, C. Kim, D. Yoo. Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.*	University of Illinois at Urbana-Champaign
56	Maciel Malgarin	Carolina	Temporal movement of type 2 porcine reproductive and respiratory syndrome virus across the maternal-fetal interface. *C.M. Malgarin1*, R. Nosach1, M. Suleman1, P. Novakovic1, A. Ladinig2, S. Detmer1, D. J. MacPhee1, J.C.S. Harding1. 1Western College of Veterinary Medicine. 2University of Veterinary Medicine Vienna.*	University of Saskatchewan
57	Rathkjen	Poul Henning	Pigs vaccinated with PRRSFLEX EU at two- or three-weeks of age that did not show sero-conversion are protected by an immunologic recall answer after artificial challenge. *P.H. Rathkjen1, R. Hennies2, K. Dreckmann2, C. Kraft2. 1Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany. 2Boehringer Ingelheim Veterinary Research Center GmbH & Co. KG.*	Boehringer Ingelheim Animal Health GmbH
58	Sang	Yongming	Antiviral potency and functional novelty of porcine Interferon-Omega subtype. *Q. Liu1, J. Lee3, W. Ma3, R.R.R. Rowland3, F. Blecha2, Y. Sang1. 1Department of Agricultural and Environmental Sciences, College of Agriculture, Human and Natural Sciences, Tennessee State University, Nashville, TN 37209. 2Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA. 3Department Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.*	Tennessee State University

59	Serão	Nick	Genetic variation for antibody response to a range of pathogens in commercial replacement gilts. *N.V.L. Serão1,2*, R.A. Kemp3, B.E. Mote4, J.C.S. Harding5, P. Willson6, S.C. Bishop7, G.S. Plastow8, J.C.M. Dekkers2. 1Department of Animal Science, North Carolina State University, Raleigh, NC. 2Department of Animal Science, Iowa State University, Ames, IA. 3Genesus Inc, Oakville, Canada. 4Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE. 5Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, Canada. 6Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Canada. 7The Roslin Institute and R(D)SVS, University of Edinburgh, Scotland. 8Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.*	North Carolina State University
60	Zhang	Qingzhan	Modulation of NF-kB activity for innate immune evasion by nonstructural protein 1 of Porcine Epidemic Diarrhea Virus. *Q. Zhang, J. Ma, D. Yoo. Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.*	University of Illinois at Urbana-Champaign
61	Zhang	Yanjin	PRRSV interference with cytokine-mediated JAK/STAT signaling. *L. Yang*, Z. Ma, Y. Zhang. Molecular Virology Laboratory, VA-MD College of Veterinary Medicine, University of Maryland, College Park, MD.*	University of Maryland
62	Zuckermann	Federico	The unfolded protein response induced by porcine reproductive and respiratory syndrome virus infection of alveolar macrophages is involved in immune dysregulation. *W.Y. Chen*, W.M. Schniztlein, G. Calzada-Nova, F.A. Zuckermann. Department of Pathobiology, University of Illinois at Urbana-Champaign. Urbana, IL.*	University of Illinois at Urbana-Champaign
101	Murgia	Maria	Identification of African swine fever virus p30 antigenic epitopes after experimental infection. *M.V. Murgia, N.N. Gaudreault, R.R.R. Rowland. Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, 1800 Denison Avenue, Manhattan, Kansas 66506, USA.*	Kansas State University
Vaccination strategies and therapeutics				
63	Chang	Chia-Jung	Evaluation of the novel subunit porcine reproductive and respiratory syndrome (PRRS) vaccine against PRRSV in piglets and sows. *C.J. Chang, Y.W. Lin, I.H. Lai, W.J. Chen, Y.C. Lin, W.I. Chou.* Reber Genetics Co., Ltd., Taipei, Taiwan.*	Reber Genetics Co., Ltd
64	Cui	Junru	A GP5 mosaic T-cell vaccine for Porcine Reproductive and Respiratory Syndrome Virus is immunogenic and confers partial protection to pigs. *J. Cui*, C.M. O'Connell, J.D. Smith, J. Smyth, P.H. Verardi, Y. Pan, A.E. Garmendia. Department of Pathobiology and Veterinary Science, College of Agriculture, Health and Natural Resources, University of Connecticut, Storrs, CT 06269, USA.*	University of Connecticut

65	de Grau	A. Francisco	Field evaluation of a modified-live PRRS vaccine in an unstable herd in Northwest Mexico. *G. Bernal1, R. García1, A.F. de Grau2*, B. Thacker3, R. Jolie4. 1MSD México, México City México. 2 Intervet Canada Corporation, Kirkland, Quebec, Canada. 3Merck Animal Health, DeSoto, KS, USA. 4 Merck Animal Health, Madison, NJ, USA.*	Intervet Canada Corp, Merck Animal Health
66	Han	Jeong Hee	Comparative serological response after an inactivated EU-typed PRRS vaccination in Korea. *K.D. Min, Y.C. Lee, E.H. Cho, J.H. Han*. College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon-Si, Kangwon-Do, 200-180, South Korea.*	Kangwon National University
67	Kraft	Jordan	Serum and mammary secretion antibody responses in PEDV-exposed gilts following PEDV vaccination. *J.B. Kraft1*, K. Woodard1, L. Giménez-Lirola1, B. Setness1, J. Ji1, P. Lasley2, E. Nelson3, J. Zhang1, D. Baum1, P. Gauger1, J. Zimmerman1, R. Main1. 1Iowa State University. 2Smithfield Hog Production. 3South Dakota State University.*	Iowa State University
68	Lee	Kyung-Tai	Interaction between ORF2 protein of porcine circovirus type 2 and C1QBP enhances phagocytic activity. *K.T. Lee1, C.Y. Choi2, E.S. Cho3, T. Chun2. 1Animal Genomics and Bioinformatics Division, National Institute of Animal Science, RDA, Wanju 55365, South Korea. 2Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Republic of Korea. 3Swine Science Division, National Institute of Animal Science, RDA, Cheonan 31000, South Korea.*	National Institute of Animal Science
69	Lee	Sunhee	Development of porcine epidemic diarrhea virus vaccines derived from a virulent Korean strain. *S. Lee*, C. Lee. Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea.*	Kyungpook National University
70	Lee	Sunhee	Pathogenic and genomic characteristics involved in porcine alveolar macrophage passages of an attenuated PRRSV nsp2 DEL strain CA-2-P100. *S.C. Lee1, S. Lee2*, H.W. Choi1, I.J. Yoon1, S.Y. Kang3, C. Lee2. 1Choongang Vaccine Laboratory, Daejeon, South Korea; 2Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea; 3College of Veterinary Medicine, Chungbuk National University, Cheongju, South Korea.*	Kyungpook National University
71	Opriessnig	Tanja	An interferon inducing PRRSV vaccine candidate protects against challenge with a heterologous virulent type 2 strain in a conventional pig model. *E. Fontanella1, Z. Ma2, Y. Zhang2, A. Castro1, H. Shen1, P. Halbur1, T. Opriessnig1,3*. 1Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA; 2VA-MD College of Veterinary Medicine and Maryland Pathogen Research Institute, University of Maryland, College Park, MD, USA; 3The Roslin Institute, University of Edinburgh, Midlothian, UK.*	University of Edinburgh, Iowa State University

72	Opriessnig	Tanja	A PRRSV candidate vaccine based on the synthetic attenuated virus engineering approach is attenuated and effective in protecting against homologous virus challenge. *D. Evenson1, P.F. Gerber2, C.T. Xiao1, P.G. Halbur1, C. Wang1, D. Tian3, Y.Y. Ni3, X.J. Meng3, T. Opriessnig1,2*. 1Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA; 2The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK; 3Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.*	University of Edinburgh, Iowa State University
73	Oswald	Hayley	Efficacy of Ingelvac PRRS® MLV against a heterologous PRRSV 1-7-4 RFLP challenge. *A. Patterson, B. Fergen, J. Hermann, G. Haiwick, R. Philips. Boehringer Ingelheim Vetmedica, Inc., Ames, USA.*	Boehringer Ingelheim Vetmedica, Inc.
74	Oswald	Hayley	Evaluation of PRRSV challenge dose in vaccinated pigs. *G. Haiwick, A. Neubauer, J. Hermann, M. Roof, B. Fergen, R. Philips.* Boehringer Ingelheim Vetmedica, Inc. MO.*	Boehringer Ingelheim Vetmedica Inc.
75	Parrillo	Matthew	How to pick the right strain of PRRSv for a vaccine... Or for an outbreak at a concentrated animal feeding operation (CAFO). *A.H. Gutiérrez1, C. Loving2, L. Moise1,3, M. Parrillo1*, W.D. Martin3, A.S. De Groot1,3. 1Institute for Immunology and Informatics, University of Rhode Island, Providence, RI; 2Virus and Prion Diseases Research Unit, NADC, USDA ARS, Ames, IA; 3EpiVax, Inc., Providence, RI.*	Institute for Immunology and Informatics at the University of Rhode Island
76	Shi	Jishu	Pigs immunized with a novel E2 subunit vaccine are protected from subgenotype heterologous Classical Swine Fever Virus challenge. *R. Madera1, W. Gong1,5, L. Wang1, Y. Burakova1,4, K. Lleellish1, A. Galliher-Beckley1, J. Nietfeld2, J. Henningson2, K. Jia3, P. Li3, J. Bai2, J. Schlup4, S. McVey6, C. Tu5, J. Shi1*. 1Department of Anatomy and Physiology; 2Department of Diagnostic Medicine and Pathobiology; 3Department of Chemistry; 4Department of Chemical Engineering, Kansas State University, Manhattan, KS 66506, USA; 5Institute of Military Veterinary Medicine, Academy of Military Medical Sciences, Changchun, China; 6United States Department of Agriculture, Agricultural Research Service, Arthropod Borne Animal Disease Research Unit, Manhattan, KS 66502, USA.*	Kansas State University
77	Stott	Christopher James	Maternally derived antibody mediated protection against porcine epidemic diarrhea virus on piglets via the different ways of immunity provides in sows following by the classical and variant strain challenges. *K. Sawattrakool, C.J. Stott*, G. Temeeyasen, D. Nilubol. Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Thailand.*	Chulalongkorn University

78	Wang	Xiuqing	Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2'3'-cGAMP VacciGradeTM adjuvant exacerbates viremia after virus challenge. *A. Van Noort1*, A. Nelsen1, A.E. Pillatzki2, D. Diel2, F. Li1,2,3, E. Nelson2, X. Wang1. 1Department of Biology and Microbiology, 2Veterinary and Biomedical Sciences Department, 3BioSNTR, South Dakota State University, Brookings, SD 57007.*	South Dakota State University
79	Wu	Shaoqin	A sow farm PRRS elimination with serum therapy and herd closure. *S. Wu,* Z. Wu, Y. Xiao, Y. Cao, F. Deng. Guangxi State Farms Yongxin Husbandry Company Nanning Hengxian 530317.*	Guangxi State Farms Yongxin Husbandry Company
80	Wu	Shaoqin	Abortions when applied serum therapy in PRRS unstable sow farms. *S. Wu, Z. Wu, Y. Xiao, Y. Qin, L. Xie. Guangxi State Farms Yongxin Husbandry Company Nanning Hengxian 530317.*	Guangxi State Farms Yongxin Husbandry Company
81	Xu	Sophia Bingling	Micro-emulsion adjuvants for swine viral vaccines: Application to a recombinant CSF vaccine. *S. Xu1*, F. Bertrand2, J. Ben Arous2, L. Dupuis2. 1SEPPIC Inc, 30 Two Bridges Road, Suite 210 – Fairfield, NJ 07004, USA. 2SEPPIC, 22 Terrasse Bellini, Paris La Défense, 92806 Puteaux Cedex, France.*	Seppic Inc
82	Zhou	Lei	Attenuate highly pathogenic Porcine Reproductive and Respiratory Syndrome virus by incorporating target site of hematopoietic-specific microRNA into viral genome. *S. Tan, R. Zhang, X. Ge, X. Guo, J. Han, H. Yang, L. Zhou*. 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.*	China Agricultural University
100	Shang	Pengcheng	Development of novel chimeric vaccine and delivery system for classical swine fever virus. *P. Shang1, A. Avila2, R. Guo1, S.K. Whitaker3, Y. Li1, J. Tomich3, R.R.R. Rowland1, Y. Fang1. 1Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; 2Department of Chemistry and Biochemistry, Auburn University, Auburn, AL; 3Department of Biochemistry and Molecular Biophysics, College of Arts and Sciences, Kansas State University, Manhattan, KS.*	Kansas State University
Virus structure and gene function				
83	Abrahamyan	Levon	Dissection of complex molecular interactions between important animal nidoviruses and the host. *L.J. Sánchez Mendoza1, 2, C.A. Valle Tejada 1, 2, C. Provost1, 2, C.A. Gagnon1, 2, F. Beaudry 2, 3, L. Abrahamyan1, 2*. 1Research Group on Infectious Diseases in Production Animals (GREMIP); 2Swine and Poultry Infectious Diseases Research Center (CRIPA); 3Department of Biomedicine, Faculty of Veterinary Medicine, Université de Montréal, St-Hyacinthe, Québec, Canada.*	Université de Montréal

84	Han	Jun	Cleavage of cytochrome c1 by the PRRSV 3C-like protease leads to shedding of an apoptosis inducer. *J. Han*, F. Zhang, X. Ge, P. Gao, L. Zhou, X. Guo, H. Yang. Key Laboratory of Animal Epidemiology and Zoonosis of the Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China.*	China Agricultural University
85	Li	Yanhua	A novel mechanism of protein-stimulated trans-activation of ribosomal frameshifting in porcine reproductive and respiratory syndrome virus: implication in improved vaccine development. *Y. Li1, S. Napthine2, E.E. Treffers3, P. Shang1, A. Tas3, A.E. Firth2, I. Brierley2, E.J. Snijder3, Y.Fang1. 1Kansas State University, Manhattan, U.S.A; 2Department of Pathology, University of Cambridge, Cambridge, U.K.; 3Leiden University Medical Center, Leiden, The Netherlands.*	Kansas State University
86	Stoian	Ana	Identification of CD163 domain involved in the infection with Type II Porcine Reproductive and Respiratory viruses. *A.M.M. Stoian*, R.R.R. Rowland. Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.*	Kansas State University
87	Zhao	Mengmeng	Cloning and sequence analysis of Nsp9 gene of Porcine Reproductive and Respiratory Syndrome Virus FS strain. *M. Zhao1,2*, G. Zhang2. 1College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China; 2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, National Engineering Research Center for Breeing Swine Industry, College of Veterinary, South China Agricultural University, Guangzhou 510642, China.*	Henan Agricultural University
88	Zhao	Mengmeng	Construction and identification of Nsp9-deficient clone of porcine reproductive and respiratory syndrome virus (PRRSV) XH-GD strain. *M. Zhao1,2*, G. Zhang2. 1College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China; 2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, National Engineering Research Center for Breeing Swine Industry, College of Veterinary, South China Agricultural University, Guangzhou 510642, China.*	Henan Agricultural University
89	Zhao	Mengmeng	Influence of Nsp9 between of highly pathogenic PRRSV and low pathogenic PRRSV to replication of PRRSV. *M. Zhao1,2*, G. Zhang2. 1College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China; 2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, National Engineering Research Center for Breeing Swine Industry, College of Veterinary, South China Agricultural University, Guangzhou 510642, China.*	Henan Agricultural University
90	Zhao	Mengmeng	Influence of Nsp9 to replication of PRRSV in MARC-145 cells. *M. Zhao1,2*, G. Zhang2. 1College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China; 2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, National Engineering Research Center for Breeing Swine Industry, College of Veterinary, South China Agricultural University, Guangzhou 510642, China.*	Henan Agricultural University

91	Zhao	Mengmeng	Subcellular localization analyisi and function prediction of PRRSV NSP9 protein. *M. Zhao1,2*, G. Zhang2. 1College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China; 2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, National Engineering Research Center for Breeing Swine Industry, College of Veterinary, South China Agricultural University, Guangzhou 510642, China.*	Henan Agricultural University
92	Zhao	Mengmeng	Substitution between highly pathogenicial PPRSV and low pathogenicial PPRSV with Reverse Genetics System. *M. Zhao1,2*, G. Zhang2. 1College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China; 2Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, National Engineering Research Center for Breeing Swine Industry, College of Veterinary, South China Agricultural University, Guangzhou 510642, China.*	Henan Agricultural University
Virus Transmission, biosecurity and area control				
93	Dee	Scott	Modeling the transboundary survival of foreign animal disease pathogens in contaminated feed ingredients. *S. Dee1*, G. Spronk1, E. Nelson2, D. Diel2, T. Clement2, A. Singrey2, F. Bauermann2, J. Hennings2, C. Jones3, R. Cochrane3, G. Patterson4. 1Pipestone Veterinary Services, Pipestone, MN; 2Animal Disease Research and Diagnostic Laboratory, Dept. of Veterinary and Biomedical Sciences, SDSU, Brookings, SD; 3Dept. of Grain Science, KSU, Manhattan, KS; 4Dept. of Veterinary Public Health & Preventative Medicine, UMN St. Paul, MN.*	Pipestone Veterinary Services
94	Gu	Jinyan	Isolation and characterization of porcine deltacoronavirus from pigs with diarrhea in China. *J. Zhou1,2,3*, J. Gu1,2*, L. Du1, S. Su1. 1Jiangsu Engineering Laboratory of Animal Immunology, Institute of Immunology, Nanjing Agricultural University, Nanjing, Jiangsu, China; 2Key Laboratory of Animal Virology of Ministry of Agriculture, Zhejiang University, Hangzhou, China; 3Collaborative Innovation Center and State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University, Hangzhou, China.*	Nanjing Agricultural University
95	Lowe	James	Leveraging data across a large geographic area to increase context and understanding of swine viral residence and transmission in smaller regions. *J.F. Lowe1*, E.J. Lowe2. 1Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL; 2Boehringer Ingelheim Vetmedica Inc, St. Joseph, MO.*	University of Illinois

96	Nathues	Christina	Estimating the costs of Porcine Reproductive & Respiratory Syndrome (PRRS) and return on investment of interventions with a PRRS economic simulator. *C. Nathues1, P. Alarcon 2, J. Rushton 2, G. Schüpbach-Regula 1, R. Jolie3*, K. Fiebig4, M. Jimenez5, V. Geurts6, H. Nathues7. 1VPH Institute, Vetsuisse Faculty, University of Bern, Switzerland; 2Veterinary Epidemiology, Economics and Public Health Group, Royal Veterinary College of London, United Kingdom; 3Merck Animal Health, New Jersey, USA; 4.-6MSD Animal Health, Germany, Spain, The Netherlands; 7Clinic for Swine, University of Bern, Switzerland.*	University of Bern
97	Suleman	Muhammad	Spatiotemporal detection and localization of type 2 porcine reproductive and respiratory syndrome virus at the maternal-fetal interface of late gestation pregnant gilts. *M. Suleman1, L. Reiter2, P. Novakovic1, C.M. Malgarin1, S.E. Detmer1, A. Ladinig2, D.J. MacPhee1, J.C.S. Harding1*. 1Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada; 2University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria.*	University of Saskatchewan
98	Valdes-Donoso	Pablo	Using machine learning to predict swine movements with application to the control of infectious diseases. *P. Valdes-Donoso1,2*, K. VanderWaal1, L.S. Jarvis2, S. Wayne3, A. Perez1. 1Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA; 2Department of Agricultural and Resource Economics, University of California Davis, Davis, CA, USA; 3Pipestone, Veterinary Service.*	University of Minnesota

S1
Update on the host genetics of resistance to porcine diseases

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The purpose of this presentation is to provide an update on ongoing research on host genetics of resistance to disease in pigs, with a focus on the grow-finish phase. Starting with experimental infections with a specific PRRS virus through the PRRS Host Genomics Consortium (PHGC), this research has evolved into experimental co-infection challenges with PRRS and PCV2, field studies of the performance of pigs under natural health challenges, and most recently, large-scale natural multi-pathogen challenges in experimental facilities. The aim of these studies is to identify pigs that are more resilient, which refers to the ability to respond to infection to minimize the impact of disease on performance. In addition to genomic analyses, the latter studies also include deep phenotyping of healthy young pigs prior to their entry in the challenge facility, with the goal to identify genetic and phenotypic predictors of pigs that are more resilient.

S2
A multi'omics approach to understanding the reproductive pathophysiology of type 2 porcine reproductive and respiratory syndrome (PRRS)

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A large-scale challenge experiment using type 2 porcine reproductive and respiratory virus (PRRSV) was performed at the University of Saskatchewan with the objective of elucidating genomic and phenotypic factors associated with the pathophysiology of reproductive PRRS. Purebred Landrace gilts in third-trimester of pregnancy were experimentally inoculated with NVSL 97-7895 (1×10^5 $TCID_{50}$ total dose; n=114) or served as sham-inoculated controls (n=19). All were humanely euthanized at 21 days post inoculation (dpi) at gestation day 106±1. Phenotypic responses in dams and fetuses were exhaustively studied following inoculation, and enabled genome-wide transcriptomic and genomic analyses of traits associated with disease progression and resilience in dams and fetuses. Fetal mortality rate ranged from 0% to 94.4% (mean 41.0±22.8%) in PRRSV-inoculated gilts. Gilt PRRSV infection resulted in a massive, acute drop in all leukocyte subsets by 2 dpi, and most prominently in subsets involved in cytotoxic and killer activities (CTL, NK) and naive B-, T-helper, and gamma-delta T cells. Levels of interferon (IFN)-alpha in gilt sera rose sharply by 2 dpi before falling to baseline by 6 dpi, in contrast to viral load in sera, which peaked at 6 dpi. PRRSV RNA concentration (log10 copies/mg) at the maternal-fetal interface was a strong predictor of viral load in fetal thymus and the odds of fetal death. However, resilience to IFN-alpha suppression (measured in supernatants of PBMC re-stimulated with PRRSV) was associated with higher fetal mortality. Even though the prevalence of fetal lesions was low (<15% of fetuses), the presence of fetal and umbilical cord lesions was positively associated with fetal compromise. Fetal death and viral load clustered in litters suggesting PRRSV transmits horizontally starting from a limited number of index fetuses. Factors associated with index fetal infection are not understood, but large fetuses appear to be at greater risk than smaller siblings. Maternal transcriptomic responses were compared in a subset of "resilient" and "susceptible" gilts. Low fetal mortality was associated with higher basal (pre-inoculation) expression of genes and/or modules involved in platelet function, IFN and pro-inflammatory signaling, and up-regulation of genes involved in T cell, pro-inflammatory and IFN responses at 2-6 dpi. Disease progression in fetuses was associated with an up-regulation of genes associated with inflammation, innate immunity, and cell death signaling, and down-regulation of genes associated with cell cycle and lymphocyte quality. Twenty-one genomic regions across 10 chromosomes, many of which that overlapped with various swine health traits, were associated with fetal viral load, death or viability.

This research provided many new insights in PRRS pathophysiology, and clear evidence that reproductive outcome depends on events occurring systemically in dams, at the maternal-fetal interface, and in the fetal compartment. In addition, the identification genomic regions associated with reproductive PRRS resilience may lead to alternative control strategies in the future. This research was supported by Genome Canada, Genome Prairie (Saskatchewan Ministry of Agriculture) and Genome Alberta.

S3
The role of the microbiome in PRRS

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Microbiome is a term used to describe the community of microorganisms that live on the skin and mucosal surfaces of animals. The gastrointestinal microbiome contains the majority of these microorganisms, which are essential for digestion of nutrients and development of the immune system. How the gastrointestinal microbiome impacts primary respiratory infections is an emerging area of study. Several infectious causes of pneumonia in humans have been found to have associations with the gastrointestinal microbiome, including factors such as cytokine expression, airway inflammation, and alveolar macrophage activity. In our research, we have focused on investigating the role of the microbiome in disease outcome of nursery pigs following co-infection with PRRSV and PCV2. Fecal microbiome composition and diversity has been associated with several outcome parameters in co-infected pigs, such as average daily gain, virus replication, presence of clinical signs, and severity of interstitial pneumonia. Microbiome characteristics shown to be beneficial for outcome have included 1) increased microbial family and species diversity, 2) increased Proteobacteria, specifically *Escherichia coli*, 3) decreased *Methanobacteriaceae,* and 4) increased *Ruminococcus* sp. This presentation will review the gut-lung axis in infectious pneumonia as well as discuss research findings on how the microbiome may play a role in outcome following co-infection with PRRSV and PCV2 in nursery pigs.

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S4
Update on novel experimental pig vaccine approaches

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Achieving consistent protection by vaccinating pigs against pathogens of importance such as porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV) or porcine epidemic diarrhea virus (PEDV) remains difficult especially when solid heterologous protection against genetically diverse strains is desired.

In this presentation, an alternative approach to protect pigs against PRRSV tested in the conventional experimental pig model will be discussed[1] which is based on the synthetic attenuated virus engineering (SAVE) approach. This approach was used to rapidly produce an attenuated farm-specific homologous vaccine strain (SAVE5) of VR-2385 and was subsequently tested in the pig model. Anti-PRRSV-IgG positive vaccinated pigs were protected from subsequent VR2385 challenge as evidenced by lack of VR2385 viremia and nasal shedding, significantly reduced macroscopic and microscopic lung lesions and significantly reduced amount of PRRSV antigen in lungs compared to the non-vaccinated VR2385-challenged positive control pigs. This work indicates that the SAVE approach was successful in attenuating PRRSV strain VR2385 and protecting against homologous virus challenge.

Novel subunit vaccines based on polyvalent vaccine platforms using the IAV M2e epitope as antigen have been very successful in the mouse model in protecting mice from lethal IAV challenge.[2,3] The same vaccine strains were tested in pigs using an intranasal vaccination route and compared to a commercial pH1N1 specific vaccine. The commercial SIV vaccine effectively protected pigs against homologous challenge as evidenced by reduced clinical signs, virus shedding in nasal secretions and oral fluids and reduced macroscopic and microscopic lesions; however, intranasal vaccination with the experimental M2e epitope-based subunit vaccines did not. The results further highlight the importance using IAV type specific vaccines in pigs and also the challenges when attempting to extrapolate data obtained in one species to another.

Finally, using less pathogenic live virus vaccines may be a fast and efficient option to protect pigs effectively against viruses where low and high virulent variants co-exist in the population such as is the case with PEDV. Since PEDV strains from the G1b cluster (Spike-gene-based phylogeny) are less pathogenic compared to the pathogenic G2b cluster,[4,5] the ability of an experimental G1b-based live vaccine and a commercial G2b-based inactivated vaccine to protect growing pigs against G2b challenge was tested in pigs. Interestingly, while a commercial inactivated G2b-based PEDV vaccine administered intramuscularly protected pigs against homologous challenge, the experimental G1b-based live virus vaccine given orally induced a high IgA response but the virus shedding pattern after shedding mimicked was similar to non-vaccinated infected pigs suggesting limited protection. This perhaps could indicate that induction of a genotype specific humoral and/or cellular immune response may be important for PEDV protection.

S5
Genome Editing for PRRSV Resistance and Beyond

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Genetic engineering has been a staple of food production, at least on the plant side. The applications have resulted in decreased pesticide and herbicide usage thus resulting in cleaner water in both rural and metropolitan communities. Recent development and application of gene editing technologies will result in even more significant applications in the plant world. The gene editing technologies include zinc-finger nucleases, TAL effector nucleases, and the CRISPR/Cas9 system. All three of these technologies have permitted genetic edits that range from the addition or removal of a single nucleotide to replacing a small region or swapping complete protein domains. What is notable is that the CRISPR/Cas9 system is relatively to use. It requires a guide RNA and the Cas9 protein. The guide RNA can easily be designed and assembled in a few days. This ease of construction is in contrast to the laborious task of assembling modules for zinc-finger nucleases and TAL effector nucleases. Not only are the guides easy to assemble they, like the other editing technologies, are highly efficient. Generally, it is thought that when the editing molecules cut the DNA the cut strand is repaired; but since the editing molecule is still there editing molecules continue to cut the DNA until a mistake is made in the repair mechanism and non-homologous end joining results. Since an error is made in the repair, the editing mechanism no longer cuts the DNA because the target sequence no longer exists. If a repair template is included at the time of editing, often the cut DNA will incorporate that molecule into the repair.

The CRISPR/Cas9 system has been used to edit *CD163* to make pigs resistant to Porcine Reproductive and Respiratory Syndrome Virus. The edits included both non-homologous end joining and repair by using a template to swap out domain 5 of the CD163 protein. In addition to *CD163*, use of a repair template has permitted introducing an Angus allele for the polled phenotype into Holstein cattle, and to introduce a sequence specific for Warthogs into domestic pig *RELA* potentially to provide resistance to African Swine Fever. Another proposed use of the editing technology would be to change a single amino acid in *CD18* in cattle to provide resistance to bovine respiratory disease caused by *Mannheimia haemolytica*.

The relative ease at which edits can be introduced into the genome of domestic animals ensures that additional applications in domestic animals for agriculture will be made to address disease resistance, animal welfare, productivity, and carcass composition. But not only agriculture will benefit, this same technology can be used to make, the pig for example, better models for studying human disease.

