2013 North American PRRS Symposium

Final Program

Intercontinental Hotel
Chicago, Illinois

December 7-8, 2013

The 2013 North American PRRS Symposium wishes to thank the following sponsors for their generous support:

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

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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 23 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 International 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 IPRRSS Student Travel Fellowships for attendance at PRRS Symposia.

2013 Student Travel Fellowship Recipients

Nanhua Chen	Kansas State University
Jenelle Dunkelberger	Iowa State University
Alyssa Evans	Iowa State University
Mingyuan Han	University of Illinois
Andrew Hess	Iowa State University
Chi Yong Kim	University of Illinois
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Nick VL Serao	Iowa State University
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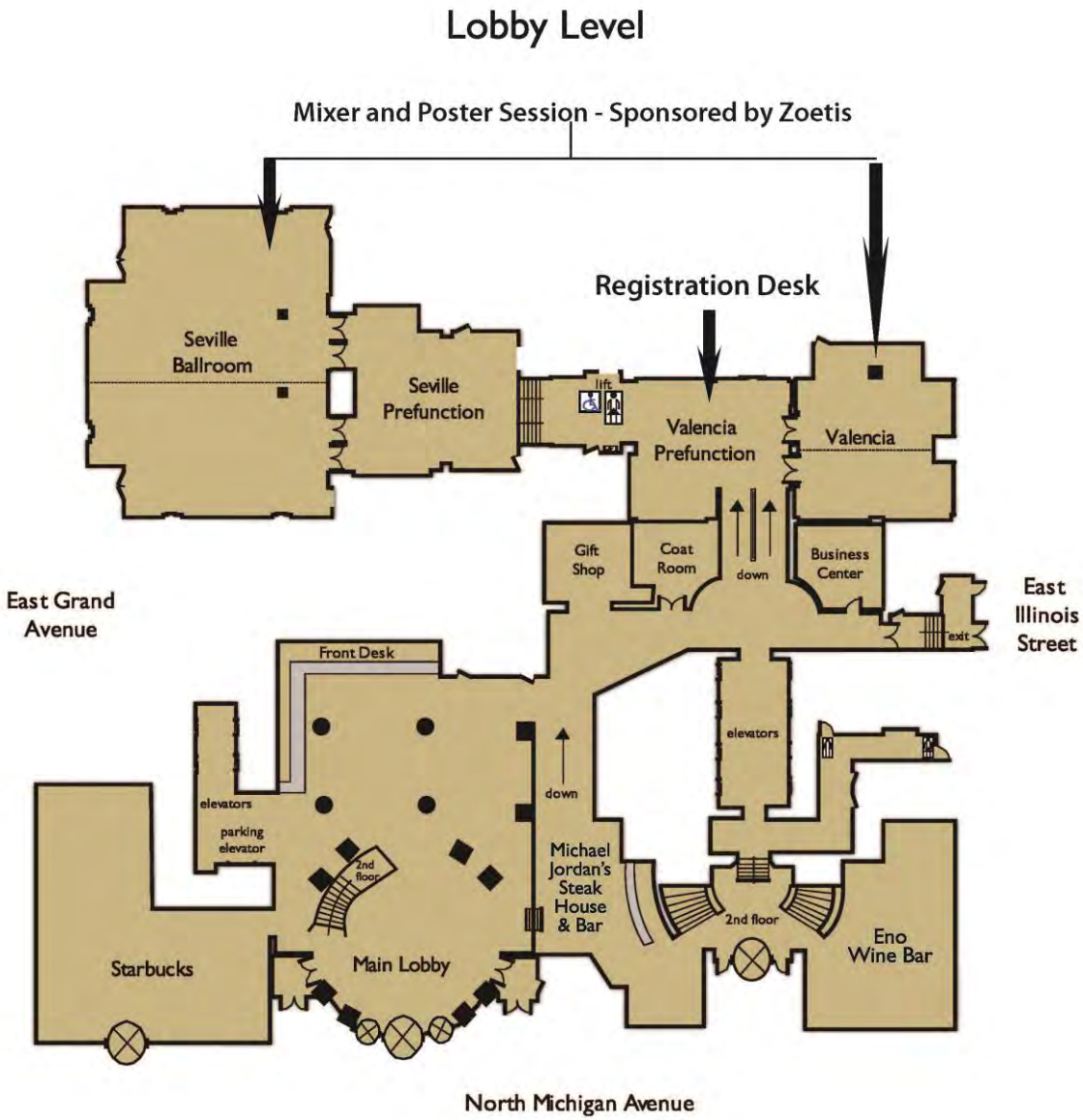
CE Credit Information

The 2013 North American PRRS Symposium Program has been approved for 12 hours of continuing education (CE) credit as approved by the College of Veterinary Medicine at Kansas State University. Kansas State University 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 will be available at the Registration Desk.

Intercontinental Hotel Maps



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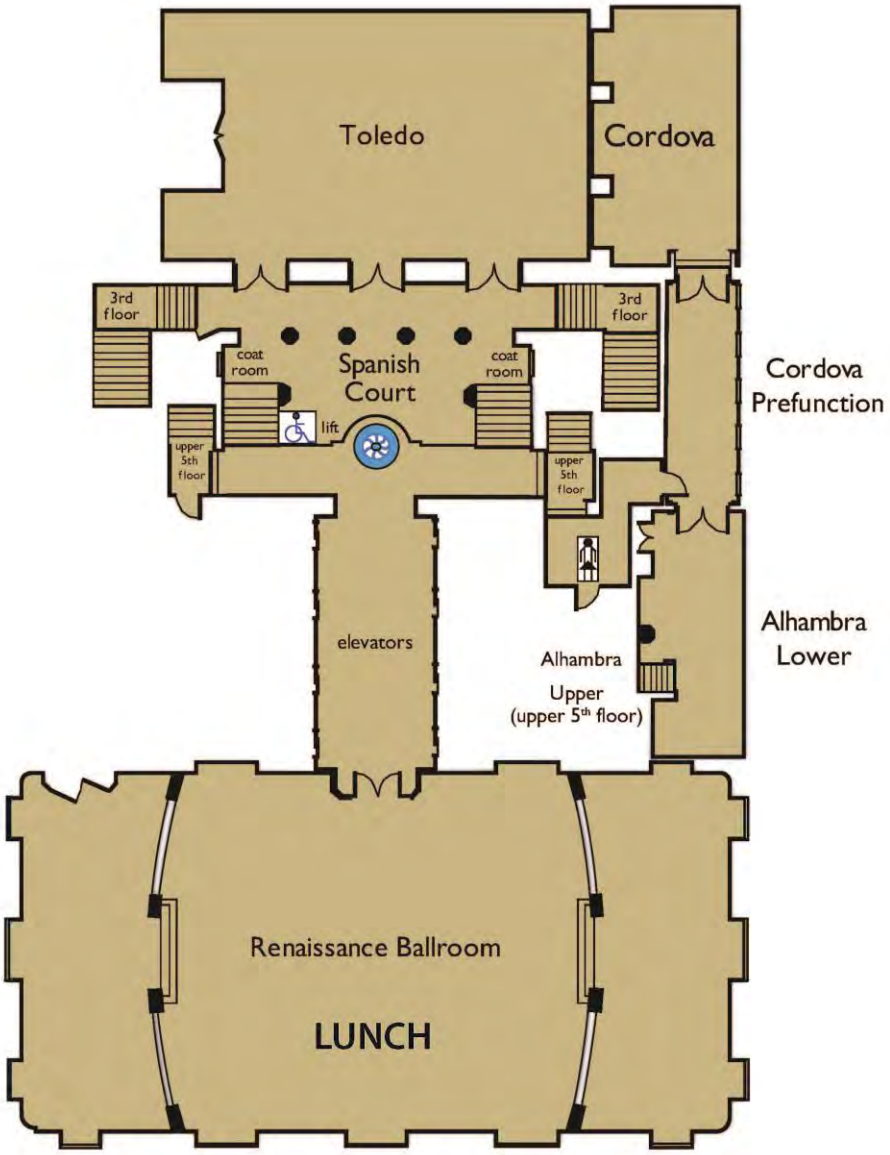
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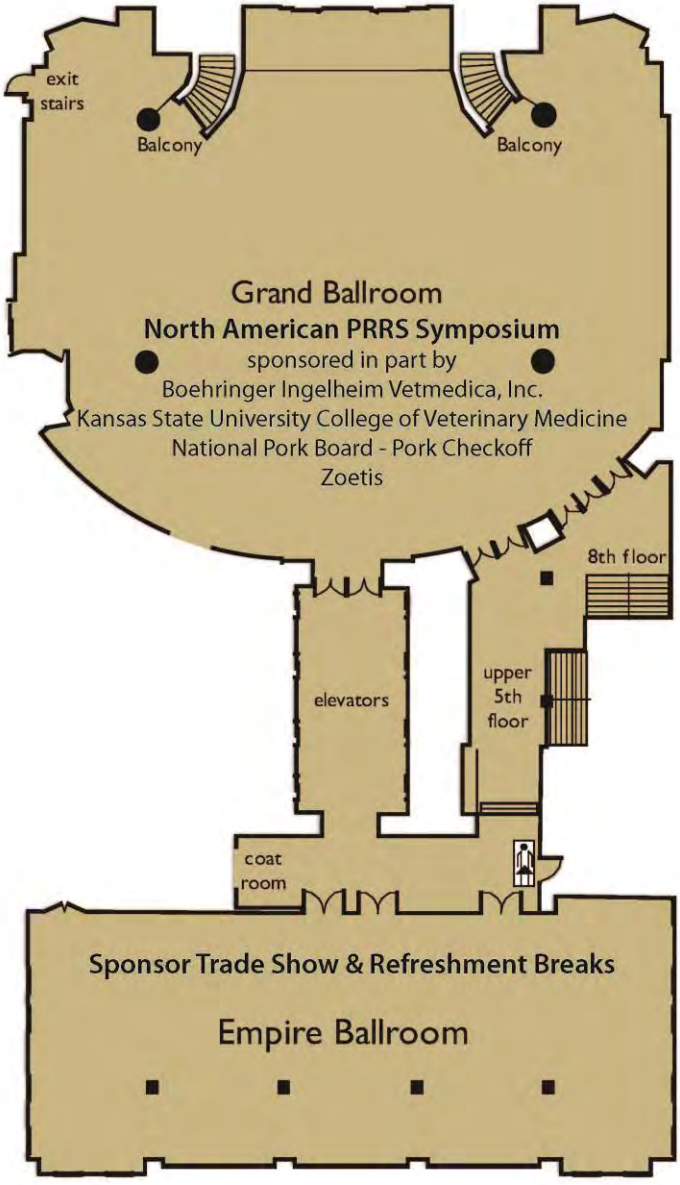
Renaissance Ballroom
LUNCH

7TH Floor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Grand Ballroom
North American PRRS Symposium
sponsored in part by
Boehringer Ingelheim Vetmedica, Inc.
Kansas State University College of Veterinary Medicine
National Pork Board - Pork Checkoff
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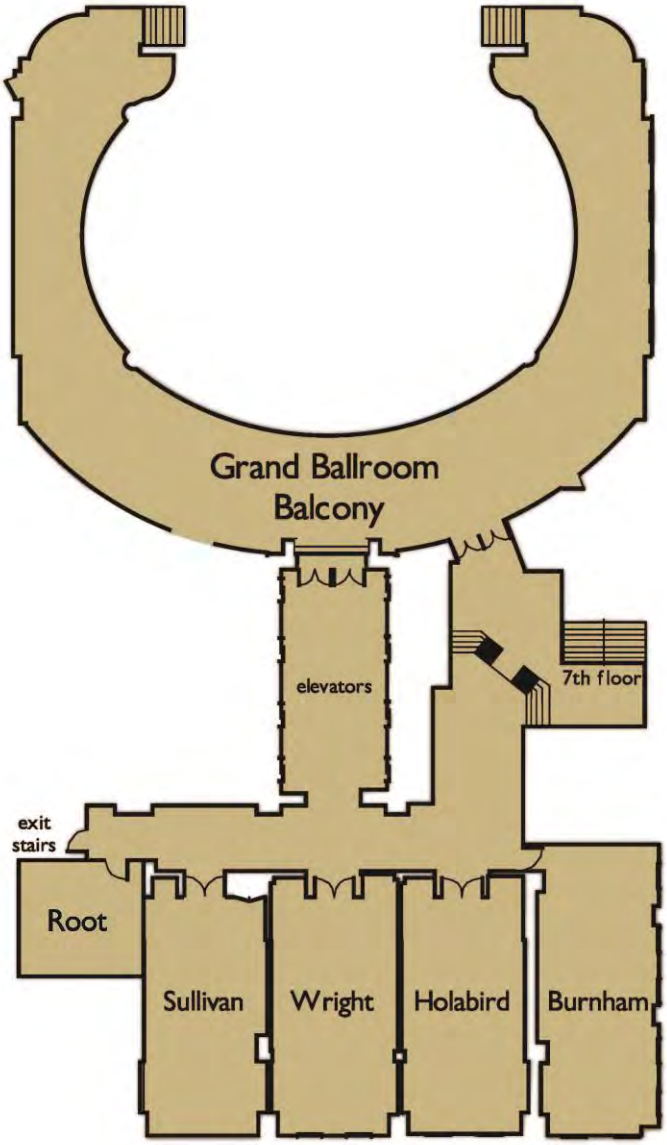
Empire Ballroom

8TH Floor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Burnham

2013 North American PRRS Symposium Program

Friday, December 6

4:30-6:00pm: Registration Open--*Lobby Level, Valencia Prefunction*

Saturday, December 7

6:45am: Registration Open--*Lobby Level, Valencia Prefunction*

Morning Session Sponsored by Boehringer Ingelheim Vetmedica, Inc.

7th Floor Grand Ballroom

PRRS Control in the Field and Area Regional Control

8:00am	Introduction: Bob Morrison, UMN
8:05am	Rodger Main, ISU: Update on current PRRS diagnostics
8:20am	Scott Dee, Pipestone Vet Clinic: Current status of PRRS and field applicable PRRS control research; what are we applying and what are we learning relevant to breeding herd stabilization and PRRS control in growing pigs?
8:50am	Daniel Linhares, PIC/Agroceres: What have we learned regarding the impact of MLV or LVI in the L-C-E protocol for Breeding Herd Stabilization?
9:20am	Clayton Johnson, The Maschhoffs: Application of PRRS control protocols; how we measure and what we've learned
9:50am	*Break: 7th Floor Empire Ballroom*
10:00am	Area Regional PRRS Control projects "4 Corners" breakout session: *In this fast paced information sharing session, projects' leaders/coordinators will have 10-minute sessions to describe their projects and any key learning they may have, with time for Q&A. To allow participants to attend as many different presentations as possible, each presentation will be given in 2 different time slots throughout the hour. Presenters will also have 1-page summaries available. Coffee and appetizers will be served during this time and the Poster Session will also be available.*

	Corner 1	Corner 2	Corner 3	Corner 4
10:00-10:10	SE_Iowa	SE South Dakota	NE_Illinois	Quebec, Canada
10:10-10:20	Buchanan Co.	NW_Iowa	Ohio	Ontario, Canada
10:20-10:30	SW_Iowa	MN N212	Pennsylvania	Sonora, Mexico
10:30-10:40	SE_Iowa	MN N212	Ohio	Quebec, Canada
10:40-10:50	Buchanan Co.	SE South Dakota	Pennsylvania	Ontario, Canada
10:50-11:00	SW_Iowa	NW_Iowa	NE_Illinois	Sonora, Mexico

11:00am	Tim Loula, SVC: PRRS ARC & beyond. Applying the principles of PRRS control, maximizing immunity – minimizing transmission, what are we learning?
11:30am	Dale Polson, BI: What's next for ARC? Where we've been and where we are heading
12:00pm	*Lunch: 5th Floor Renaissance Ballroom*

Afternoon Session Sponsored by the National Pork Board

7th Floor Grand Ballroom

The Transfer and Application of Knowledge from the Laboratory to the Field

1:00pm	Dick Hesse, Kansas State: Porcine epidemic diarrhea virus (PEDV) is here--ready or not
1:30pm	Jeff Zimmerman, Iowa State: PRRSV surveillance: The power of (good) data
2:00pm	Derald Holtkamp, Iowa State: Development of a porcine reproductive and respiratory syndrome outbreak investigations program

2:30pm	Kay Faaberg, NADC, ARS, USDA: Multifunctional role of PRRSV nonstructural protein 2
3:00pm	*Break: 7th Floor Empire Ballroom*
3:30pm	Bob Rowland, Kansas State: Not all PRRSV antibodies are created equal
4:00pm	Michael Veit, Free University Berlin: Processing of Gp5 and Gp3 of arteriviruses
4:30pm	Thomas Burkey, University of Nebraska, Nutritional management of PRRS
5:00pm	Educational Sessions Adjourn
5:30pm	Special speakers sponsored by Zoetis: *Lobby Level- Valencia Prefunction*
6:30pm	Mixer and poster session sponsored by Zoetis: *Lobby Level- Seville Ballroom and Valencia Room*

Sunday, December 8

Sunday Session Sponsored by Zoetis

7th Floor Grand Ballroom

Virus/Host Interactions in PRRS Diseases

6:30am - 8:00am	*Breakfast: 5th Floor Renaissance Ballroom*
8:00am	Paul Yeske, Swine Vet Center: Evaluation of airborne shedding of Fostera PRRS MLV vaccine from growing pigs raised under commercial conditions
8:30am	John Harding, Univ. of Saskatchewan: Predictors of fetal survival following PRRSV challenge
9:00am	Jay Calvert, Zoetis: Interferon-alpha responses following PRRS vaccination and challenge
9:30am	Dongwan Yoo, Univ. of Illinois: Type I IFN response during PRRSV infection

10:00am	*Break: 7th Floor Empire Ballroom*
10:30am	XJ Meng, Virginia Tech: Novel approaches for PRRSV vaccine development
11:00am	Michael Murtaugh, Univ. of Minnesota: New findings in anti-PRRS immunity
11:30am	Speaker Panel: Open Forum Q&A
12:00pm	Adjourn

ABSTRACTS
2013 Speakers

POSTER ABSTRACTS

Detection, Diagnostics and Surveillance

Host response to infection, including host genetics and innate/adaptive immunity

Vaccination strategies and therapeutics

Virus structure and gene function

Virus transmission, biosecurity and area control

1
Nutritional management of porcine reproductive and respiratory syndrome (PRRS)

Thomas E. Burkey[1]*, G. Mastromano[1], H. Tran[1], W. Schweer[2], J.F. Patience[2], L. Karriker[2], J.Dekkers[2], C. Sparks[3], G. Gourley[4], M. FitzSimmons[5], J. Odle[6], K. Schwartz[2], N. K. Gabler[2].

[1]University of Nebraska, Lincoln, NE; [2]Iowa State University, Ames, IA; [3]Choice Genetics, Des Moines, IA; [4]Swine Graphics Enterprises, Webster City, IA; [5]MAF Veterinary Services Inc., Mapleton, MN; [6]North Carolina State University, Raleigh, NC.

The economic losses caused by Porcine Reproductive and Respiratory Syndrome (PRRS) Virus infection are estimated to cost the U.S. swine industry in excess of $650 million annually (USDA, 2008). While significant advances have been made through research efforts to enhance our understanding of PRRSV on many levels, this disease still remains a significant issue in the U.S. swine industry because there is a major limitation in our understanding of how altered health status (as a result of pathogenic challenge) changes nutrient requirements. Therefore, the overall goal of this work is to characterize the metabolic impact immune system activation and disease has on growing pigs. Specifically, the aim of this research has been to gain a better understanding of metabolic shifts associated with immune stress in order to develop management strategies to optimize pig performance in the face of disease (i.e., PRRS) challenge.

