# 2012 International PRRS Symposium

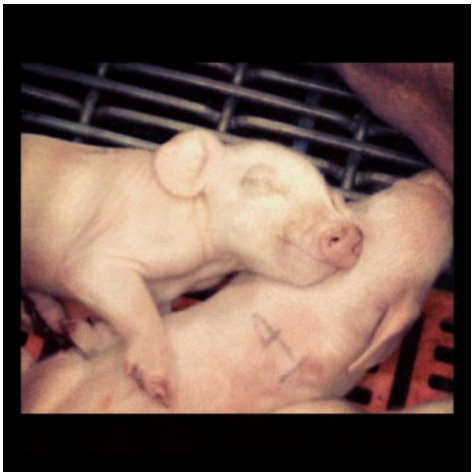
# National Swine Improvement Federation (NSIF) Conference

## BIVI PRRS Area Regional Control & Elimination Seminar

# Final Program

**Kansas City Marriott Downtown**
**Kansas City, Missouri**

**November 29th-30th, 2012**



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**Dr. David Benfield (IPRRSS travel fellowship)**
**Dr. Joan K. Lunney (IPRRSS travel fellowship)**

## *2012 IPRRSS/NSIF Conference Organizing Committee:*

| | |
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## *David A. Benfield IPRRSS Student Travel Fellowship*

David A. Benfield, who co-discovered the PRRS virus, 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 viral diseases of food animals. In 1990, he investigated the cause of "mystery swine disease", later known as PRRS. He provided the first description of the virus in 1992. For more than two decades, 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 travel 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 and related viruses.