S6
The latest on pathogenesis and control of highly pathogenic PRRSV in China

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The emergence and prevalence of the Chinese highly pathogenic PRRSV (HP-PRRSV) in 2006 caused colossal economic losses to swine production in China. The HP-PRRSV is a novel variant of type 2 PRRSV with characteristic feature of the 30-aa deletion (at the position aa481 and aa533–561) in the nsp2-coding region. Current studies have indicated that HP-PRRSV has stronger ability of causing pathological and immunosuppressive pathogenesis. The virus has been shown to gain an expanded tropism to invade multiple tissues of pigs, to replicate to very high titers, usually ranging from 10-1000 folds, and to cause acute lung injury of pigs compared to the low pathogenic strain. More significantly, the HP-PRRSV infections resulted severe thymus atrophy and thymocytes apoptosis, leading to 90% reduction in size characterized by nearly complete cortical depletion of thymocytes and a significant drop of the CD4+CD8+ subpopulation. The differential effects of HP-PRRSV on inhibiting type I interferon and TNF-alpha production, and significantly elevated levels of IL-1, IL-6, IL-10 and TNF-α in the serum of HP-PRRSV-infected pigs have been observed. HP-PRRSV could increase bacterial secondary infections of the virus-infected pigs. Our latest study has shown that the HP-PRRSV infection leads to a dramatic drop of Th17 cells, a CD4+ T subtype that is thought to be important for clearing bacterial pathogens. The HP-PRRSV has been found to possess much stronger ability to down-regulate swine leukocyte antigen class I (SLA-I) molecules on the cell surface of PAMs and target them for degradation in a manner that is dependent on the ubiquitin-proteasome system, and moreover, the nsp1α replicase protein was found to contribute to this property of HP-PRRSV. Meanwhile, the HP-PRRSV could induce significantly more CD4+CD25+FoxP3+ T_{REG} than the low pathogenic strain. By mapping the virulence-associated gene(s) using reverse genetics, the 30-aa nsp2 deletion of HP-PRRSV, the hallmark of HP-PRRSV, was first confirmed to not relate with its increased virulence. By making chimeric mutants between HP-PRRSV strain JXwn06 and LP-PRRSV HB-1/3.9, the viral nsp9 and nsp10 have been determined to be related to the fatal virulence of HP-PRRSV JXwn06. In addition, HP-PRRSV JXwn06 has higher replication efficiency both in vitro and in vivo.

Various vaccines including KV and MLV have been practiced to control HP-PRRSV infection in China. A KV vaccine based on the HP-PRRSV isolate JXA1 failed to protect pig herds from HP-PRRSV infection. Several MLV vaccines (JXA1-R, HuN4-F112, and TJM) were developed and could provide efficient protection against the lethal challenge against their respective parental HP-PRRSV isolates. Other PRRS MLV vaccines are also being used in the field, which confer partial protection against the HP-PRRSV. Massive vaccinations on pig farms using HP-PRRSV MLV vaccines clearly have benefited the reduction of the outbreak and incidence of HP-PRRS in China. However, some safety concerns have been raised and noticed in the field. Meanwhile, the diversified strains and NADC30-like PRRSV emerging recently is challenging the control of PRRS in China.

S7
Developments in infectious disease surveillance

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Since 2005, research on antibody-based and nucleic acid-based oral fluid testing in swine has focused on assay development,[6,7,8,12] optimization of sample collection,[10,13] animal behavior related to sample collection,[16] disease dynamics related to test performance,[3,11] and assay reproducibility /repeatability.[4,9] Pertinent to the Symposium's theme of Emerging and FADs, efforts have included research on CSFV[5], PEDV[1], ASFV[2,5], FMDV,[5,15] SVDV[14] and others.

The question that remains is, *How should oral fluid sampling be implemented in the field*? In this study, we developed sampling guidelines for oral fluid-based PRRSV surveys in commercial swine farms. Initially, results of PRRSV RT-rtPCR testing in wean-to-finish barns were analyzed using a piecewise exponential survival model for interval-censored, time-to-event data with misclassification. Thereafter, simulation studies were used estimate the barn-level probability of PRRSV detection as a function of sample size, sample allocation (simple random sampling vs fixed spatial sampling), assay diagnostic sensitivity and specificity, and pen-level prevalence.

These studies provided estimates of the probability of detection by sample size and within-barn prevalence. Analyses of sample allocation found that detection using fixed spatial sampling was as good as, or better than, simple random sampling. Sampling multiple barns on a site increased the probability of detection with the number of barns sampled. These results are relevant to PRRSV control/elimination projects at herd, regional, or national levels, but the guidelines are broadly applicable to pathogens of swine for which oral fluid tests are available.

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1
Differential detection, genomic characterization and phylogenetic analysis of Porcine Epidemic Diarrhea Viruses from vaccinated pig herds in Fujian, China 2016

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Porcine epidemic diarrhea virus (PEDV) causes devastating impact on global pig-breeding industry and current vaccines have become not effective against the circulating PEDV variants since 2011. In this study, a multiplex RT-PCR assay for seven swine pathogens were performed on samples collected from pig herds suffering severe diarrhea, which were immunized with commercial bivalent (PEDV and TGEV) attenuated vaccines. PEDV was identified as the major causative agent for the outbreaks. Two complete genomes (XM1-2 and XM2-4) of PEDV isolated from two pig herds in Fujian province were determined. Genomic comparison showed that the two isolates share the highest nucleotide identities (99.09% and 98.77%) with ZMDZY strain isolated in China 2011, but have only 96.65% and 96.49% nucleotide identities with CV777 vaccine strain, respectively. Amino acid alignment of spike proteins indicated that our isolates have the same insertion and deletion pattern as other Chinese PEDV variants. And also the same three substitutions in the neutralizing epitopes generating serines were found, which might change the neutralization activities against the variants. There is only one unique substitution $A_{1100}S$ in the spike protein of 2016 isolates differed themselves. Phylogenetic analysis showed that our isolates belong to the IIa subgroup of genotype II group and form a new branch. To sum up, we detected new PEDV variants in China 2016 by using a multiple RT-PCR assay. Genomic and phylogenetic analysis showed that the new isolates are clustered with PEDV variants, which could not be effectively controlled by current commercial vaccines. Our findings suggest that more effective vaccines are urgently needed for the prevention and control of Chinese PEDV variants.

2
A cost effective method for surveillance of PRRSV and IAV-S in oral fluids using a newly developed multiplex rtRT-PCR

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The objective of this study was to develop a cost effective method for respiratory disease surveillance in swine oral fluid samples, specifically for porcine reproductive and respiratory syndrome (PRRSV) and influenza A virus in swine (IAV-S). Individual real-time reverse transcription polymerase chain reaction (rtRT-PCR) tests for both PRRSV and IAV-S are well established. Here, we have developed a multiplex rtRT-PCR that detects the conserved region of the 3'untranslated region of PRRSV and the matrix region of IAV-S to cut the cost of testing to the producer and promote the continued surveillance for both PRRSV and IAV-S in a single reaction. Recently, the USDA discontinued the funding for IAV-S matrix testing making the cost of the IAV-S surveillance screening by rtRT-PCR the responsibility of the producer. The characterization of circulating IAV-S for vaccine selection remains important to swine and human health. Screening for IAV by rtRT-PCR is a vital step in surveillance. The mechanism by which PRRSV interacts with other pathogens, such as IAV-S, is still being explored but it is believed that these interactions can increase the severity of pathogenicity. The producer will see a 50% cost savings when using the multiplex testing versus the singleplex. In conclusion, having a well validated and rapid diagnostic tool such as this new multiplex rtRT-PCR will be vital for continued swine health and production.

3
ELISAs based on recombinant nsp7 proteins from PRRSV type 1 and 2 as DIVA tests

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Tests for Differentiation between Infected and Vaccinated Animals (DIVA) are of high importance for the establishment of PRRS control programs. The present study aimed to develop ELISAs based on recombinant nsp7 to assess their potential application as DIVA tests. For this purpose, RT-PCR products of nsp7 from Olot91 (type 1) and from VR2332 (type 2) PRRSV isolates were cloned into two baculovirus vectors. Then, the recombinant baculoviruses were propagated in Sf9 cells (CRL1711, ATCC). Nsp7s were purified, quantified and used to develop the ELISAs. The optimal coating conditions were determined by checkerboard titration and the background was minimized by comparing different dilution buffers. ELISAs were set up using sera from experimentally infected and non-infected animals. The positive or negative status of immunization was previously determined by commercial ELISAs based on N protein: PRRS X3 Ab test (IDEXX) and INgezim PRRS Universal (INGENASA). Cut-off of the assays were adjusted to 0.4 by ROC analysis with the MedCalc software. With this value, both the sensitivity and the specificity of the assay was 100%.

Dynamics of antibodies anti nsp7 were evaluated in 35 pigs experimentally infected with six different type 1 strains; detection started at 14-21 days post infection (dpi) and pigs remained as positive until the last dpi tested (35, 49 or 77 dpi). Also, 20 sera from pigs vaccinated under experimental conditions with PRRS type 1 or type 2 attenuated vaccines -Porcilis (MSD) and Ingelvac (Boehringer Ingelheim), respectively- were evaluated. Despite the fact that vaccinated pigs were positive in commercial ELISAs from 7 dpi onwards, antibodies against nsp7 were never noticed. Based on these results, the nsp7 ELISA assays were further analyzed as candidate ELISAs for DIVA tests using sera from infected or vaccinated animals belonging to experimental studies and field. Commercial ELISAs against N protein were used as references. For type 2, the analysis of sera from vaccinated and infected pigs under experimental conditions indicated that the nsp7 type 2 ELISA had a sensitivity of 100% and a specificity of 94.7% as a DIVA test; for PRRSV type 1, low values of sensitivity and specificity were obtained. Under field conditions, there was a clear decrease in these two parameters: sensitivity 83.3%, specificity 83.2 %; however, there were significant statistical differences ($p<0.05$) when infected and vaccinated pigs were compared at population level:

	Parameter	Infected	Vaccinated	p value
PRRSV type 1	**Mean OD nsp7**	1.54	0.27	<0.0001
	n	77	150	
PRRSV type 2	**Mean OD nsp7**	1.66	0.45	<0.0001
	n	178	103	

In conclusion, ELISAs based on nsp7 were able to differentiate between infected and vaccinated animals under experimental conditions. Regarding sera collections from the field, the results indicated that the assays were also useful as DIVA tests but at the population level.

4
Genetic characterization and phylogenetic analysis of Senecavirus A

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Senecavirus A (SVA) is an emerging picornavirus that has been associated with outbreaks of vesicular disease and neonatal mortality in the major swine producing countries of the world. Since November 2014, SVA has also been frequently reported in swine in Brazil and since July 2015 over 200 cases of SVA have been confirmed in the US. The factors that contributed for the emergence of SVA remain unknown. The overall goal of our study was to determine the genetic diversity of SVA strains circulating in the US and Brazil. The complete genome sequence of 21 contemporary SVA isolates circulating in the US or in Brazil were obtained. Complete genome sequences of seventeen SVA isolates obtained in the US and four SVA isolates obtained in Brazil were compared to other SVA sequences available on GenBank. Complete genome sequence comparisons revealed that the US contemporary isolates characterized here share 91-93% nucleotide identity with the prototype US SVA strain SVV001 and an isolate obtained in Canada in 2007 (SVA-11-55910-3), and 98-99% nt identity with other contemporary isolates recently obtained in the US, 95-97% nt identity with contemporary Brazilian isolates and 94-96% nt id with a recent Chinese isolate (CH-1-2015). Comparison of the amino acid sequences of SVA polyprotein (2181 aa) revealed that the US contemporary isolates here share 97-99% aa identity with other SVA strains. A greater genetic divergence (86-88% nt id), however, was observed when the contemporary SVA isolates were compare to historical US isolates obtained prior to 2002. Sequence comparisons between the isolates obtained here and other contemporary or historical strains available on GenBank revealed a high degree of sequence homology between contemporary isolates. Additionally, both US and Brazilian SVA isolates share a high degree of homology with a recent SVA strain obtained in China. Phylogenetic analysis using complete genome sequences of contemporary SVA isolates and historical sequences suggest that SVA is continuously evolving. Results here provide important information on the genetic diversity of contemporary SVA isolates that have been recently associated outbreaks of vesicular disease in swine.

5
Epitope mapping of M protein of PRRS virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is coding three major structural proteins – the nucleocapsid protein N, the membrane protein M and the envelope glycoprotein GP5, from which N protein is extensively used in serological diagnostics of the disease. The use of additional virus proteins in serological assays requires their detailed antigenic analysis. The goal of this work was to determine immunodominant peptide within the structure of M protein and potential for using of the peptide for serological diagnostics of the disease.
The ORF 6 gene of PRRS virus was first analyzed by software provided on the "Immune epitope database" (IEDB) web site based on previously published algorithms for the prediction of linear epitopes from protein sequence. Following protein properties were analyzed: surface accessibility, antigenicity, hydrophilicity and the presence of linear epitope. Protein sequence of ORF 6 gene was then splitted into six fragments of various length (from 20 to 30 amino acids) to cover all sites with predicted surface accessibility. Peptides of M protein were applied as antigens in indirect ELISA tests. Immunodominant peptides were identified by checking serological reactivity of all tested peptides with a panel of 91 PRRS-positive serum samples. Peptide reacting with highest number of positive sera was considered as immunodominant.
Peptide "D" located at the very N´ terminal part of the M protein (aa residues 1-30) and peptide "E" located in the central part of the protein (aa residues 51-70) reacted with the highest number of PRRS positive sera. The position of these peptides is in agreement with in silico calculated values. These two immunodominant peptides would be convenient candidates for their usage like antigens in indirect ELISA test.

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6
Assessing the value of full genome sequence analyses for genotype 1 PRRS viruses

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The expanding genetic diversity of porcine reproductive and respiratory syndrome virus (PRRSV) is well known and has been previously described. The majority of these studies have relied on data from only small portions of the genome, such as ORF5. As full PRRSV genome sequences have become increasingly obtainable, some studies have been carried out using these, but usually with genotype 2 viruses, for which many more complete sequences are published and available. This study aims to analyse the available genotype 1 sequences, including five from Great Britain , to determine what extra information the full genome sequences are able to provide beyond that already available from analyses of individual gene sequences.

The published full genome sequences of 48 genotype 1 viruses from Europe, Asia and America, including five British viruses were analysed and compared. The sequences were aligned using MUSCLE, and the phylogenies derived using the Neighbor Joining algorithm. Full length virus sequence from the inoculum (Lelystad virus) used in an experimental *in vivo* infection was also compared to that obtained in tissues 3 days post inoculation.

Comparison of all 48 nucleotide sequences revealed that their similarities ranged between 80.1% and 99.9%, while they were only 65.4% to 67.2% similar to the prototype genotype 2 virus VR2332. The genome lengths varied from 14889 to 15120 nucleotides. The phylogenetic analysis displayed geographic clusters, where for example the British viruses all belonged to a single cluster, but, as previously described, Danish viruses are found in two distinct clusters. In comparison to a phylogeny generated using whole genomes, the tree topology differed when using only ORF5 sequence data, with the geographic groupings being more disparate. The most variable area of the genome is clearly the nsp2 region, where multiple patterns of deletions were found. For the development of diagnostic reagents such as oligonucleotide primers and probes, conserved areas beyond ORF7 may also be suggested. When examining micro-evolution during the process of *in vivo* infection, genetic changes after 3 days were found only in some non-structural genes of PRRSV which would have been missed if focusing only on structural genes such as ORF5.

The use of only ORF5 sequence data for molecular epidemiology purposes seems largely satisfactory, except for the potential confounding effects of recombination. For other analyses such as those relating to the evolution of PRRSV including recombination events, for the analysis of potential antigens important for immunity, or for diagnostic test development, full genome data is much more revealing, whereas the use of only partial sequences may lead to inappropriate conclusions.

7
Development of a novel vaccine for Porcine Epidemic Diarrhea Virus

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Infections with Porcine Epidemic Diarrhea Virus (PEDV) have resulted in significant economic losses to the swine industry. Here we describe the development of a novel inactivated vaccine for PEDV that has proven highly effective in newborn piglets. When administered to sows four and two weeks prior to farrowing, high levels of neutralizing antibodies against PEDV were found in colostrum and milk, as well as in the serum of their piglets. Piglets were orally infected at 5 days of life with PEDV isolate CO 025. It was found that 95% of all piglets from vaccinated sows (n = 83) survived the infection and showed significantly reduced clinical symptoms, weight loss and viral shedding. In contrast, all piglets from unvaccinated sows displayed severe clinical symptoms including weight loss and dehydration, and 50% of these piglets died within 6 days post infection. A large field trial was performed in three commercial swine units in Saskatchewan, Canada, to assess the vaccine under conditions with different genetics, health statuses and management systems. The vaccine demonstrated to be completely safe to use; no adverse events including injection site reactions and reproductive complications were observed. Vaccine efficacy was evaluated in 8% of these animals by transporting them back to our high containment facility prior to farrowing, and infecting their piglets orally within the first week of life. Again, protection rates were significantly higher in piglets born to vaccinated sows than those from control sows. These results show that this experimental vaccine is highly effective against the neonatal form of PEDV.

8
Development of a new strategy for detection of classical swine fever virus antibodies in alphavirus based replicon particle derived E2 and E^{rns} vaccinated swine

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Introduction: Classical swine fever virus (CSFV) is a member of the *Pestivirus* genus of the family *Flaviviridae*. It is a causative agent of classical swine fever (CSF), a highly contagious disease that threatens swine production globally. CSFV is closely related to the ruminant *pestiviruses* that cause bovine viral diarrhea (BVD) in cattle and the border disease (BD) in sheep. BVD and BD viruses can naturally infect swine and antibodies generated during infections cross-react with CSFV, thus making a CSF diagnosis problematic. For effective disease surveillance, rapid and sensitive assays are needed. The fluorescent microsphere immunoassay (FMIA) or Luminex is a multiplex serological platform that can be used for the detection of multiple targets including antigenic domains of E2 and Erns with high sensitivity and specificity. Therefore, the objective of this study was to detect changes in the serum antibody response following vaccination against CSFV with an alphavirus based RP expressed E2 and E^{rns} subunit vaccine.
Methods: E2 and E^{rns} play an important role in protective immunity in the natural host. E2 and E^{rns} genes have been fragmented into 7 and 5 small pieces respectively. The recombinant protein fragments were expressed in BL-21 (DE3) *Escherichia coli* and purified proteins were covalently coupled to Luminex MagPlex® polystyrene, carboxylated microsphere beads. The target antigens were assembled into a single multiplex and tested against sera immunized with alphavirus based replicon particles (RP) expressed antigens.
Results: To determine changes in CSFV-specific IgA, IgG, and IgM overtime, animals were vaccinated with alphavirus based RP expressed E2 and E^{rns} antigens and serum samples were collected at 0, 7, 14, 21, 28, 35, 42, 49, and 56 days post-infection (dpi). The IgA, IgG, and IgM response against CSFV antigens were determined by multiplex FMIA. The results were reported as mean fluorescence intensity (MFI) and then converted to positive per sample (S/P) ratio. The results showed that vaccinated animals had CSFV-specific IgA, IgG, and IgM in serum and oral fluids. Peak IgA and IgG antibody responses were detected at 28 dpi and IgM response was found at 7 dpi. Antibody response to CSFV antigens were IgG> IgM> IgA.
Conclusion: A strong antibody response to E2 and E^{rns} suggest that these antigens are suitable targets for diagnostic tests. Therefore, the detection of infection with multiple recombinant antigen targets in FMIA is an alternative diagnostic tool over the traditional ELISA.
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9
Progress in the development of real-time PCR assays for detection of foot-and-mouth disease

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Mobile real-time reverse transcriptase PCR (rRT-PCR), provides a realistic option for accurate and timely diagnosis of foot-and-mouth disease (FMD) *in situ*. Here we describe the laboratory and field evaluation of both a pan-specific and serotype-specific rRT-PCR assay using the T-COR™ 8 (Tetracore, Inc), a robust field-ready platform. For laboratory evaluation of the pan-specific assay, analytical sensitivity was determined using an FMDV RNA standard and diagnostic sensitivity was determined using RNA extracted from a panel of clinical samples (n=32), with the T-COR 8™ assays consistently showing equivalent sensitivity comparatively to the gold-standard laboratory rRT-PCR and RT-LAMP (Genie® II). The serotype-specific assay was evaluated in the laboratory using a panel of clinical samples from East Africa (serotypes: A, O, SAT1 and SAT2), with reliable detection of all serotypes evident. Robust sample preparation methods for serum, oesophageal-pharyngeal fluid and epithelial suspensions were developed to negate the need for RNA extraction prior to rRT-PCR. The final rRT-PCR protocol and associated lyophilised reagents were field evaluated in three endemic settings (Kenya, Tanzania and Ethiopia), consistently detecting both clinical and subclinical FMD infection. The ability of the T-COR™ 8 rRT-PCR to utilise simple sample preparation, amplification and detection methods offers promise for rapid *in situ* FMD diagnosis and demonstrates an important transition for FMDV-specific molecular assays into formats suitable for field diagnostic use.

10
Development of antibody reagents & assays for Senecavirus A serodiagnosis

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Senecavirus A (SVA) is a non-enveloped, positive stranded, RNA virus belonging to the *Picornaviridae* family. Since July 2015, approximately 200 cases of SVA associated with vesicular disease and/or neonatal mortality have been reported in the US. Currently, however, there are minimal diagnostic reagents and serological assays available for herd surveillance and confirmation of disease. To address these industry needs, we developed reagents for immunohistochemistry (IHC), and fluorescent antibody (FA) staining. These reagents were applied to develop serological assays including an indirect ELISA, fluorescent microsphere immunoassay (FMIA) and a fluorescent focus neutralization (FFN) assay. Serological reagent development began with the cloning, expression and purification of SVA capsid proteins including VP1, VP2 and VP3. Recombinant VP1, VP2 and VP3 were used to immunize mice and rabbits for monoclonal and polyclonal antibody production. For the ELISA and FMIA assay development, microtiter plates were coated with 400 ng of each antigen (VP1, VP2 and VP3) while Luminex microspheres were coupled with 15 ug of protein per 3 x 10^6 microspheres. Both assays were optimized using a checkerboard titration using samples of known serostatus. Serum samples were obtained from uninfected pigs (n = 612) and from SVA-infected animals (n=171). Initial testing via FMIA showed that the reactivity of SVA positive samples with VP2 was approximately 10-fold greater than the reactivity to VP1 and VP3, thus providing the rational for further development of a VP2 indirect ELISA and FMIA. Both tests were validated and ROC analysis showed diagnostic sensitivities and specificities of 96.9, 92.0 and 96.2 and 91.9 respectively. Next, inter-rater (kappa) analysis was performed to assess testing agreement between assays. The ELISA and FMIA assays were compared to an indirect immunofluorescence assay (IFA) and kappa values were determined as 0.923 and 0.925, respectively, which demonstrates significant testing agreement. ELISA and FMIA results show the detection of SVA antibodies as soon as one to two weeks post infection. Although the sensitivity of both assays was adequate, the specificity was lower (50/612 false positives). Twenty-five false positive samples were randomly selected and subjected to FFN testing. Notably, all 25 samples were resolved as true negatives via FFN. Monoclonal antibodies against VP1, VP2 & VP3 are being used to develop a blocking ELISA to increase specificity. These new diagnostic reagents and assays should aid in improved surveillance and control strategies for SVA.

11
Evaluation of a portable real-time PCR platform (T-COR 8™) for ASF during outbreaks in an endemically infected population in Uganda

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The objective of this study was to evaluate the feasibility and performance of a diagnostic system (including sample preparation and real-time PCR assays on a portable real-time PCR thermocycler, powered by battery) for the detection of ASFV on-site during outbreak investigations or in a simple laboratory setting in remote areas. The study was carried out as part of an OIE twinning project between the OIE Collaborating Centre at the Swedish National Veterinary Institute (SVA), and the Ugandan National Animal Disease Diagnosis and Epidemiology Center (NADDEC).

Blood and/or tissue samples were collected from domestic pigs during outbreak investigations in five districts of Uganda. Sample preparations included either simple dilution in PBS or magnetic bead nucleic acid extraction. A dried-down ASFV PCR kit with internal control (IC) (Tetracore Inc., Rockville, Maryland) was used on a portable real-time PCR thermocycler T-COR 8™ (Tetracore Inc.), performed on-site in the affected villages and in a simple lab setting. As a reference, the OIE recommended UPL assay (Fernández-Pinero et al 2013) was performed on a Stratagene Mx3000P at NADDEC.

Pigs from two of the five suspected outbreak sites investigated were positive for ASFV using the ASF kit on the T-COR 8™ and these results matched those of the reference method in the lab at NADDEC. For blood diluted in PBS, inhibition was prevalent in 20-fold diluted and present in some 40-fold diluted samples. Archived samples were also tested and in total samples for twenty-two pigs were positive for ASFV out of sixty-nine tested. Overall, the portable platform performed on par with the reference method.

This study showed that confirmation of an outbreak can be performed on-site within 1.5-2 hrs, and appropriate actions can be taken. The experience of performing the PCR assays in remote areas highlighted several factors that need to be carefully considered, including biosafety issues, simplicity and effectiveness of sample preparation and turn-around time.

12
Molecular phylogenetic diversity of PRRSV Type 2 in Mexico, 2005-2013

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PRRSV Type 2 is globally distributed, and its current diversity is mainly derived from North American prototypes. While the viruses are relatively well tracked in the United States and Canada, it is still unclear where Mexico stands in the global diversity. In this study, we analyzed 445 ORF5 sequences representing samples obtained during the period 2005-2013 from all pig-producing states in Mexico. The phylogenetic analyses showed that the Mexico viruses were found in diverse locations within the tree of global type 2 PRRSV. After removing sporadic introductions, there are at least 10 well-supported monophyletic clusters representing present sustained circulations within Mexico. Among them, three clusters were likely to be the remnants of earliest PRRSV endemic in North America, whose divergence from other North America viruses were all dated back to before 1990. On the other hand, the rest of the clusters were from more recently introductions from the United States or Canada. Compared to the early endemic clusters in Mexico, these later introductions dominated the 2010-2013 sampling, with the two most prominent clusters being (i) introduced from United States and sister to China HP-PRRSV related viruses (i.e. Lineage 8), and (ii) derived (more recently) from a more pathogenic variant of Canadian-like PRRSV (i.e. Lineage 1). Within Mexico, there is frequent virus flow among the states, which resulted in the mixing of diverse viruses and subsequently recombination. Indeed, from this study there are 2.1% of the sequences identified as recombinants, despite of the fact that only ORF5 was investigated. These recombination events were either between two divergent field strains or between a field strain and a vaccine strain. In conclusion, our study reveals that Mexico harbors extensive genetic diversity of PRRSV derived from both earlier endemic viruses as well as later introductions from other North American countries.

13
Comparison of five different strategies for high level expression of soluble recombinant PRRSV-nucleocapsid protein for its use in diagnostic

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The PRRSV nucleocapsid is the most immunogenic protein and an ideal target for the detection of infected pigs and currently serological testings are widely based on PRRSV N as the antigen, for the detection of antibodies produced in response to infection with North American type II or European-like type I PRRSV. However, recombinant expression systems are often difficult to develop in procaryotic systems without a high co-expression levels of inclusion bodies.

In this study, four different systems were compared in order to obtain high expression rates of soluble PRRSV-N recombinant protein. The N gene sequence was first optimized for *E. coli* expression. The first system consist of was designed to have a N- or a C-terminal HIS-tag in the pD441 plasmid. The N-PRRSV-Cterm (pNC-term) and N-PRRSV-Nterm (pNN-term) plasmids were transformed into a BL21 *E. coli* (F– *ompT hsdS*B(rB–, mB–) *gal dcm*). In the second system pNC-term was transformed into Shuffle K12 *E. coli* and Shuffle B *E.*coli (New England Biolabs). In the third system is designed for autoinduction with rhamnose. The His-tag optimized N gene was transformed into the pD861 plasmid. The pNC-Rha plasmid, was transformed into a BL21 *E. coli* (F– *ompT hsdS*B(rB–, mB–) *gal dcm*). The fourth system consist of transformation of the pNC-term into BL21 *E. coli* that contain various combinations of the five chaperone plasmids (TaKaRa). Several times and temperatures of induction as well as and concentrations of inducers were compared. Levels of protein expression at diffrent steps were verified and analyzed by SDS-Analysis. Finally, beyond these different systems inclusion bodies were also solubilized in Urea refording solution.

Results showed that different strains (Shuffle), type of induction (rhamnose) or addition of chaperones did not drastically improve or decrease the amount of soluble N_PRRSV. However they revealed that by adding a N-terminal HIS-tag to N_PRRSV and using a strong RBS in BL21 we were able to express soluble protein at good yields (~200 mg/L of culture) and, the proteins in inclusion bodies were able to be solubilized under certain conditions giving yet another option for production. The potential use of these recombinant proteins in diagnostic will be discussed.