To accomplish this goal, two projects have been conducted in commercial settings using a live (field-strain) PRRS isolate. The objective of first project was to monitor and characterize the short to medium term impact of PRRS challenge on the pig's immune system, metabolism, and growth performance. The objective of the second project was to characterize the impact PRRS has on grow-finisher pig energy and nutrient digestibility, and to longitudinally assess body composition and tissue accretion rates. Briefly, results from the first project indicate that metabolomic, immune, and blood count biomarkers may be indicative of dynamic changes in energy requirements needed for pigs to maintain growth in the face of a PRRS challenge. Results from the second project clearly indicate that PRRS infection reduces growth performance (average daily gain, average daily feed intake, and feed efficiency) as well as coefficients of apparent total tract digestibility in grow-finisher gilts. Moreover, lean/protein and fat accretion rates all appear to be affected to a similar extent.

This research has afforded us the opportunity to integrate clinical and nutritional profiles of sick and healthy pigs. These data will aid in the development of intervention strategies to optimize pig performance in the face of PRRS as well as other disease challenges that will ultimately maximize industry profitability and efficiency. These projects have been supported by Iowa Pork Producers Association (12-113), National Pork Board (12-163), and Choice Genetics.

2
Interferon-alpha responses following PRRS vaccination and challenge

Jay G. Calvert[1]*, J. Marx[1], R.G. Ankenbauer[1], M.L. Keith[1], T.L. Martin[1], L.P. Taylor[1], D.S. Pearce[1]. M.C. Lenz[1], B. Ashton[1], P. Hoogeveen[2].
[1]Zoetis Inc., Kalamazoo, MI; [2]Zoetis Inc., Madison, NJ.

Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly infectious RNA virus which causes abortions and stillborn/mummified fetuses in sows, and respiratory distress with poor growth in young pigs. PRRSV remains one of the most economically important diseases in the global swine industry. Fostera™ PRRS received a USDA product license in February 2012, and is the only PRRSV vaccine to earn the label claim "… as an aid in preventing disease associated with PRRSV, Respiratory Form". A study was designed to evaluate the protective effect of vaccinating three week old pigs with Fostera PRRS, when challenging with virulent PRRSV strain NADC20 two weeks post-vaccination. The primary variable in determining prevention of disease was mean percent lung with lesions at 10 days post-challenge. Clinical observations and body weight were analyzed as supportive data. Cytokine data were also collected as an indicator of the immune response. The Fostera PRRS vaccinated group had a significantly lower ($P = 0.0177$) mean percent of lung involvement than the mock-vaccinated control group. In addition, significant differences ($P = 0.0001$) were seen in body weight at the day of necropsy (day 24) when comparing the Fostera PRRS vaccinated group to controls. Abnormal clinical signs were also reduced in the vaccinated group compared to the mock-vaccinated control group, though statistical comparisons were not made. In addition, interferon-alpha levels were significantly elevated ($P = 0.0001$) in the Fostera PRRS vaccinated group compared to mock-vaccinated controls just before challenge (day 13) and the vaccinated group avoided a high spike (>1000 pg/mL) in interferon-alpha levels that correlated with clinical disease following challenge with the virulent NADC20 strain ($P < 0.0001$). The data demonstrate that Fostera PRRS vaccination of three week old pigs helps provide a protective effect against challenge 14 days later. This protective effect is likely due to a combination of acquired and innate immunity. All experiments involving animals were carried out in compliance with national legislation and subject to review by the local Institutional Animal Care and Use Committee (IACUC).

3
A diagnostic investigation of the frequency, dose and diversity of airborne PRRSV surrounding filtered breeding herds

Scott A. Dee
Pipestone Applied Research, Pipestone Veterinary Clinic, Pipestone, MN.

Introduction: Air filtration is applied to commercial sow units to reduce the risk of airborne PRRSV (1). Despite data on airborne transport of PRRSV (2), no information currently exists on the dynamics of airborne challenge of PRRSV in the environment. The specific aims of this study were to estimate the frequency of airborne PRRSV challenge to filtered farms, calculate the quantity of virus in positive air samples and describe the diversity of surrounding viral populations.
Methods: Four filtered breeding herds were selected based on owner's willingness and location (NW IA, E SD, NW MN and E IA). From October 15-December 15 2012 and June 1-30 2013, daily air samples were collected outside of the facility from 7:00-7:30 AM each day. A liquid cyclonic collector was placed 30 m from the facility in the direction of the daily prevailing wind (2). All samples were screened for PRRSV RNA by PCR. All positive samples were quantified by virus titration and sequenced (ORF 5).
Results: *October-December 2012 sampling.* Across all 4 sites, 37% (80/217) of the air samples were PCR positive with mean frequencies ranging from 29% (E SD) to 42% (NW IA and E IA). Viral titers ranged from 1 x $10^{3.8}$ to 1 x $10^{5.1}$ $TCID_{50}$/mL. Titers were significantly higher in samples collected around the NW IA site versus the other 3 sites ($p < 0.0001$). ORF 5 sequencing indicated the presence of several novel, highly diverse variants around the NW IA and E IA farms in contrast to previously identified "local" variants around the SW MN and E SD sites. *June 2013 sampling:* A total of 100 samples were collected during this period. PRRSV viral RNA was detected in 11% of the samples only across 2 sites (NW IA and SW MN, 8% and 3% respectively). In contrast to the previous sampling period, viable virus was not detected. ORF 5 sequences indicated the presence of both previously recognized variants and novel variants.
Conclusions: This is the first attempt to describe the degree of airborne PRRSV challenge in the environments surrounding filtered sow farms. Based on a limited dataset, airborne challenge may be significant and seasonal in nature. These observations support the seasonal variability in PRRS outbreaks as reported by Tousignant and others (personal communication).
References

1. Dee SA et al. Evaluation of the long-term effect of air filtration on the occurrence of new PRRSV infections in large breeding herds in swine-dense regions. *Viruses*. 2012; 4:654-662.
2. Otake S et al. Long distance airborne transport of viable PRRSV and Mycoplasma hyopneumoniae from a swine population infected with multiple viral variants. *Vet Microbiol* 2010; 145: 198-208.

4
Multifunctional role of porcine reproductive and respiratory syndrome virus nonstructural protein 2

Kay S. Faaberg[1]*, M. K. Deaton[2], M. A. Kappes[1], A. A. Spear[1], K. M. Lager[1], S. D. Pegan[2].

[1]Virus and Prion Research Unit, USDA-ARS-National Animal Disease Center, Ames, IA;
[2]Department of Chemistry and Biochemistry and Eleanor Roosevelt Institute, University of Denver, Denver, CO.

Nonstructural protein 2 (nsp2) of PRRSV is the most variable region of the virus and consists of four discrete domains. One region is the N-terminal papain-like protease 2 (PLP2) domain, shown to be a member of a large family of mammalian deubiquitinating proteins (OTUs). Other identified domains are the long hypervariable region, a transmembrane-spanning region and a relatively conserved C-terminus. To further explore the functions of nsp2, three separate studies were completed.

The PLP2 domains (vOTU) of two PRRSV strains were examined for their comparative abilities to cleave different forms of ubiquitin and ubiquitin-like ISG15, rendering them capable of interfering with innate immune responses via dysregulation of ubiquitin (Ub) or ISG15 related protein signaling. PRRSV strain differences were revealed, as the vOTU expressed domain from the PRRSV strain causing overt clinical disease was markedly more active towards K63 linked polyubiquitin species. In contrast to other vOTUs and previous findings with PRRSV strains by others, no robust cleavage of mature hISG15 conjugates or proISG15 from human and porcine sources were observed. These results suggest that protein deubiquitination and proISG15 cleavage is viral strain specific.

Ultrapurified virions of PRRSV strains were also analyzed. Immunoelectron microscopy showed that nsp2 was incorporated into virus particles, as shown using anti-peptide antibodies to the PLP2 region or to the hypervariable domain. Western blot analysis, using antibodies to three nsp2 regions as well as an antibody to a c-*myc* epitope engineered into the hypervariable region of PRRSV, showed that virions contain isomers of nsp2 that vary in size depending on virus. These studies reveal that nsp2 is part of the virus (a structural protein) and can interact with the host cell during the initial stages of infection as multiple isoforms.

Last, we explored the capacity for PRRSV mutants, with deletions in the nsp2 hypervariable region replaced by specific immunogenic peptides (tags), to successfully replicate in swine. Mutant viruses were examined for viral growth kinetics and appearance of anti-PRRSV antibodies. We found most tagged viruses were stable in swine, but some were compromised in their ability to replicate. Our findings suggest that exact placement of immunogenic epitopes in nsp2, as well as the specific immunogenic epitope, have a profound ability to alter the kinetics of replication in swine.

5
Predictors of fetal survival following PRRSV challenge

John Harding[1]*, A. Ladinig[1], C. Ashley[1], J. Lunney[2], G. Plastow[3].
[1]University of Saskatchewan, Saskatoon, SK, Canada; [2]USDA, ARS, BARC, APDL, Beltsville, MD, USA; [3]University of Alberta, Edmonton, AB, Canada.

Objective

The objective of this study was to determine viral and immunological predictors of fetal death in pregnant gilts infected with PRRSV.

Methods

Purebred Landrace gilts, synchronized and bred homospermically to York boars, were experimentally infected with type 2 PRRSV (NVSL 97-7895, 10^5 TCID50 total dose) at gestation day 85 (n=114), or were sham-inoculated (CTRL; n=19). Blood, collected on 0, 2, 6, and 19 or 21 days post inoculation (dpi), provided samples for measurement of PRRSV RNA concentration using a strain-specific in-house qPCR, leukocyte counts, and lymphocyte subset typing (T-helper, CTL, B-cells, gamma-delta cells, NK cells, myeloid cells) using flow cytometry. The *in vitro* responsiveness of PBMC to homologous PRRSV stimulation were assessed by testing supernatants for innate, regulatory, T helper 1 (Th1) and Th2 cytokines/ chemokines by fluorescent microsphere immunoassay (FMIA; IL-1b, IL-8, CCL2, IFN-alpha, IL-12, IL-4 and IL-10) or ELISA (IFN-gamma). Additionally, serum levels of the same cytokines/chemokines were evaluated. Gilts were humanely euthanized 21dpi and the fetal preservation recorded. Multiple tissues were collected from each gilt enabling measurement of PRRSV RNA concentration in lung, tonsil, tracheobroncheal and reproductive LN. Multilevel modeling was used to determine significant treatment group differences in viral load, cytokine levels and PBMC subsets. Additional analyses were performed in inoculated gilts to determine if viral load, PBMC subsets and cytokine levels that differed significantly by group were associated with fetal mortality rate.

Results

Levels of IFN-alpha, CCL2 and IFN-gamma in serum, and IFN-alpha and IL-8 in supernatants of PRRSV stimulated PBMC differed significantly over time between inoculated and control gilts. Levels of IFN-alpha in serum on 2 dpi were positively associated with fetal mortality rate, as were levels of IFN-alpha produced by PRRSV stimulated PBMC collected on 2 and 6 dpi. Fetal mortality decreased as the numbers of T helper cells (represented as the sum of Th cells over the 19 dpi or area under the curve (AUC)) increased. Interestingly, absolute numbers of T helper cells and NK cells measured on 0 dpi (pre-inoculation), may be predictors and negatively related to fetal mortality ($0.05 > P < 0.1$). Viral load in reproductive lymph node, but not other tissues or sera, was positively related to fetal mortality rate.

Conclusion/Discussion

Fetal mortality was positively related to levels of serum IFN-alpha and PRRSV RNA concentration in reproductive lymph node. By contrast, fetal mortality was negatively related to T helper cell counts. These three parameters should be further investigated to determine their potential roles in the mechanisms of fetal death.

Acknowledgements

This research was enabled by funding was provided by Genome Canada, Genome Alberta and Genome Prairie. Pregnant gilts were generously supplied by Fast Genetics Inc., Spiritwood, SK.

6
Porcine epidemic diarrhea virus (PEDV) is here--ready or not

Dick Hesse
Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS.

Porcine epidemic diarrhea (PED) is a new disease to North America. It is caused by a corona virus known as porcine epidemic diarrhea virus (PEDV). The clinical presentation of PED is very similar to that caused by another coronavirus, transmissible gastroenteritis virus (TGEV) which is serologically distinct from PEDV. Severity of clinical disease caused by PEDV tends to be age related. Infection of nursing pigs usually results in extremely high mortality approaching 100% due to the malabsorption diarrhea as a result of enterocyte destruction in the small intestine. Infection of grow/finish animals results in high morbidity and low mortality with vomiting and mild to moderate diarrhea as the clinical presentation. Infection of mature swine is often overlooked due to minimal or no clinical disease.

PED was first observed in Europe in 1971 and has subsequently spread across the world. Especially severe disease was observed in Korea, Japan, and the Philippines and most recently China. There appears to be significant genetic divergence of the virus and it has been reported that vaccines based on the European virus no longer protect against the Asian strains. The PED epidemic in the United States exhibits very high viral homology with Chinese PEDV strains.

PED was first observed in the United States in April of 2013; since then it has spread to18 states with >800 diagnostic laboratory accessions testing positive for PEDV. Within days of confirmation of the presence of PEDV in the US, the swine industry rallied to combat this new disease. The American Association of Swine Veterinarians (AASV), the National Pork Board (NPB), the USDA, and several veterinary diagnostic laboratories (VDL) have all worked closely together to track the virus, to fund and conduct research, and develop diagnostic and management tools that allow the swine industry to combat PED.

KSVDL was awarded an NPB funded grant that was directed towards understanding of the PEDV infectious process and the generation of diagnostic samples. Preliminary pig infection data demonstrate prolonged fecal, nasal and oral fluid shedding, presence of viremia, lack of aerosol transmission, and the kinetics of antibody response following oral/nasal inoculation of naïve four week old pigs. In addition, over 1200 diagnostic samples have been provided to requesting diagnostic laboratories for assay development and standardization.

Numerous laboratories are presenting preliminary research results that were generated from the funding provided by the NPB and AASV. In spite of this rapid progress, there is much to learn about PED in the US: comparisons and contrasts of field and research data, future research needs and current weaknesses in tracking foreign origin viruses will be discussed.

7
Development of a porcine reproductive and respiratory syndrome outbreak investigations program

Derald J. Holtkamp[1]*, S. Radke[1], C. Mowrer[1], R.B. Baker[1], J. McKean[1], R. Main[1], D. Polson[2], J.P. Cano[2].
[1]Department of Veterinary Diagnostics and Production Animal Medicine, Iowa State University, Ames, Iowa, USA; [2]Boehringer Ingelheim Vetmedica Inc., St. Joseph, Missouri, USA.

A 2011 estimate of the cost of productivity losses attributed to porcine reproductive and respiratory syndrome virus (PRRSV) is over $664 million annually[1]. Approximately $300 million (45%) of the 2011 estimate is attributed to reproductive losses in the breeding herd. Furthermore, breeding herds in which PRRS outbreaks occur serve as a major source of virus since they typically wean PRRS virus positive pigs for several weeks after the outbreak. Therefore, the objective of this project is to establish a PRRSV outbreak investigations program for breeding herds with the aim of reducing the geographic spread of the virus and the duration of time breeding herds remain positive unstable (AASV category I) [2] following outbreaks. The program is being piloted in the Buchanan County (13 herds) and Southeast Iowa (6 herds) regional PRRSV projects in Iowa. Data collection began June 1, 2013 and includes five years of historical information on immunization protocols, PRRSV elimination events, PRRS outbreaks, PRRSV status changes, diagnostic and virus sequencing information, and Production Animal Disease Risk Assessment Program (PADRAP) surveys. As PRRS outbreaks occur, weather data six weeks prior to the outbreak will be compiled and summarized. A pre-investigation report that includes all of the relevant historical information on the herd and the weather data will be compiled by a PRRS outbreak investigations coordinator for the herd veterinarian who will conduct the outbreak investigation and complete a post-investigation report. Herds experiencing outbreaks will be monitored to determine progress toward stabilization (AASV category II) [2].

Outbreak investigations help veterinarians and producers better understand how the failure in bio-exclusion occurred. The investigations provide an opportunity for immediate feedback after an outbreak to identify areas of weakness, improve biosecurity and reduce the frequency of PRRS outbreaks in the future. The PRRSV outbreak investigations program enables veterinarians and producers to systematically observe and gather information to see associations and patterns when bio-exclusion failures and PRRS outbreaks occur and to more rapidly "learn from our mistakes." The PRRS outbreak investigations coordinator will complete the time consuming task of compiling all of the information and generating reports for the herd veterinarians which will greatly increase the likelihood that the outbreak investigations will be done in a timely manner. Success will be measured by comparing the historical frequency of outbreaks in the breeding herds in the pilot project to the frequency of outbreaks after the outbreak investigation program is implemented.

References

1) Holtkamp D.J., Kliebenstein J.B., Neumann E.J., Zimmerman J.J., Rotto H., Yoder T.K., Wang C., Yeske P., Mowrer C. Haley C. 2013. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. J. Swine Health Prod. 21:2 72-84.
2) Holtkamp D.J., Polson D.D., Torremorell M. and committee members Morrison B. (chair), Classen D.M., Becton L., Henry S., Rodibaugh M.T., Rowland R.R., Snelson H., Straw B., Yeske P., Zimmerman J. 2011. Terminology for classifying swine herds by PRRS virus status. J. Swine Health Prod. 19:1 44-56.

8
PRRS control and eradication options for breed to wean farms

Clayton Johnson[1] DVM, J.P. Cano[2] DVM, PhD.
[1]The Maschhoffs, LLC, Carlyle, Illinois; [2]Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri.

The swine veterinarian is charged with delivering optimal health management in a socially and economically sustainable manner. This expectation is complicated by the lack of available evidence for disease prevention, control and treatment forcing the swine veterinarian to regularly make decisions with less than ideal evidence. Never is this complication more evident than in the face of epidemic disease on a Breed to Wean (BTW) farm. A novel porcine reproductive and respiratory syndrome virus (PRRSV) introduction on a BTW farm results in dramatic performance losses that with or without intervention threaten farm and producer viability. In the face of this challenge the swine veterinarian must craft a health management plan which optimizes the client's biological and economic performance. To maximize BTW farm efficiency in the face of a novel PRRSV infection, the swine veterinarian must apply the best available evidence to their decision making process, and ultimately to their health management recommendations. This paper will communicate how The Maschhoffs health team has used best available evidence to control and eradicate PRRSV from BTW farms in our system, and specifically how we have applied learning's from the Time to Negative Pig Study conducted by the University of Minnesota. PRRS control and eradication options presented will focus on a Load, Close and Expose (LCE) model of PRRS management, with particular consideration given to two key decision points; the final goal of the PRRS management plan (PRRS Control vs. PRRS Eradication), and the method of BTW herd exposure (Live Virus Inoculation vs. Modified Live Virus Vaccination).