## ***2012 David A. Benfield IPRRSS Student Travel Fellowship Recipients***

**Zeenath Islam**
University of Edinburgh

**Hein Min Tun**
The University of Hong Kong

**Lianghai Wang**
China Agricultural University

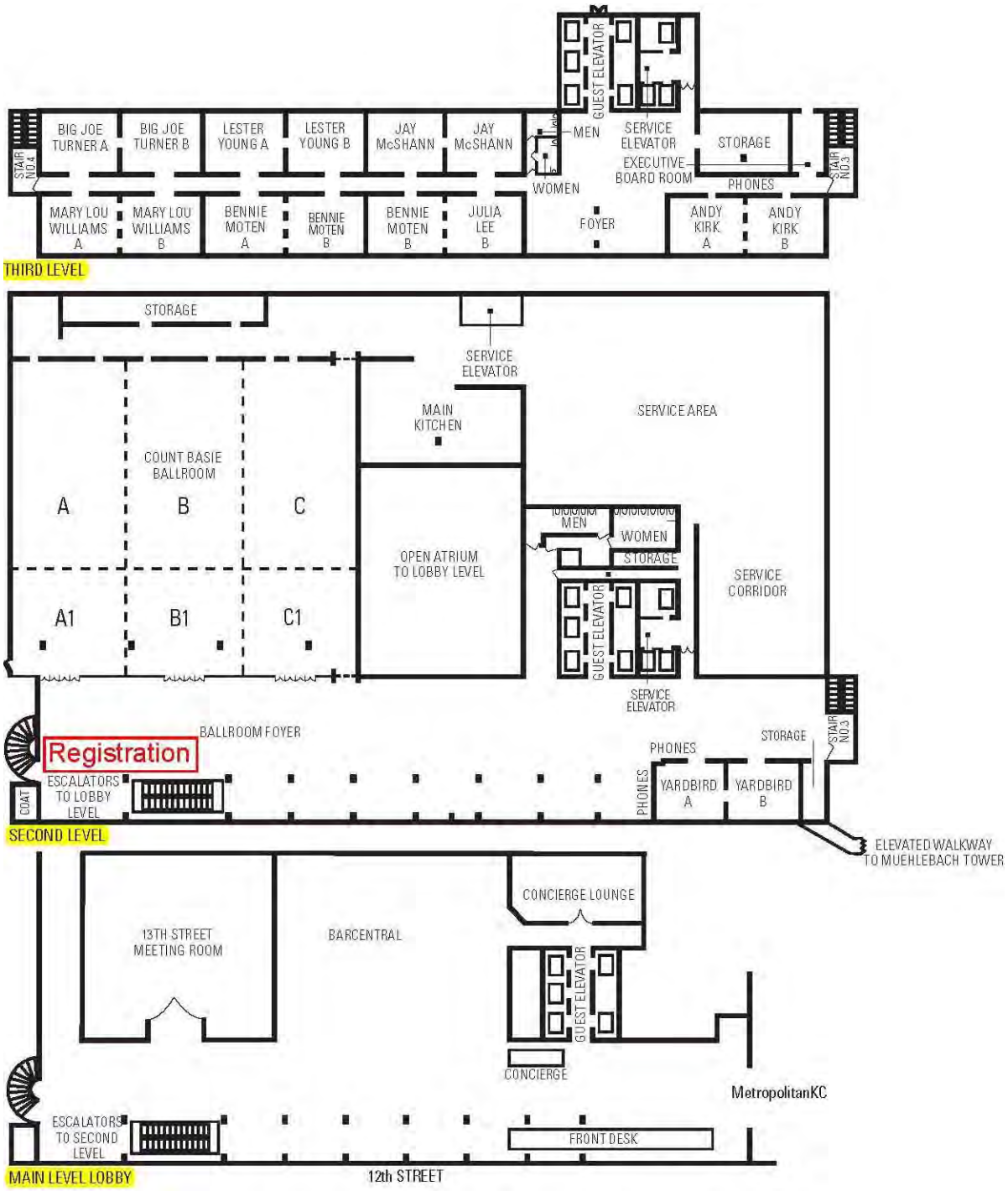
### ***CE Credit Information***

The 2012 International PRRS Symposium Program and National Swine Improvement Federation Conference have 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 IPRRSS Registration Desk.

## Kansas City Marriott Downtown

200 West 12th Street
Kansas City, Missouri, USA
Phone: 1-816-421-6800

### Meeting Rooms Map



# Joint Meetings:

# 2012 International PRRS Symposium (IPRRSS) and National Swine Improvement Federation (NSIF) Conference

## November 29 - 30, 2012 - Marriott KC Downtown, Kansas City, Missouri

### *Wednesday, November 28*

**3:00 pm - 7:00 pm - IPRRSS and NSIF Registration Opens - (***Marriott Tower, 2nd Level Foyer***)**

### *Thursday, November 29*

**7:00 am - IPRRSS and NSIF Registration Opens and Poster Set-up - (***Marriott Tower, 2nd Level Foyer***)**

**8:00 am - 5:00 pm** **NSIF - Pig Genetics and Breeding Conference**
(*Marriott Tower, 2nd Level - Basie C*)

***Session 1:*** ***Utilizing Genetic Improvement to Capture System Profitability***
***Moderator: Dr. Mark Knauer, North Carolina State University***

| | |
|---|---|
| 8:00 - 8:45 | Key Economic Drivers of an Integrated Pork Production System<br>Dr. Noel Williams, Iowa Select Farms |
| 8:45 - 9:30 | It's About Dam Time<br>Dr. Chad Hastad, New Fashion Pork |