14
Alternative sampling methods for assessing vertical transmission of PRRSV

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The objective of the present work was to test the efficiency of umbilical cord (UC) sampling and ear vein (EV) blood swabs as alternative samples to cava/jugular vein bleeding (VB) for assessing vertical transmission of PRRS in maternities. With this purpose, 21 batches of newborn were sampled on farms suspected to be unstable for PRRSV. On each batch, weak newborns were targeted and the three type of samples were collected. The final number of examined piglets was 387. The time needed to collect UC, EV and for VB was recorded as well. After collection samples were immediately shipped at 4ºC and were processed in less than 24 h. RNA was extracted with the MagMAX™ and analyzed by a commercial qRT-PCR (Thermo Fisher Scientific). Diagnostic sensitivity was calculated for individual samples and at a batch level. Agreement for batch classification according to each sample results (Cohen's Kappa, k) was calculated as well. Ct in the different samples were compared with a correlation test. 105/387 samples were positive resulting in 14/21 positive batches. By samples, 76 UC were positive compared to 55 VB and 45 EV ($p<0.05$). Apparent differences in sensitivity for batch classification -85.7% for UC, 78.6% for VB and 71.4% for EV- were non-significant. The agreement for the classification of batches were: $k = 0.52$ for UC vs. VB, $k = 0.71$ for VB vs. EV and, $k = 0.62$ for UC vs. EV. Average Ct values were 26.6±8.5 for VB, 30.8±6.4 for EV and 32.1±4.85 for UC ($p<0.01$). Correlation of Ct values between samples (27 animals positive for UC, VB and EV) was $r^2=0.63$ for VB vs. EV, $r^2=0.33$ for VB vs. UC and, $r^2=0.13$ for EV vs. UC. Some VB-negative animals UC or EV resulted positive. Average Ct values for positive UC or EV samples in those animals (34.3 and 35.5, respectively) were higher than those of the equivalent samples in viremic animals (29.7 and 28.9, respectively) ($p<0.05$). It can be hypothesized that some UC or EV samples were contaminated during the delivery, in the uterus or in the birth canal. Collection of UC was faster than any other else sample (on average 24 s vs. 55 s for EV and 72 s for VB; $p<0.05$). As a conclusion, UC testing results in a sensitive alternative that saves time of collection. This study was supported by the European PRRS Research Award 2015 from Boehringer Ingelheim.

15
Type of influenza A virus reassortants present in Quebec swine herds from 2011 to 2015 and their antiviral drugs resistance

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Data about molecular diversity of commonly circulating swine influenza A viruses (SIV) in Quebec swine are scarce. Yet, this information is essential for surveillance of animal and public health, vaccine updates and for understanding virus evolution and its large-scale spread. Different clinical samples including lung tissues, saliva and nasal swabs were obtained from 24 outbreaks of swine flu that have occurred in 2011 to 2015. Only, eighteen viruses were isolated in MDCK cells or in embryonated eggs. All eight gene segments of the isolated SIV strains were sequenced and analysed. Antiviral drugs resistance against oseltamivir carboxylate, zanamivir (SIGMA) and amantadine hydrochloride was evaluated by MUNANA (2'2′-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid sodium salt hydrate) neuraminidase activity and inhibition assay (NAIs) and plaque reduction assay. Two subtypes of SIV, H3N2 and H1N1, were identified in Quebec pig herds. Twelve SIV strains were genetically related to trH3N2 cluster IV from which at least 6 different reassortment profiles were identified. On the other hand, 6 Quebec SIV strains were found to be genetically related to the pandemic virus A(H1N1)pdm09 from which two reassortment profiles were identified. One H1N1 (1/6 = 16.7%) and one trH3N2 (1/12 = 8.3%) strains were found to be resistant against Oseltamivir. In contrast, two H1N1 (2/6 = 33.3%) and two trH3N2 (2/12 = 16.7%) strains were found to be resistant against Zanamivir. All antiviral resistant strains were from 2014 and 2015. Interestingly, SIV strains resistant against Oseltamivir were different compared to the ones resistant against Zanamivir. Overall, the SIV resistance against antiviral neuraminidase inhibitor drugs was (33.3%). In addition, it was not surprising to find that all SIV tested strains were resistant against amantadine since they were all having the amino acid sequences at the M2 protein which is known to provide resistance against amantadine. The presence of influenza A virus antiviral drug resistance in swine and the possible emergence of new SIV strains are public health concerns supporting the surveillance of influenza A virus in swine.

16
Detection of Porcine Reproductive and Respiratory Syndrome (PRRS) in Bhutan

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Bhutan is located between China and India, village backyard pig farming is characterized by small numbers of pigs reared by subsistence farmers (Timsina and Sherpa, 2005). Pig farming plays an integral role in providing nutrients and household income in many low-income countries in Bhutan. In addition, there are three government-owned pig-breeding farms in the country to supply hybridized piglets to the farmers for fattening and cross-breeding purposes. These government breeding farms have been set up to improve local breeds through cross breeding with exotic breeds. Bhutan is always at risk of introduction of exotic diseases in the country through the import of exotic pigs and more so, Bhutan shares a border with India in the south, across which there are imports of fresh pig meat and sometimes illegal live pig imports.

PRRS is a viral disease of swine that emerged in the US in the late 80s. Both genotypes of PRRSV, genotype 1 (EU type) and genotype 2 (NA type) are reported in Asia. The highly virulent strains presently circulating in China and Vietnam pose a serious threat for the pig producing countries in Asia and worldwide.

PRRS status in Bhutan was largely unknown until 2008, when it was first reported officially in Bhutan at National Pig Breeding farm with sudden abortion storm at the later stage of pregnancy. Most sows that aborted were between 3rd to 9th lactation, which indicated that all the above sows have furrowed healthy piglets before. There was also high morality in weaner piglets. Here, we report the first detection of PRRSV in pigs from in Bhutan following the sudden abortion storm.

An outbreak resembling PRRS occurred in 2008 and 2010 in a government pig breeding farms. Detection of antibodies by HerdCheck-PRRS ELISA from the IDEXX Laboratories for specific PRRSV NA type A was performed on serum samples of pigs from these farms. To confirm and determine the PRRSV genotype, RT-PCR was performed on selected sera and tissue samples and the positive samples were sequenced.

All affected sows were positive for PRRSV antibodies during the suspected outbreak. Pigs which were in contact with the affected ones also showed high levels of PRRSV antibodies. One month after the suspected outbreak, most sows and weaned piglets were positive for PRRSV antibodies. By RT-PCR, PRRSV antigen was detected in two PRRSV antibody seropositive and one seronegative sows, and in 3 of 6 tissues tested from pigs and in serum. PCR result also detected specific 90 nt deletion in nsp2 coding fragment, considered the marker of highly pathogenic strains from China and Vietnam. Sequence analysis revealed high identity among them and to that of highly pathogenic strains but low identity to the prototype PRRSV genotype 2 strain, VR2332. Furthermore, the sequences from Bhutan clustered together with those from China and Vietnam. The disease was controlled by isolation and culling of affected animals followed by decontamination and strict biosecurity measures. Acclimatization of gilts entering the farm were also followed to mainly to develop protective immunity prior to introduction into the herd.

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An indirect enzyme-linked immunosorbent assay for the identification of antibodies to Senecavirus A in swine

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Background: Seneca Valley virus (SVV), a member of the family *Picornaviridae,* genus *Senecavirus*, species *Senecavirus A (SVA)*, is a recently identified single-stranded RNA virus closely related to members of the *Cardiovirus* genus. SVV was originally identified as a cell culture contaminant and was not associated with disease until 2007 when it was first observed in pigs with Idiopathic Vesicular Disease (IVD). Vesicular disease is sporadically observed in swine, is not debilitating, but is significant due to its resemblance to foreign animals diseases, such as foot and mouth disease, whose presence would be economically devastating to the United States. IVD disrupts swine production until foreign animal diseases can be ruled out. Identification and characterization of SVA as a cause of IVD will help to quickly rule out infection by foreign animal diseases.

Results: We have developed and characterized an indirect ELISA assay to identify serum antibodies to SVA. Viral protein 1, 2 and 3 (VP1, VP2, VP3) were expressed, isolated, and purified from *E. coli* and used to coat plates for an indirect ELISA. Sera from pigs with and without IVD symptoms as well as a time course following animals from an infected farm, were analyzed to determine the antibody responses to VP1, VP2, and VP3. Antibody responses to VP2 were higher than VP1 and VP3 and showed high affinity binding on an avidity ELISA. ROC analysis of the SVA VP2 ELISA showed a sensitivity of 94.2% and a specificity of 89.7%. Compared to IFA, the quantitative ELISA showed an 89% agreement in negative samples and positive samples from 4-60 days after appearance of clinical signs.

Conclusions: A simple ELISA based on detection of antibodies to SVA VP2 helps to differentially diagnose IVD due to SVA and rule out the presence of economically devastating foreign animal diseases.

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A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure

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Porcine circovirus associated disease (PCVAD) is clinically manifested by postweaning multisystemic wasting syndrome (PMWS), respiratory and enteric disease, reproductive failure, and porcine dermatitis and nephropathy syndrome (PDNS). Porcine circovirus type 2 (PCV2) is an essential component of PCVAD although an etiologic role in PDNS is not well established. Here, a novel circovirus, designated porcine circovirus 3 (PCV3), was identified in sows that died acutely with PDNS-like clinical signs. The capsid and replicase proteins of PCV3 share only 37% and 55% identity to PCV2 and bat circoviruses, respectively. Aborted fetuses from sows with PDNS contained high levels of PCV3 ($7.57x10^7$ genomic copies/ml) and no other viruses were detected by PCR and metagenomic sequencing. Immunohistochemistry (IHC) on sow tissues identified antigen in skin, kidney, lung and lymph nodes localized in typical PDNS lesions including necrotizing vasculitis, glomerulonephritis, granulomatous lymphadenitis and bronchinterstitial pneumonia. Further study of archived PDNS tissues, that were negative for PCV2 by IHC, identified 45 of 48 were PCV3 positive by qPCR with 60% of a subset also testing positive for PCV3 by IHC. Analysis by qPCR of 271 porcine respiratory disease diagnostic submissions identified 34 PCV3 positive cases (12.5%), and ELISA detection of anti-PCV3 capsid antibodies in sera found 46 positive samples of 83 tested (55%). These results suggest PCV3 commonly circulates within U.S. swine and may play an etiologic role in reproductive failure and PDNS. Due to the high economic impact of PCV2, this novel circovirus warrants further studies to elucidate its significance and role in PCVAD.

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Development, characterization and diagnostic application of monoclonal antibodies against ASFV p30

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African swine fever virus (ASFV), the causative agent of African swine fever (ASF), is a large enveloped DNA virus and the only known member of the family *Asfaviridae*. ASFV has a complex organization, containing more than 100 proteins, including those with structural roles and enzymes that are packed in the virus core for use in early infection. Since there are currently no commercial vaccines against ASFV, the detection of ASFV specific antibodies in commercial or feral swine indicates previous infection. Many of the viral proteins are potentially useful for ASFV diagnosis; among them is p30, an early protein that is part of the inner membrane of the viral particle and a very efficient inducer of humoral responses. Although ASFV is not present in the US and no diagnostic tests are available, the current outbreaks in Eastern Europe have prompted interest in developing rapid and specific diagnostic assays for surveillance programs. The overall goal of this study was to develop, characterize and evaluate monoclonal antibodies (mAbs) against ASFV p30 that could be used in diagnostic assays.

Briefly, recombinant p30 protein, based on BA71V strain, was expressed in *E.coli* and purified. Three 6-8 week old female BALB/c mice were immunized with the purified protein (100ug/mouse/inoculation) at 2-week intervals for 4 weeks. Splenocytes from immunized mice were fused with NS-1 myeloma cells and cultured on 24-well plates. Cell culture supernatants from wells containing hybridoma colonies were screened by indirect immunofluorescence (IFA) using Vero cells infected with Alphavirus replicon particles (RP) expressing ASFV p30. Hybridoma cells from positive wells were subcloned by limited dilution and expanded. From three clones, eleven subclones were generated (47- 3, 47- 26, 47- 53, 62- 22, 62- 23, 62- 32, 62- 35, 142- 1, 142- 2, 142- 4, 142- 20) and subjected to further testing.

The reactivity of the mAbs was tested by IFA on Vero cells infected with BA71V strain. In total 8 out of 11 mAbs reacted with the infected cells, and clone 62-35 showed the highest reactivity. Next, we investigated whether the mAbs recognize different ASFV strains. Immunohistochemical (IHC) staining was performed on formalin fixed paraffin embedded (FFPE) tissues (lung, spleen, lymph nodes and tonsil) from pigs challenged with the Georgia/07 strain. Two mAbs showed weak reactivity (142-4 and 62-35) and one (47-3) had high reactivity to antigen in the FFPE tissue sections. To identify the mAbs target epitopes, eight overlapping fragments covering the p30 protein of ASFV BA71V were expressed in *E. coli* in a soluble form and purified by affinity chromatography. ELISA plates were coated with the protein fragments and with the whole protein. All mAbs recognized the whole p30 protein and three (47-3, 47-26, 47-53) reacted with a conserved region between amino acids 130-143. Similar results were obtained by Western blot analysis. Interestingly, sera from pigs immunized with Alphavirus RP-p30 recognized the same conserved region. In a competitive ELISA only one mAb (142-4) could compete off the RP-p30 sera.

In summary, this work describes the generation of p30 mAbs and their potential for use in the development of diagnostic tests.

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Oral fluids diagnostic methods for chronic Classical swine fever virus

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Classical Swine Fever virus (CSFV) is one of the most impactful swine pathogens worldwide, causing significant economic and animal welfare losses. Historically, CSFV strains caused acute disease with high mortality. Current strains cause chronic, moderate disease. This milder disease is hard to differentiate from common swine pathogens (e.g. PRRSV, PCV2, bacterial septicemia), making these newer strains the perfect candidates for infecting and persisting in naïve swine populations. Regions free of CSFV must actively survey their swine so as to avert the massive damages an unchecked outbreak would cause. In the United States the surveillance carried out by the Animal and Plant Health Inspection Service (APHIS) is limited by a lack of mass sampling techniques. Oral fluid sampling can address these limitations, and has already been shown to be effective in detecting other diseases in swine. We performed a pilot study using 10 pigs infected with the moderate CSFV strain, Paderborn. Clinical signs and rectal temperatures were evaluated daily throughout the study. Observed symptoms were typical of chronic, moderate CSFV infection. Serum was collected via jugular venipuncture at regular intervals throughout the study, while oral fluids were collected daily via cotton ropes hung in the pen. Both sets of samples were analyzed by qRT-PCR and the CSFV detection efficiency compared. Our results show that the virus could be detected in oral fluids using a single sample, and using less resources than with traditional serum sampling. This study paves the way for the validation of oral fluids for use in mass population sampling, which will greatly strengthen the surveillance capabilities of APHIS.

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Development of a new molecular method to discriminate Porcine Epidemic Diarrhea Virus infectious viral particles, from non-infectious ones, which are contaminating pig derived food additives

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Porcine epidemic diarrhea (PED) emerged in North America in April 2013. The ethological agent of this infectious disease is a coronavirus specific to swine named porcine epidemic diarrhea virus (PEDV). In affected herds, PEDV can cause significant economic losses, primarily due to a very high mortality (50% to 100%) in young piglets. It is essential to put in place biosecurity measures to assure PEDV negative status of swineherds in order to keep a good financial health of this industry. At the moment, PEDV molecular diagnostic tools do not allow us to establish if virus particles, that are detected in the environment (ex: in transportation trucks) or in food additives, are infectious and thus if they could be a biohazard threat for the swine industry. Thus, the main objective of this study is to develop a new molecular diagnostic tool that can differentiate infectious viral particles from non-infectious ones. Recently, new molecules have been reported to specifically crosslink with DNA/RNA and by doing so; inhibit reverse-transcription (RT) and PCR amplification of the viral genome. In addition, those molecules are nearly completely cell membrane-impermeable, and thus cannot pass through an intact lipid membrane. Our research hypothesis is that infectious virus particles (with an intact lipid membrane) will not allow the crossover of those new compounds to bind their inner viral genome. Consequently, only samples containing infectious viral particles will be expected to give a PEDV positive RT-qPCR reaction when pre-treated with those new molecules. Results show that pre-treatment with the molecules of spiked PEDV samples allows the distinction between infectious viral particles and non-infectious ones by using RT-qPCR reactions. Moreover, the efficiency of the new molecules combined with RT-qPCR has been also demonstrated on PEDV positive food additives. In conclusion, this molecular test will ensure the biosafety of food additives derived from swine that may be carrying some PEDV viral genome.

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Proof of concept: PRRSV IgM/IgA ELISA detects infection in the face of circulating maternal IgG antibody

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INTRODUCTION

Detection of PRRSV antibody in oral fluids (OF) is a convenient approach for PRRSV surveillance. However, in weaned pig populations originating from PRRSV-infected or vaccinated sow herds, maternal antibody (IgG) cannot be differentiated from IgG antibody produced by pigs in response to infection. Therefore, we developed and evaluated IgM- and/or IgA-specific oral fluid ELISAs as a means to detect active infection in weaned pig populations with circulating maternal IgG.

MATERIALS AND METHODS

OF samples were collected from 3 wean-to-finish (WTF) sites (A, B, C) beginning within 2 weeks of placement. Pigs originated from PRRSV vaccinated and/or exposed sow herds, but the pigs themselves were not vaccinated for PRRSV. Each site had 3 WTF barns and each barn had 36 occupied pens (~25 pigs per pen).

A total of ~2,916 OF samples were collected: 3 sites x 108 pens per site x 9 samplings. To establish the PRRSV status of each pen, all OF samples were randomized and then tested by PRRSV rRT-PCR. OF samples were subsequently tested for PRRSV IgG, IgA, IgM, and the combination of IgM/IgA using ELISAs developed in our laboratory. ELISA cutoffs, diagnostic sensitivity and specificity were determined by ROC analyses.

RESULTS

PRRSV qRT-PCR: On Site A, 3.7% of pens were positive at the 1st sampling, with all pens in all 3 barns positive ≥ 1 times by the 9th sampling. All Site B samples were negative, except for one positive pen at the 9th sampling. All Site C samples were negative.

PRRSV Antibody: Among the 3 sites, 90.6% (278/307) of the OF samples collected on the 1st sampling were IgG positive, but 273 of these were from rRT-PCR-negative pens. In samples collected over time, rising IgA, IgM, and IgM/IgA S/P values were associated with spread of PRRSV (rising rRT-PCR positivity). An ROC analysis estimated the sensitivity and specificity of the three ELISAs, at ≥ 63% and ≥ 99%, respectively.

DISCUSSION

Practitioners need both nucleic acid- and antibody-based tests to track PRRSV. This study suggested that the pigs' IgM/IgA response could be used to detect a pig's response to infection in the presence of maternal antibody.

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Spatial autocorrelation and implications for oral fluid-based PRRSV surveillance

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INTRODUCTION

Tobler's 1st Law of Geography says, "*Everything is related to everything else, but near things are more related than distant things*." "Spatial autocorrelation" can be positive (near pens have similar PRRSV status) or negative (near pens have dissimilar PRRSV status). Two questions were addressed in this study: 1) Does PRRSV infection exhibit spatial autocorrelation? 2) Is random or "fixed spatial" sampling better for PRRSV surveillance? (Fixed spatial sampling = sampling pens spaced evenly over the length of the barn.)

MATERIALS AND METHODS

In 3 wean-to-finish barns on one site, oral fluid (OF) samples were collected weekly (9 samplings) from every occupied pen (108 pens; ~25 pigs per pen) for a total of 972 OF samples. OF samples were randomized prior to PRRSV RT-PCR testing.

1. RT-PCR results were used to test for PRRSV spatial autocorrelation on the site, both globally and locally, using threshold distance as the spatial weight matrix. Moran's *I*, an indicator of global spatial autocorrelation, was used to test for clustering within barns. LISA (Local Indicators of Spatial Association) analysis, an indicator of local spatial autocorrelation, was used to identify clusters of PRRSV concentration within barns.
2. Statistical analyses were used to compare detection using random vs "fixed" spatial sampling.

RESULTS

1. Moran's *I* analysis revealed positive global spatial autocorrelation in the distribution of PRRSV virus in the swine barns. LISA analysis revealed clusters at the local level, indicating the presence of local spatial autocorrelation.
2. Analyses comparing the probability of detection showed that "fixed" spatial sampling was as good as, or better than, random sampling for the detection of PRRSV.

DISCUSSION

PRRSV (and probably most infectious diseases of swine) exhibits spatial autocorrelation. This is obvious to most swine vets, but spatial autocorrelation has not previously been tested at the barn level. This concept is important because it implies that pens' disease status exhibits spatial dependence; which helps explain why fixed spatial sampling is as good as random sampling. Overall, the results suggested that a simple, reliable, oral fluid-based surveillance strategy can be based on fixed spatial sampling.

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Construction and characterization of a full-length cDNA infectious clone of emerging porcine Senecavirus A

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A full-length cDNA infectious clone, pKS15-01-Clone, was constructed from an emerging Senecavirus A (SVA; strain KS15-01). To explore the potential use as a viral backbone for expressing marker genes, the enhanced green fluorescent protein (EGFP)-tagged reporter virus (vKS15-01-EGFP) was generated using reverse genetics. Compared to the parental virus, the pKS15-01-Clone derived virus (vKS15-01-Clone) replicated efficiently in vitro and in vivo, and induced similar levels of neutralizing antibody and cytokine responses in infected animals. In contrast, the vKS15-01-EGFP virus showed impaired growth ability and induced lower level of immune response in infected animals. Lesions on the dorsal snout and coronary bands were observed in all pigs infected by parental virus KS15-01, but not in pigs infected with vKS15- 01-Clone or vKS15-01-EGFP viruses. These results demonstrated that the infectious clone and EGFP reporter virus will be important tools in further elucidating the SVA pathogenesis and development of control measures.

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Assessment of Porcine Reproductive and Respiratory Syndrome (PRRS) impact in US sow farms

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Background: Porcine reproductive respiratory syndrome (PRRS) is an endemic swine disease causing large industry impact. The complex epidemiology of the disease, coupled with a lack of complete information and the diverse clinical outcomes observed in infected farms, have hampered efforts to quantify PRRS' impact on production over time.
Objective: To measure the impact of PRRS outbreaks in routinely vaccinated sow farms.
Methods: Longitudinal production data, regularly collected on 16 sow farms from a single system that had been affected with PRRS, were used to build a fixed-effects model to evaluate the post-outbreak production of weaned pigs. Seven indicators of farm performance (litter size, number of stillbirths, number of pre-wean piglets and sows dead, number of sows with abortions, number of sows with repetition of services, and sows farrowing) were also assessed. Pre-outbreak data were used to establish a baseline that was used to estimate the decrease in weaned pigs produced, and losses were translated into a revenue decrease assuming the average market price of $45.40 / weaned pig.
Results: Production declined one week before the outbreak was reported, and the decline was greatest between 5 and 6 weeks after. Recovery was not monotonic, and a new decay was observed between the 11th and 18th week post-outbreak. By the end of the study (35 weeks post-outbreak) a trend towards recovery was observed in all performance indicators, although baseline levels were never reached. Abortions increased significantly the week before the outbreak was reported, and stillbirths remained significantly higher than baseline even in the 35th week after the outbreak. Through the 36 weeks following an outbreak, PRRS caused a decrease of 1.9 weaned pigs per sow, leading to decrease revenue in a standard farm of this system of approximately $350,000 through the same period.
Conclusions: PRRS caused a significant decrease in weaned pig production and the negative effect lingered. Around 25% of total losses occurred within the first six weeks of the outbreak, 50% of losses by the 12th week post-outbreak. We were only able to measure production losses and not the full economic impact of PRRS. Still, our estimates indicate a larger loss than previously estimated, i.e., a decrease of 1.90 weaned pigs over 36 weeks compared with the 1.44 per sow year reported elsewhere (Holtkamp *et al.* 2013), even though farms here were performing routine PRRS vaccination.
Relevance: Metrics of PRRS-associated losses here demonstrate, in quantitative terms, the impact of the disease, and would be useful for informing cost-benefit analyses and bio-economic models of interventions. The methods presented here may also be applied to measure impacts in other type of farms, such as farms without vaccination protocols, for comparative purposes.
Acknowledgement: We thank the system, which participates in the SHMP, for sharing the information used for these analyses.

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Detection of PCV2 DNA in serum, feces and in oral fluid of pigs vaccinated against PCV2

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The aim of the study was to compare the presence of PCV2 DNA in serum, feces and oral fluid of pigs from 7 Polish farms vaccinated against PCV2.

Materials and methods

Serum, feces and oral fluid samples from piglets, weaners and fatteners, were obtained from farms with different vaccination protocols (Table 1) and analyzed with in house Real Time PCR for PCV2.

Results

PCV2 was detected in serum from 3 out of 7 farms. In farms 4 and 5 viremia was detected in fatteners and in farm 2 in weaners and fatteners. PCV2 DNA was detected in feces from all farms except from farm 1. PCV2 DNA was detected in oral fluid of weaners and fatteners from most of the farms. In farm 1 it was detected only in 12 weeks old pigs and in farm 6 only in 4 and 19 weeks old pigs.

Farm	Vaccine (age of application in weeks)	Serum			Feces			Oral fluid		
		Total no.	No. of pos.	% pos.	Total no.	No. of pos.	% pos.	Total no.	No. of pos.	% pos.
1	CircoFlex (3)	56	0	0	56	0	0	14	1	7.1
2	CircoFlex (3)	60	22	36.7	60	49.0	81.7	6	6	100
3	Porcilis PCV (6)	10	0	0	10	6.0	60.0	5	5	100
4	Suvaxyn PCV (7)	15	4	26.7	15	9.0	60.0	14	9	64.3
5	Suvaxyn PCV (6)	14	4	28.6	12	10.0	83.3	6	5	83.3
6	Suvaxyn PCV (6)	12	0	0	12	7.0	58.3	6	2	33.3
7	Suvaxyn PCV (6)	12	0	0	12	4.0	25.0	7	4	57.1
Total		179	30	16.8	177	85	48.0	58	32	55.2

Table 1: Detection of PCV2 DNA in serum, feces and oral fluid. Samples from farms 1 and 2 were tested individually. Samples from farms 3-7 were tested after pooling by 2-5.

Conclusion

On average 55.2% of oral fluid samples tested positive for PCV2 DNA, but the prevalence ranged from 7.1% in farm 1 to 100% in farms 2 and 3. PCV2 DNA was detected in 48% of fecal samples and the prevalence ranged from 0% in farm 1 to 83.3% in farm 5. PCV2 DNA was detected in serum from farms 2, 4 and 5, in 36.7%, 26.7% and 28.6% samples, respectively. On average PCV2 DNA was detected in only 16.8% of serum samples. Further studies are needed to establish the correlation between the presence of PCV2 DNA in different type of pig samples and the efficacy of vaccination protocols.

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The duration period of PRRSV Ab under serum therapy and herd closure

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Introduction Serum therapy with herd closure is a good method for control or eliminating PRRS in sow farm. The F8 farm had 1200 sows in 2014. As the status of PRRS became unstable in 2013 winter, we inoculated serum to sows and gilts on Jan. 18 and 20, and then closed the herd. In some former reports, the antibody will declined in 8 months or 12 months. In order to know the exactly duration period, we fixed some pig for check.
Materials and methods Boars and sows were fixed for monitoring Ab by IDEXX HerdChek* PRRS X3 Kit.
Results The PRRS Ab of some boars occurred negative 3 months later. It takes nearly 14 months that all boars became negative (table 1). The PRRSV Ab of Sow just like the boar, but one sow still kept Ab positive after 15 months (table 2).

Table 1 the fixed boars PRRS S/P value

ID	2/12/2014	4/23/2014	6/20/2014	8/25/2014	11/23/2014	12/29/2014	3/8/2015	3/31/2015
Boar1	1.567	0.783	0.673	0.506		0.344	0.325	0.325
Boar2	3.504	1.955	1.564	0.492	0.61	0		
Boar3	1.507	1.041	0.766	0.471	0.188			
Boar4	0.837	0.251	0.088	0.521	0.018	0.027	0.049	
Boar5	1.837	0.897	0.895	0.694	0.29	0.525		
Boar6		1.047	0.894	0.483	0.322	0.42	0.317	0.315
Boar7	3.537	1.794	1.337	0.505		0.417	0.26	0.26
Boar8		1.206	0.499	0.483	0.42			
Boar9	0.121	0.095	0.05	0.521	0.635	0	0.004	0

Table 2 the fixed sows PRRS S/P value

ID	1/17/2014	3/23/2014	5/23/2014	8/25/2014	11/23/2014	12/29/2014	2/27/2015	4/23/2015
sow4	0.223	1.225	0.62	0.306	0.225	0.357	0.242	0
sow5	0.097	1.143	0.68	0.203	0.065	0.065	0.049	0.08
sow7	0.644	1.262	0.795	0.492	0.387	0.438	0.265	0.192
sow8	0.1	2.827	1.572	0.407	0.17	0.124	0.174	0.144
sow9	0.625	2.02	0.372	0.506	0.46	0.635	0.495	0.274
sow10	0.591	1.501	0.742	0.567	0.28	0.346	0.27	0.241
sow11	0.05	0.798	0.402	0.14	0.093	0.062	0.028	0.029
sow12	0.1	0.817	1.102	0.144	0.098	0.092	0.093	0.071
sow14	0.428	1.798	1.065	0.584	0.585	0.354	0.423	0.301
sow15	0.135	2.193	0.312	0.438	0.322	0.449	0.316	0.234
sow16	0.071	0.877	0.24	0.079	0.04	0.051	0.014	0.042
sow19	0.038	2.61	1.103	0.344	0.227	0.208	0.186	0.281

Discussion The detection PRRS Ab of fixed boar and sows confirmed the previous studies are correct. But very little individual sow or boar still kept PRRS Ab positive more than 15 months. The PRRSv is negative and no PRRS clinical infection. We don't know why some pigs kept the low positive s/p value in the herd. Maybe affected by detection error or the kit or some unknown immunity.

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Development of immunochromatographic strip tests for on-site rapid and early detection of specific antibodies against porcine respiratory reproductive syndrome virus

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Objectives: Porcine reproductive and respiratory syndrome (PRRS) is considered one of the most economically significant swine diseases worldwide. Due to its increasing genetic diversity, a rapid and accurate diagnosis is crucial in laboratory as well as field situation. Although many serological diagnostic methods commonly used to detect PRRSV-specific antibodies show high sensitivity and specificity, specialized equipments and skilled personnel are needed. The objective of this study was to develop and evaluate fast and sensitive point-of-care test using immunochromatographic assay.