9
Trends in applied PRRSV diagnostics (2009 to 2013)

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PRRSV diagnostic tools, methodologies, and applications continue to evolve. The Iowa State University Veterinary Diagnostic Laboratory's (ISU VDL) proximity and service to veterinarians and pork producers throughout the United States provides a reasonable view into the changing landscape of PRRSV diagnostic tools and how they are being applied in North America. The objective of this update is to share some of the more significant trends in PRRSV diagnostics observed over the past 5 fiscal years at the ISU VDL. The number of PRRSV PCR diagnostic assays conducted on an annual basis has increased by more than 300 percent from 2009 to 2013, while PRRSV antibody testing volumes have remained relatively unchanged. PRRSV testing is increasingly being used as a preventative medicine and/or programmatic monitoring tool to determine the PRRSV status of weaned pigs, gilt or boar replacements, and boar studs. The percentage of diagnostic specimens submitted for PRRSV testing determined to be PRRSV positive has decreased from 27 to 16 percent (PCR) and 37 to 18 percent (ELISA) over this 5 year period. Veterinary diagnostic laboratories have increased their use of commercially manufactured PRRS PCR reagents in lieu of in-house or lab-specific assays. These transitions have largely been made to enhance consistency and convenience at the laboratories. The advent and subsequent wide-spread adoption of oral fluids as a diagnostic specimen has profoundly changed PRRSV diagnostic applications in North America. Oral fluids are being used for both antigen and antibody detection purposes as well as in genetic sequencing applications to diagnose, monitor, and further characterize a growing number of swine pathogens. The number of swine oral fluid diagnostics assays conducted at the ISU VDL has grown from less than 5,000 in 2010, to more than 70,000 in 2013. Increased interest in PRRSV area regional control efforts and an associated increased use of PRRSV ORF5 genetic sequencing data as a tool for monitoring the movement and diversity of PRRSV across regions and production systems over time has been another notable trend in recent years. Significant improvements have and continue to be made to the diagnostic assays, laboratory procedures, and the quality of the diagnostic systems that are putting these tools into practice at veterinary diagnostic laboratories. As one such example, all of the core PRRSV diagnostic assays and methods currently being used at the ISU VDL are either new or have been significantly modified since November 2010. Collaborative efforts are being made to develop streamlined systems that link participating veterinary diagnostic laboratory submissions, corresponding test results, and an interpreted health status of farm sites to spatiotemporal disease management tools for use in area-regional, veterinary clinic, or production system specific PRRSV monitoring and control initiatives. One differential diagnostic technology gap that remains is the inability to use antibody based testing to differentiate pigs vaccinated with modified-live PRRSV vaccine from those infected with wild-type PRRSV. Molecular techniques are presently used for DIVA diagnostic purposes.

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Novel approaches for PRRSV vaccine development

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Synthetic attenuated virus engineering (SAVE) is an emerging technology that enables to rapidly attenuate viruses. Among the synonymous codon pairs encoding the same amino acid residues, some codon pairs occur more frequently than others in certain species, which is known as codon-pair bias. Different species have different codon-pair biases, which is related to the efficiency of the protein synthesis and affects the accumulation of synthesized proteins inside cell. The principle of the SAVE is that codon-pair deoptimization of key viral gene(s) decreases the protein expression levels of the corresponding gene(s) that are related to viral virulence. By using SAVE we demonstrated rapid attenuation of PRRSV. The gp5 and nsp9 genes of PRRSV were each codon-pair deoptimized aided by a computer algorithm. Two codon-pair deoptimized viruses, SAVE5 with deoptimized gp5 gene and SAVE9 with deoptimized nsp9 gene, were successfully rescued *in vitro*. The SAVE5 and SAVE9 viruses both replicated at a lower level *in vitro* with a significant decrease of corresponding protein expression compared to the wild-type VR2385 virus. Pigs experimentally infected with the SAVE5 virus had significantly lower viremia level up to 14 days post-infection and significantly reduced gross and histological lung lesions when compared to wild-type VR2385 virus-infected pigs, indicating attenuation of the SAVE5 virus. The results proved the feasibility of rapidly attenuating PRRSV by SAVE.

Molecular breeding through DNA shuffling is another emerging technology for rapid vaccine development. Since GP3, GP4 and M genes of PRRSV induce neutralizing antibodies, we molecularly bred PRRSV through DNA shuffling of the GP3, GP4 and M genes, separately, from six genetically different strains of PRRSV in an attempt to identify chimeras with improved cross-neutralizing capability. The shuffled GP3, GP4 and M genes libraries were each cloned into the backbone of PRRSV infectious clone pIR-VR2385-CA. Four traditional-shuffled GP3 chimeras each representing all 6 parental strains and four other synthetic-shuffled chimeras were successfully rescued. One GP3 chimera GP3TS22 induced significantly higher levels of cross-neutralizing antibodies in pigs against a heterologous PRRSV strain FL-12. Additionally, three GP4-shuffled chimeras and five M-shuffled chimeras, each representing sequences from all six parental strains, were also further characterized *in vitro* and in pigs. The GP4-shuffled chimera GP4TS14 induced significantly higher cross-neutralizing antibodies against heterologous strains FL-12 and NADC20, and similarly that the M-shuffled chimera MTS57 also induced significantly higher levels of cross-neutralizing antibodies against heterologous strains MN184B and NADC20 in infected pigs. These GP3, GP4 and M-shuffled chimeric viruses showed similar levels of replication with their backbone strain VR2385 both *in vitro* and *in vivo,* indicating that the DNA shuffling of GP3, GP4 and M genes did not significantly impair the replication ability of these chimeras. The results suggest that DNA shuffling of the GP3, GP4 or M genes from different parental viruses can broaden the cross-neutralizing antibody-inducing ability of the chimeric viruses against heterologous PRRSV strains.

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New findings in anti-PRRS immunity

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Vaccination is the principal means used to prevent, control and treat porcine reproductive and respiratory syndrome virus (PRRSV) infection. An array of PRRS vaccine products is available to swine producers. However, it has been surprisingly difficult to dissect the specific elements of the anti-PRRSV immune response that are required for protective immunity. Key limitations include (a) uncertainty about the viral targets of protective immunity that prevents a research focus on individual viral structures and proteins, and frustrates efforts to design novel vaccines; (b) inability to establish clear immunological correlates of protection that requires laborious animal infection models for evaluation of protection against challenge; and (c) the great genetic diversity of PRRSV which requires that challenge experiments be interpreted cautiously since it is not possible to predict how immunological protection against one isolate will translate to broadly cross-protective immunity. Economically significant levels of cross-protection that are provided to a variety of field isolates still cannot assure that effective protection will be conferred to isolates that might emerge in the future. These enormous gaps in our understanding of porcine immunological mechanisms and functions that are necessary for protection of pigs against PRRS pose substantial barriers to new PRRSV vaccine development. Despite these impediments, new findings from producers and veterinarians in the field and scientists in the laboratory are providing new insights into the virus and the pig's immune defenses. Therefore, we are optimistic that a better understanding of PRRS immunity is underway and can be translated into improved vaccines.

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Not all PRRSV antibodies are created equal

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The different outcomes following PRRSV infection are a consequence of a complex set of interactions between the virus and the pig host. The acute phase of viremia, which covers approximately 28 days, is followed by periods of virus rebound, a result of the capacity of the virus to escape host defenses. The mechanistic basis for immunological escape is based on a combination of factors, including; 1) a complex virion structure that possesses a heavily glycosylated surface, 2) the re-direction of the humoral response towards non-surface structural proteins and non-structural proteins, 3) antigenic and genetic drift, and 4) the subversion of interferon and other innate pathways. As a result, the antibody response against the major surface component, the GP5-M heterodimer, is weak and delayed. In fact, some animals fail to mount any detectable antibody response against GP5. The weak response is linked to the presence of several N-linked glycosylation sites located within a short ectodomain region flanked by hypervariable peptide sequences. Among putative viral receptors, CD163 appears to be the major receptor, which forms an interaction with the GP2,3,4 heterotrimer. Other cell proteins may function as co-receptors. Putative linear neutralization epitopes have been identified in GP2, GP3, GP4, GP5 and M. Using a traditional virus neutralization (VN) assay, we analyzed over 1,200 serum samples from experimentally infected pigs for VN activity against four different isolates. Overall, there was an inverse correlation between virus load and homologous VN titer. Based on the breadth of VN titer, samples could be placed into one of the following groups: Group 1, no VN; Group 2, homologous VN against only the isolate used for infection; Group 3, heterologous VN with reactivity against 1 or 2 additional isolates; and Group 4, broad VN with activity against all four isolates. By definition, we call the Group 4 sera broadly neutralizing antibody (bnAb). Further analysis of bnAb showed that some serum samples were able to neutralize up to 11 different isolates, including a Type I isolate. In addition to an increased breadth of VN activity, bnAb possessed other unique properties. For example, unrelated viruses made resistant to bnAb retained the ability to be neutralized by homologous serum, and vice versa; viruses made resistant to neutralization by homologous serum retained the capacity to be neutralized by bnAb. These data support the notion that Group 2 (homologous) and Group 4 (bnAb) sera recognize different neutralization epitopes. Furthermore, pigs that produced bnAb exhibited a lower level of viremia, which persisted over an extended period of time, compared to pigs that produced only homologous VN. Together, these results have important implications in the development of the next generation of vaccines, including the presentation of neutralizing epitopes and recruitment of bnAb. This work was supported by National Pork Board Grant NPB#12-120, “Characterization of neutralizing antibody responses to PRRSV and association with host factors”.

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Processing of Gp5 and Gp3 of arteriviruses

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The envelope of arteriviruses contain two glycoprotein spikes, Gp5/M and Gp2/3/4, which are in cells retained in the ER or early-Golgi, the assembly site of virus particles. Reverse genetic experiments indicate that Gp5/M is required for virus budding and Gp2/3/4 for cell entry. Despite their pivotal role for virus replication and as target for antibodies only little information is available on the structure and processing of these glycoproteins.

Gp5, the major glycoprotein, is also an important target of neutralizing antibodies during PRRSV infection, which however appear only late in infection. This was attributed to the presence of a "decoy epitope" located near a hypervariable region of Gp5. This region also harbors the predicted signal peptide cleavage sites and (dependent on the virus strain) a variable number of potential N-glycosylation sites.

Here we report that the signal peptide of Gp5 from the American type II reference strain VR-2332 is cleaved, both during in vitro translation in the presence of microsomes and in transfected cells. This was found to be independent of neighboring glycosylation sites and occurred in a variety of porcine cell types for Gp5 sequences derived from various type II PRRSV strains. The exact signal peptide cleavage site was elucidated by mass spectrometry of virus-derived and recombinant Gp5. The results revealed that the signal peptide of Gp5 is cleaved at two sites. As a result, a mixture of Gp5 proteins exists in virus particles, some of which still contain the "decoy epitope" sequence. Heterogeneity was also observed for the use of glycosylation sites in the hypervariable region. Lastly, Gp5 mutants were engineered where one of the signal peptide cleavage sites was blocked. Wildtype Gp5 exhibited exactly the same SDS-PAGE mobility as the mutant that is cleavable at site 2 only. This indicates that the majority of all GP5 molecules does not contain the "decoy epitope".

We also report two unique findings for processing of glycoprotein 3 of equine arteritis virus (EAV). Gp3 contains an N-terminal signal peptide, which is not removed although bioinformatics predicts cleavage with high probability. There is an overlapping sequon NNTT adjacent to the signal peptide, which we show to be glycosylated at both asparagines. Exchanging the overlapping sequon and blocking glycosylation allows signal peptide cleavage, indicating that carbohydrate attachment inhibits processing of a potentially cleavable signal peptide.

Our studies also led to a new model for the membrane topology of Gp3. Membrane fractionation and secretion experiments revealed that the signal peptide of Gp3 does not act as a membrane anchor indicating that it is completely translocated into the lumen of the ER. Membrane attachment is caused by the hydrophobic C-terminus of Gp3, which however does not span the membrane, but rather attaches the protein peripherally to membranes.

A sequence comparison shows that all the Gp3 variants from various PRRSV strains contain one glycosylation sequon adjacent to the signal peptide suggesting that they follow the same unique processing scheme and exhibit the same membrane topology as Gp3 from EAV.

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Evaluation of airborne shedding of Fostera PRRS MLV vaccine from growing pigs raised under commercial conditions

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Vaccinating these pigs has shown to improve performance when challenged with field virus[1,2]. Even though previous studies demonstrated limited airborne shedding[3] there is still concern with the use of PRRS MLV that it will shed and spread to other herds.

Objectives:

Identify the amount and duration of aerosol shedding of Fostera PRRS MLV inside a commercial wean to finish barn, outside the barn directly outside the pit fan and 1 mile down wind of the barn.

Procedures to achieve Objectives:

1. Pigs in room one were vaccinated with Fostera PRRS MLV labeled dose at 4 weeks of age. Pigs in the other room were not vaccinated.
2. Air samples were tested for PRRS PCR.
3. Liquid cyclonic collectors were used to collect daily air samples for 28 days.
4. 30 sentinel pigs per room were tested periodically by PRRS PCR and ELISA.

Results:

No virus was detected in any of the air samples collected either in the barn, outside the pit fan or 1 mile in the down wind direction. Viremia was detected in vaccinated pigs. PRRS virus was detected in oral fluids post-vaccination.

Discussion:

Vaccine virus was not found in air samples at all 3 collection sites. There was no spread via aerosol that can be detected. The non-vaccinated control room turned positive following pit pumping. Implication would be less likely to have virus spread following vaccination pigs in wean to finish sites.

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Type I interferon response during PRRSV infection

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The innate immunity is the first line of defense against viral infection and in turn viruses have evolved to evade the host immune surveillance system. Host cells produce cytokines and chemokines in response to viral infection, and among such effector molecules, type I interferons (IFN-α/β) are the most potent antiviral cytokines triggered by viral infection. Cells infected with PRRSV however, do not efficiently produce type I IFNs, and in PRRSV-infected pigs, IFNs are not readily detectable in the lungs where PRRSV actively replicates by infecting alveolar macrophages. PRRSV may persist for a prolonged period in infected pigs, suggesting that the virus may carry an ability to modulate the IFN response during infection. Identification and characterization of viral proteins responsible for host cell IFN modulation may be a key to the successful control of infection. PRRSV expresses a total of 24 proteins of which 16 are non-structural proteins (nsp's) and 8 are structural components of the virus. We have screened individual viral proteins for their IFN modulatory activities and have identified four non-structural (nsp1α, nsp1β, nsp2, and nsp11) and one structural (N, nucleocapsid) proteins as viral antagonists for IFN production. Among these, nsp1α and nsp1β are two potent IFN regulators, whereas N is the NF-κB stimulator. While PRRSV replication takes place in the cytoplasm and does not require the cellular nuclear function, nsp1α, nsp1β, and N proteins were found to specifically localize in the nucleus and nucleolus, suggesting an important accessory role of these proteins in the nucleus during infection. Nsp1α degrades CREB-binding protein (CBP) in the nucleus and thus inhibit the formation of enhanceosome resulting in the suppression of IFN production. Nsp1β has been found to block the nuclear translocation of ISGF3 (interferon stimulated gene factor 3), and thus both nsp1α and nsp1β down-regulate the IFN response. Nsp2 is a viral protease and possesses ovarian tumor domain-containing deubiquitinase activity. It deconjugates ubiquitin and an ubiquitin-like modifier ISG15 from cellular targets and modifies the host antiviral response. Nsp11 is a viral endoribonuclease and has been shown to degrade the mRNA of the mitochondria antiviral-signaling (MAVS) protein [also known as VISA (virus-induced signaling adapter), IPS-1 (IFN promoter stimulator); Cardif.], resulting in inhibition of the IFN signaling. Such findings highlight that PRRSV has evolved the general plasticity of RNA virus genomes capable of coding for various multifunctional proteins providing selective advantages to evade the rigorous host innate immune defense.

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PRRSV surveillance: The power of (good) data

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In the 20th century, pig production metamorphosed from small, extensive, labor-dependent enterprises into intensive, capital-dependent, multi-site production systems. These changes produced safe, wholesome, inexpensive pork for consumers. Ironically, the same changes that led to improved efficiencies, i.e., large herds with high throughput and extensive pig movement between sites, left the industry vulnerable to the rapid dissemination of infectious diseases, including PRRSV. Under these circumstances, infectious diseases cannot be controlled without a regional perspective that integrates continuous and effective disease surveillance. Effective surveillance, however, must be able to account for the unique features of populations on swine farms:

Subpopulations: Animals on farms are spatially separated by age, production stage and/or function, with little interaction between these subpopulations. As a consequence, subpopulations may differ in disease status.

Extensive, dynamic population turnover: "Population change" is defined as "natural change" (total births minus total deaths) plus migration into (+) or out of (–) the population. This change is typically small (1-3%) in populations of humans. In contrast, a finishing barn will experience ~250% annual population change as groups of animals enter into the facility, grow, and are sent to market. In sow herds, ~40% of females in sow herd populations are replaced annually (PigChamp® Benchmark, 2011). This dynamic is important because of its destabilizing effect on herd immunity.

Metapopulations: Large numbers of pigs are routinely transported from distant farms to fill spaces (buildings) vacated as animals are marketed. Economically, it is more efficient to move young pigs to feed than the reverse. Epidemiologically, this practice connects "metapopulations" and facilitates the rapid movement of infectious agents across geographic spaces.

The purpose of disease surveillance is to control infectious diseases, assure animal health and welfare, improve producer profitability, and protect valuable national assets. Although serum is the traditional ante-mortem specimen, oral fluid samples have become a commonly used alternative in surveillance. A series of research and field studies have shown that pen-based oral fluids can meet or exceed the performance of serum for the detection of PRRSV using either PCR- or antibody-based assays. On the farm, integration of surveillance data with herd records could provide the means to: (1) identify PRRSV circulation; (2) quantify the effect of PRRSV on pig health and productivity; (3) target interventions to the correct population; and (4) time the intervention for maximum effect. At the regional level, oral fluid-based surveillance would make producer-driven PRRSV area control programs more practical and affordable.

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17
Comparison of two commercial enzyme-linked immunosorbent assays and fluorescent microbead immunoassays for detection of antibodies against porcine reproductive and respiratory syndrome virus in serum and oral fluids

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Accurate and early PRRSV diagnosis is critical to accomplish effective PRRSV control. PRRSV surveillance is particularly important and widely utilized in boar studs to minimize the risk of transmitting PRRSV through semen to breeding herds. The objective of this study was to compare the ability of two commercial enzyme-linked immunosorbent assays (ELISAs) and an *in-house* fluorescent microbead immunoassay (FMIA) to detect IgG antibodies against porcine reproductive and respiratory syndrome virus (PRRSV) types 1 and 2 isolates in serum and oral fluids from boars infected experimentally with PRRSV. Samples from uninfected control pigs and PRRSV-negative field samples were also used. Serum samples were tested by IDEXX-Se ELISA; HIPRA-Se ELISA; and an *in-house* FMIA-Se for detection of PRRSV types 1 and 2. Oral fluids were tested by IDEXX-SO ELISA and IDEXX-OF ELISA (for both PRRSV type 1 and 2); and HIPRA-OF ELISA (for type1). Among the sera, IDEXX-Se and HIPRA-Se had similar sensitivity and specificity ($p > 0.05$); however, IDEXX-Se detected positive animals earlier than HIPRA-Se ($p < 0.05$). FMIA-Se had the highest false-positive rates in known negative field samples (1/205 for IDEXX-Se, 5/205 for HIPRA-Se, and 37/205 for FMIA-Se; $p < 0.01$). Serum and oral fluid samples had similar detection rates and antibody kinetics using the IDEXX tests. There was a higher detection rate in serum than oral fluid using the HIPRA assays. In summary, IDEXX-Se had the earliest detection rates among the assays compared in this study, with similar specificity and sensitivity to HIPRA-Se. The FMIAs used in this study are not suitable for diagnostic usage at this point due to the low sensitivity and specificity. Under the conditions of this study, serum and oral fluid samples had similar detection rates and antibody kinetics using the IDEXX tests, and a higher detection rate in serum than oral fluid by HIPRA assays.

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Using litter oral fluids from weanling pigs to monitor PRRS status in commercial sow farms– field study

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Objective

The objective of this research was to assess litter OF from wean age piglets as a monitoring tool for antibody and infection status of PRRSV in sow herds.

Material and Methods

The samples were sourced from four 12,500 sow herds, considered endemically infected with PRRSV based on routine growing pig serologic monitoring. Commercial PRRSV vaccines were used in each of the 4 herds in the year prior to sampling. Gilts entering the sow farms were vaccinated with either PRRS MLV or ATP commercial vaccines. Oral fluid samples were collected from a total of 600 litters prior to weaning. Serum samples from their dams were collected 3 days after weaning. All samples were frozen at -80°C, randomized and submitted collectively for PRRSV antibodies (PRRS X3 Ab Test or PRRS Oral Fluids Ab Test (IDEXX Laboratories, Westbrook, ME)) and PRRSV (qRT-PCR and sequencing).