***Session 2:*** ***Genomics - From Obstacles for Implementation to Realized Genetic Progress***
***Moderator: Dr. Justin Fix, National Swine Registry***

| | |
|---|---|
| 9:30 - 10:00 | Technical Challenges to Implementation of Genomic Selection<br>Dr. Christian Maltecca, North Carolina State University |
| 10:00 - 10:30 | BREAK |
| 10:30 - 11:00 | Economics of Genomic Selection in Swine<br>Dr. Ken Stalder and/or Caitlyn Abell, Iowa State University |
| 11:00 - 11:30 | Accuracy of Imputation and Potential Benefits<br>Dr. Juan Pedro Steibel, Michigan State University |
| 11:30 - 12:00 | Genomic Selection - Dairy Cattle Challenges and Successes<br>Dr. John Cole - AIPL |
| 12:00 - 12:30 | Genomic Selection - Swine Challenges and Successes<br>Dr. Matt Culbertson, PIC |
| 12:30 - 2:00 | Awards Luncheon |
| 1:30 - 2:00 | Awards |
| 2:00 - 2:30 | Graduate Student Presentation |

***Session 3:*** ***Selecting for Pig Robustness***
***Moderator: Dr. David Casey, PIC***

| | |
|---|---|
| 2:30 - 3:00 | Selecting for Robustness in Pigs<br>Dr. Pramod Mathur, TOPIGS |
| 3:00 - 3:30 | Selecting for Robustness in Broilers<br>Dr. Danny Lubritz, Cobb-Vantress |
| 3:30 - 4:00 | BREAK |

## Thursday, November 29

**8:00 am - 5:00 pm** **NSIF - Pig Genetics and Breeding Conference**
(*Marriott Tower, 2nd Level - Basie C*)

***Session 4:*** ***Selecting for Sow Robustness***
***Moderator: Dr. David Casey, PIC***

4:00 - 4:30 Sow Robustness: What it is and its Importance to the Bottom Line
Dr. Benny Mote, Fast Genetics

4:30 - 5:00 Selecting for Robustness in Dairy Cattle
Dr. Bradley Heins, University of Minnesota

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**8:00 am - 12:00 pm** **PRRS CAP Vaccine Workshop: PRRS Vaccines - Past, Present, and Future**
(*Marriott Tower, 2nd Level - Basie A/B*)

8:00 The Role of Host Genetics in Bringing the Pig Closer to the Vaccine
Bob Rowland, Kansas State University

8:30 Scaling and Sustaining in Effective Large-Scale PRRS Control: It Takes a (Committed) Village, and a (Full) Toolbox
Dale Polson, Boehringer Ingelheim Vetmedica, Inc.

9:10 Alphavirus Replicon-Based PRRSV Vaccine Development
Mark Mogler, Harrisvaccines

9:50 BREAK

10:10 Molecular Breeding of PRRSV for Novel Vaccine Development
X.J. Meng, Virginia Tech

10:50 PRRS - Progress Against a Challenging Disease
Jay Calvert, Pfizer Animal Health, Fostera

11:30 Roundtable Discussion on the Future of PRRS Vaccines

12 Noon LUNCH - provided by NSIF, IPRRSS and BIVI

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**1:00 pm - 5:00 pm** **Boehringer Ingelheim Vetmdica, Inc. PRRS ARC & E Seminar**
(*Marriott Tower, 2nd Level - Basie A/B*)

1:00 - 1:30 Sufficient Surveillance for ARC&E: Getting the Big-Picture Without Missing the Important Stuff
Dr. Dale Polson, Boehringer Ingelheim Vetmedica, Inc

1:30 - 2:00 Disease Bioportal: Visualization and Analytic Tools for PRRS Epidemiology
Dr. Andres Perez, University of California, Davis

2:00 - 2:30 Application of Disease Bioportal for Decision Making in a Production System
Dr. Jean-Paul Cano, Boehringer Ingelheim Vetmedica, Inc

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## *Thursday, November 29*

**1:00 pm - 5:00 pm** **Boehringer Ingelheim Vetmdica, Inc. PRRS ARC & E Seminar**
(*Marriott Tower, 2nd Level - Basie A/B*)