Methods: The immunochromatographic strip test (ICST) was developed by type1 and 2 N proteins labeled with colloidal gold to detect PRRSV-specific antibodies. Diagnostic performance was demonstrated with 991 sera from 71 local swine farms and 66 sera from 12 experimentally inoculated pigs by comparing the validity with that of commercial ELISA (IDEXX PRRS X3 ab) using IFA and IgM ELISA as reference standards. To elucidate temporal humoral responses, sera from pigs challenged with field viruses (LMY, PL97-1, and E38) were collected at 0, 7, 14, 28, 39, 52 days post infection (dpi). Additionally, to identify how early these test can detect PRRSV-specific antibodies, sera challenged with prototype viruses (VR2332 and LV) were collected at 0,1, 3, 5, 7, 14 dpi.

Results: The sensitivities of our ICST and commercial ELISA were 97.5% and 97.8%, respectively and the specificities were 91.1% and 92.4%, respectively. More importantly, the ICST could detect PRRSV-specific antibodies as early as 3 dpi, while commercial ELISA could detect PRRSV antibodies from 7 or 14 dpi.

Conclusions: The test results of ICST are generated within approximately 15 min with only 20ul of swine sera. The ICST shows similar performance compared with commercial ELISA and can detect PRRSV-specific antibody in earlier stages of infection. As a simple and accurate test for screening swine sera against PRRSV, we suggest ICST would successfully replace the current commercial ELISA for on-farm use in near future.

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Complete genome sequencing of a genotype 1 subtype 2 PRRSV isolate obtained in Western Siberia

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Purpose. In the present study, we report the complete genome sequencing of a subtype 2 of a Type 1 PRRSV strain. The virus was isolated from lung tissue of aborted foetus from the endemically infected farm in West Siberia, Russia in 2013 and was named Siberian.

Methods. Amplification and sequencing primers were designed by us on the basis of existing GenBank PRRSV sequences available for subtypes 1 and 3 of genotype 1. The resulting PCR fragments covered the entire genome of the virus. Total RNA was extracted and RT-PCR was completed. Sanger sequencing was performed, the coverage at least 2 times. 3' and 5'ends of the genome were amplified.

Results. The full sequence of the PRRSV Siberian isolate genome was 15050 nucleotides in length excluding the poly (A) tail. The genome sequence of the Siberian PRRSV isolate was compared to those of the prototype strains of genotype 1 subtypes 1 and 3, Lelystad and Lena respectively. Full sequence alignment revealed 80,0 and 81,8 % homology, respectively. The Siberian isolate was found to form a monophyletic group with subtype 3 strains. Comparison of ORF1a sequences of the Siberian and Lelystad isolates revealed a deletion 18 amino acids long in the variable region of the NSP2 protein (positions 741-759) and a deletion of one amino acid at position 901. The 3' end of the ORF3 sequence of the Siberian isolate is 10 amino acids shorter than that of the Lena strain and 26 amino acids shorter compared to the Lelystad strain.

Conclusions. We sequenced and analyzed the complete genome sequence of the PRRSV Siberian isolate, which belongs to subtype 2 of European type PRRSV. To our knowledge, this is the first complete genome sequence of a subtype 2 PRRSV-1. This fact is of interest for the perspective of understanding PRRSV evolution and its correlation with geographic distribution of different PRRSV subtypes.

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Identification of immunodominant B-cell linear epitopes present in the nucleocapsid protein of Porcine Epidemic Diarrhea Virus

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Porcine epidemic diarrhea virus (PEDV) is a Coronavirus that causes acute vomiting, anorexia and watery diarrhea in pigs of all ages, with up to 90-95% mortality in suckling piglets. Coronaviruses contain four major structural proteins: spike (S) envelope (E), membrane (M), and nucleocapsid (N). The S protein is further divided into S1 and S2 domains and includes neutralizing epitopes. The N protein binds to viral RNA and provides a structural basis for the nucleocapsid. It is the most abundant and conserved viral protein. In other coronaviruses, N is a target for antibodies, but these antibodies do not possess neutralizing activity. The current study aimed to characterize antibody immune responses to PEDV S1 and N and to identify linear B-cell epitopes within the N protein.

First, we produced recombinant N and S1 proteins in *E. coli* and eukaryotic cells respectively. Second, in-house ELISA has been developed for detection of IgG antibodies against these proteins in pig serum samples. Third, a purified peptide library containing 109 overlapping biotinylated peptides and covering complete N protein sequence was produced. This peptide library was used in PEPSCAN assay to identify linear antigenic epitopes in the N protein.

Twenty serum samples were obtained from a PEDV-positive pig farm in Ontario. All these samples were tested in ELISA using purified recombinant S1 and N proteins and found to be positive. Average ELISA titer against S1 was 1360, and average titer against N was 646. Five serum samples with the highest titer against N were used in PEPSCAN assay. Three epitopes were identified on the N protein sequence that consistently recognized by immune sera of all five pigs.

These findings have direct implications for PEDV diagnostics and eventual eradication as the identified epitopes may represent serological marker candidates for differential (DIVA) PEDV vaccines, derived from infectious cDNA clones.

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Establishment of a SYBR Green I based real time PCR for rapid detection of PRRSV Nsp9 gene and its expression in PRRSV infected cells

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A pair of specific primers targeted to non-structure gene 9(*Nsp*9)of PRRSV was designed and a SYBR Green I fluorescent based real time PCR was developed for the quantization of PRRSV. The melting curve analysis using SYBR Green I dye showed one specific peak, a melting temperature, and no primer dimers peak was observed. No amplification was detected from unrelated virus samples by this method, such as HEV, SIV, PRV. Fine reproducibility was obtained for detecting plasmid DNA with intra-assay. The real-time PCR method developed in this study will be useful for rapid laboratory diagnosis and epidemiology investigation for PRRSV. During the process of virus infecting cells, the expression level of *Nsp*9 increase gradually, it got the highest at 36 h.

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Influence of antiserum of PRRSV to replication in Marc-145 cells

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In order to verify whether hyper-immune serum of PRRSV have an influence on the replication of PRRSV , hyper-immune serum was prepared, PRRSV XH-GD strain were cultured, then centrifuged with high speed, the purified viruses were used to immunize the New Zealand rabbits to raise antibody. New Zealand rabbits were immunized intraperitoneally (i.p.) plus Freund's complete adjuvant. Antiserum was collected from the rabbits that had been placed under terminal halothane anesthesia. Antiserum titer was 1:640, the antiserum was incubated with virus, and then qPCR and TCID50 used to evaluate the titer of virus. The results show that antiserum could inhibit the replication of PRRSV at the mRNA lever, so hyper-immune serum of PRRSV is able to inhibit the replication of PRRSV in Marc-145 cells.

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Preparation and identification of the monoclonal antibodies against Nsp9 protein of Porcine Reproductive and Respiratory Syndrome Virus

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In order to generate monoclonal antibody against the NSP9 protein of PRRSV, the NSP9 protein was expressed in Escherichia coli and subsequently used as an antigen to immunize mice and for the initial screening of hybridomas prepared from the mice for their ability to produce anti NSP9 protein Mabs via an indirect ELISA. Three positive hybridomas were identified in this manner and verified based on the ability of their released Mab to react specifically with both naturally and artificially expressed NSP9 protein in Western Blots. The three hybridomas named 3B17, 3J13, 3F19 were subcloned three times before being introduced intraperitoneally into mice. The three hybridomas could occur with PRRSV-infected Marc-145 cells by indirect immunofluorescence verification. During the process of viral infection, the protein levels of NSP9 gene keep rising, it get highest at 48 h. These antibodies would be of great assistance to elucidate the function of Nsp9 gene.

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Effect of PEDV and PRRSV outbreaks on reproductive performance of commercial sows

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Historically, porcine reproductive and respiratory syndrome (PRRS) has caused major economic impact to the US swine industry from decreased performance in growing pigs and reproductive sows. Since its first reported outbreak in the U.S. in 2013, porcine epidemic diarrhea (PED) has been considered a major problem in the swine industry because of its high mortality rate in piglets. However, little is known about the impact of PED on reproductive performance of sows. Therefore, the objective of this study was to evaluate the impact of PRRS virus (PRRSV) and PED virus (PEDV) outbreaks on the reproductive performance of commercial sows.

The data analyzed for this project were from ten commercial farms located in North Carolina. A total of 21,060 farrowing records on 5,448 multiparous commercial F1 (Landrace x Large White) sows were used. The dataset included phenotypic records on number of piglets born alive (NBA), mummified piglets (MUM), stillborn piglets (NSB), born dead piglets (NBD), piglets weaned (NW) and abortion (AB). Occurrences of PEDV and PRRSV breaks were confirmed by serological results. However, the dates of the PEDV and PRRSV outbreak phases on each farm were determined based on the herd-year-week estimates of the reproductive performance. A total of 6 and 15 outbreaks were identified for PRRSV and PEDV, respectively. The impact of the disease phases on reproductive performance of sows was evaluated in SAS 9.4 in a model including the fixed effects of disease phase (Clean, PRRSV, and PEDV), season, parity, year and farm, and the random effect of sow. An additional model was used to assess the interaction between disease phase and season.

Disease phase had a significant effect on all traits analyzed ($P<0.0001$). Reproductive performance during Clean and PEDV phases was the same ($P>0.05$) for all traits analyzed, except for NW which averaged 9.8±0.05 and 3.2±0.1 piglets weaned for Clean and PEDV phases, respectively. In contrast, reproductive performance during PRRSV was different ($P<0.001$) than the Clean and PEDV phases for all traits. Interestingly, there were 2 occurrences of simultaneous PEDV and PRRSV breaks at the same farm. For all traits but NW, performance during simultaneous breaks was similar to PRRSV ($P>0.05$). For NW, simultaneous breaks showed the lowest ($P<0.0001$) performance, with only 1.8±0.3 piglets weaned. When the interaction of disease phase and season was analyzed, this effect was statistically significant for AB, MUM and NW ($P<0.05$), but not NBA, SB, or NBD ($P>0.05$). However, differences within disease phases were only found for NW, and NW was highest in summer and lowest in fall for both PRRSV and PEDV outbreaks.

These results indicate that PEDV does not have an effect on NBA, SB, MUM, NBD or AB, but does decrease NW. PRRSV affects all reproductive performance traits, although PEDV has a greater effect on NW. When a farm breaks with both PRRSV and PEDV, NBA and SB are statistically similar to a PRRSV break, but MUM, NBD, and NW show a larger effect than either a PRRSV or PEDV break. In addition, these results indicate that there is a compound effect between infection and season of the break.

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Senecavirus A infection in sows, neonates, and market weight gilts with subsequent protective immunity

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Objective: The objectives of this study were to 1) characterize SVA infection in market weight pigs, late-gestation sows, and neonates and 2) examine protective immunity in late-gestation gilts

Materials and Methods: For Part 1 of the study 15 market weight gilts were inoculated with SVA, bled regularly for 2 weeks, and clinical observations were made daily. In addition 10 sows were split into 2 groups. Group A was inoculated at various times pre-farrow (-17, -12, -10, -4, -3) and Group B was inoculated various times post-farrow (2, 3, 7, 7, 14) with their piglets. All animals were bled and swabbed at 0, 7, and 14 days post inoculation (dpi). In addition, piglets were bled pre-colostrum intake. For Part 2 of the study, 12 of the 15 gilts inoculated in Part 1 were bred and challenged again in late gestation. Serum samples were collected from the gilts and piglets along with milk samples. Serum and swab samples were tested for SVA by PCR and a virus neutralization assay was used for serum antibody testing

Results: During Part 1, all market weight gilts developed coronary band vesicles by 5 dpi. Snout lesions appeared 2 days after the first coronary band lesions and only developed in 40% of the gilts. Viremia lasted about a week in most gilts. Lameness was apparent starting on 2 dpi and lasted around 1 week, though feed intake was not affected. On the contrary, of the ten sows inoculated, only one sow developed a vesicle on the snout, though all had evidence of viral replication by PCR. Piglets born to sows infected on -17 and -12 were negative for SVA. Piglets born to the sow infected at -10 were positive for SVA before suckling and in subsequent sampling. Finally, piglets born to sows infected at -4 and -3 were negative at pre-suckle sampling, but positive by PCR in subsequent samplings. Two litters developed a yellow diarrhea often seen with enteric colibacillosis just after birth, but recovered completely in 2-3 days. All sows and piglets infected post-farrowing showed evidence of viral replication, but did not develop any clinical signs. All gilts and sows had increased antibody titers post exposure to SVA. During Part 2, there was no evidence of viral replication in any of the gilts or their piglets. In addition, all milk samples were negative for SVA.

Conclusions: Market weight gilts experimentally infected with SVA developed a clinical picture similar to reports from the field. On the other hand, we were not able to experimentally reproduce clinical signs observed in sow farms naturally infected with SVA, namely neonatal mortality. We did show evidence of infection and viral replication in neonates, but we did not observe increased pre-weaning mortality. Gilts were challenged 5 months after initial exposure in late gestation and we were able to demonstrate protective immunity. Continued experimental studies with SVA will improve understanding of the pathogenesis of SVA and help shape control and prevention measures in the swine industry.

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Phenotypic characterization of a novel HP Italian PRRSV-1 isolate in experimentally infected pigs

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Highly pathogenic (HP) PRRSV isolates are characterized by high viral loads, severe general clinical signs and high mortality. Their genomes share a discontinuous aa deletion in the non-structural protein 2 (nsp2). This investigation is aimed at characterizing clinical, virological, pathological and serological outcomes in conventional pigs experimentally infected with a potential highly pathogenic, Italian PRRSV-1 subtype 1 isolate. The isolate (PR-402014) was obtained from nursery pigs in the course of an outbreak of high post-weaning mortality (up to 50%) associated with severe systemic and respiratory disease. The nucleotide sequence of the ORF5 showed 85.9% homology to LV. The full genome sequence revealed a discontinuous deletion of 42nt and 366nt in the nsp2 region, and a 6nt deletion in the ORF4. To confirm the high pathogenicity of the virus, five conventional pigs of six weeks of age from a PRRSV negative herd were intranasally inoculated with 10^6 $TCID_{50}$/pig PR-402014 (HP) in 2 ml of PBS, 1 ml/nostril (HP group). Moreover, five pigs from the same origin were inoculated with the same dose of a recent Italian PRRSV (PR-012014) isolate. This group (NP group) served as a reference group for the comparison of the obtained findings. At day 3 p.i., two more pigs were added to each group to act as "in contact" pigs. In total 7 pigs/group were considered. Thus, a group of 3 pigs intranasally inoculated with 2 ml of PBS served as negative control. Body temperature and clinical signs (respiratory disorders scored from 0 to 6, appetite, level of consciousness) were monitored daily. Blood and nasal swabs were collected at 0, 3, 7, 10, 14, 21, 28, 35, 42 days p.i. in all experimental pigs. Clinical and virological differences were observed among animals inoculated with the HP and the NP isolates. In particular, high fever (the average temperature was >40°C from day 2 to 24 p.i. peaking up to 41,5°C in some animals), anorexia and depression of the level of consciousness were the prominent signs in pigs inoculated with the HP from day 3 to 28 p.i. Fever in NP animals was constantly lower. Four out of 7 (57%) pigs died in the HP group. The ADWG was 264 g/day for the survivors (3 pigs) in group HP, 345 g/day for those of NP group and 497 in the negative control group. Viremia in HP infected pigs was higher and longer in duration as compared to the NP infected animals. Dead animals from HP group showed severe lymphocyte depletion in the lymphoid tissues and organs and severe interstitial pneumonia. Taken together a) the clinical outcomes of the PRRSV isolate in the field, b) the genome deletions and 3) the experimentally induced severe clinical signs associated with high viremia and pathological lesions in challenged pigs, we can assume that the isolate PR-402014 could be defined as a highly pathogenic PRRSV-1, subtype 1.

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Comparison of morbidity and mortality after challenge with two North American PRRS virus isolates shows marked variation in time course and prevalence of clinical disease between isolates

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Porcine reproductive and respiratory syndrome (PRRS) is the most economically devastating disease of swine worldwide. Significant genetic variation among PRRS virus isolates can correlate to differences in clinical signs and disease progression. The objective of this study was to compare morbidity and mortality of two heterologous North American (Type 2) PRRSV isolates after experimental challenge in nursery pigs. Two experimental populations of approximately 200 commercial crossbred pigs (Pietrain × LW) each were infected with either NVSL-97-7985 (NVSL) or KS-2006-72109 (KS06) and followed for 42 days post-infection (dpi). Overall morbidity after challenge with NVSL or KS06 was 39.2 and 21.6%, respectively ($p = 0.0002$). Mortality rates were also higher after infection with NVSL when compared to KS06 (11.5 and 9.0%, respectively); however, this difference was not statistically significant ($p = 0.51$). The time course of clinical disease post-infection with NVSL was chronic, with clinical signs occurring at a similar rate throughout the course of the trial. In contrast, clinical disease post-infection with KS06 was acute, with clinical signs primarily occurring between 4 and 10 dpi. The mean duration of clinical disease also reflected the chronic and acute disease progression of the two isolates; 11.5 ± 6.0 dpi with NVSL and 4.0 ± 2.1 dpi with KS06 ($p < 0.0001$). Clinical signs of respiratory disease, such as open mouth breathing, dyspnea, tachypnea and nasal discharge, were 11.3 times more likely to occur after challenge with NVSL than after challenge with KS06 ($p < 0.0001$). Infection with NVSL was 3.9 times more likely to reduce body condition during the course of the trial compared to infection with KS06 ($p = 0.001$). Severity of clinical disease correlated with an overall increase in PRRSV replication after challenge with NVSL; total virus load was 153.7 ± 20.3 and 140.3 ± 17.2 for NVSL and KS06, respectively ($p < 0.0001$). Average daily gain (ADG) during the 42 day trial was also significantly lower in pigs challenged with NVSL; 0.43 ± 0.09 kg and 0.45 ± 0.10 kg in pigs challenged with NVSL and KS06, respectively ($p = 0.03$). In this model, challenge with NVSL had significantly higher morbidity, greater virus replication, and decreased ADG due to the chronic duration of disease. However, challenge with KS06 resulted in similar mortality due to acute and severe disease within the first week post-infection. These results provide evidence for the acute and chronic forms of clinical disease that can occur with different PRRSV isolates.

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Cross-reactivity of immune responses against Porcine Reproductive and Respiratory Syndrome virus

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The extent of cross-reactivity of humoral and cell-mediated immunity (CMI) against porcine reproductive and respiratory syndrome virus (PRRSV) are yet to be fully understood. CMI against PRRSV has been described as slow and weak, and it has been hypothesized that expression of interferon (IFN)-gamma by T-cells correlates with reduced viremia and lung lesions after challenge. Protection against challenge is also afforded by neutralizing antibodies. While the overall antibody response against PRRSV is very broad, neutralizing antibodies show high specificity. The objective of our work was to determine the cross-reactivity of T-cells and its correlation with humoral immunity and genetic variability.

To that purpose, two groups of four-week old pigs were either inoculated intramuscularly with PRRSV isolate FL12 (n=12) or left uninfected (n=12). All animals were bled at 0, 14, 28, 42, 63 and 77 days post-infection, serum was collected and peripheral blood mononuclear cells (PBMCs) were isolated. The number of IFN-gamma secreting cells (SC) per million PBMCs was determined by ELISpot using FL12 and nine different PRRSV isolates of varying genome-wide pairwise distance as recall antigens. Antibody responses, total and neutralizing, were evaluated by immunofluorescence and serum viral neutralization assays against five of these PRRSV isolates.

The IFN-gamma SC responses were highly variable between animals against both homologous and heterologous isolates. The overall IFN-gamma SC response was not significantly different among PRRSV isolates, regardless of genetic distance to FL12, and time post-infection. This suggests that the T-cell response against PRRSV is broadly cross-reactive. No association could be drawn between PRRSV isolate genome-wide pairwise distance and the number of IFN-gamma SC. The total antibody response was broad, while the neutralizing antibodies were specific to FL12. Additional studies into PRRSV CMI should be useful in determining whether a highly specific subset of T-cells exists, analogous to neutralizing antibodies in the humoral immune response.

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Changes in the genetic composition of PRRSV quasispecies and its relationship with long and short viral infections

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Replication of RNA viruses is usually mediated by an error-prone RNA-polimerase. The high mutation rate of such RNA-polimerases produces a cloud of mutants in each round of replication. This leads to the formation of quasispecies; namely to groups of related viral genomes competing with other groups in a high mutation environment. This concept of quasispecies is important to understand viral evolution. In PRRSV it is known that vaccination only provides partial protection against the heterologous challenge. This means that vaccinated pigs can be infected although replication of the virus in the vaccinated animals is shorter and of lesser intensity compared to naïve pigs. When this examined in detail for type 1 PRRSV, it can be seen that some vaccinated infected animals have very short viremias (1-3 days) while other pigs develop viremias lasting one or two weeks. It is known that the host's genetic background may play a role on this. The objective of the present work was to assess the variation in the virus across the infection period in vaccinated pigs. To do so, three groups of infected pigs: non-vaccinated (n=6, vaccinated presenting a short-viremia (1-3 days after challenge, n=7) and vaccinated showing a long-viremia (>3 days after challenge, n=4) have been analyzed. After setting up a specific protocol to directly sequence RNA avoiding intermediate PCR steps, pig sera samples were used to infect macrophages. The ARN extracted from the cell culture supernatants were characterized by Next-Generation Sequencing (NGS) using the MiSeq Illumina platform in order to characterize the population diversity and structure of PRRSV. A high proportion of reads (>85%) showed good Phred Quality scores (q>20), coupled with the high coverages reported (>100) indicated the reliability of the reads obtained. Nucleotide and aminoacid differences among groups and with the initial inoculum were screened comparing the nucleotide frequency differences for every polymorphic position by means of a hierarchical nested analysis of molecular variance (AMOVA). A large proportion of nucleotide mutations were not silent, inducing aminoacid changes. Those differences were not evenly distributed along the PRRSV genome, being concentrated in the nsp2, nsp2TF and nsp9 genes, pointing that virus-based factors may influence the differential susceptibility against PRRSV infection within a herd.

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The progression to T cell immunity after infection with porcine reproductive and respiratory syndrome virus

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Although the adaptive response to infection with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) has been analyzed *in vivo*, there has not yet been an explanation for the delay in the occurrence of IFN-gamma secreting cells. Given that PRRSV is able to infect macrophages (Mϕs) as well as CD163+ dendritic cells (DCs), it seems plausible that different antigen presenting cells (APCs) will evoke specific lymphocyte proliferative responses. We hypothesize that macrophages are responsible for the delay in immune protection, whereas DCs are responsible for stimulating IFN-y secreting lymphocyte proliferation. Using GMCSF-derived Mϕs, monocyte derived DCs (MoDCs), and Flt3L-derived bone-marrow dendritic cells (BMDCs), we first compared these cells in vitro in response to infection with PRRSV. Using flow-cytometry, we observed an up-regulation of MHC molecules on CD163+ BMDCs and MoDCs, suggesting their importance in the progression to adaptive immunity. In comparison, the CD163- DCs showed a down-regulation of both MHC molecules. Additionally, we found that levels of MHCI and MHCII expression on the surface of Mϕs infected with PRRSV are both down-regulated. To further characterize the progression to adaptive immunity during PRRSV infection *in vivo*, animals were infected with PRRSV VR-2332 and T cells were isolated over the course of the infection on different dates. By co-culturing MoDCs, macrophages, and BMDCs with T cells from the respective dates, we will analyze the differences in the T cell response to antigen-presentation by either macrophages or DCs. We hypothesize that only the 163+ DCs will be able to stimulate a significant IFN-y cell mediated response. Results from a pilot study in which animals were immunized with the MLV support our hypothesis. ELISPOTs in which PBMCs, from the immunized animals, treated with either inactivated PRRSV or live PRRSV both evoked IFN-gamma responses. However, in the T cell co-cultures, T lymphocyte proliferation was only observed when macrophages were cultured with live but not inactivated PRRSV. This indicates that there is a cell population present in PBMCs that promotes an IFN gamma response to inactivated virus. It seems likely that a subset of dendritic cells is responsible for such a response, which could also provide a theory for the delay in cell-mediated immunity observed *in vivo.*

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Pathological findings at the maternal-fetal interface during the early type 2 PRRS virus infection of late gestation pregnant gilts

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Understanding the pathophysiological mechanisms responsible for fetal death related to porcine reproductive and respiratory syndrome (PRRS) is essential step in designing effective control measures against this costly disease. Our recently completed study of type 2 PRRSV infection at maternal-fetal interface (MFI; uterus plus adherent fetal placenta) 21 days post-inoculation (dpi) revealed that pathological lesions were not compatible with fetal death. However, due to the large number of autolyzed and decomposed fetuses in that study, an association between lesions at the MFI and fetal preservation status was not confirmed. The aim of this present study is to evaluate progression of PRRSV induced pathology in the uterus and fetal placenta during early time points of infection. A total of 15 PRRS virus-naïve, high-health pregnant gilts were intramuscularly and intranasally inoculated with PRRSV (NLSV 97-7895; 1 x 10^5 $TCID_{50}$ total dose) on gestation day 85±1. Five negative control gilts were sham inoculated. Three gilts challenged with PRRSV and one control gilt were euthanized on each of five days post inoculation (dpi 2, 5, 8, 12 and 14). At each time point, MFI samples corresponding to each fetus were formalin-fixed and paraffin-embedded. Histopathology of the MFI was assessed by a pathologist blinded to inoculation status and time points. Severity of inflammation in uterus and fetal placenta was assessed based on percentage of affected tissue: normal, minimal (<10%), mild (10-25%), moderate (25-50%) and severe (>50%). Results of pathological assessment revealed large numbers of PRRSV-induced lesions in the endometrium (Table 1), with the earliest lesion detected on 2 dpi consisting of focal vasculitis. Detachment of fetal placenta from uterus was observed more often in PRRSV infected compared to non-infected samples. On 14 dpi, 64% of PRRSV-infected uterine samples were severely inflamed with diffuse vasculitis and 22% of uterine samples exhibited detachment of fetal placenta. Pathological findings from our study revealed a progression of lesion severity in the maternal-fetal interface starting from 2 dpi to 14 dpi. This project was supported by Genome Canada, Genome Prairie (Saskatchewan Ministry of Agriculture) and Genome Alberta.

Table 1. Pathological lesion severity in endometrium by days of post-inoculation

	PRRSV-infected gilts						**Non-infected gilts**					
dpi	Severity of inflammation in endometrium					Detachments[1]	Severity of inflammation in endometrium					Detachments[1]
	N	Min	Mild	Mod	Sev		N	Min	Mild	Mod	Sev	
2	0/34	23/34	11/34	0/34	0/34	3/34	6/14	7/14	1/14	0/14	0/14	3/14
5	1/43	16/43	23/43	3/43	0/43	6/43	11/13	2/13	0/13	0/13	0/13	0/13
8	0/43	1/43	7/43	27/43	8/43	6/43	10/14	4/14	0/14	0/14	0/14	1/14
12	0/49	0/49	2/49	25/49	22/49	7/49	12/16	4/16	0/16	0/16	0/16	0/16
14	0/36	0/36	2/36	11/36	23/36	8/36	5/14	8/14	0/14	1/14	0/14	1/14

[1] Presence of fetal placental detachments from the uterus. N=normal, Min=minimal, Mod=moderate, Sev=severe

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Pathogenesis and infection dynamics of Senecavirus A in pigs

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Senecavirus A (SVA), a picornavirus of the genus *Senecavirus*, has been recently associated with vesicular disease and neonatal mortality in swine the US and in Brazil. However, many aspects of SVA infection biology and pathogenesis remain unknown. Here, the pathogenesis and infection dynamics and of SVA were investigated in pigs. Twelve finishing pigs (~100 lb) were randomly allocated into two experimental groups as follows: Group 1: Mock-control group ($n = 4$) and Group 2: SVA-inoculated group ($n = 8$). Animals were inoculated oronasally with a contemporary SVA strain and monitored daily for characteristic clinical signs and lesions associated with SVA infection. Viremia was assessed in serum and virus shedding was monitored in oral and nasal secretions and feces. Samples collected on days 0, 3, 5, 7, 10, 14, 21, 28 and 35 post-inoculation (pi) were tested by real-time reverse transcriptase PCR (qRT-PCR) and virus isolation. Clinical signs characterized by lameness and lethargy were first observed on day 4 pi and persisted for 2-10 days. Vesicular lesions were observed on the snout and feet, including coronary bands, dewclaws and sole of inoculated animals. Vesicular lesions were observed between days 4 and 14 pi. Viremia was detected between days 3 and 10 pi, whereas virus shedding was detected between days 1 and 28 pi in oral and nasal secretions and feces. Notably, rRT-PCR and *in situ* hybridization performed on tissues collected on day 38 pi revealed the presence of viral RNA on the tonsil of all SVA infected animals. Serological responses were monitored by virus neutralization and indirect immunofluorescence assays. Animals developed an early neutralizing antibody (NA) response to SVA, with NAs being first detected on day 5 pi and peaking on day 10 pi. High levels of NAs were still detected on day 38 pi. SVA specific IgG antibodies were first detected by IFA on day 10 pi, peaked on day 14 pi and presented a slight decline on days 35 and 38 pi. Results of this study provide important insights about the pathogenesis, infection dynamics, and shedding patterns of SVA in swine.