Results

Over all farms and samples, mean sera ELISA S/P ratios were approximately 1.00, while corresponding mean litter OF ELISA S/P ratios were approximately 4.62 (Table 1). Zero of 600 sow serum samples were positive for PRRSV by qRT-PCR. 8 of 600 litter OF samples were confirmed positive by qRT-PCR testing at two laboratories. Two of these were successfully sequenced (Orf 5) and identified as wild type PRRSV. Oral fluid S/P ratios from the PCR positive samples ranged from 1.92 to 8.83. Testing of corresponding dam sera showed S/P ratios ranging from 0.92 to 2.91 (Table 2).

Table 1. Commercial PRRS Ab kit results - sow sera and corresponding litter OF by farm and parity

Farm	n	Sow Serum s/p	Litter OF s/p
1	153	0.98	5.27[a]
2	145	1.09	5.06[a,b]
3	152	0.91	3.86[c]
4	150	0.99	4.30[b,c]

Parity	n	Sow Serum s/p	Litter OF s/p
1	169	1.04[a]	4.49
2	113	0.83[c]	4.46
3	91	1.05[a,b]	4.70
4	93	0.86[b,c]	4.51
5	57	1.21[a]	5.12
≥6	77	1.05[a,b,c]	4.82

[a] differences in superscripts identify statistical differences ($p<0.05$)

Table 2. Antibody and qRT-PCR results of the 8 sows and corresponding litters where litter OF was qRT-PCR positive

Farm	Sow parity	Litter size	Sow serum s/p	OPCR xCt	Litter OF s/p
1	1	11	0.92	34.6	7.75
1	2	9	1.75	27.2	8.83
1	1	10	2.68	35.3	6.98
2	5	11	2.14	35.0	8.26
2	5	10	2.91	29.8	4.22
3	5	11	1.11	34.1	1.92
3	1	9	2.75	28.5	8.21
3	1	11	2.61	30.1	6.84

Conclusions

Herd PRRSV monitoring programs can be improved by evaluating the dynamics of PRRSV transmission using oral fluid samples collected from litters of pigs prior to weaning. In endemically infected and/or vaccinated herds, piglet oral fluids are a useful tool for surveillance of both PRRSV exposure and shedding in sow herds.

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Development of an indirect ELISA for detection of antibodies against porcine epidemic diarrhea virus (PEDV)

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Porcine epidemic diarrhea virus (PEDV) was first identified in the U.S. in May 2013 and has since been confirmed in multiple states. PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the *Coronaviridae* family. PEDV was first recognized in the United Kingdom in the early 1970s and spread through much of Europe and Asia. Recent outbreaks with high mortality in China have been associated with new variant strains of the virus. In the U.S., PCR assays were quickly developed to detect the presence of PEDV RNA in intestinal contents or fecal material and these assays provide an important tool in control of the virus. However, well-validated, high-throughput assays to detect antibodies developed following infection would provide a valuable additional diagnostic tool for the swine industry. The first generation of indirect fluorescent antibody (IFA) serological assays are now being offered by several U.S. diagnostic laboratories but they have drawbacks including challenges propagating the virus, somewhat subjective interpretation, difficulty adapting to high throughput testing and limited ability for adaption to oral fluid testing.

In response to these needs, we developed a serological enzyme-linked immunosorbent assay (ELISA) based on recombinant expression of a full length PEDV nucleoprotein (NP). The NP gene was cloned and expressed as a 51 kDa, 6x His tag protein which reacted to PEDV positive sera and a 6x His-specific monoclonal antibody via immunoblotting. The test was evaluated for sensitivity and specificity for the serodiagnosis of PEDV antibodies in serum samples of known status. Known PEDV negative sample sets included samples from selected high biosecurity herds with no history of PEDV and archived serum samples collected prior to the emergence of PEDV in the U.S., including samples testing positive for the related swine coronaviruses, TGEV and PRCV. Known positive samples were collected from pigs that were naturally infected at least 3 weeks prior to collection and were previously positive by PCR. Based on samples of known serostatus (n=>600), a receiver operating characteristic (ROC) curve analysis of the ELISA results shows estimated sensitivity and specificity of over 93%. Additionally, none of the known positive TGEV or PRCV samples tested (n=>50) were shown to cross-react on the PEDV NP-ELISA.

These results indicate that the purified nucleoprotein may be a useful antigen for the serodiagnosis of PEDV and also suggest that the ELISA may be a sensitive and specific test for detecting antibodies to PEDV. This assay may prove to be of value in controlling the spread of the disease in North America, as well as in seroprevalence studies. Ongoing studies include adapting the ELISA to oral fluid and milk testing and to a fluorescent microsphere immunoassay (FMIA) format.

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Development of a new IDEXX ELISA for the detection of PRRS antibodies in swine oral fluids

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Introduction
Testing of swine oral provides a convenient and cost-effective tool for disease surveillance in commercial pig herds. Recently, detection of antibodies to PRRS in oral fluids has been described using an adapted overnight sample incubation format adaptation of the HerdChek® PRRS X3 ELISA, (IDEXX Laboratories, Inc.).[1] In this study, we describe a new PRRS oral fluids ELISA (IDEXX PRSS OF Ab Test) for same-day detection of PRRS antibodies in swine oral fluids. An S/P $\geq$ 0.40 is considered a positive result.

Materials and Methods
Sample set 1 consisted of a temporal series of pooled oral fluids from a barn of 1200 PRRSV vaccinated pigs (PRRS MLV, Boehringer Ingelheim Vetmedica Inc.).
Sample set 2 consisted of a temporal series of paired oral fluids and serum collected from individual boars 0-7 days before (negative exposures status) and 21 days after (positive exposure status) vaccination or experimental infection with type I or type II PRRSV.[2]
Sample set 3 consisted of pen-based oral fluid samples collected at various levels of prevalence (0%, 4%, 12%, 20%, and 36%) of antibody-positive vaccinated pigs introduced at 14 days post-vaccination into pens of PRRS-negative pigs.[3]

Results
A comparison between the new protocol and the standard overnight protocol (SOP) using sample set 1 indicated 100% agreement between the two tests, with average S/P values 1.4 to 1.5-fold higher than SOP. Analysis of Sample set 2 indicated a specificity of 98.7% and a sensitivity of 100% with new IDEXX PRRS OF Ab Test. A temporal analysis of oral fluids from the pigs inoculated with either type I or type II PRRSV revealed a similar ability to detect both strains. Finally, evaluation of sample set 3 indicated that the estimated probability of detecting antibody to the PRRS virus using the PRRS OF Ab Test Kit is 91% in pens with a prevalence of 20% and increased to over 99% in pens with at least 32% sero-positive pigs. These results were obtained using a statistical model with pen considered as a random effect.

Conclusion
These results describe the first commercially available test for antibodies to PRRSV, in support the emerging use oral fluids for frequent surveillance of pig herds by the swine industry.

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Presence of porcine circovirus type 2 antibodies and virus in finishing pigs after widespread use of PCV2 vaccination

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Porcine circovirus 2 (PCV2), one of the most economically important pathogens of pigs, is the causative agent of porcine circovirus associated disease (PCVAD). Widespread availability and use of PCV2 vaccines, starting in 2006, ameliorated PCVAD in finishing pigs so successfully that nearly all pigs in the US are currently vaccinated around weaning. Vaccination of piglets eliminates disease, decreases the level of PCV2 in serum, and increases production performance, but does not eliminate infection. Thus, it is possible that nearly all finishing pigs are infected with PCV2 at this time. Alternatively, widespread use of PCV2 vaccination may decrease the PCV2 viral load in pigs, thus leading to generation of PCV2-negative animals over time. The aim of this study is to examine and compare the PCV2 viral load and antibody levels in finishing pigs today, following widespread adoption of vaccination in 2007, to that of samples obtained in 2006, prior to vaccine availability. Serum samples were collected as part of the USDA NAHMS Swine 2012 study and a subset were examined for both PCV2-specific antibody levels and PCV2 viral levels. PCV2 viral loads were similar between animals on the same farm, but between farms, viral loads varied from barely detectable to low viral levels present. High viral levels were not observed in animals from any of the farms, contrary to viral loads observed in 2006 samples. PCV2 capsid-specific antibodies were present in all animals, but at lower levels than were observed in 2006. Antibodies to the PCV2 replicase protein were mainly observed at low levels, with high levels of PCV2 replicase antibodies in a small number of animals. Widespread use of PCV2 vaccines has greatly decreased, but not eliminated PCV2 virus in swine herds throughout the US. PCV2 viremia today is at low or undetectable levels in finishing pigs, whereas in 2006 high levels of viremia were observed in all finishing farms in the majority of animals. PCV2-specific antibodies remain present in the majority of animals, but at lower levels than were observed previously. Thus, widespread PCV2 vaccination has decreased the PCV2 viral load in the US finishing herd, in addition to providing solid protection against PCVAD.

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Development and validation of ELISA testing for serological monitoring and surveillance of porcine epidemic diarrhea virus exposure

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Porcine epidemic diarrhea virus (PEDV), a member of the Coronaviridae family, causes acute outbreaks of severe diarrhea and vomiting in suckling pigs leading to significant mortality. PEDV was first observed in Europe in 1971 and became endemic in Asia by 1982. Its emergence in the United States was first confirmed in May, 2013, and had been identified in 18 states as of October 6. Due to this explosive emergence, the United States has an immediate need for rapid, high-throughput diagnostic tests for serological monitoring and surveillance of PEDV. To that end, we have cloned, expressed and purified PEDV proteins to be used in an ELISA assay to detect the presence of antibodies to PEDV in swine serum. Sequences from multiple US PEDV isolates were used to design primers to clone the matrix, nucleocapsid, and spike domain 1 and 2 protein-coding genes from infected fecal samples. PCR products were then cloned into both the pET-25b and pMAL-p5X plasmid vectors for protein expression. Proteins were successfully expressed and purified for nucleocapsid and both spike domains. Proteins were used to coat plates in a direct ELISA to detect anti-PEDV antibodies present in swine serum samples. The nucleocapsid protein showed the highest reactivity with serum samples from PEDV-infected pigs. A PEDV research ELISA is now available to identify PEDV-infected serum samples. This ELISA will be further optimized for diagnostic use and can be modified for use with other sample types such as oral fluids and feces for detection and surveillance of PEDV in swine farms across the United States.

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Development of diagnostic reagents and assays for detection of porcine epidemic diarrhea virus infection

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The porcine epidemic diarrhea disease virus (PEDV) has been recently detected in the US swine herd. Rapid diagnosis of PEDV infection is critical to control the spreading of the disease. Although PEDV has been endemic in Europe and Asia, due to biosecurity reasons, diagnostic reagents and materials are difficult to import to the US. The PEDV control and prevention in the US is hindered by lacking of key reagents and validated diagnostic assays. Therefore, the objective of this study is to develop a panel of selected diagnostic reagents and assays for PEDV detection. Three PEDV antigens have expressed in *E. coli* expression system, including nucleocapsid (N), membrane (M) and spike (partial, S1) proteins. A rabbit polyclonal antibody against M protein is generated. Using the N protein, a fluorescent immunomicrosphere assay (FMIA) has been developed. Based on a preliminary evaluation of 171 serum samples from experimental infected animals, the N protein-based FMIA achieved 95.5% sensitivity and 96.5% specificity. Serum antibodies specific to N proteins were detected as early as 14 days post infection. At the meantime, parallel ELISA and immunofluorescent assay (IFA) have also been developed. Results from these three serological assays are correlated well (kappa values greater than 0.9). Currently, we are adapting our established technologies to develop a multiplex FMIA to simultaneously detect PEDV, porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV) and porcine circovirus (PCV2). This panel of reagents and assays provide important tools for PEDV diagnosis and surveillance in the US.

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An improved solution strategy to control PRRSV

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PRRSV outbreaks are feared in the swine industry because of the devastating economical impact. Even though various diagnostic tests for PRRSV including ELISA and PCR systems exist correct result interpretation with respect to the actual PRRSV status pose a major challenge. Noteworthy are the genetic diversity of PRRSV isolates, the prolonged PRRSV persistence and the complex immunological behavior of the virus. Vaccination against PRRS has shown to be an effective tool to reduce clinical disease however PRRSV infection is not prevented. Modified Live Virus (MLV) vaccine may be shed and transmitted to non-vaccinated contact pigs and vaccine virus can persist in boars and be disseminated through semen .
The complex nature of the PRRSV disease indicates that single diagnostic tests may not be enough to successfully diagnose PRRS virus but rather comprehensive solution strategies are needed for the effective control of the disease.
Here we demonstrate the reliability and robustness of the PRRSV detection tools PrioCHECK®-PRRSV RT-PCR and PrioCHECK®-PRRSV Ab porcine.
Validation studies using the PrioCHECK®-PRRSV RT-PCR showed a highly specific and sensitive detection of PRRS virus in different tissues. Most importantly, the PrioCHECK®-PRRSV RT-PCR was able to detect EU subtypes 2, 3, 4 and atypical virus isolates which are difficult to detect with other tests.
Over 1300 pig serum samples derived from different herds and countries were tested in validation studies using the PrioCHECK®-PRRSV Ab porcine ELISA system. With a calculated specificity of 99.5% and sensitivity of 96.3% as well as a high agreement to competitor ELISA systems the test was shown to be fit for field testing.
In combination with herd / site information, the results of molecular tools (RT PCR) as well as immunological detection systems (ELISA) translate to an optimized diagnostic system for sustainable PRRSV control.

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Next generation sequencing of the porcine reproductive and respiratory syndrome virus genome

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The aim of this study is to develop a next generation sequencing protocol to sequence the whole PRRS Virus genome in order to generate sequence information on multiple geographically diverse strains. Using the Ion PGM™ system in conjunction with the latest bioinformatics techniques, sequence information can be quickly generated, analyzed and compared to determine strain similarity based on different ORFs and potentially determine new regions of interest for robust assay development.

This study uses commercially available kits to isolate and purify PRRSV nucleic acid from animal sourced matrices. Techniques such as ribosomal RNA removal are then used to further enhance PRRSV RNA recovery and to remove endogenous porcine rRNA. PRRSV genome libraries are then prepared and amplified to capture all representative portions of the genome. inally, these libraries are attached to Ion Sphere™ Particles that are loaded onto a sequencing chip and placed on the Ion PGM™ for sequence generation. Bioinformaticians then analyze the data based on the researchers needs.

Although this is an ongoing project, initial results are promising. Contigs obtained from de novo assembly of sequence reads span over 99% of the PRRSV genome based on comparison to a reference sequence. Less than 2% of the sequence information generated comes from endogenous porcine origin and that is likely to be reduced as more optimization is performed. We are collaborating with labs in the US and in Europe to obtain strains that are currently in circulation. The information generated by this study will increase robustness and effectiveness of future assay designs.

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Real time RT-PCR detection of porcine reproductive and respiratory syndrome virus comparison study

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has presented a challenge to the swine industry for over a decade. The virus causes weight loss and death in pigs costing farmers and driving up pork prices. Early detection is for PRRSV is crucial, and cost effective means of accurate detections are also integral.

Three commercially available PRRSV Taqman based Real time RT-PCR including a previously developed in-house SYBR green assay were analyzed for their relative sensitivity and specificity. In this study a diverse sample set was selected to compare the multiple commercial kits' performance relative to the sample type. A total of 287 samples were tested including serum, oral fluid, and semen as well as lung, lymph node, spleen, and tonsil tissue homogenates. All samples were submitted to Animal Disease Diagnostic Lab (ADDL) for testing between 2009-2013.

Overall the findings of this study show that kits designated A, B, C, and D had average sensitivities of 90.2%, 95.9%, 96.8%, and 78.2%, with average specificities of 95.0%, 95.7%, 85.2%, and 96.8% respectively across all sample types. Furthermore kit B demonstrated highest positive predictive value (PPV), and kit C displayed the highest negative predictive value (NPV) among all the sample types.

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Development of monoclonal antibodies and other reagents for detection of porcine epidemic diarrhea virus (PEDV)

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Porcine epidemic diarrhea virus (PEDV) was first identified in the U.S. in May 2013 and has since been confirmed in multiple states. PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the *Coronaviridae* family. Due to the recent emergence of PEDV in North America, availability of specific monoclonal antibodies and hyperimmune antisera is limited. Therefore, the purpose of this study was to develop high quality, readily available reagents for detection of PEDV antigen in diagnostic tests, such as virus isolation, immunohistochemistry and fluorescent antibody techniques.

Rabbits and mice were immunized with selected recombinant PEDV proteins including a 51 kDa full length PEDV nucleoprotein (NP) or portions of the spike (S) protein containing multiple putative neutralizing epitopes. Rabbits received two booster immunizations at 2 to 3 week intervals prior to blood collection and processing. BALB/c mice were also boosted twice at 2 to 3 week intervals with selected antigens. Splenocytes from immunized mice were fused with NS-1 myeloma cells and cultured on 24-well plates with selective HAT medium. Cell culture supernatants were screened by ELISA and IFA then positive wells were subcloned, expanded and retested.

To date, six different hybridoma clones producing monoclonal antibodies against the PEDV nucleoprotein have been isolated and we are currently in the process of screening multiple PEDV spike-specific monoclonal antibodies. Immunoglobulin isotyping of the monoclonal antibodies is being performed using a commercial lateral flow assay. The concentration of PEDV epitope-specific antibody within rabbit serum was quantified via IFA and shown to give titers of >1: 1280 for PEDV-NP and >1:320 for PEDV spike protein. Further characterization of epitope specificity for both monoclonal and polyclonal antibodies is being performed using immunoprecipitation and immunoblotting methods. Fluorescein conjugated antibodies are being prepared for direct FA applications.

The monoclonal antibodies, monospecific hyperimmune serum and related reagents produced in this project should prove of substantial value in the detection of PEDV antigen following virus isolation attempts as well as in a variety of diagnostic methods such as immunohistochemistry, antigen capture assays and fluorescent antibody technologies. They are currently being utilized in PEDV environmental stability studies and in fluorescent focus neutralization (FFN) assays for assessment of neutralizing antibodies produced following PEDV infection.

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A simplified serum neutralization assay based on EGFP-tagged PRRSV

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Serum neutralization assays provide a method for measuring the level of host protection to PRRSV infection. The current gold standard for measuring PRRSV-specific serum neutralizing activity involves reacting virus with serial dilutions of sera, incubation for 96 hours, then determining the endpoint titration as the last well without PRRSV induced cytopathic effects. Assay results can be difficult to interpret, due to the subjective nature of endpoint determination. Further, there is a high degree of standard error, which is likely introduced during sera dilutions. In this study, we describe the development of a single dilution serum neutralizing assay incorporating EGFP tagged-PRRSV. The recombinant PRRSV expresses EGFP from an independent subgenomic mRNA. The assay involves reacting a single dilution of serum with a standard amount of EGFP-PRRSV, in triplicate. Following one hour incubation, contents are transferred to confluent MARC-145 cells in a 96-well tissue culture plate. Cells are then incubated for 36-42 hours. The plate is then analyzed for GFP fluorescence using a FLUOstar Omega plate reader (BMG Labtech), in scanning mode. To standardize the assay to traditional methods, a set of standards, with nAb titers ranging from <8 to 1024 were used to make a standard curve (8XLog2(inverse dilution) vs. mean GFP fluorescent intensity). Standard curves from four independent assays resulted in Pearson Coefficient of Determination values (R^2) ranging from 0.97-0.99. A comparison of the results from measuring neutralizing activity in unknown samples using the new EGFP-based assay and the traditional assay showed agreement. Overall, the results suggest the new method provides a quicker, more reproducible, and potentially a more accurate approach for measuring nAb titers compared to traditional assays. Current efforts are focused on optimizing the assay. Future efforts will determine how accurate the assay is compared to traditional methods.