2:30 - 3:30 Area Regional PRRS Control Projects Breakout Session

| | Corner 1 | Corner 2 | Corner 3 | Corner 4 |
|---|---|---|---|---|
| **2:30-2:40** | Project_W1 | Project_E1 | Project_E4 | Posters |
| **2:40-2:50** | Project_W2 | Project_E2 | Project_W4 | Posters |
| **2:50-3:00** | Project_W3 | Project_E3 | MN N212 | Posters |
| **3:00-3:10** | Project_W1 | Project_E1 | Project_E4 | Posters |
| **3:10-3:20** | Project_W2 | Project_E2 | Project_W4 | Posters |
| **3:20-3:30** | Project_W3 | Project_E3 | MN N212 | Posters |

-All presenters will hand out 1-page summaries

3:30 - 4:00 Update on Tools for PRRS Control: Filtration, Biosecurity, Vaccination
Dr. Scott Dee, Pipestone Veterinary Clinic

4:00 - 4:30 Emergency Measures for Disease Control Programs
Dr. Jean-Pierre Vaillancourt, University of Montreal

4:30 - 5:00 Applying Epidemiology to Animal Disease Control: A Review on Personal Experience
Dr. Preben Willeberg, University of California, Davis

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**5:00 pm - 7:00 pm** **Reception and Poster Session (cash bar)**
(*Marriott Tower, 2nd Level Foyer*)

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## *Friday, November 30*

**7:00 am -** **IPRRSS and NSIF Registration Opens - (***Marriott Tower, 2nd Level Foyer***)**

**7:00 am - 8:00 am** **Breakfast Provided by IPRRSS and NSIF - (***Marriott Tower, 2nd Level Foyer***)**

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**8:00 am - 12:00 pm** **NSIF - IPRRSS Sponsored Talks on the Host Response to PRRSV Infection**
**(***Marriott Tower, 2nd Level - Basie A/B***)**

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|---|---|
| 8:00 | Progress of the PRRS Host Genetics Consortium: Variation in Gene and Protein Expression in Response to PRRSV Infection<br>Joan Lunney, USDA ARS - Beltsville |
| 8:30 | Phenotypic Variability to PRRS in the Reproductive Model: A Potential Opportunity<br>John Harding, University of Saskatchewan |
| 9:00 | Genetic Basis of Host Response to PRRSV Infection<br>Nick Boddicker, Iowa State University |
| 9:30 | Perspectives on Efforts in the EU and Beyond<br>Stephen Bishop, The Roslin Institute, University of Edinburgh |
| 10:00 | BREAK |
| 10:30 | Genomic Analysis of the Differential Response in Experimental Infection with Porcine Circovirus 2<br>Daniel Ciobanu, University of Nebraska |
| 11:00 | Genetic Engineering of Pigs for PRRSV Resistance<br>Randy Prather, University of Missouri |
| 11:30 | Roundtable Discussion<br>Graham Plastow, University of Alberta |
| 12 Noon | LUNCH - provided by IPRRSS |

**1:00 pm - 5:00 pm** **Ten Years of PRRS CAP and National Pork Board Research Progress in PRRS Control**
**(***Marriott Tower, 2nd Level - Basie A/B***)**

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|---|---|
| 1:00 | Ten Years of Advancements in PRRSV Immunity<br>Michael Murtaugh, University of Minnesota |
| 1:45 | 10 Years of Research in PRRSV Epidemiology/Ecology - An Overview<br>Jeff Zimmerman |
| 2:30 | PRRSV Vaccine and Diagnostic Assay Development:<br>From Basic to Applied Sciences<br>Ying Fang, South Dakota State University |
| 3:15 | BREAK - POSTER REMOVAL |
| 3:30 | Ten Years of Research in Structure and Function of PRRSV Proteins<br>Dongwan Yoo, University of Illinois |
| 4:15 | Progress in PRRS Area Regional Control and Elimination<br>Robert Morrison, University of Minnesota |
| 5:00 | ADJOURN |