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The effect of PRRS viral level and isolate on tonsil gene expression 42 days after infection

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The objective of this research was to identify differentially expressed genes (DEGs) and gene ontology-biological process (GO-BP) in tonsils from pigs with either extreme high or low tonsil Porcine Reproductive and Respiratory Syndrome virus (PRRSV) levels 42 days post infection (dpi) with one of two viral isolates, NVSL-97-7985 (NVSL) and KS-2006-72109 (KS06). The data analyzed for this study were from two PRRS Host Genetics Consortium PRRSV infection trials of ~200 commercial nursery pigs each from the same genetic source. At 42 dpi, pigs were euthanized and tonsil samples were collected. Tonsil samples were chosen for RNA-seq based on high or low levels (at least one standard deviation from the mean) of PRRSV present in the tonsil, evaluated by a semi-quantitative PCR assay for PRRSV RNA, resulting in 15 NVSL-high, 13 NVSL-low, 12 KSO6-high, and 10 KSO6-low. After removing transcripts with mean read counts across all samples less than 8 and less than 4 samples with read counts larger than zero, 11,909 genes were determined to be expressed in the tonsil. After backward variable selection built on QuasiSeq, the statistical model for read counts for each gene included PRRSV isolate, tonsil virus class (high/low), the interaction between PRRSV isolate and tonsil virus class, genotype at marker WUR10000125 (WUR), which has been reported to be strongly associated with both weight gain and serum viremia in pigs after infection with PRRSV, and sex as fixed class variables, and RNA integrity number (RIN) as a covariate. Using the QuasiSeq package, 258 DEGs were identified ($q \leq 0.1$) between the two PRRSV isolates, 105 DEGs ($q \leq 0.1$) were identified between the high and low tonsil virus class, and 7 genes were significant ($q \leq 0.1$) for the interaction between PRRSV isolate and tonsil virus class. Using all genes expressed in the tonsil as the reference, GO-BP terms were tested for enrichment in the DEGs using PANTHER. Overrepresented ($p<0.05$) terms among DEGs between the two isolates included immune system process, cell-matrix adhesion, cell-cell adhesion, ectoderm development, cellular component morphogenesis, and signal transduction. The term immune system process was also overrepresented (p=5.97E-03) among DEGs between the high and low tonsil virus class. Taken together, this study suggests that genes involved in the immune system process are correlated with the tonsil virus level for both PRRSV isolates. In future work, the identified DEGs will be further validated and selected to build a biomarker-based classifier for PRRSV resistance. This project was funded by Genome Canada, USDA-ARS, USDA-NIFA grant 2013-68004-20362 and National Pork Board grants #12-061 and #14-223. We would also like to acknowledge contributions from members of the PRRS Host Genetics Consortium.

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A major gene for host response under PRRS challenge is not negatively associated with overall performance in commercial pig lines under non-challenged conditions

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Previous research identified the *GBP5* gene as a major gene for host response to PRRS, for which the single nucleotide polymorphism (SNP) WUR10000125 (WUR) can be used as a tag SNP. The effect of WUR has been validated across breeds, genetic sources, and with different PRRS virus (PRRSV) isolates. However, questions that remain unanswered are: what is the effect of the favorable (B) allele for WUR on economically important traits under non-challenged conditions and why does the B allele have a low frequency (~0.2) in commercial populations? Thus, the objective of this study was to estimate the effect of WUR genotype on selected index traits in commercial pig lines under normal, non-challenged conditions.
Data originated from records collected on four Topigs Norsvin lines: a Landrace maternal line (A), a Large White maternal line (B), a synthetic boar line (C), and a Pietrain boar line (D). Frequency of the B allele ranged from 10 to 22% by line. Pigs were genotyped using either the Porcine SNP60 or SNP80 BeadChip, which included the WUR SNP. Nine traits were analyzed, including total number born, number stillborn, farrowing survival, lactation survival, litter mortality, backfat, daily feed intake, lifetime daily gain, and daily gain during test. The Topigs Selection Index (TSI), indicative of overall performance, was also analyzed. De-regressed estimated breeding values for each trait (except for TSI) were analyzed within line using univariate animal models in ASReml 4.0. PRRS vaccination status (PRRS_vacc; whether/not pigs were vaccinated for PRRS), WUR, and PRRS_vacc*WUR were fitted as fixed effects and genetic relationships were accounted for using pedigree.
For the B line, no difference in farrowing survival was detected between genotypes within the non-vaccinated group, but farrowing survival was numerically greater for AB/BB pigs than for AA pigs within the vaccinated group (PRRS_vacc*WUR; P=0.05). A significant effect of WUR (P<0.001) on litter mortality was detected in the D line, where BB pigs had significantly greater litter mortality than AB or AA pigs. No significant effect of WUR genotype was detected on any of the finishing traits for the maternal lines. In the C line, the B allele was associated with significantly lower lifetime growth rate (P=0.001) and test growth rate (P=0.002), but also lower feed intake (P=0.004). Conversely, the B allele was associated with significantly higher feed intake (P<0.001) and a tendency for higher test growth rate (P=0.09) in the D line. The effect of WUR on TSI was not significant for any of the lines ($P\geq$0.15).
In conclusion, the direction of the WUR genotype effect differed between traits and lines. The favorable (B) allele for host response to PRRS had a favorable effect on farrowing survival in the B line when pigs were vaccinated for PRRS. The B allele was also associated with greater feed intake and a tendency for greater growth in the D line, but the opposite direction of effect was detected for the C line. However, regardless of the effect on individual traits, no effect of WUR on the overall index value was detected for any of the lines. Therefore, selecting for the B allele may result in progeny with increased performance under PRRS challenge without adversely affecting overall TSI. This work was supported by the USDA ARS and NIFA award 2012-38420-19286 and by Topigs Norsvin.

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Porcine reproductive and respiratory syndrome virus (PRRSV) up-regulates IL-8 expression through TAK-1/JNK/AP-1 pathway

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The acute phase of respiratory distress caused by porcine reproductive and respiratory syndrome virus (PRRSV) infection is likely a consequence of the release of inflammatory cytokines in the lungs. IL-8, the main chemokine and activator of neutrophils, might be related in the lung lesions upon PRRSV infection. In this study, we first confirmed that HP-PRRSV induced IL-8 expression in vivo and in vitro. Subsequently, we showed that JNK and NF-κB pathways were required for the enhancement of IL-8 expression. JNK and NF-κB pathways were indeed activated upon PRRSV infection, as evidenced by JNK and IκB phosphorylation. We further verified that PRRSV activated TAK-1 was essential in JNK and NF-κB pathway activation and IL-8 expression. Moreover, we revealed an AP-1 binding motif in the cloned porcine IL-8 (pIL-8) promoter, and deletion of this motif abrogated the pIL-8 promoter activity. Additionally, the AP-1 subunit c-Jun was found to be critical for the activation of porcine IL-8 promoter by PRRSV. Our findings suggest that PRRSV-induced IL-8 production is likely through the activation of TAK-1/JNK/AP-1 pathway.

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Genetic evaluation of reproductive performance during PRRS/PED outbreaks

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most infectious swine diseases in the world, resulting in over $600 million dollars of economic loss in the U.S. alone. More recently, the U.S. swine industry has been having additional major economic losses due to the spread of porcine epidemic diarrhea (PED). However, information regarding the amount of genetic variation for response to diseases in reproductive sows is still very limited. Therefore, the purpose of this study was to quantify differences in genetic parameters for reproductive performance within PRRS and/or PED challenged and non-challenged environments.

Performance data and a five-generation pedigree were available from 10 commercial farms in North Carolina. A total of 21,060 farrows from 5,448 crossbred (Large White x Landrace) multiparous sows were used for analyses. Traits analyzed included abortions (AB), number of piglets born alive (NBA), number of stillborn piglets (SB), number of mummified piglets (MUM), number born dead (NBD), and number weaned (NW). Occurrences of PED and PRRS breaks were confirmed by serological results. However, the dates of the PED and PRRS outbreak phases on each farm were determined based on the herd-year-week estimates of the reproductive performance. A total of 6 and 15 outbreaks were identified for PRRS and PED, respectively. Genetic parameters (heritabilities and correlations) were estimated for each phase (Clean, PRRS, and PED) separately, in a model including the fixed effects of season, year, and farm, and the random effect of animal. A covariate of number of weaning events was added for the NW trait. For the Clean phase, a random permanent environmental effect was fit in the model to account for the repeated records. Genetic correlations between traits were estimated within and between disease phases. Traits with a large number of zeros (SB, MUM, and NBD) were analyzed as the natural log of the phenotype + 1. Analyses were done in ASReml4.

Heritability estimates for the Clean phase ranged from 0.01±0.003 (MUM) to 0.23±0.04 (AB), for the PRRS phase ranged from 0.05±0.07 (SB) to 0.29±0.06 (AB), and for the PED phase ranged from 0.01±0.02 (SB) to 0.38±0.06 (AB). Genetic correlations within the PRRS phase ranged from -0.97±0.46 to 0.82±0.44 for NBD with NBA and MUM, respectively. Within the PED phase, genetic correlations were estimated from -0.62±0.82 to 0.98±0.38 for MUM with NW and NBD, respectively. In general, estimation of genetic correlations between phases for the same traits showed convergence problems. However, interesting genetic correlations were found, such as the low estimate (0.36±0.17) for NW between the Clean and PED phases, indicating that NW in these phases are two genetically different traits, and the overall high estimates (>0.80) between PRRS and PED phases for most traits, indicating that, although different diseases, response to these diseases are genetically similar.

These are the first reports on genetic variation for response to PED infection in reproductive sows. These results indicate that there is a sizable genetic variation for response to PED, and that the selection for increased NW during PED outbreaks will not impact NW performance in clean environments. In addition, response to PED and PRRS seems to be genetically the same for reproductive traits.

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Genetically edited pigs lacking CD163 show no resistance following infection with the African Swine Fever Virus isolate, Georgia 2007/1

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African swine fever is a highly contagious, often fatal disease of swine for which there is no vaccine or other curative treatment. The macrophage marker, CD163, is a putative receptor for African swine fever virus (ASFV). The objective of this study was to evaluate the requirement of CD163 using a genetically edited pig model. Pigs possessing a complete knockout of CD163 on macrophages were inoculated with Georgia 2007/1, a genotype 2 isolate. Knockout and wild type pen mates became infected and showed no differences in clinical signs, mortality, pathology or viremia. There was also no difference following *in vitro* infection of macrophages. The results do not rule out the possibility that other ASFV strains utilize CD163, but demonstrate that CD163 is not necessary for infection with the Georgia 2007/1 isolate. This work creates opportunities to focus on alternative ASFV receptors and entry mechanisms. Understanding these virus-host interactions is important for designing better antiviral strategies against ASF.

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Porcine reproductive and respiratory syndrome virus takes advantage of host intercellular mitochondria transferring pathway for cell to cell spreading of the infection

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Our recent study showed that intercellular nanotube connections can serve as an alternative pathway for cell to cell spreading of PRRSV infection (Guo et al., 2016, J. Virology, 90(10):5163-75). Here, we further studied the mechanisms of nanotube formation during PRRSV infection and cellular organelles involved in the transportation of PRRSV infectious materials. We found that PRRSV infection can induce the formation of intercellular nanotube connections between infected and uninfected MARC-145 cells in the early stage of infection. Co-culturing PRRSV-infected cells with uninfected cells rescued PRRSV-induced cell necrosis. Mitochondria, an important regulator for virus-induced danger signaling, can be observed transferring from uninfected cells to PRRSV-infected cells. Importantly, impaired formation of nanotubes or ethidium bromide and uridine treated cells carrying defective mitochondria were unable to rescue infected cells from necrosis in the co-culture system. Confocal microscopy analysis showed that mitochondria-associated molecules in the antiviral signaling pathways, including MAVS and RIG-I, were co-localized with the mitochondria and transported through the nanotubes from uninfected to infect cells. Furthermore, PRRSV nsp1alpha, nsp1beta, and nsp4 were detected to be associated with mitochondria and transported from infected to uninfected cells through the nanotubes. Our results suggest that transferring of functional mitochondria through nanotubes rescued the PRRSV-induced cell necrosis in the early stage of infection. On the other hand, PRRSV takes advantage of this intercellular pathway, in which mitochondria could be utilized as a cargo to transport viral infectious materials for cell to cell spreading of the infection.

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Evaluation of fetal and maternal gene expression responses to reproductive porcine reproductive and respiratory syndrome virus infection

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Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infection causes reproductive failure in pregnant females. It is mainly characterized by late-term abortions, early farrowing, fetal death, and an increase of weak born fetuses which can result in elevated pre-weaning mortality. Our group has used a pregnant gilt model (PGM) to probe the mechanisms of PRRSV-induced reproductive failure. Our original model (PGM1) tested effects at 21 days post infection (dpi) in pregnant gilts and their fetuses, inoculated at 85 +/- 1 day gestation (DG). Our new PGM2 uses 85 DG gilts that were sham (control; n=5) or type 2 PRRSV (strain NVSL 97) inoculated (n=15). Gilts were euthanized at 2, 5, 8, 12, and 14 dpi and fetal preservation status evaluated. Blood and tissue samples were collected to measure viral load to determine when the virus crosses the placental barrier and infects the fetuses. For each fetus, the thymus was collected as well as the maternal-fetal interface (MFI) which was separated into maternal endometrium and fetal placenta; all samples were snap frozen. To characterize factors regulating the effects of temporal movement of PRRSV across the MFI and gain insight into the mechanisms of PRRSV pathogenesis we will analyze gene expression in these tissues using Nanostring gene expression technology, testing samples from 4 time points (5, 8, 12, 14 dpi). We will compare data from sets of fetuses from each negative control gilt (NEG) and from each of the 3 PRRSV inoculated gilts [matching PRRSV negative, uninfected (UNINF) with infected (INF) from the same litter] based on fetal serum and thymus PRRSV viral load. RNA will be prepared from maternal endometrium, fetal placenta and thymus from each selected fetus. For Nanostring PCR, >220 genes were selected for expression analyses. These gene codesets were based on data from past PRRSV infection response studies, in cell cultures, growing pigs, and PGM1 RNAseq studies, analyzed using Ingenuity Pathway Analysis (IPA) and KEGG Pathway Database. The critical gene sets and pathways that were significantly associated with post-PRRSV infection response included signaling pathways associated with interferons, TREM1, HMGB1, B cell/T cell receptors, Toll-like receptors, and apoptosis/mitosis, etc. The codeset also tested for genes related to endometrium and placenta tissue remodeling and epithelial integrity and permeability. Overall this data should reveal mechanisms involved in PRRSV transmission from the gilt to some, but not all, of her fetuses and highlight genes which predict prevention of in utero fetal PRRSV infection. Funding provided by Genome Canada, Genome Prairie (Saskatchewan Ministry Agriculture), Chinese Scholarship Council fellowship, and USDA ARS.

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SAP domain in nsp1-beta of porcine reproductive and respiratory syndrome virus (PRRSV) correlates with interferon suppression in cells and pathogenesis in pigs

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an arterivirus whose translation is cap-dependent and occurs in the cytoplasm of infected cell. In the present study, we show the subversion of host cell mRNAs translation by PRRSV during infection. PRRSV blocked the host mRNA nuclear export to the cytoplasm, and it was common in both European genotype and North American genotype of PRRSV. For PRRSV, nsp1-alpha, nsp1-beta, and N were nuclear proteins, and of these, nsp1-beta was found to play a pivotal role for host mRNA nuclear retention. A SAF-A/B, Acinus, and PIAS (SAP) motif was identified in nsp1-beta with a consensus sequence of 126-LQxxLxxxGL-135. Mutations were introduced in the SAP domain and the mutants were examined for their subcellular localization, inhibition of host cell mRNA nuclear export, and suppression of host protein synthesis. In site hybridization unveiled that L126A, R128A, R129A, L130A, and L135A were unable to cause nuclear retention of host cell mRNAs. PRRSV nsp1-beta was shown to inhibit the type I IFN response, and the above mutants were unable to inhibit the IFN production and signaling pathways. Using the infectious clone for PRRSV and reverse genetics techniques, SAP mutant viruses were generated. Infectious viruses were recovered for vK124A, vL126A, vG134A, and vL135A, but vR128A, vR129A, and vL130A were non-viable. Among the viable mutants, vL126A and vL135A lost the ability to suppress the IFN production in cells, suggesting that the SAP domain in nsp1-beta of PRRSV is crucial for type I IFN suppression. To investigate the role of SAP domain in nsp1-beta of PRRSV for viral pathogenesis, piglets of 3-week-old were infected intramuscularly with SAP mutant viruses. Pigs infected with vL126A or vL135A exhibited less severe clinical signs with lower and shorter durations of viremia comparing to pigs infected with wild-type PRRSV. The titers of neutralization antibody were higher in vL126A- or vL135A-infected pigs than those of control pigs. When examined for viral persistence in the tonsils, two of ten pigs in the vL135A-infection group were PRRSV-negative, and the remainders were positive for PRRSV. Reversion to wild-type sequence occurred in all ten pigs in the vL126A group and seven of eight pigs of the vL135A group. Only one of the eight pigs in the vL135A group remained unchanged. These data show that the SAP domain in nsp1-beta of PRRSV contributes to viral pathogenesis.

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Contribution of PRRSV minor glycoproteins to a protective immune response in swine

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Determining the role of PRRSV minor glycoproteins in the induction of a protective immune response is an area of PRRSV research that may have an impact on vaccine development. GP2, GP3, and GP4 form a trimer on the surface of the virion. GP2 and GP4 interact with CD163, the main host cell receptor for PRRSV infection, and because GP3 is part of a trimer it may be interacting with the receptor as well. Previous work by many laboratories has shown minimal cross-protection between Type 1 and Type 2 PRRSV. With this knowledge, GP2, GP3 and GP4 of a Type 2 infectious clone were cloned into a Type 1 infectious clone, and this virus was used to determine the contribution of the minor glycoproteins to a protective immune response. Chimeric virus was recovered, and we determined that the chimeric virus was sensitive to neutralization, indicating that neutralizing antibodies are directed against GP2, GP3, and GP4. This chimeric virus was then used in an animal experiment to evaluate the contribution of GP2, GP3, and GP4 to protective immunity in pigs. Neutralizing antibodies against the chimeric virus and the Type 2 parental virus, and GP2, GP3, and GP4 specific IFN-gamma secreting cells were detected prior to challenge and increased after challenge. Viremia after challenge was lower in the chimeric virus group compared to the PBS and Type 1 PRRSV controls. Tissue viral load in the lung was lower for the chimeric virus group compared to the control groups, while the tonsil and lymph node viral loads were similar across all groups. The chimeric virus group had lower lung pathology scores than the control groups. Animals immunized with chimeric virus developed neutralizing antibodies and antigen-specific T cells that recognized GP2, GP3, and GP4 peptides; however, GP2, GP3, and GP4 conferred only partial protection against challenge.

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Porcine deltacoronavirus induces caspase-dependent programmed cell death

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Porcine deltavoronavirus (PDCoV), a member of the genus *Deltacoronavirus* in the family *Coronaviridae* of the order *Nidovirales*, is a newly emerged swine enteric coronavirus causing severe clinical diarrhea and intestinal pathological damage in piglets. As a novel identified viral pathogen, numerous aspects of virus-host interactions have been largely undeciphered. As a first step toward understating the effect of PDCoV on cells, we sought to investigate whether PDCoV induces programmed cell death (PCD) *in vitro* and to elucidate mechanisms associated with the process of PCD after PDCoV infection. PDCoV-infected cells showed evidence of apoptosis including DNA fragmentation and phosphatidylserine exposure. In addition, caspase-3, the main effector caspase, was activated in PDCoV-infected cells up to 24 h post-infection (hpi), indicating the cascade of caspase activation in relation to PDCoV-induced apoptotic cell death. Furthermore, the use with Z-VAD-FMK, a pan-caspase inhibitor, affected PDCoV replication and inhibited virus-induced apoptosis, suggesting that a caspase-dependent pathway is involved in the process. Since caspases appear to be essential factors in PDCoV-induced cell death, we tried to assess the release of cytochrome c (CytC), which is proapoptotic protein to execute caspase proteolytic cascade-dependent intrinsic apoptosis, from mitochondria to the cytosol by mitochondrial outer membrane permeabilization. A number of coronaviruses, including porcine epidemic diarrhea virus, are known to trigger PCD through an apoptosis inducing factor (AIF)-mediated pathway. Thus, we further aimed to investigate whether mitochondrial AIF translocate to the nucleus during the course of PDCoV infection. We also examined effects of cyclosporin A (CsA), an inhibitor of cyclophilin D (CypD) that is the major component of the mitochondrial permeabilization transition pore (mPTP), and *N*-phenylmaleimide, an AIF inhibitor, on PDCoV infection and virus-induced apoptosis. Results and discussion of *in vitro* assessment for those pharmacological treatments will be presented. Taken together, our results indicate that a caspase-dependent pathway plays a central role in PDCoV-induced apoptotic cell death.

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Resolution of genotype 1 PRRSV attachment on bone marrow-derived dendritic cells

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Porcine sialoadhesin (pSn) and CD163 have been thought the two main receptors mediating uptake and genome release of porcine reproductive and respiratory syndrome virus (PRRSV), and heparan sulphate (HS) facilitating initial attachment. To further evaluate the role of HS/pSn/CD163, the attachment on bone marrow-derived dendritic cells (BMDCs) by three genotype 1 PRRSV isolates 3249, 3262 and 3267 (with distinct immunobiological properties) was conducted. Firstly, BMDCs were digested with 10 U/ml heparinase I for 60 min at 37 ℃ to remove cell surface HS. Thereafter, each viral isolate was added at a multiplicity of infection (m.o.i.) of 3, incubating for 90 min on the ice. Attachment was subsequently quantified by flow cytometry (FC), and colocalization of virus and receptors was analysed by confocal microscopy. For FC, cells were fixed and underwent an indirect staining for PRRSV. According to the relative mean fluorescence index (MFI), isolates 3249 and 3267 generated higher MFIs while isolate 3262 scarcely attached on BMDCs. After HS removal, binding of 3249 was not significantly changed compared to reduction by 92% and 87.5% of 3262 and 3267, respectively. For single-cell imaging, a four-color fluorescence confocal staining PRRSV/pSn/CD163/DAPI was developed. As a result, attachment of 3249 was seen on $pSn^{+}CD163^{+}$, $pSn^{+}CD163^{-}$, $pSn^{-}CD163^{+}$ as well as $pSn^{-}CD163^{-}$ BMDCs, without obvious difference in fluorescence intensity between each subset. Even if on pSn^{+} cells, many virions did not colocalize with pSn. This pattern was not affected by HS removal. Isolate 3267 also attached on the four subsets but HS removal sharply decreased its attachment. For isolate 3262, very few viral particles were detected even without heparinase treatment, which conflicted with its efficient replication on BMDCs. These preliminary results indicate 1) HS plays different roles in mediating attachment of different type 1 PRRSV isolates on BMDCs. 2) Besides HS/pSn/CD163, BMDCs probably possess other potential receptors for type 1 PRRSV entry. 3) Alternative entry pathways independent of receptors for isolate 3262 may exist on BMDCs.

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IFN-lambda preferably inhibits PEDV infection of porcine intestinal epithelial cells compared to IFN-alpha

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Unlike type I interferons targeting many types of cells and organs in host, interferon lambda (IFN-L) is recently ascribed to primarily act on the mucosal epithelial cells and exhibits robust antiviral activity on mucosal surface. Porcine epidemic diarrhea virus (PEDV) causing high morbidity and mortality in piglets is an economically important enteropathogenic coronavirus. Here we demonstrated that both recombinant porcine IFN-L1 (rpIFN-L1) and rpIFN-L3 displayed powerful antiviral activity against PEDV in Vero E6 and intestine epithelial cell line-IPECJ2, and IFN-L1 inhibited both two genotypes of PEDV (genotype 1 CV777 strain and genotype 2 LNCT2 strain). rpIFN-L1 primarily controlled viral infection at the early stage of infection and showed more antiviral activity in IPEC-J2 than Vero E6. The anti-PEDV activity accounted for the antiviral IFN-stimulated genes (ISGs) (ISG15, OASL, MxA, and IFITMs) in IEC induced by rpIFN-L1. In addition, rpIFN-L1 exhibited more antiviral activity against PEDV in IEC than porcine IFN-alpha, which was consistent with the results that rpIFN-L1 triggered higher levels of ISGs (ISG15, OASL, MxA) in IEC than porcine IFN-alpha. Therefore, our data provide the first experimental evidence that porcine IFN-L has the ability to suppress PEDV infection of IPEC-J2 and might provide a new therapeutic to control PEDV infection in piglets.

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PRRSV nsp5 downregulates expression of antiviral genes by suppressing phosphorylation of STAT2

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Porcine reproductive and respiratory syndrome (PRRS) is an important disease causing severe economic losses in the swine industry worldwide. PRRSV has the ability to suppress innate immune signaling during infection, and elucidation of viral mechanism for innate immune modulation has been of interest. In the present study, we found that nonstructural protein 5 (nsp5) of PRRSV was an additional antagonist for antiviral innate immunity. The luciferase reporter assays using the ISRE-luc reporter, nsp5 was found to inhibit the ISRE promoter activity. When activation of STATs was examined by immunofluorescence in nsp5-expressing cells, STAT2 nuclear localization was specifically inhibited and this inhibition was mediated by the reduced phosphorylation of STAT2 as shown by Western blot. In contrast, STAT1 and STAT3 were normally translocated to the nucleus even in the presence of nsp5, suggesting that the STAT2 activity was specifically modified by nsp5. These results suggest that PRRSV nsp5 is involved in interfering the host antiviral innate immune response during infection. The present study contributes to a better understanding of the molecular basis of host cells process manipulated by PRRSV nsp5.

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Temporal movement of type 2 porcine reproductive and respiratory syndrome virus across the maternal-fetal interface

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection within the uterus and at the maternal-fetal interface (MFI) is poorly understood. The aim of this study was to characterize the temporal movement of PRRSV by measuring viral load in the MFI, fetal tissues, and fetal and gilt serum at five time points after experimental inoculation of pregnant gilts. Using 20 gilts on day 85±1 of gestation, 15 were inoculated with 1 x 10^5 $TCID_{50}$ PRRSV (NVSL 97-7895) and 5 were sham-inoculated with sterile media (CTRL). At 2, 5, 8, 12, and 14 days post infection (dpi) three of the inoculated gilts plus one control were euthanized. Pre-and post-inoculation serum, and reproductive lymph node were collected from gilts, along with a section of MFI adjacent to the umbilical stump of each fetus. The placenta was manually separated from the endometrium for testing. After the preservation status of each fetus was determined, serum, thymus, spleen, mesenteric lymph node, umbilical cord, and amniotic fluid were collected. Viral RNA was extracted from samples using commercial kits. Probe based reverse-transcriptase quantitative PCR (qRT-PCR) targeting PRRSV NVSL 97-7895 was performed to assess viral load in samples. Gilt serum at 0 dpi, and all samples from CTRL gilts and their fetuses were negative for PRRSV. Average PRRSV RNA concentration (target log_{10} copies/uL) in gilt serum was 4.4 (2 dpi), 5.9 (5 dpi), 5.7 (8 dpi), 5.0 (12 dpi), and 3.1 (14 dpi). In the endometrium and fetal serum, qRT-PCR results were categorized as: RNA not detected (NEG; Cq>40), detectable but not quantifiable (DNQ), positive (POS: concentration >1.1 log_{10} copies/uL), and compared across day of termination (Table 1). The main findings were that the endometrium is largely infected by 2 dpi and the first evidence of fetal infection (based on serum PCR) was on 5 dpi. Fetal compromise was first observed on 8 dpi and increased progressively through 14 dpi.

Table 1: PCR and fetal preservation results by day of termination.

DPI	ENDOMETRIUM			FETAL SERUM			FETAL PRESERVATION		
	NEG	DNQ	POS	NEG	DNQ	POS	VIA	MEC	DEC
2	5/34 15%	7/34 20%	22/34 65%	34/34 100%	0/34	0/34	34/34 100%	0/34	0/34
5	0/43	2/43 5%	41/43 95%	24/43 56%	18/43 42%	1/43 2%	43/43 100%	0/43	0/43
8	0/43	1/43 2%	42/43 98%	23/43 53%	14/43 33%	6/43 14%	42/43 98%	0/43	1/43 2%
12	0/49	0/49	49/49 100%	7/47 15%	13/47 28%	27/47 57%	45/49 92%	0/49	4/49 8%
14	9/36 25%	0/36	27/36 75%	14/34 41%	9/34 26%	11/34 33%	21/36 58%	13/36 36%	2/36 6%

POS = positive; DNQ = detectable; but not quantifiable; NEG = negative; VIA = viable; MEC = meconium stained; DEC = decomposed; DPI = day post infection.

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Pigs vaccinated with PRRSFLEX EU at two- or three-weeks of age that did not show sero-conversion are protected by an immunologic recall answer after artificial challenge

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Porcine Reproductive and Respiratory Syndrome (PRRS) Virus is one of the major pathogens in pigs that have a significant economic impact on the swine industry worldwide. Vaccination against PRRSV has been demonstrated as an effective tool to control clinical signs related to infection. In the field sero-conversion is a common readout of successful vaccination, and animals that do not sero-convert have a questionable status in terms of protection.
In this study piglets were vaccinated on a commercial farm at two- or three-weeks of life, or not vaccinated as control animals. At 10-weeks of life 25/26 pigs of each group were artificially challenged with a heterologous field strain. Ten days post challenge the animals were euthanized and necropsied. At necropsy pigs PRRS induced lung lesions were scored according to previously established methods. Piglets were blood sampled on the day of vaccination, before challenge and the day of necropsy. All serum samples were analysed for PRRSV antibodies assayed by IDEXX 3x-ELISA. RNA of PRRSV was quantitatively detected in serum by Real Time PCR. A subset of 10 animals per group was tested for cellular immunity.