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Comparative serum immune responses of pigs after a challenge with porcine reproductive and respiratory syndrome virus (PRRSV)

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As part of our PRRS Host Genetics Consortium (PHGC) project, serum cytokine levels in PRRSV infected pigs were evaluated using Fluorescent Microsphere Immunoassay (FMIA). Serum samples were collected at 0, 4, 7, 11, 14, 21, 28, 35 and 42 days post infection (dpi) with PRRSV. Samples for 147 pigs from PHGC trial 3, 5 and 7 were selected for testing based on serum viral load and growth rate during the sampling period. The FMIA simultaneously detected 7 cytokines, representing early innate (IL-1b, IL-8, IFN-alpha), T helper 1 (Th1) (IL-12), Th2 (IL-4), and regulatory (IL-10) immune responses and macrophage migration chemokine (CCL2). The log 10 transformed cytokine measurements were analyzed as repeated measures per animal over 9 different time points using SAS software. Pigs were assigned into four phenotypic groups; high viremia-high growth (HvHg), high viremia-low growth (HvLg), low viremia-high growth (LvHg) and low viremia-low growth (LvLg). The least-squares means of 4 different groups at each dpi were calculated and multiple pairwise comparisons were adjusted using the Tukey-Kramer method. In addition, least-squares means of high and low viral level were derived from (HvHg+HvLg) - (LvHg+LvLg). After PRRSV infection, all cytokine levels except IL-4 were altered across 9 DPIs ($P<0.05$). The level of IL-8 at 10 and 14 DPI in HvHg pigs were significantly higher than LvLg pigs ($P<0.05$), and the level of IFN-alpha at 14 DPI in HvLg pigs were significantly higher than LvHg pigs ($P<0.05$). In high viremia (HvHg+HvLg) pigs, the levels of IL-8 (at 4, 7, 10 and 14 DPI), IFN-alpha (at 7, 10, 14 and 28 DPI) and CCL2 (at 14, 21 and 35 DPI) were significantly higher than low viremia (LvHg+LvLg) pigs ($P<0.05$). These results indicate that the serum concentrations of IL-8, IFN-alpha and CCL2 are significantly altered after PRRSV infection and reflect potentially different viral control mechanisms. We are continuing to probe correlations of cytokine profiles with serum viral levels, growth performance and genetic background. Support: Genome Canada, Genome Alberta, NPB grant #09-244, and CNPq Brazil fellowship (200602/2010-1) to KPCA.

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Understanding genome-enabled selection

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The common method to estimate breeding values and rank animals based on genetic merit is known as traditional BLUP (best linear unbiased prediction) selection. This method relies on phenotypic information on animals and their relatives to determine the expected genetic potential for all animals. Researchers have established ways to incorporate information of the genetic make-up of individuals into breeding programs. The process of integrating this genomic information into a selection strategy is known as genome-enabled selection.

For genome-enabled selection, a tissue or blood sample is collected and used to obtain genetic information; the genetic information from an individual animal's sample is decoded or sequenced to determine the specific genetic code at certain points along the entire DNA sequence. This information, specifically the areas where the sequence is different at a single location (called a single nucleotide polymorphism, or SNP, which simply means at a single point there are differences in the DNA code) can be used to enhance traditional breeding value estimation. There are two approaches to genome-enabled selection: genome-enhanced BLUP and SNP-effect models.

Genome-enhanced BLUP utilizes genomic information by modifying the relationship among animals involved in the analyses (relationship matrix) that is used in traditional BLUP selection. Typically, the relationship between animals used in genetic evaluations is based on pedigree relationships. Genome-enhanced BLUP utilizes the actual genomic relationship among relatives rather than just using the average relationship based on pedigree. This approach can be extended to incorporate phenotypes from individuals that are not genotyped in what is called single-step genome-enhanced BLUP.

In SNP effect models, the effect of each position (or SNP) on phenotype is simultaneously estimated for all genotyped SNPs in what is called a training population. The resulting SNP-key is then used to predict the breeding value of animals that are genotyped but have no phenotype by summing the estimated effects at each genotyped SNP. To incorporate information from individuals that are not genotyped, the resulting genomic prediction is 'blended' with an estimate of the breeding value derived using traditional BLUP. This blended estimate is used as the final genetic index value for each animal, combining both traditional BLUP and genomic information.

The expected benefit from genome-enabled selection is improved accuracy of EBV and obtaining more accurate EBV at a younger age, since there is no need to wait for phenotypes. Increasing EBV accuracy will proportionally increase the rate of genetic gain expected. Having more accurate EBV at a younger age allows selection decisions to made earlier (if possible given reproductive age), which reduces the generation interval and increases genetic gain per unit of time.

Genome-enabled selection has been implemented in some pig breeding programs (e.g. PIC). Because generation intervals are already low in pigs, the greatest impact is on increasing accuracy of selection. The greatest impact of genome-enabled selection is expected for lowly heritable traits that are hard to measure or measured late in life such as disease resistance, feed efficiency, and longevity. Disease resistance is not easily defined and systematically measured. Feed efficiency is expensive to measure directly and sow longevity is a sex limited trait that is not recorded until the sow is culled from the herd.

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Identification of a putative quantitative trait nucleotide (QTN) that may control variation in host response to porcine respiratory and reproductive syndrome (PRRS) virus infection

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Previously, we identified a major quantitative trait locus (QTL) on Sus scrofa chromosome 4 (SSC4) for host response to PRRS virus infection as part of the PRRS Host Genetics Consortium (PHGC). Recent additional RNAseq work identified a strong candidate gene in the QTL region based on differential expression (DE) due to QTL genotype (WUR0000125; with genotypes AA and AB) and allele specific expression at several days post infection (dpi) with the NVSL97-7985 PRRS virus isolate in nursery pigs. The objective of this study was to identify the causative mutation in this gene that is responsible for these effects. A whole transcriptome assembly generated by Velvet-Oases revealed three splice isoforms for the candidate gene (wild-type, frame-shifted and retained intron isoforms). In addition, an intronic mutation was identified in a previously uncategorized splice site that was predicted to alter splicing. This mutation was in perfect linkage disequilibrium with the SSC4 QTL and explained one isoform that encodes a frame-shifted protein and an early stop codon. By evaluation of RNAseq reads from 8 AA versus 8 littermate AB genotype individuals, altered splicing was observed to segregate consistently with genotype at the mutation. Expression of the three isoforms was quantified using RNAseq data and validated by quantitative capillary electrophoresis. Statistical analysis of the RNAseq data revealed that differential splicing was associated with the splice variant genotype at 14 dpi ($p<0.0001$). This result was further validated in a combined analysis across five dpi ($p<0.0001$). The wild-type isoform was identified as over-expressed in AB vs. AA genotype animals. In contrast, the frame-shifted allele was identified as over-expressed in the AA vs. AB genotype animals. These findings make this mutation a strong positional and biological candidate quantitative trait nucleotide for host response to PRRS virus infection. Acknowledgements: PHGC for the samples and PIC/Genus for funding the RNAseq.

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Pigs selected for increased feed efficiency are less affected by experimental infection with the PRRS virus

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Feed efficiency is of great importance to the swine industry, yet little research has been performed to evaluate the robustness of pigs selected for increased feed efficiency to disease. Recent resource allocation theories suggest that such animals may be more susceptible to disease as a result of having less available energy to mount an immune response. Since Porcine Reproductive and Respiratory Syndrome (PRRS) is the most economically devastating disease to the US swine industry, the objective of this study was to analyze the effect of PRRS on lines of pigs divergently selected for low and high residual feed intake (RFI), a measure of feed efficiency. We hypothesized that the efficient low RFI pigs will have a greater reduction in average daily gain (ADG) and higher serum viremia upon infection with the PRRS virus, compared to pigs selected for high RFI (reduced feed efficiency).

Two hundred piglets, of the ISU high and low RFI selection lines, were shipped to Kansas State University upon weaning (between 18 and 28 days of age) and received an intranasal/oral dose of the NVSL 97-7985 PRRS strain one week post-arrival. Serum samples were collected on days 0, 4, 7, 11, and 14 post-infection and weekly thereafter. Body weights were collected weekly and used to calculate ADG as the slope of weight regressed on day. For comparison, body weights were also collected on 489 non-challenged pigs, which were either full or half siblings of the pigs in the PRRS-challenged data set. At least two weights were collected for each piglet before leaving the nursery and ADG was then calculated as the slope of weight regressed on age. Viremia was quantified as viral load (VL) as area under the curve for 0-21 days post-infection of the log of PCR-based serum viremia.

In the challenged pigs, VL tended to be slightly lower and ADG slightly higher for the more efficient low RFI line ($p=0.12$ and 0.10, respectively). In contrast, for the non-challenged pigs, ADG was slightly lower for the low RFI line, but again not significantly ($p=0.20$). A joint analysis of challenged and non-challenged pigs showed a significant interaction between RFI line and challenge status ($p=0.038$), demonstrating that growth of the low RFI line was less affected by PRRS-challenge than growth of the high RFI line.

These findings demonstrate that selection for increased feed efficiency based on RFI does not increase the impact of PRRS infection on serum viremia and weight gain. In fact, our evidence shows that growth of the more efficient pigs was less affected by PRRS infection than that of the inefficient line. These findings may provide commercial farmers with additional incentives to invest in feed-efficient pigs.

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Predicting swine MHC haplotypes from high-density SNP genotypes

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The major histocompatibility complex (MHC) region in swine, the swine leukocyte antigen (SLA) complex, is rich in genes associated with disease resistance. Many of these genes have haplotype-specific expression. There are various ways to determine MHC haplotypes, including wet lab assays such as using PCR-sequence-specific primers (PCRSSP) for low-resolution (Lr) SLA haplotyping, which can be time intensive and costly. Alternatively, high-density single nucleotide polymorphism (SNP) panels can be used to infer haplotypes in a genomic region. The objective of this study was to determine whether SNP genotypes from a commercial high-density SNP chip can be used to predict haplotypes within the MHC region of the swine genome.

A total of 140 pigs from four PRRS Host Genetics Consortium trials, and from multiple companies and various breeds, were haplotyped using the PCRSSP method. Pigs were selected, in roughly equal numbers per trial, based on extreme (high/low) viremia and growth following inoculation with the NVSL 97-7985 PRRS strain.

All pigs were genotyped using the Illumina SNP60 BeadChip. Seventy-five SNPs located within the 23-27 Mb SLA I region and 64 SNPs within the 29-31 Mb SLA II region of chromosome 7 were used to analyze MHC class I and II, respectively. SNP genotypes in each region were phased into haplotypes using BEAGLE software. Resulting haplotypes in the 23-27 Mb window (the entire window used for phasing) or the 24 Mb window were analyzed for SLA class I. Similarly, SNP haplotypes in either the 29-31 Mb window or 29 Mb window were analyzed for SLA class II. Identical SNP haplotypes for a given window were grouped to determine which Lr SLA haplotype they shared. Accuracy of prediction was calculated as the percentage of SNP haplotypes that could be assigned to a Lr SLA haplotype.

When analyzing SNP haplotypes using the entire window used for phasing to group haplotypes, accuracy of prediction was 85% for class I and 88% for class II. Greater haplotype prediction accuracy was obtained when grouping SNP haplotypes using the 1 Mb approach (91% and 95%, for class I and II, respectively). Therefore, observing a narrower SNP window to predict haplotypes was more accurate than utilizing the entire chromosomal region used for phasing, possibly because additional SNPs in these windows introduced noise.

In conclusion, BEAGLE software can be used to predict MHC haplotypes from high-density SNP genotype data with fairly high accuracy. These results indicate that the Illumina SNP60 BeadChip can be used to predict SLA haplotypes as an alternative to wet lab methods. This will enable us to investigate the role of SLA class I and II genes in PRRS resistance/susceptibility.

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Analysis of gene expression in a region associated with host response to porcine reproductive and respiratory syndrome virus challenge

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Previous work by Boddicker et al. (2012) established a 1 Mb QTL region on *Sus scrofa* chromosome 4 (SSC4) associated with response to PRRS virus infection based on viremia from 0 to 21 days post infection (dpi) and weight gain through 42 dpi. The region exhibits high linkage disequilibrium, which complicates further fine mapping. The objective of this study was to identify positional candidate genes in the 1 Mb region based on gene expression analysis.

Whole blood samples were collected in Tempus tubes at 0, 4, 7, 11, and 14 dpi from 8 littermate pairs from trial 3 of the PRRS Host Genetics Consortium (PHGC). Each littermate pair included one pig with the favorable (AB) and one pig with the unfavorable (AA) genotype for the SSC4 region. Sample RNA extraction and preparation included depletion of globin transcripts, preparation of sequencing libraries, and sequencing using the Illumina HiSeq platform. The reads generated were aligned to version 10.2 of the pig genome using Tophat2 in conjunction with Bowtie2. The aligned reads were then analyzed using Cufflinks to generate a consensus transcript list, which was used with HTSeq to generate transcript level read counts. The count data were normalized and transformed before being fit in a linear model to assess differential expression between the AB and AA genotypes. In addition, Single Nucleotide Polymorphisms (SNPs) were called using the Genome Analysis Toolkit (GATK). There were 136 SNPs in the SSC4 region that were in linkage disequilibrium with the previously identified genotype. Allele counts from the SNP data were used to do allele specific expression (ASE) analysis for the 136 SNPs.

Of the 12 Ensembl reference transcripts in the region, representing 8 genes, one strong candidate gene showed higher expression in AB compared to AA individuals at all dpi except 0; this was significant ($P<0.05$) at 7 and 10 dpi. Other genes showed statistically significant differential expression at only one time-point. The ASE analysis showed that the exonic SNPs for the differentially expressed gene had a higher ($P <0.05$) expression level of the B versus the A allele, confirming the greater expression in AB individuals.

These results provide evidence of a promising candidate gene for further functional work to validate and detail its role in the host response to PRRS infection.

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Acute viremia levels of porcine reproductive and respiratory syndrome virus are altered in infected piglets with severe combined immunodeficiency

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is the most problematic and economically significant disease impacting the swine industry worldwide. The causative agent, PRRS virus, was identified over twenty years ago, but its mechanism of infection and its ability to subvert the immune response is still an active area of research. Currently, viral replication has focused on two potential models: 1) the permissiveness of porcine alveolar macrophages (PAMs) in young pigs, characterized by an inherent anti-inflammatory phenotype, or 2) the induction of suppressive regulatory T cells that may influence the permissiveness of PAMs. However, appropriate animal models have remained elusive in clarifying these fundamental questions. Severe Combined Immunodeficiency (SCID) is a group of genetically-linked disorders that are characterized by deficiencies in the adaptive immune repertoire. The disease has been observed in humans, horses, dogs, and mice, and afflicted animals are severely immunocompromised, and cannot mount antigen-specific immune responses. However, murine SCID models have been utilized extensively in research, and made significant contributions towards understanding host-pathogen interactions. Specifically, they have proven extremely valuable in understanding innate immunity to viral infections, and have provided insights regarding adaptive immunity through the use of immune reconstituted animals. Recently, we discovered a naturally occurring SCID line of pigs. These animals are devoid of T and B cells and have striking alterations within various immune compartments. Therefore, we pursued the SCID pig line as a unique opportunity to examine viral replication, in the absence of adaptive immunity and suppressor T cells, using the highly relevant PRRSV infection model.
Objective: To assess early viremia levels of PRRSV in infected SCID and normal piglets, as a preliminary assessment of piglet susceptibility to the virus in animals lacking adaptive immunity and suppressive regulatory T cells.
Methods: Nursery-aged normal and SCID piglets were infected with PRRSV and viremia levels were monitored for 21 days post infection using a PCR-based detection system.
Results: Lower levels of viremia were observed in infected SCID animals during the first 14 days post-infection compared to normal littermates.
Conclusions: These data indicated that SCID animals potentially have fewer permissive macrophages, resulting in lower viremia levels compared to normal littermates. Further studies will be conducted to assess whether SCID macrophages are inherently less permissive than PAMs derived from normal animals, and whether the cells of the adaptive immune compartment are modulating the PAMs toward an anti-inflammatory and PRRSV permissive phenotype.

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Highly pathogenic PRRSV induces prostaglandin E2 production through cyclooxygenase-1 dependent on ERK1/2-p-C/EBP-beta pathway

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Atypical porcine reproductive and respiratory syndrome (PRRS) caused by highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) is characterized by high fever and high mortality. However, the mechanism underlying the fever induction is still unknown. Prostaglandin E_2 (PGE_2) synthesized by cyclooxygenase type 1/2 (COX-1/2) enzymes is essential for inducing fever. In this study, we found that PGE_2, together with COX-1 was significantly elevated by HP-PRRSV. We subsequently demonstrated that ERK1/2 and p-ERK were the key nodes to trigger COX-1 expression after HP-PRRSV infection. Furthermore, we proved the direct binding of p-C/EBP-β to the COX-1 promoter by luciferase and ChIP assay. In addition, silencing C/EBP-β remarkably impaired the enhancement of COX-1 production induced by HP-PRRSV infection. Taken together, these results indicate that HP-PPRSV elicits the expression of COX-1 through ERK1/2-p-C/EBP-β signaling pathway, resulting in the increase of PGE_2, which might be the cause of high fever in infected pigs. Our findings provide new insights into the molecular mechanisms underlying the pathogenesis of HP-PRRSV infection.

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Biogenesis of non-structural protein 1 of arteriviruses and their role in host innate immunity

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Type I interferons (IFNs) play a key role for the antiviral state of host, but the porcine arterivirus PRRSV has been shown to down-regulate the production of IFNs during infection. Non-structural protein (nsp) 1 of PRRSV is the first viral protein synthesized during infection and has been identified as a viral antagonist for IFN production. Subsequently, the nsp1-alpha subunit of nsp1 has been shown to degrade the CREB (cyclic AMP responsive element binding)-binding protein (CBP). As the major component of the IFN enhanceosome, CBP participates in IFN gene expression, and thus the CBP degradation inhibits the formation of enhanceosome resulting in the suppression of IFN production. The current study was conducted to determine whether the IFN modulation was a common strategy used by other members in the family Arteriviridae. The family is consisted of PRRSV, equine arteritis virus (EAV), murine lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV).Individual nsp1 genes were cloned from EAV, LDV, and SHFV and expressed in cells. While PRRSV nsp1 and LDV nsp1 were auto-cleaved into the nsp1-alpha and nsp1-beta subunits, EAV nsp1 remained uncleaved. The SHFV nsp1 was initially predicted to generate three subunits (alpha, beta, and gamma), but only two subunits nsp1-alpha/beta and nsp1-gamma was found to be generated. The auto-cleavage of SHFV nsp1 was mediated by papain-like cysteine protease-beta (PCP-beta), and PCP-alpha appeared to be inactive. All subunits of nsp1 from all arteriviruses were localized in the both nucleus and cytoplasm, but a predominant nuclear localization was observed for PRRSV nsp1-beta, LDV nsp1-beta, SHFV nsp1-gamma, and EAV nsp1. When their IFN modulatory activity was examined using the luciferase reporter assays, nsp1 of all arteriviruses exhibited the IFN suppressive activity, and the suppression was mediated through the interferon regulatory factor 3 (IRF3) and NF-kappa B pathways. The total amount of IRF3 protein remained unchanged, and the IRF3 was normally phosphorylated upon stimulation, suggesting that the IFN suppression by nsp1 of arteriviruses was independent from IRF3. The CBP degradation was evident in cells expressing LDV nsp1-alpha and SHFV nsp1-gamma, whereas no degradation was observed for EAV nsp1. Our data demonstrate that the nsp1-mediated IFN modulation is a common strategy for all arteriviruses, but their mechanism of action may differ from each other.