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# ABSTRACTS
# 2012 Speakers



# POSTER ABSTRACTS
# PRRS Epidemiology, Heterogeneity, and Evolution

## PRRS Molecular Biology: Structural and Functional Relationship

‡ Denotes NSIF abstract

## PRRS Vaccines and Immunity

## PRRS Virus-Host Interaction

‡ Denotes NSIF Abstract

# 1
# Perspectives on PRRS research in Europe

S.C. Bishop[1]*, T. Ait-Ali[1], A.B. Doeschl-Wilson[1], C.M. Orrett[1], Z. U. Islam[1], D.G. McLaren[2], N. Deeb[3] and A.L. Archibald[1]
[1]The Roslin Institute & R(D)SVS, University of Edinburgh, Midlothian, UK, [2]Genus PIC, De Forest, WI, USA, [3]Genus PIC, Hendersonville, TN, USA

Europe has a large and active PRRS research community, reflecting the importance of the disease to the European pig industry, which mirrors many of the activities underway in the US and Canada. A framework for coordinating these activities and communicating the outcomes of the research is given by EuroPRRSnet (www.europrrs.net), an EU-funded network. This initiative aims to develop more effective multidisciplinary collaborative PRRS research in Europe centred on PRRSV epidemiology, immunopathology, vaccine development and diagnostics tools. Major research topics currently include extensive efforts in the field of vaccine development, with a focus on exploring the potential for protection across PRRSV strains. Allied to this, much effort focusses on the definition and diagnosis of PRRSV subtypes, their pathogenicity and strain-specific immune responses. The EU-Asia project PoRRScon (www.porrscon.ugent.be/index.html) coordinates much of this research. Further, resources are put into devising sustainable methods of control, particularly developing combined control approaches including biosecurity and attempts at eradication.

Several groups within Europe are also actively involved in various aspects of host genetics work, exploring options for exploiting genetic differences in host responses to infection to assist in disease control. This research involves the elucidation of breed differences in resistance to PRRSV infections, as well as genome scans for PRRS resistance in commercial pigs, in both the growing pig and the pregnant sow. In particular, The Roslin Institute has a cohesive and wide-ranging research program in PRRS resistance, much of which involves close collaboration with academic and industrial partners in North America. These studies range from elucidation of host functional responses to infection, through efforts to genetically improve resistance through natural variation and engineered resistance, to population-level studies and the development of mathematical models to describe the impacts of infection within hosts and the transmission of infection between hosts.

A major focus of research on host genetic resistance has been the comparison of host resistance in the pregnant sow and the growing pig. Roslin-led research, in collaboration with Genus, has involved detailed interrogation of field data from a Chinese herd with 60k SNP chip data available. The dataset has revealed differential impacts of PRRS on reproductive outcomes, dependent on when during pregnancy the sow faced infection, and has given indications of a locus on SSC4 associated with reproductive losses. Currently we are assessing this result in independent commercial populations and determining whether this QTL is consistent with the SSC4 QTL, identified by the PHGC, which affects viremia in growing pigs. Using Bayesian inference techniques we are also obtaining detailed interpretations of the viremia profiles seen in the pigs challenged with PRRSV by the PHGC. Further, we are using *in vitro* techniques to determine mechanisms of host cell permissiveness to PRRSV. The analysis of experimental data is complemented by the development of mathematical host-pathogen interaction models to explore alternative hypotheses underlying infection dynamics. Our ambition is to use such models to help design and explore alternative disease control strategies.