Before challenge, at 10 weeks of live, a total of 13 out of 51 vaccinated animals (25%) were tested negative for PRRS antibodies by ELISA (cutoff SP < 0,4) (‘non-responders‘; 6 animals vx. at 2-weeks of life and 7 animals vx. at 3-weeks of life). By chance, only three of the ‘non-responders’ were pre-selected for immunologic analysis. All but one control animals were tested negative for PRRS antibodies as the maternally derived antibodies had phased out.

At necropsy the vaccinated ‘non-responders’ did show a recall answer both for humoral and cellular immunity and had significant higher amounts of antibodies and PRRS specific IFN-gamma producing cells. Also, the mean amount of lung lesions was reduced by 40% and to semilar extent in both “responding” and “non-responding pigs in both of the vaccinated groups.

In conclusion, although some vaccinated animals did not show a sero-conversion after vaccination at two or three weeks of life, they were protected by the vaccine upon a heterologous challenge with a virulent field strain, both, by means of antibodies and cellular immunity.

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Antiviral potency and functional novelty of porcine Interferon-Omega subtype

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Innate immune interferons (IFNs), particularly type I IFNs, are primary mediators regulating antiviral immunity. These antiviral cytokines have evolved remarkable molecular and functional diversity to confront ever-evolving viral threats. Pigs have the largest and an expanding type I IFN family consisting of nearly 60 functional genes that encode seven IFN subtypes including multigene subtypes of IFN-alpha and omega. Whereas subtypes such as IFN-alpha and beta have been widely studied, the unconventional IFN-omega subtype has barely been investigated. We have evolutionarily defined the porcine IFN family, and preliminarily showed that porcine IFN-omega subtype has evolved several novel features including, (1) a signature multi-gene subtype expanding particularly in bats and ungulates, (2) emerging isoforms that have much higher antiviral potency than typical IFN-alpha, (3) cross-species high antiviral (but little anti-proliferative) activity in cells of humans and other mammalian species, and (4) potential action through non-canonical signaling pathways. This study is focused on antiviral potency of porcine IFN-omegas investigating their evolutionary and functional diversity, signaling specificity, and optimization of novel antivirals against devastating viral diseases. This project will, for the first time in an animal species, establish state-of-the-art procedures for efficient characterization of the molecular and functional spectrums of unconventional IFNs, which will further IFN-based novel antiviral design.

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Genetic variation for antibody response to a range of pathogens in commercial replacement gilts

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Replacement gilts sourced from multiplier herds are usually introduced into commercial herds following acclimation and vaccination procedures that aim to expose these naïve animals to pathogens (or antigens), such as the strains of PRRSV, PCV2, and other typical swine diseases that are present in the herd. The objective of this study was to estimate genetic parameters for antibody response to several pathogens measured at different stages of production in commercial replacement gilts.

The data set used included phenotypes and genotypes (40K SNPs) on 2479 F1 replacement gilts that were sourced from 17 high-health multipliers from 7 genetic sources. Gilts, in groups of 10 to 63 in size, were introduced into 22 commercial farms with historical cases of natural disease challenges. Blood samples were collected at arrival (D0) on the farms, at 40.1±14 days after arrival (post-acclimation; PA), and at parities 1 (P1) and 2 (P2). Serum was extracted and used for quantification of antibodies to PCV2, swine influenza virus (SIV), *Mycoplasma hyopneumoniae* (MH), and 8 serotypes of *Actinobacillus pleuropneumoniae* (APP; 1, 2, 3, 5, 7, 10, 12, and 13) using commercially available tests. For each serological test, the data set was split into 5 subsets based on the proportion of seroconverted animals within each group: 0, 25, 50, 75, and 100%. Heritabilities were estimated for each trait, time point, and proportion of seroconverted animals within each group. The model used for estimation of heritabilities included the fixed effect of group and a genomic relationship matrix of 3535 individuals. All analyses were performed in ASReml4.

Heritability estimates ranged from 0.03 (PA, 0%) to 0.32 (D0, 75%) for SIV, and from 0.07 (D0, 0%) to 0.35 (P2, 75%) for MH. For APP, the only scenario analyzed was for 0%, as few animals were positive for a given serotype. Heritability estimates ranged from 0.1 (PA) to 0.24 (P2) for APP1, 0.06 (P1) to 0.13 (D0) for APP2, 0.18 (P2) to 0.31 (D0) for APP3, 0.07 (P1) to 0.31 (PA) for APP5, 0.11 (P2) to 0.22 (PA) for APP7, 0.19 (P2) to 0.25 (PA) for APP10, 0.05 (P1) to 0.18 (D0) for APP12, and 0.15 (P1) to 0.29 (P2) for APP13. Heritability estimates for PCV2 were close to zero.

These results indicate that there is substantial genetic variation in response to a range of pathogens in commercial replacement gilts following standard acclimation procedures. The only that was not heritable in this study was antibody response to PCV2. Overall, heritability estimates for all other traits increased with the proportion of seroconverted animals but estimates varied by stage of production and serology test. Thus, selection for changing the immune responsiveness in replacement gilts is possible. Financial support from Genome Canada, the Canadian Swine Health Board, and PigGen Canada is appreciated.

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Modulation of NF-kB activity for innate immune evasion by nonstructural protein 1 of Porcine Epidemic Diarrhea Virus

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Porcine epidemic diarrhea virus (PEDV) is an emerged virus in the US and has become endemic posing significant economic concerns. We previously showed that PEDV possessed an ability to suppress type I IFN production. Of the identified ten viral IFN antagonists, nonstructural protein 1 (nsp1) was one of the most potent viral proteins. Nsp1 inhibited IRF3 signaling pathway for IFN suppression by degradation of CREB-binding protein in a proteasome-dependent manner in the nucleus. In the present study, we found that PEDV efficiently replicated in a porcine epithelial cell line and confirmed the suppression of type I IFN production in these cells. PEDV blocked p65 nuclear localization in virus-infected cells and did not activate the NF-κB pathway at early time post-infection. PEDV was further found to significantly suppress the PRDII promoter activity, which suggests that PEDV inhibits NF-kB activity and suppress type I IFN production. PEDV also suppress the expression of NF-kB-mediated pro-inflammatory cytokines at early time post-infection, but induced at later time post-infection. Of the ten IFN antagonists, nsp1, nsp3, nsp14, nsp15, ORF3, E, and N protein were found to inhibit NF-kB-mediated IFN production. Nsp1 inhibited NF-kB activity and potently suppressed the production of pro-inflammatory cytokines. Nsp1 was further found to block the p65 nuclear localization and interfered the phosphorylation and degradation of IKB-alpha. A series of nsp1 mutants were made according to the high-order structure prediction to identify motifs important for suppression of NF-kB activity. Interestingly, mutations of the conserved residues altered their cellular distributions and subverted their NF-κB suppressive activity, which suggests that conserved high-order structures are crucial for nsp1-mediated NF-kB activity. Our study shows that PEDV modulates NF-κB activity in a time-dependent manner and nsp1 is the NF-kB antagonist for suppression of both IFN and early production of pro-inflammatory cytokines.

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PRRSV interference with cytokine-mediated JAK/STAT signaling

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PRRSV infection is characterized by prolonged viremia and elicitation of inadequate protective immune responses featured by delayed onset and low level of neutralizing antibodies and weak cell-mediated immunity. One of the possible reasons for the weak protective immune response is that PRRSV interferes with cytokine signaling and innate immunity. Many cytokines initiate function by binding to specific receptors on cells to activate Janus kinases (JAK)-signal transducers and activators of transcription (STAT) signal pathway. STATs are a family of transcription factors that regulate cell growth, differentiation, proliferation, apoptosis, immunity, inflammatory responses, and angiogenesis. There are seven mammalian STAT proteins (STAT1, 2, 3, 4, 5a, 5b, and 6). Each STAT member responds to a defined set of cytokines. It is known that lymphocyte development and differentiation rely on cytokines, many of which signal via JAK/STAT pathway to exert their biological effects. Due to the importance of JAK/STAT signaling in the immune response, it is often antagonized by viruses, including PRRSV. The objective of this research was to define the mechanisms of PRRSV interference with JAK/STAK signaling. We discovered that PRRSV inhibits interferon-activated JAK/STAT signaling by blocking nuclear translocation of STAT1/STAT2/IRF9 heterotrimer, and IL-6/IL-10-activated JAK/STAT3 signaling by inducing degradation of STAT3 protein. PRRSV nsp1beta is responsible for the inhibition of JAK/STAT1/STAT2 signaling by downregulating importin alpha5, which is known to mediate nuclear import of the STAT1/STAT2/IRF9 heterotrimer. Interestingly, substitution of the amino acid residue valine 19 of nsp1beta with isoleucine abolished its capability to reduce the importin, whereas nsp1beta of a vaccine MLV strain has no effect on the importin but gains the function after mutation of isoleucine 19 to valine. PRRSV nsp5 is found to reduce STAT3 protein level via ubiquitin-proteosome degradation pathway. The C-terminal portion of nsp5 is required for the inhibition of STAT3 signaling. STAT3 is known to play critical roles in cell growth, proliferation, differentiation, immunity and inflammatory responses. These results indicate that PRRSV may evade the host innate antiviral response and thwart the development of protective adaptive immunity by interfering with cytokine-mediated JAK/STAT signaling.

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The unfolded protein response induced by porcine reproductive and respiratory syndrome virus infection of alveolar macrophages is involved in immune dysregulation

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Porcine reproductive and respiratory syndrome virus (PRRSV), an important swine pathogen, infects alveolar macrophages causing dysregulated interferon (IFN)-alpha and tumor necrosis factor (TNF)-alpha production through a mechanism(s) yet to be resolved. We show that alveolar macrophages infected with PRRSV secrete reduced quantities of IFN-alpha following the cells' subsequent exposure to synthetic dsRNA. This diminution did not correlate with less IFNA1 gene transcription but rather with two events occurring late in infection indicative of translational attenuation, namely the activation of eukaryotic translation initiation factor 2 (eIF2)alpha and the appearance of stress granules. In contrast, the rapid production of TNF-alpha in response to lipopolysaccharide (LPS) was suppressed or enhanced depending on when the stimulation of the PRRSV-infected alveolar macrophages was initiated. If introduction of this agonist was delayed until 6 h post-infection (hpi), to enable eIF2alpha phosphorylation by the stress sensor RNA-like endoplasmic reticulum kinase (PERK), inhibition of TNF-alpha synthesis was observed, presumably due to translational repression. However, a synergistic response, due to earlier NFkB activation apparently via another stress sensor, inositol-requiring enzyme (IRE)-1alpha, was noted if LPS exposure began 4 h earlier at 2 hpi, prior to the onset of eIF2alpha phosphorylation. These results indicate that, depending on when after PRRSV infection an alveolar macrophage encounters LPS, the asynchronous actions of two distinct branches of the unfolded protein response (UPR), IRE-1alpha and PERK, to virus-infection is associated with an increase or decrease TNF-α production via the activation of NFkB or eIF2alpha, respectively. PPRSV pneumonias frequently become complicated with secondary bacterial infections triggering severe inflammation, lung dysfunction and death. The presence of high levels of TNF-alpha in lungs afflicted with PRRSV-bacterial co-infections might be the result of the activation of NFkB in PRRSV-infected alveolar macrophages via the IRE-1alpha. The nonconforming robust TNF-alpha response to bacterial products promoted by PRRSV would mediate severe inflammation and lung damage.

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Evaluation of the novel subunit porcine reproductive and respiratory syndrome (PRRS) vaccine against PRRSV in piglets and sows

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A Pseudomonas exotoxin (PE)-based chimeric subunit vaccine system was developed using a reverse vaccinology (RV) technique. The plasmids chosen for this vaccine contain four PE-PRRS chimeric sequences from the porcine reproductive and respiratory syndrome virus (PRRSV) combined with a ligand moiety and Pseudomonas exotoxin deleted domain III (PE (DIII)), and a carboxyl terminal moiety that includes a polypeptide with amino acid sequence KDEL (K3). PRRSV-negative piglets vaccinated with this construct demonstrated significantly lower ($P < 0.05$) mean rectal temperatures, respiratory scores, lung lesions and presence of PRRSV nucleic acids within interstitial pneumonia, as well as reduced type 1 or type 2 PRRSV viremia compared to unvaccinated challenged pigs. The PE-PRRS combination vaccine induced PRRSV-specific INF-gamma cellular immunity and complement neutralizing antibody in pigs. Further, a field trial of this vaccine to was conducted to evaluate the immune response of pregnant sows following vaccination in a PRRSV positive farm. Results indicate increased sow reproductive performance associated with reduced viremia assessed by RT-PCR analysis. And it also stimulated maternal immune response associated with reduced viremia in the piglets. The data presented supports the claims that the commercialized novel PRRS sub-unit vaccine is an effective tool in the control of the disease based on the presentation of the Pseudomonas exotoxin (PE-K3) carrier in combination with PRRSV conserved epitopes against heterologous PRRS viruses.

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A GP5 mosaic T-cell vaccine for Porcine Reproductive and Respiratory Syndrome Virus is immunogenic and confers partial protection to pigs

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Objectives: Evaluate T-cell epitope mosaic DNA vaccines to protect swine against genetically and antigenically diverse PRRSV strains.

Methods: Two mosaic DNA vaccines that encode mosaic PRRSV GP5 derived from 748 genotype II ORF5 sequences were constructed. The mosaic DNA vaccines were tested in pigs in three pilot vaccination/challenge trials. Gene gun, electroporation and complexed to liposomes were utilized as delivery systems in these trials. T-cell responses were evaluated by lymphocyte proliferation assay using the MTT method and the expression of interferon-gamma and IL-10 mRNA measured in virus-stimulated PBMCs by real-time PCR using the delta-delta method. Antibody responses were monitored in vaccinated animals by indirect-ELISA. Protection was evaluated by measuring viral copy numbers in serum and tissue samples and lung lesion scores.

Results: Mosaic vaccines were shown to be functional and immugenic. Significantly higher levels of proliferative responses were detected in virus-stimulated peripheral blood mononuclear cells of GP5-Mosaic-vaccinated pigs compared to control pigs in both Trials 1&2. In Trial 2, significantly higher levels of interferon-γ mRNA and lower levels of IL-10 mRNA were detected in GP5-Mosaic-vaccinated pigs as compared to control pigs. Virus-specific antibodies were higher in GP5-Mosaic-vaccinated animals than in control animals in Trials 2&3. The antibodies were neutralizing. In Trial 3, there were significant differences between mosaic-vaccinated and control animals in the expression of interferon-gamma mRNA by virus-stimulated PBMCs at 21, 35 and 48 day post vaccination ($p<0.05$). Expression levels of mRNA of other cytokines are being investigated. Significantly higher levels of interferon-gamma mRNA were detected in PBMCs collected from mosaic-vaccinated animals than those in control animals by divergent strains stimulation.

Conclusions: The data shows that vaccination induced both humoral and cellular immune responses in both positive control and mosaic-vaccinated pigs but not in control animals, confirming their immunogenicity. Significantly higher levels of interferon-gamma mRNA were detected in PBMCs collected from mosaic-vaccinated animals compared to those of control animals against diverse PRRSV strain stimulation, which indicated that mosaic vaccines could induced broad immune responses towards diverse strains but not controls.

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Field evaluation of a modified-live PRRS vaccine in an unstable herd in Northwest Mexico

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Introduction: For more than two decades, porcine reproductive and respiratory syndrome virus (PRRS) has caused severe economic losses to the Mexican pork industry. The disease is widespread and serological surveys demonstrated that farms have been infected since 1992. Herd-to-herd biosecurity and PRRS specific control programs vary among swine operations in northwestern Mexico. Efforts to eradicate the disease through regional control and depopulation of herds have yielded mixed results and acute outbreaks are still common. Furthermore, viral dissemination between farms is high, resulting in the emergence of unique strains due to recombination and evolution. The preferred approach for PRRS control in this region has been to maintain a uniform level of immunity through a combination of vaccination and serum inoculation. However, these practices have been unable to uniformly stop PRRS circulation and disease outbreaks still occur.

Objective: To evaluate the efficacy in young pigs of a recently approved PRRS vaccine (Prime Pac® PRRS, MSD Animal Health, Boxmeer, Netherlands) in a highly PRRS challenged, multi-strain affected operation located in northwestern Mexico by comparison with the farm's previous vaccination program. The specific goal for the Prime Pac PRRS vaccination was to improve mortality and productivity parameters of nursery pigs raised in this operation.

Material and Methods: The study was conducted in a nursery site that received the weekly production from a 1,800 sow farm. The study involved 14 weekly groups and a total of 6,923 pigs; 7 groups and 3,415 pigs that were vaccinated at 3 weeks of age with a competitor vaccine (previous program) and 7 subsequent groups and 3,408 pigs that received Prime PAC PRRS at 14 days of age. The pigs were housed in the nursery for 6-7 weeks. Production parameters evaluated in the nursery phase included mortality and cull rates, daily weight gain and feed conversion.

Results: Mortality rate improved following the implementation of Prime Pac PRRS from 18.65% to 3.91%, a difference of 14.74%, and a 79.0% reduction. Daily gain improved by 18.7% (353 to 419 grams/day) while feed conversion improved by 15.8% (1.65 to 1.39). The rate of cull pigs declined from 3.73% to 0.90%, a difference of 2.83% and a 75.9% reduction.

Conclusion: Vaccination is an important tool for controlling PRRS in endemically infected herds. In this operation, Prime Pac® PRRS significantly impacted farm profitability due to the reduced mortality and cull pig rates, increased daily weight gain and improved feed conversion compared with the previous program.

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Comparative serological response after an inactivated EU-typed PRRS vaccination in Korea

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in sows and respiratory disease in young pigs. Prevention of PRRS neonatal infections by passive colostrum and lactogenic immunity transferred from the sow has been shown to be dose dependant. The aim of this study was to compare with and assess the PRRS-specific serologic response after sow vaccination with an inactivated EU-typed PRRS vaccine and its passive transfer to piglets, by serum neutralization (SN) test, enzyme-linked immunosorbent assay (ELISA), and indirect fluorescent antibody (IFA) test in Korean field conditions.

Materials and methods: The study was carried out in an EU-typed PRRSV negative 600-sow farm located in Bo-ryeong city South-Korea. Eight sows randomly chosen were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a non-vaccinated control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 sows, 5 piglets per sow were selected to be tested. All sows were bled on day D-63, D-42, D0 (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. SN test antibody titers of all sera were analyzed, with PRRSV Lelystad strain, and MARC-145 cells. ELISA antibody titers of all sera were analyzed using an ELISA kit(IDEXX Laboratories. Inc., Westbrook, USA). IFA antibody titers of all sera were analyzed, with Lelystad strain, and MA104 cells. T-test and Mann-Whitney U test of SPSS statistics 21 (IBM Corp., USA) were used for statistical significance($P<0.05$).

Results

The results are as follows: Table 1 and 2.

Table 1. EU-PRRSV-specific antibody titers of the sows (M±SD)

Sows		Day -63	Day -42	Day 0	Day 26
SN	V	0 (±0)	1.13 (±0.81)	2.63 (±0.49)	1.88 (±0.81)
	C	0.25 (±0.46)	0 (±0)	0.25 (±0.46)	0 (±0)
	P value	0.167	0.105	0.001	0.010
ELISA	V	0.12 (±0.08)	0.67 (±0.46)	0.81 (±0.56)	0.75 (±0.52)
	C	0.16 (±0.12)	0.17 (±0.12)	0.17 (±0.12)	0.19 (±0.13)
	P value	0.382	0.009	0.005	0.000
IFA	V	1.81 (±0.14)	2.08 (±0.15)	2.62 (±0.14)	2.34 (±0.16)
	C	1.83 (±0.12)	1.83 (±0.12)	1.85 (±0.12)	1.85 (±0.13)
	P value	0.721	0.130	0.000	0.010

Table 2. EU-PRRSV-specific antibody titers of the piglets (M±SD)

Piglets		Day 7	Day 14	Day 26
SN	V	2.62(±0.26)	1.59(±0.20)	0.32(±0.20)
	C	0.32(±0.24)	0(±0)	0(±0)
	P value	0.000	0.000	0.011
ELISA	V	1.08(±0.21)	1.01(±0.20)	0.60(±0.20)
	C	0.20(±0.11)	0.22(±0.12)	0.19(±0.12)
	P value	0.000	0.000	0.000
IFA	V	2.67(±0.21)	2.23(±0.20)	2.00(±0.20)
	C	1.83(±0.11)	1.83(±0.12)	1.78(±0.12)
	P value	0.000	0.000	0.003

Discussion and conclusion

All three methods' antibody titers of the vaccinated sows significantly increased to Day0 and then slightly decreased. Otherwise, the control sows remained low. Antibody titers of piglets of the vaccinated group were significantly higher than those of the control group at every sampling point and decreased over time as expected. In this study, PROGRESSIS® was shown to induce high level of antibodies in the sows that are well transferred to their piglets, and SN test, ELISA, and IFA were useful to detect EU-PRRSV antibody.

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Serum and mammary secretion antibody responses in PEDV-exposed gilts following PEDV vaccination

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Purpose: Since there is little possibility that PEDV will be eradicated in the near future, reliable methods to prevent and/or control its effects are needed. In the sow herd, this implies monitoring/maintaining levels of immunity sufficient to protect neonatal pigs. The objective of this study was to compare antibody responses in previously PEDV-exposed gilts to vaccination.

Methods: PEDV antibody-positive gilts (n = 36) in a commercial production system were randomly assigned to 1 of 5 vaccination treatments and sampled as described in Table 1.

Trt*	Vaccination treatment	Animals	Sampling
1	Unvaccinated (control)	5 gilts	1. Serum at 5 weeks pre-farrow
2	1 ml IM - 2 weeks pre-farrow	6 gilts	2. Serum and colostrum ≤ 24 hr post
3	1 ml IM - 5 and 2 weeks pre-farrow	8 gilts	farrowing
4	2 ml IM - 2 weeks pre-farrow	7 gilts	3. Milk at 3, 10, and 21 days
5	2 ml IM - 5 and 2 weeks pre-farrow	7 gilts	post farrowing

*Treatment 2, 3 - *Harrisvaccines™ PEDV Vaccine;* 4, 5 - *Zoetis PEDV Vaccine, Killed Virus*

Thirty-three gilts completed the study, i.e., farrowed viable litters and provided a full complement of samples. Serum, colostrum, and milk samples were tested by PEDV whole virus (WV) IgG and IgA ELISAs and for neutralizing antibody by PEDV fluorescent focus neutralization assay (FFN).

Initial analyses found no significant differences in outcomes between 1 vs 2 doses of either Vaccine A or B. Therefore, the data were analyzed on the basis of 3 treatment groups: 1) no vaccine (controls); 2) PEDV vaccine A; and 3) PEDV vaccine B. Thereafter, a nonparametric one-way ANOVA was used to test for differences among treatments for IgG, IgA, and FFN by sample type (serum, colostrum, milk). A mixed-effects repeated measures model was used to analyze the difference between treatment groups for IgG, IgA, and FFN by sample type.

Results: Gilt serum antibody responses at 5 weeks pre-farrow, i.e., pre-vaccination, were not different ($p > 0.05$). Controls (no vaccine) had significantly lower antibody responses than vaccinates for most tests and specimens. IgG responses in serum and colostrum from gilts vaccinated with B were higher than controls and gilts vaccinated with A ($p < 0.01$). IgA and neutralizing antibody responses were not different between gilts vaccinated with Vaccine A and B.

Conclusions: Compared to unvaccinated controls, vaccination increased IgG, IgA, and neutralizing antibody levels in gilts in all diagnostic specimens tested.

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Interaction between ORF2 protein of porcine circovirus type 2 and C1QBP enhances phagocytic activity

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Porcine circovirus type 2 (PCV2) is non-enveloped, icosahedral, small DNA virus with a single-stranded circular DNA genome. PCV2 infection often impairs host immunity, with the subsequent development of multifactorial postweaning multisystemic wasting syndrome (PMWS) due to secondary infections. Among four major open reading frames (ORFs) of PCV2, ORF1, which is also termed the "rep" gene, encodes viral replicase and ORF2 (the "cap" gene) encodes capsid protein. ORF3 and ORF4 encode non-structural genes that may modulate the phenotype of host cells. In this study, we cloned pig C1QBP cDNA and determined its complete cDNA sequence. Then, we showed that the direct interaction between the C1QBP and PCV2 ORF2 led to increasing stability of C1QBP by inhibiting ubiquitin-mediated proteasomal degradation of C1QBP. Increased stability of the C1QBP by the interaction with PCV2 ORF2 further enhanced phagocytic activity of porcine macrophages through the phosphoinositol-3-kinase (PI3K) signaling pathway. This evidence may explain the molecular basis of how PCV2 ORF2 enhances phagocytic activity of host macrophages.

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Development of porcine epidemic diarrhea virus vaccines derived from a virulent Korean strain

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Massive outbreaks of PEDV re-emerged in South Korea in 2013–2014 and rapidly swept across the country, causing tremendous financial losses to producers and customers. Despite the availability of PEDV vaccines in the domestic market, their protective efficacy in the field is still being debated. The unsatisfied effectiveness of current vaccines appears to result from antigenic and genetic differences between vaccine and field epidemic strains, raising the need for new vaccine development using the PEDV isolate prevalent in the field. We first aimed to produce an inactivated killed vaccine using a cell culture-propagated KNU-141112 epidemic strain and evaluate its effectiveness in nursery piglets. Pregnant sows were immunized intramuscularly with the inactivated adjuvanted monovalent vaccine at 6 and 3 weeks prior to farrowing. Six-day-old piglets born to vaccinated or unvaccinated sows were challenged with the homogeneous virus. The administration of the inactivated vaccine to sows greatly increased the survival rate of piglets challenged with the virulent strain, from 0% to approximately 92% (22/24), and significantly reduced diarrhea severity including viral shedding in feces. In addition, litters from unvaccinated sows continued to lose body weight throughout the experiment, whereas litters from vaccinated sows started recovering their daily weight gain at 7 days after the challenge. Furthermore, strong neutralizing antibody responses to PEDV were verified in immunized sows and their offspring, but were absent in the unvaccinated controls. Altogether, our data demonstrated that durable lactogenic immunity was present in dams administrated with the inactivated vaccine and subsequently conferred critical passive immune protection to their own litters against virulent PEDV infection. In addition, a virulent Korean PEDV strain was serially propagated in Vero cells for up to 100 passages for attenuation to develop a modified live virus vaccine. During cell adaptation process, we were able to isolate 4 deletion (DEL) mutants with 8 to 10 amino acid changes distributed throughout the genome. Results of animal inoculation studies to assess the virulence of the cell-adapted PEDV DEL strains will be discussed.

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Pathogenic and genomic characteristics involved in porcine alveolar macrophage passages of an attenuated PRRSV nsp2 DEL strain CA-2-P100

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PRRSV is a globally ubiquitous swine viral pathogen and a major economic plague worldwide. We previously reported the attenuated phenotype in inoculated pigs and amino acid mutations of a cell-adapted CA-2-P100 (100th passage of the virulent type 2 Korean PRRSV nsp2 DEL strain CA-2 in MARC-145 cells) However, some of pigs challenged with CA-2-P100 remained viremia-negative and seronegative to PRRSV throughout the trial, suggesting that the virus might be over-attenuated. In the present study, a high-passage derivative of CA-2, CA-2-P100, was serially propagated in cultured porcine alveolar macrophage (PAM) cells for up to 20 passages (CA-2-P100+PAM20). Animal inoculation studies were conducted to assess and compare *in vivo* effects of CA-2-P100 and CA-2-P100+PAM20 strains in the natural host. There were no noteworthy differences in virulence between two cell-adapted viruses, with exhibiting normal weight gain, body temperatures, and lung lesions comparable to the control group. No pigs in the virus-infected groups shed virus nasally, orally or rectally throughout the experiment. However, CA-2-P100+PAM20 infection resulted in consistently higher levels of viremia in pigs compared to CA-2-P100 infection. Furthermore, all pigs inoculated with CA-2-P100+PAM20 developed viremia and seroconverted to PRRSV. In addition, we determined the whole genome sequences of PAM-passage derivatives of CA-2-P100. The nsp2 111-1-19 DEL signature was completely retained for 20 passages in PAM cells, whereas no other deletions or insertions arose during the additional PAM adaptation process. However, CA-2-P100+PAM20 contained 36 random nucleotide mutations that resulted in 14 amino acid changes throughout the genome, suggesting that these genetic drifts provide a possible molecular basis correlated with the PAM-adapted phenotype *in vivo*. Altogether, our data indicate that the PAM-passage CA-2-P100+PAM20 strain is a promising candidate for developing a safe and effective live PRRSV vaccine.