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Validation of the effects of a SNP on SSC4 associated with viral load and weight gain in piglets experimentally infected with PRRS virus

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Porcine reproductive and respiratory syndrome (PRRS) is the most costly disease to the US pork industry, and vaccines, biosecurity measures, and proposed methods for eradication have had limited success. The aim of the PRRS Host Genetics Consortium (PHGC) is to identify genomic markers and pathways associated with host response to PRRS virus (PRRSV), which could potentially be used for genetic selection of pigs for increased resistance or reduced susceptibility to the virus. Boddicker et al. (2012) identified a SNP on SSC4 (WUR10000125) for which the favorable allele (B) was associated with reduced viral load (VL) and increased weight gain (WG) under infection with the NVSL-97-7895 PRRSV isolate. The objective of this study was to test the effects of this SNP when infecting pigs with a genetically different isolate of PRRSV (KS-2006-72109), which has 89% amino acid sequence identity with NVSL-97-7895 in GP5. Following the same experimental design, ~200 commercial crossbred piglets per trial for a total of 5 trials were experimentally infected with PRRSV at 28-35 days of age. Blood samples and weights were collected periodically for up to 42 days post infection (dpi). Viremia was measured using a qPCR assay for PRRSV RNA, and VL was defined as the area under the curve of Log viremia from 0-21 dpi. WG was defined as weight gain from 0 to 42 dpi. Analyses were carried out using PROC MIXED in SAS (v9.2), with trial nested with parity and the number of B alleles for the SSC4 SNP as fixed effects, weight and age at infection as covariates, and litter and pen nested within trial as random effects. Consistent with previous findings, individuals that were heterozygous for the SSC4 SNP had greater WG (0.95±0.32 kg, $p=0.0031$, $n_{AA}=682$, $n_{AB}=167$) and lower VL (-3.89±0.78 units, $p<0.0001$, $n_{AA}=431$, $n_{AB}=86$) compared to their AA counterparts. The size of the effect for the SSC4 SNP was approximately half the reported value for the NVSL-97-7895 isolate for WG (0.95±0.32 vs 2.0±0.4 kg), but comparable for VL (-3.89±0.78 vs -4.1±0.6 units). These results suggest this SSC4 marker may be useful for genetic selection of pigs for increased resistance or reduced susceptibility to PRRSV isolates that differ genetically and possibly in pathogenicity. This work was supported by Genome Canada, USDA ARS, and breeding companies of the PHGC and PigGen Canada.

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Quantitative analysis of porcine reproductive and respiratory syndrome (PRRS) viremia profiles from experimental infection: a statistical modelling approach

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Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant viral disease facing the global swine industry. Viremia profiles of PRRS virus challenged pigs reflect the severity and progression of the infection within the host and provide crucial information for subsequent control measures. In this study we analyse the largest longitudinal PRRS viremia dataset from an in-vivo experiment. The primary objective was to provide a suitable mathematical description of all viremia profiles with biologically meaningful parameters for quantitative analysis of profile characteristics. The Wood's function, a gamma-type function, and a biphasic extended Wood's function were fit to the individual profiles using Bayesian inference with a likelihood framework. Using maximum likelihood inference and numerous fit criteria, we established that the broad spectrum of viremia trends could be adequately represented by either the uni- or biphasic Wood's functions. Three viremic categories emerged: cleared (uni-modal and below detection within 42 days post infection(dpi)), persistent (transient experimental persistence over 42dpi) and rebound (biphasic within 42dpi). The convenient biological interpretation of the model parameters estimates, allowed us not only to quantify inter-host variation, but also to establish common viremia curve characteristics and their predictability. Statistical analysis of the profile characteristics revealed that persistent profiles were distinguishable already within the first 21dpi, whereas it is not possible to predict the onset of viremia rebound. Analysis of the neutralizing antibody(nAb) data indicated that there was a ubiquitous strong response to the homologous PRRSV challenge, but high variability in the range of cross-protection of the nAbs. Persistent pigs were found to have a significantly higher nAb cross-protectivity than pigs that either cleared viremia or experienced rebound within 42dpi. Our study provides novel insights into the nature and degree of variation of hosts' responses to infection as well as new informative traits for subsequent genomic and modelling studies.

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Exploring cytokine profiles and PBMC subsets following PRRSV infection in pregnant gilts

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Objective: We have undertaken a large-scale project investigating the genomic and phenotypic predictors of PRRS resistance in pregnant gilts. One of the objectives was to evaluate cytokine levels in gilt sera and in supernatants of PRRSV stimulated PBMC, as well as temporal changes in gilt peripheral blood mononuclear cell (PBMC) subsets following infection.
Methods: At gestation day 85 (±1), 114 gilts were inoculated with a Type 2 PRRSV strain, 50% IM and 50% IN (10^5 $TCID_{50}$ total dose). Nineteen gilts were similarly sham inoculated (control). Heparinized blood and serum samples were collected on 0, 2, 6, and 19 dpi. Automated white blood cell counts (Cell-Dyn 3500, Abbott Diagnostics) and manual differential counts were performed (300 cells total). PBMC isolated from whole blood were stained with fluorochrome-conjugated, monoclonal antibodies to define cell subsets using flow cytometry. The *in vitro* responsiveness of PBMC to homologous PRRSV stimulation was assessed by testing the supernatants for the following innate, regulatory, T helper 1 (Th1) and Th2 cytokines/chemokines by fluorescent microsphere immunoassay (FMIA): IL-1b, IL-8, CCL2, IFN-alpha, IL-12, IL-4 and IL-10, or ELISA (IFN-gamma). Additionally, serum levels of the same cytokines/chemokines were evaluated. Multilevel, repeated measures mixed models were used to compare responses between inoculated and control gilts over time.
Results: IFN-alpha, CCL2 and IFN-gamma in serum, and IFN-alpha and IL-8 in supernatants of PRRSV stimulated PBMC differed significantly over time between inoculated and control gilts. IFN-alpha and CCL2 were significantly higher in serum of inoculated gilts on 2 and 6 dpi, whereas IFN-gamma was significantly higher on 2 dpi only. IFN-alpha expression in PRRSV stimulated PBMC supernatants was significantly lower in inoculated gilts compared to controls at all investigated time points after infection. By contrast, IL-8 expression was significantly higher in PBMC supernatants of inoculated gilts on 2, 6, and 19 dpi.
The total numbers of all investigated PBMC subsets, which included T-helper cells, cytotoxic T cells, gamma-delta T cells, B cells, natural killer cells and myeloid cells, differed significantly over time between inoculated and control gilts. While T-helper cells, CTL, B cells and myeloid cells were significantly lower in inoculated compared to control gilts on 2 and 6 dpi, gamma-delta T cells were significantly lower on 2 dpi only. Inoculated gilts had significantly higher numbers of NK cells than control gilts on 0 and 19 dpi, but numbers were significantly reduced on 2 and 6 dpi.
C**onclusion/Discussion:** Some cytokines and all PBMC subsets were found to be significantly different in inoculated gilts compared to control gilts over time. These variables could be associated with clinical severity of PRRS. We plan to use multilevel models to investigate these associations with viral levels and fetal survival in order to determine predictors of PRRSV susceptibility in pregnant gilts and their fetuses.

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Immunogenic potential of the E protein of porcine respiratory and respiratory syndrome virus

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The E protein is a non-glycosylated minor structural protein of the Porcine Reproductive and Respiratory Syndrome virus (PRRSV). The amino acid sequence of this protein is highly conserved among all PRRSV strains. A published study demonstrated that the E protein possesses ion channel protein-like properties and is essential for viral uncoating. As per the predicted topology, this protein possesses one membrane spanning region in the middle with the N-terminus end exposed on the virus extracellular surface, and the C-terminus end localized in the virus interior. Our previous studies with custom-synthesized peptides that correspond to the N-terminus and C-terminus amino acid sequences indicated that all infected pigs develop C-terminus specific antibodies, but only a low proportion of the animals develop N-terminus specific antibodies. The goal of the current study was to determine the virus neutralizing potential of the N-terminus and C-terminus specific antibodies. In our first approach, peptide competition was used in *in vitro* neutralization assay with pig serum samples containing high levels of antibodies to the N- and C-terminus peptides. Unexpectedly, instead of the expected reduction in neutralizing activity in the presence of peptide competition, N-terminus peptides by themselves interfered with the virus infectivity. This phenomenon was not observed in the case of the C-terminus peptides. In our second approach, peptide-specific antibodies were generated in mice by immunization with either the C-terminus or the N-terminus peptides mixed with alum adjuvant. Mice immunized with the C-terminus, but not the N-terminus peptides developed antigen-specific antibodies. *In vitro* neutralization studies indicated that the mouse C-terminus peptide-specific antibodies are not capable of preventing PRRSV from infecting permissive cells. Ongoing studies are focused on induction of N-terminus peptide-specific immune responses in pigs and determining their role in protection against PRRSV infection.

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Current status of the swine leukocyte antigen (SLA) system

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The swine leukocyte antigen (SLA) system is among the most well characterized MHC systems in non-human animal species. The International Society for Animal Genetics (ISAG) and International Union of Immunological Societies Veterinary Immunology Committee (IUIS VIC), SLA Nomenclature Committee was formed in 2002. The committee's primary objectives are: 1) to validate newly identified SLA sequences according to the guidelines established for maintaining high quality standards of the accepted sequences; 2) to assign appropriate nomenclatures for new alleles as they are validated; and 3) to serve as a curator of the IPD-MHC SLA sequence database (http://www.ebi.ac.uk/ipd/mhc/sla/), which is the repository for all recognized SLA genes, their allelic sequences and haplotypes. To date, there are 131 classical class I (SLA-1, SLA-2, SLA-3), 13 non-classical class I (SLA-6, SLA-7 and SLA-8) and 174 class II (DRA, DRB1, DQA, DQB1, DMA) alleles officially designated. There are 34 class I and 27 class II haplotypes at the high-resolution (allele) level designation. Recent evidence has suggested certain loci in the SLA system previously recognized as pseudogenes (e.g. SLA-9, SLA-11, DQB2 and DOB2) may be expressed at the transcript level for some haplotypes; the committee will determine if designation of the alleles of these loci is warranted as more evidence accumulates. A systematic nomenclature for the genes, alleles and haplotypes of the swine MHC is critical to the research in swine genetic diversity, immunology, health, vaccinology, and organ or cell transplantation. Continuous efforts on characterizing SLA alleles and haplotypes and studying of their diversity in various pig populations will further our understanding of the architecture and polymorphism of the SLA system and their role in disease, vaccine and allo- or xeno-grafts responses.

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Swine toolkit progress for the US Veterinary Immune Reagent Network

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The US Veterinary Immune Reagent Network (US VIRN, www.vetimm.org) was established to address the lack of immunological reagents specific for veterinary species. Efforts are targeted at swine, ruminants, poultry, equine and aquaculture species. Our goal is to produce reagents that function in ELISA, multiplex bead assays, ELISA and flow cytometric applications. Numerous swine chemokines and cytokines were cloned, expressed in *Pichia,* purified and most shown to be bioactive using chemotaxis, upregulation of marker expression or cell stimulation assays. We have recently expressed and proven bioactivity of swine immunoregulatory cytokines, IL-17A and IL-17F. Hybridoma fusions for monoclonal antibodies (mAb) to interleukin-13 (IL-13), IL-17A, interferon-alpha (IFNa) and IFNb were completed at Univ. Massachusetts and Cornell Univ. A sensitive fluorescent microsphere, Luminex bead, immunoassay for CCL2 was developed with US VIRN produced mAb and included in the 6-plex swine cytokine assay we had previously developed. At Cornell Univ. a fusion protein expression system was used to generate material for immunizations for cell surface antigens, IFNAR, CD19, and NK cell marker NKp44 (NCR2). Immunizations and fusions were performed; screening of potential positive mAbs are continuing. The US VIRN website www.vetimm.org has a progress update for swine. Since many swine cytokine and CD reagents are available commercially the website includes a listing of those reagents and their sources. Products developed in this proposal are available to collaborators and have been made commercially available through Kingfisher Biotech, Inc. http://www.kingfisherbiotech.com/. This project was funded by USDA NIFA proposal #2010-65121-20649, USDA NIFA/DHS #2010-39559-21860 grants and USDA ARS funds.

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Broadly neutralizing antibodies against porcine reproductive and respiratory syndrome virus, a rapidly evolving RNA virus

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Introduction Neutralizing antibodies are a critical part of the immune armory for defense against viruses, and are the mechanism by which many effective vaccines work to protect against viral infections. However, infections by rapidly evolving and genetically diverse viruses are often characterized by ineffective neutralizing antibody responses. Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly genetically diverse RNA virus that causes the most significant disease (PRRS) of pigs worldwide. The prevailing view of immunity to PRRSV is characterized by delayed and ineffectual production of neutralizing antibodies lacking cross-reactivity that is necessary for vaccine efficacy.

Objective We sought to examine PRRSV neutralization characteristics from serum of animals from herds with a history of multiple exposures to PRRSV over time, either through natural infection, modified live virus vaccination, or serum inoculation

Methods Fluorescent focus neutralization (FFN) and ELISA-based serum neutralization (SN) assays were used to screen sow serum against a panel of diverse PRRSV isolates for quantification of anti-PRRSV cross-neutralizing activity.

Results Sera from previously infected commercial sows had high levels of neutralizing activity against diverse PRRSV strains, including genotypically distinct type 1 PRRSV. Fifty percent cross-neutralization titers in excess of 1/1024 were observed. Cross-neutralization activity was dose-dependent and was maintained in the immunoglobulin fraction.

Conclusions The presence of high-titered, anti-PRRSV cross-neutralizing antibodies in pigs is strong evidence that highly conserved neutralization epitopes are present in genetically disparate PRRSV. These findings provide a new model to help elucidate mechanisms of antibody production and maturation that target inapparent conserved neutralization epitopes in rapidly evolving viruses.

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Age-related susceptibility of macrophages to porcine reproductive and respiratory syndrome virus infection

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Introduction Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes the most economically important disease affecting swine production in the United States. Age-dependent resistance to PRRSV has been observed, with young pigs exhibiting longer periods and higher levels of viremia compared to older pigs. PRRSV targets macrophages, and cellular surface receptors CD163 and CD169 have been identified or implicated as important for infection. However, mechanisms underlying age-related differences in permissiveness remain unclear.

Purpose Preliminary evidence suggests pulmonary alveolar macrophages (PAMs) belonging to older pigs are more resistant to PRRSV compared to those from younger pigs. We sought to determine if age-related resistance to PAM infection results from decreased cell permissivity due to differential expression of surface receptors for PRRSV.

Methods PAMs isolated from six pigs of various age groups (3 days old, 10-12 weeks, and adult) were infected with virulent PRRSV field strain MN184. PRRSV infection and cellular expression of CD163 and CD169 were analyzed by flow cytometry at 12 hours post-infection (hpi). Viral replication was compared at 12, 24, and 48 hpi by quantitative RT-PCR.

Results Infection percentage was higher in PAMs from younger pigs and infection yielded greater amounts of virus compared to those from older pigs. Level of infection for PAMs from 10-12 week old pigs was more similar to adult PAMs. CD163 and CD169 expression was uniformly high in all experiments and did not differ among age groups.

Conclusions PAMs isolated from older pigs are more resistant to PRRSV infection compared to those from younger pigs. Age-related PAM resistance to PRRSV infection is not due to differential levels of CD163 and CD169 expression. In the future, we hope to identify mechanisms responsible for age-related PRRSV resistance. These may include cellular receptor polymorphisms, innate anti-viral gene response, and differential macrophage polarization.

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Favorable allele on chromosome 4 is associated with resilience to PCVAD

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Porcine Circovirus Associated Disease (PCVAD) describes several disease syndromes associated with porcine circovirus type 2 (PCV2). The level of PCV2 viremia is increased in the presence of PRRSV. These co-infections can result in high morbidity and mortality in swine production and cause a significant economic impact. To evaluate the host response of different genotypes to co-infection, approximately 230 8-week old pigs were challenged with PCV2 and PRRSV and followed for 42-days post-challenge. Half of these pigs had a favorable B allele (AB) on chromosome 4 which was previously associated with increased weight gain and decreased viral load; the remaining pigs were AA. During the experimental infection, veterinary directed-health evaluations and symptomatic therapy were completed. Between day 22 and day 35 post-challenge, a significant increase in mortality and morbidity occurred. Clinical signs appeared consistent with PCVAD, including tachypnea, dyspnea, loss of condition, mucoid nasal discharge, lethargy, pale discoloration of skin, and ear cyanosis. Lung lesions were evaluated in 18 pigs with and without clinical signs. Four groups of pigs (AA clinical, AA nonclinical, AB clinical, AB nonclinical) were evaluated. Pigs were assigned a score or designation of severity for gross lung lesions, microscopic lung lesions, and lymphoid depletion. Viremia levels for PCV2 and PRRSV at the time of necropsy were quantified for each animal using real-time PCR. Average daily weight gains between day 0 and day 28 post-challenge were 0.42 kg/d, 0.55 kg/d, 0.56 kg/d, and 0.95 kg/d for the AA clinical, AA nonclinical, AB clinical, and AB nonclinical groups respectively. Pigs with the favorable AB genotype showed increased average daily weight gain when compared to the AA genotype despite the presence of gross and microscopic lung lesions, PCV2 related lymphoid depletion, and clinical signs. These results suggest that the presence of the favorable allele on chromosome 4 is associated with continued weight gain in the presence of clinical disease.

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Replication efficiency of porcine circovirus (PCV) is genotype dependent in co-infection with porcine reproductive and respiratory syndrome virus (PRRSV)

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Both PRRS virus (PRRSV) and porcine circovirus type 2 (PCV2) are major pathogens in the swine industry. Viral co-infections can play a crucial role in the initiation of PCV2 diseases such as postweaning multisystemic wasting syndrome (PMWS). Furthermore, existence of pathogenic synergy was demonstrated in field conditions, but the pathogenesis mechanisms are unknown. PCV2 and PRRSV have been found in aerosol form in pig farms and are transmitted through the respiratory tract. Following aerosol virus transmission, two cell types are in contact with PCV2 and PRRSV in order to allow the viruses to enter and disseminate in the host organism: 1) the alveolar macrophages (PAM) and 2) the epithelial cells of the respiratory tract. PAM cells are extensively used in the literature. Thus, in this study, a recent cellular *in vitro* model of respiratory tract epithelial cells (NPTr-CD163 cells). Those cells are known to be permissive to both PCV and PRRSV replication and were used to investigate PCV/PRRSV co-infections. Results demonstrated that in NPTr-CD163 cells, PCV2a genotype was replicating less efficiently than PCV1/2a, PCV1 and PCV2b. During co-infections with PRRSV, the replications of each virus were affected differently. PCV1 virus titer was decreased in co-infected cells compare to PCV1 single infection. PCV2b titer was increased in cell co-infected compared with cell infected with PCV2b alone. PCV1/2a and PCV2a titers were not affected by co-infection with PRRSV. PRRSV titer was decreased in PCV1/2a, PCV2a or PCV2b co-infected cells but was increased in PCV1 co-infected cells compared to PRRSV infected cells alone. Cell growth was also evaluated for all single or co-infection combinations. Cell growth was decreased in PRRSV or PCV2a single infection and in all co-infected cells compared to mock infected cells. Cytokines mRNA expression was also determined in PCV/PRRSV co-infected cells. IFN-alpha mRNA was not modulated in single or co-infected cells when compared to mock infected cells. INF-beta, IL-10 and TNF-alpha mRNA were increased in cells infected by PRRSV compare to mock infected cells. INF-beta mRNA was increased in PCV1/PRRSV co-infected cells compared to PCV1 and mock infected cells. IL-10 mRNA was decreased in PCV2b and PCV2b/PRRSV infected cells compared to PRRSV infected cells. PCV1/PRRSV co-infection increased TNF-alpha mRNA compared to single virus infections. Those results suggest that the less efficient replication of PCV2a might be related to the slower growth rate of the NPTr-CD163 infected cells. Also, the decrease of PCV1 replication in PCV1/PRRSV co-infected cells might be due to higher quantity of INF-beta mRNA. In conclusion, cells response to PCV/PRRSV co-infections and viruses' replication are PCV genotype dependent.