# 2
# Genetic basis of host response to PRRSV infection

N. J. Boddicker[1], J. K. Lunney[2], R. R. R. Rowland[3], D. J. Garrick[1], J. M. Reecy[1], and J. C. M. Dekkers[1]
[1]Department of Animal Science, Iowa State University, [2]USDA, ARS, BARC, [3]College of Veterinary Medicine, Kansas State University

Improvement of animal health, specifically for the economically important disease of PRRS, has been facilitated by improvements in biosecurity, vaccines and, most recently, the potential to use host genetics to select for more PRRS resilient pigs. The objective of this study was to discover the genetic basis of host response to PRRS virus infection using data from the PRRS Host Genetics Consortium PRRS-CAP project by conducting a genome-wide association analysis and estimating genetic parameters. Eight groups of ~200 commercial crossbred pigs were infected between 18 and 28 days of age with virus isolate NVSL 97-7985. Breeds represented in the crosses included Large White, Landrace, Yorkshire, Duroc, and Pietrain. Blood samples and body weights were collected up to 42 days post infection (dpi). Experimental pigs and their parents were genotyped with the Illumina Porcine 60k Beadchip that includes over 60,000 Single Nucleotide Polymorphisms (SNPs) across the genome. Whole genome analyses focused on viral load (VL = area under the curve for log-transformed RT-PCR based serum virus from 0-21 dpi) and weight gain (WG = gain from 0-42 dpi). Viral load data was only available for the first 7 trials. Across trials, VL and WG heritabilities that were estimated using pedigree information were 0.41 and 0.29, respectively. Single nucleotide polymorphisms that were associated with VL and WG were identified using Bayes-B of GenSel software. A 38-SNP (~ 1 Mb) region on chromosome 4 explained 14.6% and 9.1% of the genetic variance for VL and WG, respectively. The effect of this region acted in a dominant manner, with the favorable allele at a tag SNP that captured most of the effect of the 1 Mb region estimated to decrease VL by 4 units (0.53 phenotypic sd) and increase WG by 2 kg (0.49 phenotypic sd). The effect of this region was in the same direction and of similar magnitude within each trial. Furthermore, the favorable allele at the tag SNP was present at low frequency (0.04 to 0.34 %) in all breeds involved in the crosses and had an effect on phenotype regardless of breed or parental origin. Across all trials, the genotyped SNPs in the 1 Mb region were present in 77 unique haplotypes, of which 11 carried the favorable allele at the tag SNP. Furthermore, all haplotypes that carried the favorable allele were associated with improved viral clearance. In conclusion, the 1 Mb region on chromosome 4 explained a substantial proportion of genetic variation in response to experimental challenge with a specific strain of the PRRS virus. Estimates of heritability were substantial and, with a minor allele frequency of 0.15 across all trials, there is room for genetic improvement in PRRSV clearance. The SNPs in this region are in high linkage disequilibrium, which makes further fine mapping difficult. This work was supported by PRRS CAP, USDA NIFA Award 2008-55620-19132, NRSP-8 Swine Genome and Bioinformatics projects, the National Pork Board and breeding companies of the PRRS Host Genetics Consortium.