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An interferon inducing PRRSV vaccine candidate protects against challenge with a heterologous virulent type 2 strain in a conventional pig model

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Recently, an interferon inducing PRRSV vaccine candidate strain A2MC2 was demonstrated to be attenuated and induce neutralizing antibodies. The objective of this study was to determine the efficacy of passage 90 of A2MC2 (A2P90) to protect pigs against challenge with moderately virulent PRRSV strain VR-2385 (92.3% nucleic acid identity with A2MC2) and highly virulent atypical PRRSV MN184 (84.5% nucleic acid identity with A2MC2). Forty 3-week old pigs were randomly assigned to five groups including a NEG-CONTROL group (non-vaccinated, non-challenged), VAC-VR2385 (vaccinated, challenged with strain VR-2385), VR2385 (challenged with strain VR-2385), VAC-MN184 (vaccinated, challenged with strain MN184) and MN184 group (challenged with MN184 virus). Vaccination was done at 3 weeks of age followed by challenge at 8 weeks of age. No viremia was detectable in any of the vaccinated pigs, however, by the time of challenge, 15/16 vaccinated pigs had seroconverted based on ELISA and had neutralizing antibodies against a homologous strain with titers ranging from 8 to 128. Infection with VR-2385 resulted in mild-to-moderate clinical disease and lesions. Vaccination reduced VR-2385 viremia and nasal shedding, which was significantly lower than VR2385 group. Vaccination also reduced macroscopic and microscopic lung lesions associated with PRRSV VR-2385. Infection with MN184 resulted in moderate-to-severe clinical disease and lesions with significantly higher levels of viremia and virus shedding and reduced weight gain compared to NEG-CONTROLS and VAC-VR2385 pigs. Vaccination had little protective effect against MN184 challenge. Under the study conditions, the A2P90 vaccine strain was attenuated without detectable shedding and provided protection against VR-2385 challenge.

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A PRRSV candidate vaccine based on the synthetic attenuated virus engineering approach is attenuated and effective in protecting against homologous virus challenge

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Rapid production of attenuated farm-specific homologous vaccines is a feasible alternative to commercial vaccines. In this study, attenuation and efficacy of a codon-pair de-optimized candidate vaccine generated by synthetic attenuated virus engineering approach (SAVE5) were tested in a conventional growing pig model. Forty pigs were vaccinated intranasally or intramuscularly with SAVE5 at day 0 (D0). The remaining 28 pigs were sham-vaccinated with saline. At D42, 30 vaccinated and 19 sham-vaccinated pigs were challenged with the homologous PRRSV strain VR2385. The experiment was terminated at D54. The SAVE5 virus was effectively attenuated as evidenced by a low magnitude of SAVE5 viremia for 1-5 consecutive weeks in 35.9% (14/39) of the vaccinated pigs, lack of detectable nasal SAVE5 shedding and failure to transmit the vaccine virus from pig to pig. By D42, all vaccinated pigs with detectable SAVE5 viremia also had detectable anti-PRRSV IgG. Anti-IgG positive vaccinated pigs were protected from subsequent VR2385 challenge as evidenced by lack of VR2385 viremia and nasal shedding, significantly reduced macroscopic and microscopic lung lesions and significantly reduced amount of PRRSV antigen in lungs compared to the non-vaccinated VR2385-challenged positive control pigs. The nasal vaccination route appeared to be more effective in inducing protective immunity in a larger number of pigs compared to the intramuscular route. Vaccinated pigs without detectable SAVE5 viremia did not seroconvert and were fully susceptible to VR2385 challenge. The SAVE approach was successful in attenuating PRRSV strain VR2385 and protected against homologous virus challenge. Virus dosage likely needs to be adjusted to induce replication and protection in a higher percentage of vaccinated pigs.

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Efficacy of Ingelvac PRRS® MLV against a heterologous PRRSV 1-7-4 RFLP challenge

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The use of Ingelvac PRRS® vaccines can significantly reduce lung lesions following challenge with heterologous isolates (86-94% ORF5 nucleotide similarity) in the three-week-old pig respiratory challenge model. However, the efficacy of Ingelvac PRRS® MLV vaccine against current virulent PRRSV isolates, such as RFLP 1-7-4, has not been reported to date. This experiment was designed to evaluate the efficacy of two commercially available PRRSV vaccines in a three-week-old pig respiratory challenge model, using a heterologous RFLP 1-7-4 field isolate from 2014.

Materials and Methods: At approximately three weeks of age (Day 0 of the study), 154 PRRSV naïve piglets pigs were randomized into groups, and intramuscularly vaccinated with 2 ml of either a placebo (challenge controls n=64), Ingelvac PRRS® MLV (n=45) or Fostera® PRRS (n=45). Pigs were housed in rooms by group during the vaccination period. At day 28 of the study (D28), all pigs were comingled and challenged with 2.0 mL intramuscularly and 2.0 mL intranasally (1 mL per nostril) with $10^{4.6}$ $TCID_{50}$/mL of PRRSV RFLP 1-7-4. Serum samples, weights, and temperatures were collected periodically from D0 through termination of the study on D42. On D42 (14 days post-challenge), all pigs were necropsied and lungs were scored for the presence of macroscopic lesions and BALF samples were collected. Serum samples were tested by RT-PCR for the presence of viremia and by ELISA for the presence of anti-PRRSV antibody. A subset of samples were assayed by bead-based multiplex assay for multiple cytokines including IFN-alpha. Data were analyzed using Generalized and Linear Mixed Models. Pairwise comparisons between groups were conducted as appropriate using a level of confidence of 0.05 to indicate statistical significance.

Results: Table 1 summarizes lung lesions (percentage) for each group. Table 2 summaries average daily weight gain (ADWG) for the post-challenge period by group. The percentage of vaccinated animals with detectable amounts of INF-alpha at D29 and D35 was significantly lower than the controls ($p \leq 0.05$). Additional data analysis is in progress at the time of abstract preparation.

Table 1. Day 42 Percent Lung Lesions (Median)

Group	Treatment	Lung Lesions (%)
1	Ingelvac PRRS® MLV	8.4[a]
2	Fostera® PRRS	12.9[a]
3	Placebo	25.4[b]

[a] significantly different from the placebo at $P \leq 0.05$

Table 2. Post-challenge ADWG

Group	Treatment	ADWG (lbs)
1	Ingelvac PRRS® MLV	0.61[a]
2	Fostera® PRRS	0.49[a]
3	Placebo	0.24[b]

[a] significantly different from the placebo at $P \leq 0.05$

Conclusion: The pigs vaccinated with Ingelvac PRRS® MLV had significantly reduced lung lesions, and increased ADWG, in comparison to placebo vaccinated pigs, following challenge with a recent PRRSV RFLP 1-7-4 isolate. In addition, vaccination with Ingelvac PRRS® MLV resulted in a significantly lower percentage of animals with an IFN-alpha response as compared to placebos at D29 and D35.

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Evaluation of PRRSV challenge dose in vaccinated pigs

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The infectious dose of PRRSV has been shown to be very low, therefore highly infectious. The objective of this study was to evaluate the effect of PRRSV challenge dose in vaccinated pigs.

Materials and Methods: The study was performed in ninety, three-week-old pigs from a PRRS naïve and PCR negative source. Groups 1-5 (n=10) were vaccinated (Day 0) with Ingelvac PRRS® MLV (2ml IM). Forty pigs served as matched non-vaccinated challenge controls (NVC-Groups 1-4; n=10 per group). Groups 1-4 were challenged on Day 28 intranasally with 2.0 ml of virulent PRRSV SDSU-73 at 4log, 3log, 2log or 1log$_{10}$TCID$_{50}$/ml, respectively. Group 5 was not challenged. Temperature (Day 28-42), viremia and ADWG (Day 28-70) were evaluated and statistically analyzed.

Results: At all challenge doses, Ingelvac PRRS® MLV vaccinated pigs demonstrated a significant decrease in days pyrexic compared to NVC groups ($P<0.05$). At PRRSV challenge doses of ≤2logs, the average temperatures and days pyrexic of the vaccinated challenged pigs were similar to the non-challenge control (Table 1).

Table 1. Mean Number Days Pyrexic Post-Challenge

Group	4log	3log	2log	1log	Non-Challenge
Ingelvac PRRS® MLV	4.4[1]	4.2[1]	1.0[1]	1.4[1]	1.8
NVC	11.2	8.8	10.0	6.0	-

1 Statistically significant difference ($P < 0.05$) in number days pyrexic between Ingelvac PRRS® MLV and Challenge Control groups based on model prediction.

As compared to the NVC, there was a significant increase in ADWG ($P<0.05$) in the 3, 2 and 1log groups, and at $P<0.07$ in the 4log group. ADWG in vaccinated groups challenged with ≤2logs of PRRSV were numerically similar to the ADWG of control. There was a measurable negative impact on ADWG in the NVC groups with no difference across all challenge doses (Table 2).

Table 2. Average Daily Weight Gain (lbs)

Group	4log	3log	2log	1log	Non-Challenge
Ingelvac PRRS® MLV	1.41	1.29[1]	1.70[1]	1.64[1]	1.67
NVC	1.18	1.06	1.15	1.23	-

1 Statistically significant difference ($P < 0.05$) in ADWG between Ingelvac PRRS® MLV and Challenge Control groups based on model prediction.

Vaccinated pigs demonstrated fewer percent PCR positive pigs than NVC pigs at all challenge doses. As challenge dose decreased the percentage of viremic pigs in vaccinated groups decreased, with viremia in vaccinated pigs challenged with ≤2logs similar to the non-challenge control. At all challenge doses, the NVC pigs show similar post-challenge viremia profile.

Discussion: In this study, at all challenge doses, Ingelvac PRRS® MLV vaccinated pigs demonstrated a reduction in post-challenge viremia, temperature and increased ADWG as compared to NVC pigs. Based on challenge dose (≤2logs), the consequences in vaccinated pigs were similar to non-challenged pigs. The post-challenge viremia and ADWG of NVC pigs were similar across all challenge doses, indicating a measurable negative impact. Implementation of vaccine for PRRS control can mitigate the consequences of PRRSV infection subsequently improving health and performance.

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How to pick the right strain of PRRSv for a vaccine... Or for an outbreak at a concentrated animal feeding operation (CAFO)

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We have developed an immunoinformatics tool to identify the best PRRSv vaccine to use for herd-specific PRRSv outbreaks. Like many RNA viruses, PRRSv has considerable genetic and antigenic variability that has resulted in the vaccine and the outbreak strain being not well matched. The widely used 'whole gene' approach fails to gauge cross-reactivity. This is because it does not consider the T cell epitopes that are presented to the immune system, and whether they are conserved between the vaccine and the challenge strain. We developed an Epitope Content Comparison (EpiCC) tool to solve this issue and better define the degree of conservation between PRRSv vaccines and circulating strains. This tool will be used to identify the best vaccine to use for herd-specific PRRSv outbreaks.

We have previously developed a set of Swine Leukocyte Antigen (SLA)-restricted epitope prediction tools (PigMatrix). We further modified this tool to define relatedness based on T cell epitope content. Using this new tool (EpiCC) we screened 20 complete genomes from PRRSv and three modified live virus (MLV) vaccines. We identified epitopes predicted to bind to common class I and class II SLA alleles. These epitopes were compared and an epitope-based relatedness score (EpiCC score) was calculated. We observed epitope content variability across proteins and these EpiCC scores can be used to classify PRRSv strains based on their T cell epitope content.

EpiCC gives pork producers and vaccine researches an objective approach to aid in vaccine selection when a PRRSv strain is introduced into a herd, and to select the appropriate viral epitopes for incorporation into a MLV vaccine.

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Pigs immunized with a novel E2 subunit vaccine are protected from subgenotype heterologous Classical Swine Fever Virus challenge

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Background: Classical swine fever (CSF) or hog cholera is a highly contagious swine viral disease. CSF endemic countries have to use routine vaccination with modified live virus (MLV) vaccines to prevent and control CSF. However, it is impossible to serologically differentiate MLV vaccinated pigs from those infected with CSF virus (CSFV). The aim of this study is to develop a one-dose E2-subunit vaccine that can provide protection against CSFV challenge. We hypothesize that a vaccine consisting of a suitable adjuvant and recombinant E2 with natural conformation may induce a similar level of protection as the MLV vaccine.

Methods: Our experimental vaccine KNB-E2 was formulated with the recombinant E2 protein (Genotype 1.1) expressed by insect cells and an oil-in-water emulsion based adjuvant. 10 pigs (3 weeks old pigs/group) were immunized intramuscularly with one dose or two doses (3 weeks apart) KNB-E2, and 10 more control pigs were administered normal saline solution only. Two weeks after the second vaccination, all KNB-E2 vaccinated pigs and 5 control pigs were challenged with 5x105 TCID50 CSFV Honduras/1997 (Genotype 1.3, 1 ml intramuscular, 1 ml intranasal).

Results: It was found that while control pigs infected with CSFV stopped growing and developed high fever (>40^{o}C), high level CSFV load in blood and nasal fluid, and severe leukopenia 3 – 14 days post challenge, all KNB-E2 vaccinated pigs continued to grow as control pigs without CSFV exposure, did not show any fever, had low or undetectable level of CSFV in blood and nasal fluid. At the time of CSFV challenge, only pigs immunized with KNB-E2 developed high levels of E2-specific antibodies and anti-CSFV neutralizing antibodies.

Conclusions: Our studies provide direct evidence that pigs immunized with one dose KNB-E2 can be protected clinically from CSFV challenge. This protection is likely mediated by high levels of E2-specific and anti-CSFV neutralizing antibodies.

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Maternally derived antibody mediated protection against porcine epidemic diarrhea virus on piglets via the different ways of immunity provides in sows following by the classical and variant strain challenges

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The study was conducted to investigate the efficacy of the maternally derived antibody (MDA) raised against two different routes of PEDV immunization in the induction of lactogenic immunity as measured by ELISA IgG/IgA and VN titers of colostrum at 0 day-post-parturition (DPP) and milk at 3 DPP. In addition, protection in piglets against oral challenge was measured using severity of clinical disease, shedding periods with RT-PCR, and, villous height to crypt ratio (VCR) at 4 day-post-challenge (DPC). Thirty primiparous sows free of PEDV were randomly allocated into three groups of 10 sows each. Three groups included Negative (Neg), Feedback (FB) using piglet intestines feedback, and intramuscular vaccinated (IV). Neg was left as control. FB was orally administrated with feedback at 11 and 13 weeks of gestation (WG). IV was intramuscularly vaccinated with attenuated PEDV vaccine at 11 and 13 WG. Colostrum and milk samples were collected at 0 and 3 DPP and assayed for antibody response by viral neutralization assay (VN) and ELISA IgG/IgA. In each group, 2 piglets per sow were weaned at 3 days of age and orally challenged with either genogroup 1a (G1; n = 10) or genogroup 2a (G2; n = 10) isolates of PEDV. Mortality was recorded and all pigs were necropsied at 4 DPC. VCR was determined. The MDA results demonstrated that the IV group had significantly higher VN titers than FB and Neg in both colostrum and milk samples (Table 1). In contrast, FB had significantly higher level of IgA compared to other two groups. Neg had no detectable antibody response in colostrum and milk samples throughout the study. All piglets in Neg died at 4 DPC following challenge. All piglets in IV group displayed severe clinical disease leading to necropsied at 4 DPC. In contrast, following G1 and G2 challenge, mortality in FB was 80 and 30%, respectively. Piglets in FB group had significantly lower VCR in G2 challenge that that of G1 challenge. These results indicate that the G2 variants, compared to the G1 variants, were more virulence to those negative pigs. Intramuscular vaccinated with the G1 was only partial protection against G1 challenge, but not the G2 challenge. In contrast, the feedback of G2 variants are more suitable to protect against both variants.

Experimental groups		Villous height/ crypt depth ratio (VCR)			VN		ELISA IgG		ELISA IgA	
		Duodenum	Jejunum	Ileum	0 DPP	3 DPP	0 DPP	3 DPP	0 DPP	3 DPP
Neg	G1	1.59^{a} (0.17)	1.19^{a} (0.08)	1.24^{a} (0.12)	0^{c} (0)	0^{c} (0)	0.02^{c} (0.01)	0^{c} (0)	0.01^{c} (0)	0.01^{c} (0.01)
	G2	0.79^{b} (0.08)	1.02^{a} (0.07)	0.99^{b} (0.10)						
FB	G1	0.76^{b} (0.03)	0.58^{b} (0.08)	0.87^{b} (0.03)	1.8^{b} (0.39)	1.8^{b} (0.33)	0.87^{b} (0.19)	0.60^{b} (0.18)	1.16^{a} (0.08)	0.88^{a} (0.11)
	G2	1.16^{a} (0.04)	1.15^{a} (0.07)	1.13^{a} (0.07)						
IV	G1	1.49^{a} (0.06)	0.97^{b} (0.05)	1.13^{a} (0.08)	6^{a} (0.21)	5.4^{a} (0.27)	1.62^{a} (0.08)	0.62^{b} (0.10)	0.56^{b} (0.04)	0.39^{b} (0.05)
	G2	1.55^{a} (0.08)	1.41^{a} (0.07)	1.18^{a} (0.08)						

Table 1 VCR, VN, IgG and IgA values of each experimental groups (Neg, FB and IV) and treatments (G1 and G2) in this study.

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Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2'3'-cGAMP VacciGrade™ adjuvant exacerbates viremia after virus challenge

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in pregnant sows and acute respiratory disease in young pigs. It is a leading infectious agent of swine respiratory complex, which has significant negative economic impact on the swine industry. Commercial markets currently offer both live attenuated and killed vaccines; however, increasing controversy exists about their efficacy providing complete protection. Therefore, there is a pressing need for a safe and effective vaccine to control and prevent this devastating disease.

We generated VLPs by expressing the glycoprotein 5 (Gp5), envelope protein (E), membrane protein (M) and nucleocapsid protein of PRRSV using the flashBAC™ baculovirus expression system. After two intranasal immunizations of pigs with phosphate-buffered saline (PBS), VLPs, or VLPs with 2'3'-cGAMP VacciGrade™ adjuvant, immunogenicity and protection efficacy were evaluated after virus challenge at two weeks after boost immunization. No PRRSV N specific antibody or Gp5 epitope specific antibody was detected in all animals prior to challenge. N protein specific antibody was detected in all animals at day 10 after challenge, but no significant difference was observed between the vaccinated and control groups. However, a significant increase in serum IgG and IgA specific to VLPs was observed at day 7 after challenge only in the VLPs plus the 2'3'-cGAMP VacciGrade™ group, suggesting that the adjuvant may boost the recall immune response against VLPs. It seemed to correlate with a higher interferon-alpha in the serum in the VLPs plus the adjuvant group. Surprisingly, a significantly higher viremia was observed in the VLPs and VLPs plus the adjuvant groups compared to the control group. A transient slight increase in the interferon-gamma and IL-10 in the serum was observed in all groups at day 3 after challenge, but no significant difference between the groups was observed. The average rectal temperature was significantly higher at day 10 after virus challenge in the control group than the VLPs plus adjuvant group. Similarly, a slightly milder lung histological lesion was observed in the VLPs plus adjuvant group compared to the VLPs group. Overall, the intranasal immunization of PRRSV VLPs plus adjuvant exacerbates viremia. A stronger interferon-alpha and a milder histological lesion were observed in the VLPs plus 2',3'cGAMP adjuvant group. Future studies should be focused on incorporating other viral proteins of PRRSV into VLPs and improving VLPs assembly efficiency. Additionally, different dose of VLPs, different adjuvant and route of vaccination such as intramuscular injection should be explored further to fully assess the feasibility of such a vaccine platform for PRRSV control and prevention.

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A sow farm PRRS elimination with serum therapy and herd closure

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Introduction Serum inoculation (therapy) with herd closure is a good method for control or eliminating PRRS in sow farm. The F8 farm had 1200 sows in 2014. The status of PRRS became unstable in 2013 winter. According with clinical signs, we decided inoculate serum to sows and gilts, and then closed the herd.

Materials and methods The PRRSV positive serum from F8 farm, was detected no classical swine fever and pseudorabies virus. We introduced 6 months gilt before serum acclimation. The herd was inoculated (10μl serum/head) by intramuscular injection on Jan. 18 and 20, respectively. Then we closed the herd and monitor the serum by ELISA.

Results There was little effect on the breeding performance during the acclimation period. Sow abortion began at Jan.19 and ceased at Jan.23, there was only 10 sow abortion together. The PRRS antibody positive rate of boar, sow herd, nursey and finish gradually decreased when the time passed. The S/P ratio of PRRS antibody levels also gradually decreased ,the rate of PRRS Ab negative pigs risen, the boar, sow, nursery and finish became PRRS Ab negative (Table 1).

Table 1 the detection of PRRS S/P value

item	year	boar	sow	gilt	piglet	nursey	finish
samples	2014	266	471	75	52	264	530
	2015	247	408	221	725	441	221
	2016(6)	75	231	283	538	391	1002
s/p>0.4 positive	2014	60%	52%	45%	19%	14%	13%
	2015	2%	24%	1%	5%	0%	0%
	2016(6)	3%	13%	1%	1%	0%	0%
s/p average value	2014	0.71	0.62	0.48	0.30	0.19	0.23
	2015	0.06	0.27	0.04	0.10	0.04	0.01
	2016(6)	0.05	0.16	0.05	0.06	0.04	0.02

Discussion Serum therapy and herd closure are useful tool for control and eliminating PRRS. In order to improve the success, we should introduce more than 6 months gilt and acclimate the gilts at the same time. The serum must be free of PRV and CSFV, or it will cause greater loss. Serum therapy and herd closure is not always successful, there are many unpredictable factors. Sometime it is a dilemma when we decide whether utilize serum inoculation for the herd or not. However, as long as really know the health status of the farm and planned well, strengthen farm biosecurity at the same time, the opportunity of PRRS elimination will become much higher, just like this case.

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Abortions when applied serum therapy in PRRS unstable sow farms

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Introduction Serum inoculation (therapy) is a good method to control and maintain the PRRSV stable. We usually use it for gilt acclimation. But sometimes the sow farm becomes PRRS unstable, such as much higher abortion rate than normal in gestation sows, and surveillance the PRRSV from weak piglets in farrowing room and/or abortion sows. If we continuously monitor the virus shed from weak piglet and off-feed sows for 3 weeks, and get the sequence of PRRSV. We should adopt some strategies for controlling the PRRS.

Materials and methods The PRRSV positive serum from every own farm, was detected no classical swine fever and pseudorabies virus. The herd was inoculated 1 or 2 times (1-10μl serum/head/time) by intramuscular injection. We applied serum therapy in PRRS positive unstable sow farm 11 times from 2007 to 2106. The sow scale of 8 farms are ranging from 400 to 2200.

Results We collect the abortion data form 2007 to 2016. The data include when we applied the serum injection, how many times injection we applied, when the abortion took place, which day was the peak day and when the abortion was ceased. We also calculated the number of days corresponding to the abortion events (see table1). Analyzed the 11 time abortions, we can see the abortion will begin from 0 to 4 days after injecting the serum, the average days is 1; the peak abortion range from 4 to 9 days, the average days is 6.2; the ends abortion from 6 to 13 days, the average days is 10.4.

Table 1 serum inoculation and the duration period of sow abortion

Farm code	Date of happening					days		
	SI*1 date	SI*2 date	AT# start	AT# peak	AT# end	start	peak	duration
F1	7/14/2007	7/16/2007	7/16/2007	7/20/2007	7/27/2007	2	6	13
F1	6/30/2013	7/2/2013	6/30/2013	7/6/2013	7/12/2013	0	6	12
F5	2/26/2011	2/28/2011	2/26/2011	3/4/2011	3/11/2011	0	6	13
F4	8/7/2007	\	8/7/2007	8/14/2007	8/18/2007	0	7	11
F4	3/8/2011	3/10/2011	3/12/2011	3/17/2011	3/21/2011	4	9	13
F3	9/12/2007	\	9/12/2007	9/17/2007	9/23/2007	0	5	11
F2	9/14/2007	\	9/15/2007	9/20/2007	9/23/2007	1	6	9
F8	1/18/2014	1/20/2014	1/19/2014	1/22/2014	1/24/2014	1	4	6
F6	4/9/2014	4/11/2014	4/12/2014	4/15/2014	4/17/2014	3	6	8
F6	3/26/2016	3/30/2016	3/26/2016	4/1/2016	4/2/2016	0	6	7
F7	3/30/2016	4/2/2016	3/30/2016	4/6/2016	4/10/2016	0	7	11
#AT=abortion *SI=serum inoculation					average	1.0	6.2	10.4

Discussion Serum therapy is a good method for controlling PRRS .The duration period of abortion induced by serum therapy is nearly 10.4 days. Compared to some reports, it is much shorter than using MLV. Whether we use serum therapy for sow herd or not, it is a dilemma. Sometime the abortion is as higher as 20%, but sometime as lower as 0.2%.

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Micro-emulsion adjuvants for swine viral vaccines: Application to a recombinant CSF vaccine

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Classical swine fever (CSF) is a highly virulent disease in pigs caused by a Pestivirus, which causes high mortality and can therefore have dramatic impacts on the productivity of pig industry. Vaccination against CSF has been successfully implemented using live attenuated vaccines. Recombinant viral vaccines are a safe alternative which allow the differentiation between infected and vaccinated animals. However they usually also have a decreased efficacy and thus need strong adjuvants. Here we demonstrate the safety and efficacy of a recombinant E2 CSF vaccine based on a micro-emulsion adjuvant.

3 groups of 10 40-day-old pigs received 2 injections of 1ml 20 days apart. Group 1 was immunized with a recombinant E2 vaccine formulated with the microemulsion adjuvant Montanide IMS 1313 VG (IMS), Group 2 with a recombinant E2 vaccine formulated with the polymer adjuvant Montanide Gel 01 (Gel). The control group received 2 injections of saline solution. For safety assessment, clinical signs after vaccination were recorded in all groups. Body temperature after vaccination and body weight during the trial were measured. For efficacy assessment, blood samples were collected at D0, 20, 40, 80 and 120 after injection and ELISA titrations and virus neutralization (VN) tests were performed on the pig sera. Animals were slaughtered at D120. This trial was repeated in 3 different farms in South Korea.

No severe clinical signs were observed. Animals showed some degree of depression right after vaccination. Pyrogenicity was lower than 0.7°C, and temperature and behavior of all animals were back to normal 24h after vaccination. There were no significant difference in the body weight of vaccinated and control animals at any time during the trial.

Antibody and virus neutralization titers were significantly higher in the IMS vaccinated group than in other groups at all time points during the trial in all farms. ELISA measurements showed that 100% of IMS vaccinated animals had protective levels of IgG at 40 days post injection. At 120 days post injection, 97% of IMS vaccinated animals showed protective levels of IgG. Virus neutralization tests showed that 100% of IMS vaccinated animals had protective VN titers at 40 dpi. At 120 dpi 70% of IMS vaccinated animals had protective VN titers.

This study shows that Montanide™ IMS adjuvants combined with recombinant CSF swine vaccine can induce lasting protective immune response until market age in pigs, while preserving the safety properties of the vaccine.

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Attenuate highly pathogenic Porcine Reproductive and Respiratory Syndrome virus by incorporating target site of hematopoietic-specific microRNA into viral genome

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Incorporation of target sequences of tissue- or cell-enriched microRNAs into the viral genome can be used to restrict off-target spread of virus. And infection in pulmonary alveolar macrophage (PAM), the host cell of porcine reproductive and respiratory syndrome virus (PRRSV), is critical for PRRSV pathogenicity and immunosuppression. To attenuate the highly pathogenic PRRSV by restricting viral replication in PAM, the target sequence of hematopoietic-specific miR-142 that is highly-expressed in PAMs was incorporated into PRRSV genome by using reverse genetic operation. Then, the in *vitro* and in *vivo* replication capability and pathogenicity for piglets of the rescued chimeric virus were systematically analyzed and compared with its parental backbone virus RvJXwn.

A viable chimeric virus designed as RvJX-miR-142t, with 22nt miRNA-142 target sequence replacing same length fragment of Nsp2 coding region, was first successfully rescued. And the multistep growth curve showed that the chimeric RvJX-miR-142t and RvJXwn have the same growth prosperities in MARC-145 cells. However, as expected, the replication of RvJX-miR-142t was seriously impaired in PAMs with significantly lower titers compared with that of RvJXwn at all time points. Especially, as much as 10^4 times peak titers difference could be observed between these two viruses.

Then the pathogenicity of the chimeric virus was further investigated in animal inoculation test. The results showed that the body temperature in RvJX-miR-142t-infected group raised slowly, and did not reach 41 ℃ until the 16 day post inoculation (dpi), accompanied by a significantly higher ADG than that of RvJXwn-infected group ($P < 0.001$). The clinical symptoms of piglets in RvJX-miR-142t-infected group were also significantly lower than that of RvJXwn-infected group ($P < 0.001$), and the mortality decreased to 2/6, with only two piglets died at 19 dpi. As well, the virus loads of RvJX-miR-142t-infected piglets were obviously lower than that of RvJXwn group on 3, 5, 7 dpi ($P < 0.001$). Meanwhile, the seroconversion for antibodies to PRRSV was later than that of RvJXwn group. Moreover, during the acute phase in the first 7 dpi, lung lesions of piglets in RvJX-miR-142t-infected group were much milder than that of RvJXwn group, with significantly lower lung lesions scores than that of RvJXwn group ($P < 0.01$).

Taken together, the results above demonstrated that incorporation of miR-142 target sequence into RvJXwn impaired the replication of PRRSV in its host cells and reduce its pathogenicity in piglets, correspondingly. This study provided a novel method for limiting PRRSV's tropism and driving viral attenuation.