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Genetic analysis of reproductive traits and antibody response in a commercial sow herd before and after a PRRS outbreak

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The objective of this work was to study the genetic architecture of reproductive traits and antibody response underlying a PRRS outbreak in a sow herd. The dataset available for analysis was obtained from a commercial multiplication herd that experienced a PRRS outbreak during the winter of 2011/12. Farrowing data (number born alive [NBA], number stillborn [NSB] and mummified [NBM], number alive at 24 hours, number weaned, and number fostered) of 5,227 litters from 1,967 purebred Landrace sows, and a pedigree with 2,995 individuals, were used for the analysis. The PRRS outbreak date was estimated based on rolling averages of farrowing traits and used to split the data in a Pre- and a Post-PRRS phase. All 641 sows that were in the herd during the outbreak had blood samples collected 46 days after the estimated outbreak date, and were tested for the PRRSV using ELISA (expressed as sample-to-positive [SP] ratio). Genetic parameters of traits were estimated separately for the Pre- and Post-PRRS data sets. All sows were genotyped using the PorcineSNP60 BeadChip. The genotype data were used to perform genome-wide association (GWA) analyses separately for each trait and each PRRS phase using method Bayes-B. For the pre-PRRS phase analyses, repeated records of a sow were pre-adjusted for the fixed effects of contemporary group, parity, breed of sire (Yorkshire and Landrace) and averaged by sow before the analyses. For the post-PRRS phase, the model included the fixed effects of parity, breed of sire, the genotype at a SNP (WUR) on SSC4 that has been found to be associated with PRRS response in growing pigs, and the rolling average for the trait being analyzed (except for SP ratio). Estimated heritabilities for the reproductive traits ranged from 1% (NBM) to 12% (NSB) in the pre-PRRS phase, and from 6% (NSB) to 12% (number born dead, NBD) for the Post-PRRS phase. SP ratio showed the highest heritability (45%) and had substantial genetic correlations with most traits, ranging from -0.72 (NBM) to 0.73 (NBA). The GWA analysis detected genomic regions associated with NSB and proportion born dead in the pre-PRRS phase, and for NBD, NSB, and SP ratio in the post-PRRS phase. For SP ratio, two regions on SSC7 separated by 100 Mb accounted for 40% of the genetic variation, including a region encompassing the Major Histocompatibility Complex (MHC), which explained 25% of the genetic variance. These results indicate that there is a significant genomic component associated with PRRS antibody response and NSB in this PRRS outbreak sow herd. In addition, the high heritability and genetic correlation estimates for SP ratio during the outbreak suggest that SP ratio could be used as an indicator of PRRS impact on reproductive traits. This work was supported by grants from Genome Canada, the Canadian Swine Health Board, and PigGen Canada.

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Epitope differences between homologous and broadly neutralizing antibodies to PRRSV

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Neutralizing antibody (NA) is an important component of host defense against PRRSV infection. NA activity during infection or after vaccination with modified live virus (MLV) is described as weak and delayed. Furthermore, NA is primarily directed against the isolate used for infection/vaccination. These properties have hampered the development of the next generation of vaccines. Using a traditional virus neutralization (VN) assay, we analyzed over 1,200 serum samples from experimentally infected pigs for VN activity against four different isolates. Overall, there was an inverse correlation between virus load and homologous VN titer. Based on the breadth of VN titer, samples were placed into one of the following groups: Group 1, no VN; Group 2, homologous VN against only the isolate used for infection; Group 3, heterologous VN with reactivity against 1 or 2 additional isolates; and Group 4, broad VN with activity against all four isolates. By definition, we call the Group 4 sera broadly neutralizing antibody (bnAb). Based on these differences, we predict that the different groups of VN recognize different epitopes. To test this hypothesis, VN escape mutants were prepared by selecting for viruses that were resistant to homologous antibody (Group 2) or bnAb (Group 4). Selection was accomplished using a two-step process. The first step was to perform a checkerboard VN titration of virus versus antibody on a 24 well plate of MARC-145 cells. The last well in each row showing CPE were pooled. In a second step, the pooled virus was expanded on a T-25 flask of MARC cells and the titration repeated on a 24 well plate until VN activity was no longer present. The results showed that virus passaged in the presence of Group 2 serum lost homologous VN activity, but retained the ability to be neutralized by bnAb. Conversely, the bnAb escape mutant virus was still susceptible to neutralization by homologous VN. These results suggest that broad nAb and homologous nAb recognize different epitopes within the PRRSV proteome. Preliminary sequencing results for the homologous nAb escape mutant virus showed changes in the ectodomain of GP5. In future experiments, reverse genetics and infectious cDNA clones will be used to confirm these results. Together, these results have important applications in the development of the next generation of vaccines, including vaccines that illicit a robust and rapid bnAb response. This work was supported by National Pork Board Grant NPB#12-120, “Characterization of neutralizing antibody responses to PRRSV and association with host factors”.

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Modified-live PRRS virus vaccine at weaning reduces shedding of wild-type virus in aerosol of growing pigs

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The risk of area-spread of porcine reproductive and respiratory syndrome virus (PRRSV) continues to be high in swine-dense regions potentially because of PRRSV shedding from large populations of growing pigs. The therapeutic use of modified-live virus (MLV) vaccine in infected pigs has been shown to reduce the duration of wild-type virus (WTV) shedding to sentinels and in aerosol. The objective of this study was to quantify the effect of MLV vaccine on performance and measure WTV shedding in pigs vaccinated at weaning and challenged 4 weeks later. A total of 2100 PRRS-negative weaned pigs were randomly allocated to either a non-vaccinated control (NVC) or to a MLV vaccinated group, each housed in separated rooms. Biosecurity protocols were implemented to avoid PRRSV transmission between rooms. Pigs in the MLV group were IM vaccinated with Ingelvac PRRS® MLV (Boehringer Ingelheim Vetmedica, Inc.) at 4 weeks of age. Four weeks post-vaccination 10% of the pigs in each group were IM inoculated with 1 mL of PRRS WTV RFLP pattern 1-18-2 at a concentration of $4.2x10^7$ RNAc/mL. Infection dynamics was monitored by PCR and ELISA tests on serum and oral fluid (OF) samples. Daily air samples were collected from each group at 8 AM using Liquid Cyclonic Collectors (Midwest MicroTek, Brookings, SD) placed in front of exhaust fans for 30 minutes and tested by PCR. Mortality, cull rate, ADG and feed conversion (FC) were recorded for wean to finish performance. Mild clinical signs developed in both groups following inoculation with PRRS WTV. No differences ($P>0.10$) were detected in PCR or ELISA results in serum or OF samples between the two groups. MLV vaccine was not detected in the NVC group. The frequency of detection of PRRSV RNA in air samples was higher ($P<0.0001$) in the NVC (21/118 days) than in the MLV group (4/118 days). The duration of detection of PRRSV RNA in air samples was numerically shorter in the MLV group (6 days) than in the NVC group (36 days). Performance of the MLV barn was the following: mortality, 5.1%; cull rate, 2.8%; ADG, 1.63 lbs/d. Performance of the NVC barn was the following: mortality, 4.8%; cull rate, 5.9%; ADG, 1.57 lbs/d. The prophylactic use of PRRS MLV vaccine in growing pigs at risk of infection represents a valuable tool to reduce the risk of transmission between herds in swine-dense-areas. The observed performance benefits as well as shedding reduction in MLV vaccinated pigs challenged with WTV support the recommendation of MLV vaccination of growing pigs at risk of infection.

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Attenuation of PRRSV by inactivating expression of ribosomal frameshifting products: implication for the rational design of PRRS vaccines

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Attenuated viral vaccines could be generated by targeted deletion of a gene encoding the virulent factor. Recently, we discovered a novel PRRSV protein (nsp2TF), which is expressed through a unique ribosomal frameshift mechanism. In this study, the nsp2TF was determined to associate with innate immune suppression function of the virus, as characterized by its effect on viral de-Ubiquitination (de-UB) and de-ISGylation activities. Two recombinant viruses, vSD95-21-mut1 and vSD95-21-mut2, were generated by partial or completely knocking out the expression of nsp2TF. The *in vitro* de-Ubiquitination and de-ISGylation assays showed that these mutants displayed an impaired ability to interfere with cellular protein ubiquitination and ISGylation. When tested in swine alveolar macrophages, gene expression levels of IFN-α and ISG15 were up-regulated in cells that were infected with the mut1 or mut2 compared with those infected with wild-type virus. These two mutants were further tested in a nursery pig model. Viable viruses were recovered from the serum samples of infected pigs at 7, 14 and 21 days post infection (dpi), and sequence result confirmed that both mut1 and mut2 viruses retained their corresponding mutations in the nsp2 region, indicating active replication of these mutant viruses in pigs. The duration and peak amount of viremia were further quantified, and result showed that both groups of pigs infected with mut1 and mut2 had significant lower viral load than those pigs infected with wild-type virus at 3, 7 and 21 dpi. To access the lung pathology during the acute infection, three pigs from each of the group 2-6 were euthanized at 10 dpi. Gross lung lesions were absent in mutant virus-infected pigs, and mild lesions (2-6%) were observed in wild-type virus infected pigs. To investigate whether these nsp2TF-deficient mutants are capable to induce protection against PRRSV challenge, pigs were challenged with a virulent virus at 28 dpi. At 3 and 7 days post challenge (dpc), viral loads significantly decreased in pigs infected with mut1 or mut2, in comparison with those pigs infected with wild-type virus. Lung pathology was evaluated at necropsy. Pigs from mut2 infected/challenged group showed the lowest mean lung lesion score of 7.6, while mut1 infected/challenged group of pigs had mean lesion score of 12. In contrast, apparent lung lesions were observed in two of the wild type virus infected/challenge group of pigs, with mean lesion score of 25.6. Taken together, our studies demonstrate that the newly identified nsp2TF has important function in modulation of host immune response and nsp2TF-deficient viruses are attenuated *in vivo*. Manipulating the expression levels of ribosomal frameshifting products may provide a rational basis for PRRS vaccine development.

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PRRSV field exposure and mortality relationship analysis in non-vaccinated and vaccinated finisher pig barns

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Modified live vaccination has been used as a primary tool for minimizing mortality rate due to PRRSv[1,2]. Vaccination reduces shedding and excretion of wild-type virus in vaccinated pigs, but did not prevent infection[2]. The objective of this study was to analyze mortality rate in non-vaccinated control and vaccinated finisher barns with different levels of wild type PRRS (WT PRRSv) exposure in a large production system. A barn-level side by side study was conducted in a large production system located in the USA. The pig flow was selected from PRRSv Stable Sow Farms, defined as PRRSv negative or ≤20% positive PRRS PCR 5:1 pooled serum samples at weaning. Pigs in the vaccinated groups were vaccinated with Ingelvac PRRS® MLV (2ml) at nursery placement. Treatment group and matched pair integrity was maintained upon movement of pigs from the nursery to the finisher sites. The study evaluated 60 vaccinated finishing barns and 59 non-vaccinated finishing barns within the same finisher sites. The sampling protocol during the finisher phase utilized oral fluids from 6 ropes per barn, for PRRS PCR at 15-16 wks of age. All PCR positive samples were sequenced (ORF5). Percent mortality from the Finisher group/barn closeouts was used for the analysis. For each finisher barn in the study, the proportion of positives at 15-16 weeks of age and its respective mortality were analyzed through a run chart using MINITAB 16.2 with WT PRRSv proportion on axis X and mortality on axis Y. The run chart included two tests for randomness (No. of runs about the median & No. of runs up and down) to indicate whether there were any statistically significant patterns or evidence of non-randomness. Results showed that in control barns, the higher the percentage of OF sample positive for WT PRRSv, the higher the mortality (clustering P-value=0.022), however, vaccinated barns showed no indication of a relationship between high mortality and PRRSv WT proportion (clustering P value 0.910) confirming that vaccination does not prevent infection, but significantly reduces mortality due to PRRSv.

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PRRSV control in finisher pigs, a large scale barn study in a high dense area in USA

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Controlling Porcine Reproductive and Respiratory Syndrome (PRRS) in large production systems is challenging, but considered one of the most important drivers for keeping systems producing at target levels. The objective of this study was to improve finishing pig survivability, growth, and/or cull rate through the strategic use of Ingelvac PRRS® MLV as compared to contemporary non-vaccinated control pigs. The study was implemented in a large commercial production system located in North Carolina, USA. The pig flow for this study was selected according to the following criteria: 1. Pigs sourced from a PRRSv Stable Sow Farm, defined as PRRSv negative or ≤20% positive PRRS PCR 5:1 pooled serum samples at weaning, and 2. Placement of nursery pigs in sites utilizing an all-out flow (n=2600 pigs/site). Treatment groups; vaccinated and non-vaccinated pigs, were allocated to alternating nursery sites. Both vaccinated and non-vaccinated nursery sites/treatment groups were moved and matched to a finishing site while maintaining integrity of each treatment group within the same finishing site. Pigs allocated to vaccinated nursery sites were vaccinated with Ingelvac PRRS® MLV per label recommendations within 7-10 days of placement in the nursery. All groups vaccinated with Ingelvac PRRS® MLV met the criteria of <20% serum-pooled PRRSv PCR positive results, (low PRRSv prevalence at weaning). The study evaluated 60 vaccinated finishing barns and 59 non-vaccinated finishing barns within the same finisher sites, with each barn housing 1224 pigs. A total of 32 matched pair finisher sites were included in the study. The experimental unit was the finisher barn and the replication number was established considering an improvement of 0.05 lbs. in gain per day (GPD) or a 2 % improvement in mortality (each independent), with a priori alpha value of 0.15 and beta value of 0.80. Oral fluids were collected with 6 ropes per barn at two periods: 15-16 wks of age and 22-23 wks of age. All PCR positive samples were further sequenced for ORF5 genomic analysis. Finisher group/barn closeouts were used for the analysis of Gain per Day (GPD); Livability %; Mortality %; Cull% and Average Weight. The data was analyzed using the fit model platform in JMP ANOVA. Results showed improvements in key production parameters: gain per day (P=0.003), livability (P=0.062) and mortality rate (P=0.008). These improvements represent the major economic drivers in finishing performance. The exposure level to PRRS wild type virus during the finisher was similar in both treatment groups during the two sampling points, with weeks 5-6 placed (15-16 wks of age) having the highest level of exposure. Under the conditions of this study, Ingelvac PRRS® MLV demonstrated significant benefit in improved weight gain per day, livability %, and a significant reduction in mortality % in finisher pigs. The results from this study support the use of vaccine for control of PRRSv in growing pigs as a reliable tool to reduce the negative impact of PRRSv in growing pig performance of pigs placed in high dense areas.

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Modified live PRRSV vaccination is efficacious following challenge with eight genetically diverse PRRSV isolates

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Modified-live vaccine (MLV) can significantly reduce lung lesions following a heterologous PRRSV challenge. It is necessary for PRRS MLV vaccines to be effective against newly emerging PRRSV field isolates. The objective of this report is to summarize experimental data on PRRSV MLV efficacy following challenge with eight genetically diverse PRRSV isolates. Field isolates 1 (1-18-2) and 2 (1-4-4) were isolated in 2008 and 2011, respectively, from herds in the upper Midwest. Field isolate 3 had a RFLP type of 1-5-2. All strains were associated with reproductive problems and high mortality in growing pigs. Conventional pigs were vaccinated at approximately 3 weeks of age and challenged at either 21 or 28 days post vaccination. Pigs were challenged with a 2ml intranasal dose of 5.92 log $TCID_{50}$/ml, 5.13 log $TCID_{50}$/ml, or 4.78 log $TCID_{50}$/ml viral stock, respectively for Isolate 1, 2 and 3. All pigs were humanely euthanized and necropsied 14 days following challenge. Lungs were collected and scored for percent pneumonia associated with PRRSV exposure. Studies involving the remaining challenge isolates [NADC, VR2332, SDSU 73, 1-8-4 and 1-4-2] have been previously described.[1] The challenge isolates varied in lineage with lineages of 1, 5.1, 8, and 9 being represented.[2] Additionally, the nucleotide similarity of the challenge isolates to Ingelvac PRRS® ATP varied from 86-91% based on ORF 5 sequence. The nucleotide similarity of the challenge isolates to Ingelvac PRRS® MLV varied from 86-100% based on the ORF 5 sequence. Differences between vaccinated and challenge control animals for all 21 studies were statistically significant ($P<0.05$). Vaccination significantly ($P<0.05$) reduced the percent of lung lesions induced by a PRRSV challenge compared to non-vaccinated pigs (select isolates shown in Table 1). Both Ingelvac PRRS® MLV and ATP were equally effective in reducing lung lesions following a PRRSV challenge. Use of a modified-live PRRSV vaccine significantly reduced lung lesions in multiple challenge models which used genetically diverse heterologous PRRSV isolates.

Table 1. Lung lesions (%) in vaccinated and non-vaccinated pigs challenged with PRRSV.

		Lung lesions, %		
Study	Ingelvac PRRS® Vaccine	Vaccinated	Non-Vaccinated	Challenge isolate
3	ATP	17.8	70.1	SDSU 73
12	MLV	8.0	47.0	SDSU 73
11	ATP	8.7	62.5	MN 1-8-4
16	MLV	4.5	32.9	MN 1-8-4
17	ATP	13.8	58.1	FI 1 1-18-2
20	MLV	37.4	52.9	FI 2 (1-4-4)
18	MLV	1.4	18.0	FI 3 (1-5-2)

FI=Field isolate

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Isolation and characterization of porcine epidemic diarrhea virus (PEDV) for development of serologic tests and vaccine

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The highly contagious and deadly porcine epidemic diarrhea virus (PEDV) was first discovered in the US in Iowa in April 2013. Since then the virus has spread rapidly nationwide. The high mortality (70-100%) among nursing pigs has led to significant economic losses. An effective vaccine is urgently needed to control the disease, but no vaccine is available in the US. Therefore, development of an effective US PEDV vaccine should be a high priority to control this disease. Since most viruses will accumulate mutations and become attenuated (reduced pathogenicity) after continuous passage in cell culture, the isolation of PEDV in cell culture is the first step toward the development of an attenuated live vaccine. We attempted to isolate a US PEDV strain in different cell lines from about thirty PEDV-positive field fecal and serum samples and germfree pig-passaged PEDV. One Ohio PEDV strain PC22A has been propagated successfully in Vero cells for 10 passages in the presence of trypsin. In the Vero cells infected with PEDV, we observed distinct cytopathogenic effects (CPE) at 2-4 days post-inoculation (dpi) depending on inoculation doses. Immunofluorescent microscopy using antibody against PEDV showed that PEDV antigens were in the cytoplasm of syncytia containing multiple nuclei. PEDV particles on the cell surface and in vesicles inside infected Vero cells were observed by transmission electron microscopy. By quantitative real time RT-PCR, viral RNA titers increased steadily up to 5-6 $\log_{10}$-fold compared to the inoculum after the 4th passage. Viral passages 7-9 were titrated as 5-6 $\log_{10}$ 50% tissue culture infectious dose ($TCID_{50}$)/mL or plaque forming units (PFU)/mL by CPE and plaque assays, respectively. We will continue to passage PEDV in Vero cells to generate an attenuated vaccine candidate. We also plan to develop a viral neutralization test for the detection of neutralizing antibodies in swine sera. Attenuation of PEDV will be a major achievement for development of an oral PEDV vaccine. Since PEDV is so deadly in nursing pigs and can cause major economic losses to the US swine industry, the research results will help to prevent and control PED outbreaks in the US, thus promoting animal health and the sustainability of the swine industry. This work was supported by NPB Project #13-222.