# 3
# Fostera™ PRRS –progress against a challenging disease

J.G. Calvert
Pfizer Animal Health, Kalamazoo, MI

More than 20 years have passed since Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) appeared in the swine industry and it still remains one of the major health challenges in pig production worldwide. Using a novel cell line Pfizer Animal Health recently introduced a new genotype 2 (North American genotype) modified-live virus (MLV) vaccine to the US and Canadian markets. Fostera™ PRRS contains a novel strain, and differs from the monkey-cell attenuated vaccines currently available. As with other genetically variable viruses, the extent to which immunity against one strain will also protect against others is a key question. Natural infection yields solid immunity against the homologous strain but only partial protection against heterologous strains. The level of protection of a vaccine will depend on both the overall immune response and the antigenic relatedness of the infecting strain. Unfortunately, however, the latter is difficult to predict. The ever-increasing genetic diversity amongst PRRS isolates takes the form of a continuous spectrum that persists over time, rather than a series of discrete steps in which new types emerge and quickly replace older types. Although it is possible to measure genetic homology, this may not fully correlate with the degree of cross-protection. In addition to its ability to mutate, the PRRS virus encodes a number of immune system evasion mechanisms that reduce the ability of infected pigs to mount an effective immune response. Despite the challenges mentioned above, both killed and modified-live vaccines against PRRS have been developed and are widely used. It is generally believed that the latter have proved much more efficacious, but the use of a modified-live virus for vaccination introduces possible complications. The vaccine virus may shed and spread from animal to animal, raising the question of reversion to virulence. Certain populations, for example naive pregnant sows, may be susceptible to pathogenic effects, such as abortion, from some otherwise well attenuated strains. These potential risks are intrinsic to modified-live virus vaccines and if necessary can be managed, but they are a complication and may pose some restrictions on how such vaccines can be used. Pfizer Animal Health's PRRSV research team has achieved scientific advances in our understanding of the genetic makeup of the virus and the importance of its structural proteins. From a practical perspective, however, the most significant breakthrough was the identification and isolation of the CD163 PRRS receptor and consequent development of novel cell lines to support virus growth. CD163 is normally expressed exclusively in macrophages, such as PAMs. Although other proteins have also been shown to be involved in the propagation of the PRRS virus by PAM cells, we have shown that transfection with the CD163 gene alone is sufficient to render a variety of non-permissive cell lines fully permissive to PRRSV, with production of progeny virus. The development of a new, non-simian cell line opened the way for Pfizer Animal Health to work in previously restricted areas, but it has scientific as well as commercial significance. The development of MLV vaccines involves attenuation by multiple passages through cell culture. During this process the virus gradually accumulates mutations that allow it to grow better in its new host cell, simultaneously losing properties that helped its survival and caused virulence in the pig. There is an element of randomness such that no two MLV vaccines are ever likely to be identical. Using different cell lines, however, greatly increases the likelihood of major differences in the mutations accumulated, as selective pressures will be different. Thus, the introduction of Fostera™ PRRS in the US and Canada offers pork producers a new and innovative tool for the control of PRRS.

# 4
# Genomic analysis of the differential response in experimental infection with Porcine Circovirus 2

E.E. Jobman[1], A.M. McKnite[1], T.W. Moural[2], J.K. Tart[1], J.W. Bundy[1], T.P. Bohnert[1], S.Y. Barnes[1], C.A. Kreikemeier[1], T.B. Engle[1], E.H. Davis[1], J.K. Qiu[1], J.A. Galeota[2], S.P. Harris[2], M.F. Rothschild[3], T.E. Burkey[1], R.K. Johnson[1], S.D. Kachman[4], D.C. Ciobanu[1*]

[1]Animal Science Department, University of Nebraska, Lincoln, NE, [2]Veterinary Diagnostic Center, School of Veterinary Medicine and Biological Sciences, University of Nebraska, Lincoln, NE, [3]Department of Animal Science, Iowa State University, Ames, IA, [4]Department of Statistics, University of Nebraska, Lincoln, NE

Porcine Circovirus 2 (PCV2) is the etiological agent of several associated diseases that impact health, efficiency and could lead to mortality. Disease progression is influenced by several factors of which host genetics and co-infection play important roles. Using various crossbred lines of pigs experimentally infected with PCV2b, we explored the genetic variation of the main indicators of disease progression and immune response. Growth, viral load and specific antibody response were profiled weekly during the 28 day challenge period. The initial results provide evidence of host variation in magnitude and time of immune response to PCV2b. A higher viral load was correlated with slower growth (r = - 0.29) and higher level of PCV2 - specific antibodies, IgM (r = 0.32 to 0.43) and IgG (r = 0.30 to 0.43). Tumor necrosis factor-alpha, haptoglobin and total antioxidant capacity were increased in pigs that expressed high viral load and low growth. A genome wide association study that included the genotypes of 56,433 SNPs uncovered genomic regions that influence the variation of disease phenotypes. Major clusters of SNPs that impacted viral load were found on SSC6 (36.2 Mb), SSC7 (30.4 - 30.8 Mb, 134.1 - 134.4 Mb) and SSC12 (18.2 - 18.6 Mb). The same region located on SSC7 had also an effect on growth during challenge. The cluster of genes from the Swine Leukocyte Antigen class II, is located in this region and could explain the observed phenotypic variation. These results represent the initial efforts to establish an extensive set of phenotypes and samples that will be used to uncover genes and genetic variants associated with PCVAD susceptibility. Application of DNA markers associated with disease susceptibility in selection programs has the potential to improve animal health, welfare and reduce production costs.