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Dissection of complex molecular interactions between important animal nidoviruses and the host

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The long-term goal of our research program is to better understand molecular mechanisms of interactions between animal nidoviruses and their hosts in order to develop new strategies for effective control of viral infections. The present research project is focused on emerging swine pathogens of unprecedented economic significance, such as the porcine reproductive and respiratory syndrome virus (PRRSV) and the porcine epidemic diarrhea virus (PEDV). The PEDV and PRRSV are responsible for severe economic losses and considered as the primary emerging livestock pathogens worldwide. Insufficient understanding of virus-host interactions impedes the development of effective animal vaccines against PRRSV and PEDV.

Host-virus interactions are highly dynamic and may involve multiprotein complexes. Earlier, our group employed biochemical and proteomics approaches to identify virus-host multiprotein complexes, and showed that their composition is controlled by the virus either by direct recruitment of or by binding to host proteins. Consequently, characterization of the composition of PRRSV and PEDV and identification of the host proteins that are specifically encapsidated into or bound to virions are important for our further understanding of virus-host interactions. To accomplish this objective, we produced and purified PRRSV and PEDV using both the simian cell cultures that are routinely used for virus production and PRRSV/PEDV natural target cells. We hypothesized that the composition of PRRSV and PEDV virions and virus-host molecular complexes will reflect changes in environmental conditions (e.g., pH, activities of host proteases, and tissue-specificity). Furthermore, we hypothesized that the tight homeostatic balance between host cell and virus defines the fate of infection and pathogenesis. Proteomics is the best method to directly characterize the multimolecular complexes important for virus entry and pathogenesis. We examined the composition of progeny virions in order to identify cellular proteins that are associated with or encapsidated into viral particles using state-of-the-art mass spectrometry (MS) strategies, including a high-resolution hybrid Quadrupole-Orbitrap MS. The present study has demonstrated the incorporation of cellular proteins in PRRSV and PEDV virions. Further investigations are needed to evaluate the role of individual cellular proteins in the viral replication, assembly, and pathogenesis.

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Cleavage of cytochrome c1 by the PRRSV 3C-like protease leads to shedding of an apoptosis inducer

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Porcine reproductive and respiratory syndrome (PRRSV) is an important pathogen of swine and represents a major threat to the worldwide swine production. PRRSV-induced cell apoptosis contribute critically to viral pathogenesis but the molecular details have remained yet understood. In this report, we show that the 3C-like protease of PRRSV interacts with the mitochondrial inner membrane protein cytochrome c1 (cyto.c1) and induces its proteolytic cleavage. Importantly, the cleavage of cyto.c1 results in release of a strong cell apoptosis inducer that causes mitochondrial fragmentation, leading to cell apoptosis. Time course analysis of PRRSV infection revealed that this cleavage is consistent with the timing of the 3C-like protease expression and the onset of caspase-3 activation. Further RNAi silencing experiments revealed that cyto.c1 is critical for PRRSV-induced apoptosis. Taken together, the cleavage of cyto.c1 by PRRSV 3C-like protease is a critical trigger of cell apoptosis. Our studies provide an important piece of mechanistic clues of PRRSV-induced cell apoptosis and also indicate a novel mechanism for the 3C-like proteases of positive-stranded RNA viruses in induction of cell apoptosis.

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A novel mechanism of protein-stimulated trans-activation of ribosomal frameshifting in porcine reproductive and respiratory syndrome virus: implication in improved vaccine development

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Viruses have evolved various non-canonical translation mechanisms, like programmed ribosomal frameshifting (PRF), to overcome some of the restrictions posed by host cell ribosomes and - for example - express multiple proteins from a single mRNA. Arteriviruses utilise PRF to direct the efficient expression of two alternative proteins from the nsp2-coding region of their replicase gene. The PRF signal involved, unusually, lacks an obvious stimulatory RNA secondary structure and induces both –2 and –1 PRF, to produce an nsp2 variant with an alternative C-terminal domain (nsp2TF) and a truncated version of nsp2 (nsp2N), respectively. In the genome of porcine reproductive and respiratory syndrome virus (PRRSV), the minimal PRF signal maps to a 34-nt region including a slippery sequence (GG_GUU_UUU) and downstream conserved C-rich motif. Strikingly, efficient –2/–1 PRF also depends on the nsp1β replicase subunit [Fang et al. (2012), Proc. Natl. Acad. Sci. USA 109:e2920 and Li et al. (2014), Proc. Natl. Acad. Sci. 111:e2172-81]. In addition to this viral trans-activator, PRF requires the participation of cellular poly(C) binding proteins (PCBPs). *In vitro* translation and RNA binding assays revealed that a complex of nsp1β and PCBP binds to the genomic mRNA downstream of the slippery sequence, where it mimics the action of the more typical RNA pseudoknot-type of PRF stimulators [Napthine et al. (2016), Nucleic Acids Res., 44(12):5491-503]. This unprecedented viral PRF signal provides new insights on the modulation of ribosomal elongation by *trans*-acting protein factors. On the other hand, it prototypes a new class of virus-host interactions. Frameshift knockout mutants of PRRSV were attenuated *in vivo*. In comparison with wild-type virus, the frameshift knockout mutants stimulated earlier IFN-α production in infected pigs, and the upregulation of innate immune gene expression was correlated with reduced viral load in animals. Our data strongly implicate the PRF products in viral immune evasion and provides a basis to explore –2/–1 PRF inactivation in vaccine development.

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Identification of CD163 domain involved in the infection with Type II Porcine Reproductive and Respiratory viruses

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Porcine Reproductive and Respiratory Syndrome has been shown to be one of the most economically destructive diseases affecting swine worldwide. In the U.S. alone, recent estimates indicate losses to the national swine herds of $664 million annually from 2005-2010. Efforts to solve this issue have granted a breadth of knowledge revealing details of how the virus infects the host. CD163, present on macrophages and cells of the monocyte lineage, has been shown to play a critical role in infection. Physiologically, CD163 functions as a scavenger receptor for the hemoglobin-haptoglobin complex. CD163 is composed of nine scavenger receptor cysteine-rich (SRCR) domains. There are two 35-amino-acid proline-serine-threonine (PST)-rich regions, one between domains six and seven, and one between domain nine and the transmembrane region and cytoplasmic tail.

In this study, CD163 constructs containing an EGFP fluorescent tag and bearing serial deletions of SRCR domains were produced in order to determine which domains are important for permissiveness to Type II PRRSV. HEK293T cells, which do not support PRRSV infection, were transfected with the CD163 constructs. Transfection was confirmed by visible expression of the CD163-EGFP fusion protein. The proper expression of each CD163 construct was also evaluated by Western blot, using an anti-EGFP antibody for the detection of the fusion protein. The permissiveness of transfected cells for PRRSV was tested using recombinant PRRS viruses expressing a red fluorescent protein (RFP). Successful infection was detected by the presence of red fluorescence in a green fluorescing cell.

The infection experiments revealed that all the constructs containing domain 5 were able to sustain infection by Type II PRRSV while all the constructs that lacked domain 5 were not permissive to infection. This included constructs that had the first 5, 6, and 8 N-terminal SRCR domains deleted. Thus, the presence of domain 5 is a key component in achieving viral infection. Finer mapping studies need to be conducted by making smaller and smaller deletions in this target region to determine the smallest deletion needed to block infection by Type II PRRS viruses.

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Cloning and sequence analysis of Nsp9 gene of Porcine Reproductive and Respiratory Syndrome Virus FS strain

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In order to evaluate function of Nsp9 gene, the target gene of porcine reproductive and respiratory syndrome virus (PRRSV) FS strain was amplificated and sequenced after being cloned into the pMD18-T vector. The physical and chemical properties, homology, hydrophilicity, surface probability plot, antigenic index, secondary structure and subcellular localization were predicted by various softwares. The results showed that the length of Nsp9 was 1 929 bp, its predicted molecular weight was 70.5 ku and pI was 8.04, and it was unstable protein. There were many antigen sites, and the flexibility and hydrophilicity of Nsp9 were ideal. The study showed that Nsp9 possessed potential antigenicity, and it fits for preparation of monoclonal antibodies. The results of subcellular location showed that it may exist in the cytoplasm. Nsp9 of FS strain shared 96.9 % to 98.9% amino acid homology with other strains. Some amino acid mutations were found between the parent strains and vaccine strains, and insertions and deletions could be found among the European strains and the American strains. Whether these insertions and deletions correlate with the virulence and polymerase needed further research.

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Construction and identification of Nsp9-deficient clone of porcine reproductive and respiratory syndrome virus (PRRSV) XH-GD strain

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Nonstructural protein 9 (Nsp9) is one of the most conserved nonstructural proteins in porcine reproductive and respiratory syndrome virus (PRRSV). The major role of NSP9 remains unclear. To further identify the function of Nsp9 in PRRSV, reverse genetic manipulation was performed and an infectious PRRSV cDNA clone with Nsp9-deficient mutants were constructed to verify the function of Nsp9. The results revealed that the PRRSV clones deficient of Nsp9, cannot be rescued suggesting that Nsp9 is critical to the replication of PRRSV. These results may provide the basis for the functional study of this nonstructural protein.

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Influence of Nsp9 between of highly pathogenic PRRSV and low pathogenic PRRSV to replication of PRRSV

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In order to verify the influence of Nsp9 gene between highly pathogenic PRRSV and low pathogenic PRRSV to replication of PRRSV, plasmids pIRes2-EGFP-Nsp9-XH-GD and pIRes2-EGFP-Nsp9-CH-1R containing whole Nsp9 gene were transfected into Marc-145 cells, then cells were infected with PRRSV XH-GD strain at a MOI of 1, TCID50 was used to evaluate viral titer, qPCR and western blot were conducted to evaluate expression of PRRSV N protein. The results showed that the level of N protein in the cells that were transfected with typical PRRSV CH-1R Nsp9 gene was higher than that of the highly pathogenic PRRSV group in the mRNA level, in the protein level, it was same as the mRNA level, and virus titer was also higher than of highly pathogenic PRRSV group. So the conclusion is that Nsp9 gene of low pathogenic PRRSV strain CH-1R is more beneficial to the replication of PRRSV than that of highly pathogenic PRRSV strain XH-GD in the Marc-145 cells.

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Influence of Nsp9 to replication of PRRSV in MARC-145 cells

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In order to evaluate whether Nsp9 could enhance the replication of PRRSV, plasmids pIRES2-EGFP-Nsp9 containing whole Nsp9 genome were transfected into Marc-145 cells, qPCR and western blot were used to evaluate its titer and expression after PRRSV was inoculated, the results showed that level of N protein in the transfected Nsp9 cells was higher than control group, 1.5times of the control group. Meanwhile, the protein level was same as the mRNA level, N protein both at the mRNA and protein level were increased with the increase of plasmids. So the conclusion is that Nsp9 gene could enhance replication of PRRSV, and it was closely related with replication of PRRSV.

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Subcellular localization analyisi and function prediction of PRRSV NSP9 protein

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PRRS is one of the most serious diseases that are harmful to pig industry and NSP9 gene encodes RNA dependent RNA polymerase (RdRp) of PRRSV. Plasmids containing NSP9 gene were transfected into BHK cells and Marc-145 cells in order to confirm the subcellular localization of NSP9 gene and verify whether PRRSV infection could affect its localization through indirect immunofluorescence microscopy. The results show that NSP9 gene was located in the cytoplasm in BHK cells and Marc-145 cells. NSP9 genes are mainly distributed in the cytoplasm ; NSP9 gene gradually transfers to the nucleus after PRRSV infection, and the area was also gradually increased. The research laid a foundation for the replication of PRRSV.

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Substitution between highly pathogenicial PPRSV and low pathogenicial PPRSV with Reverse Genetics System

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The Nsp9 gene of porcine reproductive and respiratory syndrome virus (PRRSV) codes the RNA dependent RNA polymerase, which play a big part in the virus replication. However, whether Nsp9 gene influences the virulence is unclear. To determine the interaction of Nsp9 with virulence and speed of viral replication, cell tropism, the Nsp9 sequences of vaccine strain CH-1R were cloned, ligated it to a virulent strain of the experimental XH-GD infectious clone after restriction enzyme digestion, and transformed the virus under the basis of the reverse genetics. Following by virus rescue, the virus can be successfully saved. Biological characteristics of the virus were explored, titers of saved virus were significantly higher than the growth rate and the parental strain XH-GD, but lower than vaccine strain CH-1R, while plaques similar in size, the polymerase activity of the vaccine virus CH-1R is higher than the virulent strain XH-GD.

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Modeling the transboundary survival of foreign animal disease pathogens in contaminated feed ingredients

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Objective: To model the survival of foreign animal diseases in feed ingredients shipped from Asia to the US using surrogate viruses.
Materials & Methods: Based on the Swine Health Information Center pathogen matrix, 10 FAD viral pathogens were identified as significant risks to the US swine industry. For the purpose of the study, a surrogate virus was selected to represent each pathogen: FMDV (SVA), CFV (BVDV), PRV (BHV-1), ASFV (Vaccinia), Nipah virus (CDV), SVDV (PEV) and VEV (FCV). Other selected pathogens included PRRSV 174, PCV2 and VSV. Using a transboundary model (1), feed ingredients known to be imported from China to the US (organic & conventional soybean meal, soy oil cake, DDGS, lysine, choline, vitamin D, pork sausage casings, and various pet foods) were inoculated (5g ingredient+100uL virus). Controls included complete feed (surrogate or saline) and stock virus (positive control, no feed matrix). Samples were incubated in an environmental chamber for 37 days programmed with T and % RH data recorded from China to the US during December 2012 through January 2013 (SeaRates.com). Samples were tested by PCR, VI and bioassay at day 2, 8, 25 and 37 PI.
Results: Testing of the FMDV, CSFV and PRV surrogates indicated the survival of SVA and BHV-1 at 37 DPI. Both surrogates survived in conventional soybean meal and soy oil cake. SVA also survived in lysine, pet food, Vit D, complete feed and casings. Positive controls did not survive. BVDV was negative at 37 DPI, independent of ingredient.
Discussion: These preliminary results suggest that contaminated feed ingredients could serve as vehicles for FAD introduction to the US, supporting our PEDV data. Phase 2 has begun, consisting of surrogates for ASFV, VEV and Nipah Virus along with PRRSV (174). New data will be shared if selected for presentation.

(1). Dee S, et al. Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus *BMC Vet Res,* 2016, 12:51

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Isolation and characterization of porcine deltacoronavirus from pigs with diarrhea in China

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Porcine deltacoronavirus (PdCoV) (family Coronaviridae, genus Deltacoronavirus) is an emerging swine enteropathogenic coronavirus that causes symptoms of acute diarrhea and vomiting. It is a significant threat to the pig industry. In this study, a molecular-based method was used to investigate the prevalence of PdCoV and characterize the aetiologic agents that induce diarrhea in pigs in China. The RT-PCR results indicated that porcine epidemic diarrhea virus (PEDV) is the most common porcine enteric virus isolated with PdCoV. Furthermore, the prevalence of PEDV is more common than PdCoV in 12 provinces of China. A total of 4 different PdCoV strains were isolated using swine testicular (ST) cells. Phylogenetic analysis of the complete genome revealed that the N and S protein sequences of the four PdCoV isolates were most closely related to PdCoVs from Korea and the US. With no way of predicting an outbreak of PdCoV in China and there being no vaccine available to prevent infection, extensive surveillance for PdCoV is critical in order to define the epidemiology and evolution of PdCoV. An effective vaccine is urgently needed to prevent widespread emergence of this disease in China.

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Leveraging data across a large geographic area to increase context and understanding of swine viral residence and transmission in smaller regions

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Area Regional Control (ARC) projects have been successful helping producers and veterinarians understand the local risk for PRRSV introduction into herds and coordinating disease control efforts at a local level. One weakness of ARC projects is that while they provide granularity at the local level, they do not provide a broad context for current and potential sources of new viruses to that region. To address that information gap we established a network (coined Illinois Swine Health Network or ISHN) of ARC projects, producers that had sites both within and outside of ARC projects and other producers that are in proximity to ARC projects. The goals of the network are: 1) to develop the data collection methods, infrastructure and collaborations between producers, veterinarians, and technical experts, 2) to facilitate dynamic, real time estimates of the risk of disease introduction into a pig farm, 3) to facilitate the development and deployment of optimum prevention strategies for herds, local production ecosystems and regional production ecosystems.

Since inception in June of 2014, ISHN has enrolled 572 individual sites. Each site has a robust set of metadata including, geo-location, production type, size, and Prem ID. The focus has been on collecting all PRRSV and Influenza Virus A sequences obtained from the enrolled sites along with their associated metadata (Date, etc.). Each participant (ARC, veterinary clinic or production system) maintains its own dataset of sequences to allow for individual local analysis. Using Disease BioPortal (www.bioportal.ucdavis.edu, Univ. CA-Davis), the ISHN aggregates these datasets and provides analytics for the entire network. Standard reporting is provided to participating veterinarians routinely. As of July 1, 2016, ISHN contains 1978 unique PRRS sequences and 508 unique IAV - HA sequences. Additional target gene sequences (NA, M, etc.) are also contained in the database but are not routinely analyzed. Future work includes capturing animal movement data to provide better context for disease movement patterns and integrating poultry sites to understand cross species IAV movement.

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Estimating the costs of Porcine Reproductive & Respiratory Syndrome (PRRS) and return on investment of interventions with a PRRS economic simulator

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Porcine reproductive and respiratory syndrome (PRRS) is among the diseases with the highest economic impact in pig production worldwide. Holtkamp et al. (2013) estimated annual losses due to the disease in the USA as high as $664 Mio. Yet, the economic impact of the disease at farm level is not well understood as, especially in pig herds chronically infected with PRRS virus, losses caused are often not obvious for farmers and veterinarians. Furthermore, the vast number of options to control PRRS makes it difficult for farmers and veterinarians to decide on the economically most efficient strategy for their farm. Aim of this study was to develop an economic model to estimate the costs of PRRS and the cost-efficiency of different control strategies for an individual farm. In a production model simulating the production of different farm types, batch systems, etc., an epidemiological model was integrated to estimate the impact of PRRS infection on health and productivity parameters, depending on PRRS severity. Based on this, financial losses were calculated in gross margin and partial budget analysis. This cost model was extended to incorporate different intervention strategies: a) depopulation / repopulation (D/R), b) close & roll-over (C&R), c) test & removal (T&R), d) mass vaccination of sows (MS), e) MS and vaccination of piglets (MSP), f) vaccination of sows according to the status of reproduction (6-60), g) 6-60 and vaccination of piglets (6-60P), h) improvement of biosecurity and management (BSM), and combinations of h) with d) – g). Data on the effects of PRRS infection and of each intervention were obtained through literature review and expert opinion. Economic efficiency of the different control strategies was assessed over a period of 5 years through investment appraisals, and the resulting expected value (EV) indicated the most cost-effective strategy. The final calculator was coded as a stochastic model in Excel add-in @RISK 6.3.1 (Palisade Corporation, Newfield, New York, USA). In a moderately affected herd (moderate deviations in all health and productivity parameters from what could be expected in an average negative herd), total median losses per year were estimated as being $-495,775. The intervention strategies with the highest median EV were 1) C&R ($1,261,120), 2) MSP ($1,247,520) and 3) 6-60P ($1,226,990). In a slightly affected herd, median annual losses were $-249,915, and the most cost-efficient strategies according to their median EV were 1) MSP ($807,777), 2) 6-60P ($797,442) and 3) MS ($743,273). Results indicate that losses in affected herds can be considerable and that the expected benefits of interventions and the most efficient strategy depend on the farm individual situation, e.g. disease severity. The model can provide a better understanding of the economic impact of PRRS in a farm and the need for interventions. It is a valuable tool for farmers and veterinarians to facilitate decision on the most economically efficient intervention strategy.

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Spatiotemporal detection and localization of type 2 porcine reproductive and respiratory syndrome virus at the maternal-fetal interface of late gestation pregnant gilts

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection causes severe reproductive failure characterized by high fetal mortality leading to substantial economic losses to the swine industry. The mechanism of PRRSV-induced fetal death needs further studies to explore PRRSV transmission across the maternal-fetal interface (MFI). The localization of PRRSV antigen at various time points post-inoculation is important to identify the mechanisms used by the virus to breach the MFI. An immunofluorescence (IF) technique was used for the spatiotemporal detection of type 2 PRRSV across the MFI in 20 pregnant gilts on day 85±1 of gestation. Type 2 PRRSV (NVSL 97-7895) was used to inoculate 15 gilts (1 x 10^5 $TCID_{50}$ total dose) at 85 days of gestation. Five gilts were sham-inoculated with sterile culture media (CTRL). Chronological IF detection of PRRSV in MFI was conducted in three inoculated gilts and one control gilt euthanized at 2, 5, 8, 12, and 14 days post infection (dpi). Samples of MFI were collected from the area adjacent to the umbilical stump of each fetus and frozen for cryosectioning. The images were obtained using CellSens® software and an Olympus® IX83 microscope equipped with a high resolution Zyla sCMOS camera. To date we have analysed transplacental PRRSV transmission in randomly selected fetuses obtained at day 5, 8, 12 and 14 dpi in both PRRSV inoculated and corresponding CTRL gilts. Five-micrometer cryosections were used for single IF detection and localization of PRRSV-positive cells in the MFI. Three major focus areas were categorized: endometrial connective tissues (including uterine glands and blood vessels), the interface (junction of endometrium and fetal trophoblasts) and fetal placental structures (mesenchymal cells and placental blood vessels). Results obtained from our preliminary work show that the majority of PRRSV was localized in the endometrial connective tissues and the interface at 5 and 8 dpi, whereas the detection of PRRSV was more localized in the fetal placental side at 12 and 14 dpi. IF labelling at 14 dpi revealed the majority of fetal placental blood vessels were infected with PRRSV-positive cells, which was not observed at 5, 8 and 12 dpi. The PRRSV infected cells at 12 and 14 dpi were also more abundant than 5 and 8 dpi and localized in a very consistent “lined up” fashion along the fetal placental border. Further investigation is in progress to perform double and triple IF staining to focus on specific maternal/fetal cells and connective tissue structures that PRRSV is potentially exploiting for active transmission from dam to fetuses leading to reproductive failure. This project was supported by Genome Canada, Genome Alberta and Genome Prairie (Saskatchewan Ministry of Agriculture).

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Using machine learning to predict swine movements with application to the control of infectious diseases

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Background: Direct or indirect contact between farms is one of the most important factors influencing the spread of infectious diseases in food animals, including the US swine industry. Understanding an industry's structural network of contacts is prerequisite to plan for efficient production strategies and, for effective disease control measures. Unfortunately, systematic collection of contact data between farms is difficult and, thus, such information is often unavailable.

Objectives: Here, we first summarized frequency and distance of movements within two partial networks of swine producing facilities in Minnesota, and, second, developed a methodology utilizing that incomplete information to estimate a complete contact network in a region in which a voluntary swine disease control program has been implemented.

Methods: A machine learning technique, referred to as Random Forest (RF), which is an ensemble of independent classification trees, was used to estimate the probability of pig movements between farms and/or market sites located in two counties in MN. Once calibrated and tested, the model was used to predict animal movements in sites located across 34 Minnesota counties (RCP-N212).

Results: Agreement between observed and expected movements in the model was maximized using a 0.85 probability threshold. Variables that were important in predicting pig movements included distance between sites, ownership, and production type of the origin and destination. Using a weighted-Kernel approach to describe spatial variation in centrality measures of the predicted network, we also show that the south-central region of the study area exhibited high spatial-aggregation of pig entries and exits. This area also seemed to be a hot spot for some swine diseases, which would be expected for an area engaging in large quantities of animal movements.

Conclusions: The information provided here will help to design and implement control strategies in the region. Additionally, the methodology here may be used to estimate contact networks for other livestock systems when only incomplete information is available.

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Epitope mapping of monoclonal antibodies against emerging porcine circovirus subtype 3

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Porcine circovirus (PCV) has been causing significant economic losses to the swine industry worldwide. Recently, novel subtype 3 (PCV3) has been identified in field cases with porcine circovirus-associated disease (PCVAD). Development of specific diagnostic reagents and assays are important for controlling this emerging pathogen. In this study, we generated a panel of monoclonal antibodies (mAbs) against the capsid proteins of PCV3. Four mAbs were selected for characterization in various diagnostic assays. The antigenic epitopes recognized by these mAbs were further mapped with full-length and C-terminal truncated capsid proteins of PCV3. MARC-145 cells were transfected with a plasmid DNA expressing full-length or a fragment of capsid protein, and indirect immunofluorescence assay was used for detecting the mAb reactivity. The results showed that mAbs 29-1, 40-84, and 47-1 recognized epitopes located at amino acid (aa) 59–140 of the capsid protein. These mAbs were further tested in Western blot using cell lysates of MARC-145 cells that express full-length of PCV3 capsid protein. The mAbs 14-1 and 40-84 reacted with the capsid proteins from cell lysates, but no reactivity was detected for other mAbs, suggesting that mAbs 14-1 and 40-84 recognized linear epitopes, while the others mAbs recognized the conformational epitopes. Further mapping of the individual epitope and testing the mAb cross-reactivity with other PCV subtypes are currently going on in our laboratory. This panel of mAbs provides a useful tool for PCV diagnostics and pathogenesis studies.

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Development of novel chimeric vaccine and delivery system for classical swine fever virus

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Classical swine fever virus (CSFV) is highly contagious to pigs. Although it has been eradicated from the US and many other countries, biosecurity measures, including efficient vaccines, are critical to assure a CSFV-free status. CSFV vaccines are available, but modified live virus (MLV) vaccines may pose concerns on biosecurity issues in CSFV-free countries, while subunit vaccines have limitations on stimulation of sufficient levels of immunity. A viral vector vaccine would be able to overcome these limitations. In this study, a modified live porcine reproductive and respiratory syndrome virus (PRRSV SD95-21 MLV) was used as a viral vector backbone to express immunogenic E2 protein of CSFV. Using the infectious clone of SD95-21 MLV, the CSFV E2 gene was inserted between non-structural and structural genes, which was transcribed as an additional subgenomic RNA. Upon transfection of cells with this plasmid, the progeny virus "PRRSV-E2" was obtained. To eliminate the need for a cold chain, DNA vaccine approach was used, in which nanoparticles composed of branched amphiphilic peptide capsules (BAPCs) were employed as the DNA delivery agent. Transfection of DNA-BAPC nanoparticles in MARC-145 cells recovered infectious viruses. Subsequently, DNA vaccination and BAPCs delivery approach were evaluated using a nursery pig model. Pigs were immunized by PRRSV-E2 chimera in a form of DNA-BAPC nanoparticles (group 1), DNA without nanoparticles (group 2), live recombinant chimeric virus (group 3), or mock-inoculated as a control (group 4). Viral RNA was detected by nested RT-PCR from group 1 and group 3 pigs at 7 days post inoculation, while virus specific antibody response was detected by 3 weeks post-infection. This study developed a chimeric dual user candidate vaccine for both PRRSV and CSFV. The chimeric vaccine platform and DNA delivery system established in this study could be applied to other emerging and transboundary swine pathogens in the future.

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Identification of African swine fever virus p30 antigenic epitopes after experimental infection

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African swine fever virus (ASFV) is the etiologic agent of a devastating disease of swine for which there is no available commercial vaccine. The ASFV has a multilayer virion composed of at least 50 proteins and contains a double stranded DNA genome of 170-190 Kbp encoding for more than 100 polypeptides. Kollnberger and colleagues identified p30 among the 12 most antigenic proteins, and Gomez-Puertas and colleagues showed that p30 induces neutralizing antibodies. There is little information on the p30 antigenic epitopes recognized by sera from ASFV infected swine. Identification of epitopes is important for the development of diagnostic tools and vaccines. The aim of our study was to identify antigenic epitopes on p30 using sera from pigs immunized with an alphavirus based replicon particles expressing p30 (RP-30) and then infected with the attenuated ASFV strain, OURT88/3. Ten pigs were immunized with RP-30 and boosted after three weeks. Six non-immunized pigs were included as controls. All sixteen pigs were inoculated by intramuscular injection with OURT88/3 at 10^4 $TCID_{50}$/ml one week after the last immunization. Pigs were euthanized by 21 days post infection (DPI) with OURT88/3. Temperature and clinical signs were recorded daily until the end of the study. For epitope mapping, five overlapping p30 fragments and p30 whole protein expressed in *E.coli* were used as antigen for ELISA, and sera from pigs at days 0 (before inoculation with OURT88/3) and at 17 DPI were tested. All day 0 sera from the 10 immunized pigs showed high reactivity with the p30 whole protein, 3 sera showed reactivity above background with the p30 region between amino acids (aa) 61 and 91, and 1 sera reacted with the region between aa 91 and 110. Overall, no strong immune dominant epitopes are elicited after vaccination with RP-30.

As expected, no reactivity against p30 fragments nor the whole protein was detected for sera at day 0 from the non-immunized pigs. All sera from 17 DPI reacted with p30 whole protein, and 5 sera from the immunized group reacted with the p30 region between aa 91 and 130, while 1 reacted weakly with the region between aa 111 and 130. One out of 6 sera from the non-immunized group reacted with the region between aa 91 and 130. Among 8 sera that did not react with any p30 fragment, 2 of those pigs had high average clinical score and temperature starting from the second week post infection and were euthanized 2 days before the end of the study. Three pigs had low average clinical score and temperature throughout the study, and the remaining three had average clinical score of around 2 or below. In conclusion, we have identified p30 epitopes recognized after ASFV experimental infection, however a clear role for those epitopes in the pathogenesis of ASFV has not yet been established. Future studies will be conducted to test the serum samples for neutralizing activities, as well as to investigate the role of other immunogenic proteins.

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