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Enhanced neutralizing antibody generation by an interferon-inducing porcine reproductive and respiratory syndrome virus strain

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PRRSV appears to inhibit synthesis of type I interferons (IFNs), such as IFN-alpha and -beta, which are critical to innate immunity against viruses and play an important role in the modulation of adaptive immunity. An exceptional PRRSV strain, A2MC2, is able to induce type I IFNs in the cultured cells. Here, we report the results of an animal study comparing A2MC2, Ingelvac PRRS MLV and VR-2385 (a moderate virulent strain). Three-week-old pigs were exposed to these viruses via intranasal and intramuscular routes, separately. The interferon-inducing A2MC2 induced earlier appearance and higher levels of neutralizing antibodies than MLV. In addition, the A2MC2-induced neutralizing antibodies were capable of neutralizing VR-2385, a heterologous strain. The pigs exposed via intranasal route had higher titers of neutralizing antibodies than those injected via intramuscular route. Macroscopic and microscopic lung lesions 14 days post-exposure indicated that A2MC2 had similar virulence *in vivo* as VR-2385. Pulmonary alveolar macrophages (PAMs) collected during the necropsy 14 days post-exposure in the A2MC2 group had higher level expression of IFN-gamma than the MLV group. These results indicate that A2MC2 can be further explored for development of an improved vaccine against PRRS.

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Characterization of genetic variation in non-structural protein 2 in pigs during experimental infection with porcine reproductive and respiratory syndrome virus

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The non-structural protein 2 (nsp2) of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has been implicated in a number of important functions for PRRSV. It inhibits interferon (IFN) responses in the host during infection. T-cell and B-cell epitopes have been identified across the protein, and a strong antibody response against nsp2 has been observed in PRRSV-infected pigs. In addition, genetic variation in nsp2 contributes to the genetic diversity between PRRSV types and is associated with differences in pathogenicity among diverse isolates. Recently, a study identified nsp2 as a structural component of the PRRSV virion. This important discovery, in conjunction with the high number of B-cell epitopes and large antibody response, raises the question if nsp2 is under selective pressures from the host's antibody response. To test this, we analyzed nsp2 sequences in seven pigs from the PRRS Host Genetics Consortium (PHGC). The selected pigs differed in disease course: two that successfully cleared the virus, two with persistent viremia, and three that initially cleared the virus but rebounded in viral load by day 42. A 2700 nucleotide region spanning the large hypervariable region of nsp2 was amplified from the inoculum, day 7 (early) of all pigs, and a late day from the persistent and rebound pigs. Amplicons were cloned and 7-30 clones from each sample were sequenced. The sequences were evaluated by discriminant analysis of principal components (DAPC) to determine how nsp2 populations changed over time, and in respect to the different disease courses. The inoculum population was distinct from both early and late populations. The next most distinct virus populations were those from persistent and rebound pigs. So, although both persistent and rebound virus changed relative to the inoculum virus, they changed in different ways. In addition, we identified identical nucleotide changes that arose at day 7 and became the dominant variant in the majority of infected pigs. Together, these results suggest that nsp2 may be under selective pressure for growth *in vivo*. We also analyzed variation within epitopes and non-epitope regions by computing the average entropy across sites within and outside of known B-cell epitopes. There was no significant difference in variation in epitope and non-epitope regions, indicating no strong evidence of immune selection over the course of the infection.

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Genetic characterization of a field strain of murine arterivirus lactate dehydrogenase-elevating virus and comparative analysis with other member viruses in the family Arteriviridae

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PRRS virus belongs to the family Arteriviridae which also includes equine arterivirus (EAV), lactate dehydrogenase-elevating virus (LDV) of mice, and simian hemorrhagic fever virus (SHFV). These viruses infect macrophages as a primary target and tend to undergo persistence in infected hosts. The genomic RNA of arteriviruses varies between 12-16 kb in size but their genome organization and coding strategies are relatively consistent with some minor variations. At least 10 functional open reading frames (ORFs) are found in the PRRSV genome: ORF1a, ORF1b, ORF2a, ORF2b, and ORFs 3 through 7, plus recently identified ORF5a within ORF5. For SHFV, ORFs 2a, 2b, and 3 residing downstream of ORF1b was reported to be duplicated, and the gene duplication has recently been confirmed by sequencing two field isolates. Recently, a -2 ribosomal frame-shifting has been identified for PRRSV for expression of nsp2TF (transframe) in the non-structural protein (nsp) 2-coding region of ORF1a, but the nsp2TF gene is absent in EAV. Of EAV, LDV, and SHFV within the family, LDV is the closest to PRRSV in its genomic similarity but a little is known for this virus. In an attempt to expand our understanding on the genetics of PRRSV, a field isolate of LDV was obtained from a mouse breeding colony and was designated LDV-Urbana. Then the full-length genomic sequence was determined from the virus without cell culture amplification. In comparison with the LDV-P laboratory strain, the LDV-Urbana sequence was only similar by 87 % at the full-genomic sequence. As with PRRSV, all ORFs were identified in the genome, and nsp2TF, ORF5a, and E protein genes were retained in the Urbana strain. At the amino acid sequence levels, genetic similarities vary between 91-97 % in comparison with the LDV-P strain. The critical motifs for polyproteins processing including papain-like cysteine proteases, 3C-like protease, and serine protease were conserved, suggesting appropriate nsp biogenesis from pp1a and pp1ab. The structural genes were similar to the P strain by 91%, suggesting diverse genetic evolution of the virus. Based on the genetic information of the field strain of LDV, an infectious cDNA clone is being constructed. The LDV infectious clone will be a valuable genetic tool to study the molecular biology and pathogenesis of PRRSV.

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Deep sequencing analysis of PRRSV genetic variation among cell types

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus with an extremely high mutation rate estimated at ~10^{-2}/site/year. Mutations can arise from viral RNA polymerase infidelity, genomic recombination, and host cell mutator activity. However, the frequency of nucleotide variations across individual sites in the viral genome, which might help address the contribution of various mutational mechanisms, has not been investigated in permissive cells or host animals. Since biological and antigenic variation arising from these mutations may contribute to disease severity, incomplete effectiveness of vaccination, and prolonged infection, we examined this question using ultra-deep sequencing. Strain variation has been examined extensively through consensus ORF5 sequencing analysis, however, nucleotide sequence variation across the entire genome of individual viral genomes in a viral population has not been evaluated. We sequenced three independent virulent PRRSV strains grown in two different permissive cell types at an average redundancy between 6,000- and 50,000-fold. Fifty bp, paired-end reads were mapping to the corresponding reference genome and single nucleotide polymorphisms were detected across the genome. Our preliminary results show that the highest mutation frequencies were detected in nonstructural protein 2 coding region (nsp2), nsp3, and nsp11. Overall nucleotide substitution patterns were random, but at frequencies higher than 1%, A to G and G to A substitutions were over-represented, suggesting the potential editing activities of a cytoplasmic form of the apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3 (APOBEC3) family. PRRSV whole genome SNP analysis showed that the mutational spectrum was dependent on both virus strain and permissive cell type, either porcine macrophages or MA-104, a simian epitheliod cell line. Overall, host cellular anti-viral mechanisms appear to have a limited effect on the PRRSV mutation rate, suggesting that antigen-specific adaptive immunological responses may play a dominant role in driving PRRSV mutation.

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Amino acid and phylogenetic analysis of some PRRSV strains from Romania

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The aim of this study was to evaluate the genetic diversity and amino acid analysis of PRRSV isolates from different areas in Romania by comparing the nucleotide sequences obtained for ORF5 gene and ORF7 gene with reference sequences from GenBank. This study was conducted on national scale and for establishing the PRRSV genetic diversity in the swine farms 605 samples were collected from different individuals (3 months – 4 years).
The detection of the virus was made by Real Time PCR using specific kits and protocols, followed up by Sanger sequencing and data interpretation.
The amino acid sequences of both genes were aligned using BioEdit program, resulting in a 201 amino acids alignment corresponding to ORF5 gene and a 128 amino acid alignment corresponding to ORF7 gene. We identified for ORF5 two hypervariable regions, one in the signal peptide and one in the beginning of the mature chain. Interestingly, for ORF7, the amino acid sequence for the isolate Rom22 has an asparagine inserted at position 12 of the sequence and one substitution in position 42, for the isolate Rom26, three amino acid substitutions in positions 4, 8 and 16 and for the isolate Rom30 one amino acid substitution in position 124.
The analysis of amino acid sequences evidenced for both GP5 and N-nucleocapsid proteins the belonging Romanian virus to type 1.
One of our aims was to investigate the amino acid difference among the subtype 1 of Romanian isolates. In particular, some known functional domains of GP5 such as the signal peptide, mature chain with transmembrane regions, some motifs in GP5 - like primary neutralizing epitope and decoy epitope were also analyzed according to a previous paper published.
Secondly, phylogenetic analysis was performed for both genes and the sequences revealed that the Romanian PRRSV nucleotide sequences clustered in three groups within the subtype 1.
In conclusion, the results obtained from the amino acid sequences and from phylogenetic trees confirm the affiliation of all Romanian isolates to the subtype 1 of PRRSV.

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Porcine respiratory and reproductive virus structural proteins GP2a and N activates transcription factor AP1 and NF-kB

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Objective: Porcine reproductive and respiratory syndrome virus (PRRSV) is a threat to the pork industry, and its pathogenesis needs further investigations. The structural and nonstructural proteins of the virus act in concert with the host cellular proteins for its successful infection, propagation and maintenance. Host transcription factors (TFs) are one of the major intermediate players in this process. Here, we investigated the role of two structural proteins of PRRSV, the glycoprotein 2 (GP2) and N, in mediating virus-host interactions.

Method: Two stable cell lines (MARC-2a and MARC-N) expressing GP2 and N proteins respectively were established for this codon optimized N and GP2 gene (PRRSV strain VR2332) was cloned to pIREShyIA plasmid and transfected to MARC-145 cell line. Positive clones expressing viral proteins were selected by hygromycin B (300 ug/ml) and named as MARC-N and MARC-2a cells. The activation of the NF-κB and AP-1 in these cell lines were evaluated by SEAP reporter assay. Nuclear fraction was separated from the cell lines and western blot was performed to verify the ESAP reporter assay result. Apoptosis was induced in these cells by staurosporine treatment. Annexin-V, PI staining and Hoechst 33342 staining was performed for evaluation of apoptosis. Relative quantification of anti-apoptotic genes MCL-1, HSP-90 and APAF-1 m-RNA was performed by real time PCR.

Result: Significantly high activities of NF-κB and AP-1 in MARC-N and MARC-2a cells were found. Staurosporine induced apoptosis in the cells showed apoptotic resistance in MARC-2a in comparison to MARC-N and MARC-145 cells. Upon anti-apoptotic investigation in MARC-2a cells, we found an increased m-RNA level of HSP-90 and down regulation of APAF-1 (member of apoptosome complex), which has an inverse relation with HSP-90 level.

Conclusion: Overall, our data lead us to a conclusion that although both stable cell lines activate NF-κB and AP-1, GP2 triggers the anti-apoptotic process through another intermediate step that needs to be further investigated.

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Electrostatic particle ionization (EPI) system as a means to decrease artificially generated PRRSV aerosols

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Introduction: Porcine reproductive respiratory syndrome virus (PRRSV) is arguably the most significant swine pathogen for which airborne transmission appears important in pig dense regions. The EPI system is a technology capable of reducing airborne agents due to its ability to clump and settle airborne particles and thereby improve air quality and potentially decrease the risk of pathogen dissemination. However, the magnitude of this "air cleaning" effect is influenced by both distance to the source of ions and by environmental characteristics. The objectives of this study were to determine, under experimental conditions, the effect of the EPI system on particle size distribution and quantity of PRRSV in artificially generated aerosols.
Materials & methods: The EPI system was installed at 3 levels (1, 2, 3m from the ground) in a 35 m^3 BSL-2 room isolation room located in the animal isolation units at the University of Minnesota, St. Paul Campus. Artificially generated PRRSV aerosols were sampled using an Andersen Cascade Impactor capable of collecting particles by size ranging from 9 to 0.4µ (8 stages). Air samples were collected with the EPI system on and off for 30 min and analyzed by quantitative RT-PCR. Three replicates per level were performed. Differences in viral quantities (RNA copies/m^3) obtained for each of the particle size ranges were analyzed. All results were fit into a regression model to predict the efficiency of the system in removing virus particles from the air and adjusted for the conditions of this study.
Results: A total of 144 air samples were analyzed. The PRRSV qRT-PCR analysis of each Andersen Impactor stage demonstrated a decrease in the quantity of PRRSV from the air when the EPI system was on. The reduction was significant for the largest particles and the increase in the distance from the EPI lines (Table 1).

Particle sizes	9.0-10.0µ	5.8-9.0µ	4.7-5.8µ	3.3-4.7µ	2.1-3.3µ	1.1-2.1µ	0.7-1.1µ	0.4-0.7µ
EPI 3m	*3.0(1.3,4.7)	*3.7(2.1,5.4)	*3.8(2.2,5.5)	*2.6(1.0,4.2)	1.3(-0.3,3.0)	1.1(-0.6,2.7)	1.1(-0.6,2.7)	1.1(-0.6,2.7)
EPI 2m	*4.1(2.5,5.8)	*3.4(1.7,5.0)	0.39(1.8,2.0)	-0.1(-1.7,1.6)	-0.1(-1.7,2.0)	0.5(-1.1,2.1)	1.0(-0.7,2.6)	1.1(-0.6,2.7)
EPI 1m	1.1(-0.5,2.8)	1.2(-0.4,2.9)	-1.5(-3.1,0.2)	-0.4(-2.0,1.3)	-0.2(-1.8,1.5)	-0.1(1.7,1.6)	0.9(-0.7,2.6)	0.3(-1.3,2.0)

Table 1. Concentration difference in log scale (logistic regression model LSMeans) of ORF6 RNA copies/m3 of artificial aerosol (EPI system off minus EPI system ON) for the different particle sizes measured with the Andersen Impactor at different distances from the EPI lines.

Conclusions: Our results with artificially generated PRRSV aerosols indicated that particle size and distance to the source of ions interacted in the efficiency of the system. The most pronounced reductions were observed at 3m and particle sizes of 10 to 5 microns. Further studies are needed to corroborate these results with aerosolized viral particles generated by infected pigs. Decreasing the infectious viral load of PRRSV in the air of positive pig farms could decrease the likelihood of dissemination of airborne pathogens to neighboring pig sites.

Acknowledgments
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Porcine epidemic diarrhea virus (PEDV): origin of the U.S. strains and current status in China

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The sudden emergence of porcine epidemic diarrhea virus (PEDV), for the first time in the United States causes significant economic and public health concerns. Since its recognition in May 2013, PEDV has rapidly spread across the United States, resulting in high mortality in piglets in 18 States. Three emergent strains of PEDV isolated from outbreaks in Minnesota and Iowa were characterized. Genetic and phylogenetic analyses of the three U.S. strains revealed a close relationship with recent Chinese PEDV strains and their likely Chinese origin. All PEDV strains with known full-length genomic sequences fall into two distinct genogroups, designated genogroup 1 (G1) and genogroup 2 (G2). The U.S. PEDV strains belong to G2 and underwent evolutionary divergence, which can be classified into two sublineages. Molecular clock analysis of the divergent time based on the complete genomic sequences is consistent with the actual time difference, approximately 2 to 3 years, of the PED outbreaks between China (December 2010) and the United States (May 2013). The emergent U.S. PEDV strains share unique genetic features at the 5-untranslated region with a bat coronavirus provided further support of the evolutionary origin of PEDV from bats and potential cross-species transmission. Currently, attenuated vaccines based on the classical G1 strains (CV777, DR13, SM98, etc.) are not effective against the circulating G2 strains in China. Feedback is still a practical "vaccination" protocol in Chinese pig farms. However, the issue of lack of cross-protection against heterologous strains is challenging, possibly due to the antigenic variations on the spike gene. Our results provide much needed information to devise effective preventive and control strategies against PEDV.

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Pigs immunized with modified live Chinese high pathogenic PRRSV vaccine are protected from North American PRRSV strain NADC-20

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Porcine reproductive and respiratory syndrome (PRRS) causes huge economic loss to the swine industry worldwide, and vaccination is the most effective way to control the disease. Recently, strains of highly pathogenic PRRSV (HP-PRRSV) have appeared in China and Southeast Asia. Traditional type 2 modified live virus (MLV) vaccines developed in the United States offer no protection to these HP-PRRSV strains. Modified live vaccines specific to HP-PRRSV strains available in China are reported to provide protection to the Chinese strains of HP-PRRSV, however, the efficacy of Chinese HP-PRRSV vaccines to current circulating North American PRRSV viruses has not been reported. The aim of this study is to investigate whether pigs challenged with the North American NADC-20 strain are protected by vaccination with Chinese MLV HP-PRRSV vaccines. On day 0, pigs were vaccinated with Chinese JXA1-R-MLV vaccine or a mock vaccine. After 28 day post vaccination, pigs were challenged with $2x10^5$ $TCID_{50}$ NADC-20 PRRSV. The MLV-HP-PRRSV vaccinated pigs showed good protection to NADC-20 challenge as shown by reduced virus-induced-fever, reduced lung pathology scores, and lower NADC-20 virus load in the blood. PRRSV-specific Ab, as measured by IDEXX ELISA, appeared one week after vaccination and virus neutralizing Abs were detected 4 weeks post vaccination. Vaccinated pigs developed high titers of viral neutralizing Abs to NADC-20, JXA1-R, and HV-HP-PRRSV (a highly pathogenic strain of PRRSV). The secretion of innate cytokines IFN-alpha and IFN-beta were elevated in the lung tissue at necropsy, but the level TNF-alpha was decreased in the lung tissue of MLV-HP-PRRSV vaccinated animals. The level of adaptive cytokine IFN-gamma was enhanced in the serum and more IFN-gamma-secreting PBMCs were generated in pigs vaccinated with MLV-HP-PRRSV. In summary, our study provides the first evidence that Chinese HP-PRRSV vaccines confer protection to the North American PRRSV strain NADC-20. Therefore, the availability of Chinese HP-PRRSV vaccines in North America may not only act to increase the preparedness of possible transmission of HP-PRRSV to North America but also help protect pigs against PRRSV strains native to North America.

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Porcine circovirus type 2 in China: an update to insight on its prevalence and control

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Currently, porcine circovirus type 2 (PCV2) is considered as the major pathogen of porcine circovirus associated-diseases (PCVAD) that causes large economic losses for swine industry in the world annually, including China. Since the first report of PCV2 in 1998, it has been attracting tremendous attention for the government, farming enterprises, farmers, and veterinary practitioners. Chinese researchers have conducted a number of molecular epidemiological works on PCV2 by using molecular approaches in the past several years, resulting in identification of novel PCV2 genotypes and PCV2-like agents, as well as the description of new prevalence patterns. Since late 2009, commercial PCV2 vaccines, including subunit vaccines and inactivated vaccines, have already been used in Chinese swine farms. The aim of the review is to update the insight on the prevalence and control of PCV2 in China, which would help to understand epidemiology, control measures, and design of novel vaccines for PCV2.

Meetings of Interest

The **2013 Conference of Research Workers in Animal Diseases (CRWAD)** Meeting will be held December 8-10, 2013, at the Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois. http://www.cvmbs.colostate.edu/mip/crwad/

The 45th annual meeting of the **American Association of Swine Veterinarians** (AASV) is scheduled for March 1-4, 2014 in Dallas, Texas, USA. http://www.aasv.org/annmtg/

World Pork Expo will be held June 4-6, 2014 in Des Moines, Iowa. https://www.worldpork.org/

The **23rd International Pig Veterinary Society Congress** is scheduled for June 8-11, 2014 in Cancun, Mexico. http://www.ipvs2014.org

European Federation of Animal Science 65th Annual Meeting will be held August 25-28, 2014 in Copenhagen, Denmark. www.eaap.org

Allen D. Leman Swine Conference will be held September 13-16, 2014. http://www.cvm.umn.edu/vetmedce/events/adl/

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