# 5
# PRRSV vaccine and diagnostic assay development: from basic to applied sciences

Ying Fang
Department of Veterinary and Biomedical Sciences, Department of Biology/Microbiology,
South Dakota State University, Brookings, South Dakota, USA

PRRS is still the most economically devastating swine disease worldwide. The challenge in developing a broadly effective PRRS vaccine is mainly due to natural characteristics shared with all RNA viruses: PRRSV undergoes rapid evolution, which creates genetically and antigenically heterogeneous populations. This is also reflected on our inadequate knowledge of mechanisms employed by the virus in evading host innate and adaptive immune responses. In an effort to overcome these major obstacles, our studies have been directed to identify the virulence determinants and develop strategies to enhance the viral specific immunity. With the development of PRRSV reverse genetic system and advanced knowledge on the structure and function of nonstructural proteins, the rationale design of genetically engineered PRRS vaccine has been explored. On the other hand, with the absence of a completely effective vaccine, one of the key approaches to achieve PRRS control and elimination is to timely identify PRRSV infection and prevent the spreading of viruses to large animal populations. Thus, the availability of rapid, highly sensitive and specific diagnostic assays is essential. Currently available PRRSV diagnostic assays provide good sensitivity and specificity in controlled settings. However, the field experience with PRRSV outbreaks suggests that substantial improvement is needed in almost all of the areas. The PRRSV nonstructural proteins have been tested as potential new antigens for the development of diagnostic assays. To assist field epidemiological surveillance, oral fluid-based diagnostic assays are being developed as an alternative to serum-based assays. In addition, high throughput, multiplexing Luminex technology is being adapted for PRRSV antigen and antibody detection. In parallel with PRRS marker vaccine development, differential diagnostic assays have been explored for the ability to differentiate the vaccinated animals from the naturally infected animals. These studies represent our initial effort to develop a new generation of PRRS vaccines and diagnostic assays. Future advancements in our understanding of PRRSV biology and host immunity will provide a basis for additional and improved approaches to the development of vaccines and diagnostic assays for PRRS control and elimination.

# 6
# Phenotypic variability in response to the PRRS virus in the reproductive model: a potential opportunity

J. Harding[1]*, C. Ashley[1], A. Ladinig[1], K. Clarke[1], Z. Ye[1], T. Chang[1], S. Detmer[1], P. Novakovic[1], D. Wilson[2], S. Walker[2], S. Napper[2], J. Wilkinson[3], R. Yates[4], N. McKenna[4], J. Lunney[5], J. Dekkers[6], R. Rowland[7], R. Kemp[8], G. Plastow[3]

[1]Western College of Veterinary Medicine, Univ. of Saskatchewan, Saskatoon, SK, [2]Vaccine and Infectious Disease Organization, Univ. of Saskatchewan, Saskatoon, SK, [3]Dept. Agricultural, Food and Nutritional Science, Univ. of Alberta, Edmonton, AB, [4]Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, [5]USDA, ARS, BARC, APDL, Beltsville, MD, [6]Dept. Animal Science, Iowa State Univ., Ames, IA, [7]Dept. Diagnostic Medicine and Pathobiology, Kansas State Univ., Manhattan, KS, [8]PigGen Canada, Guelph, ON

A large scale, multi-institutional project investigating genomic and phenotypic predictors of PRRSV resistance/susceptibility in pregnant gilts is underway at the University of Saskatchewan. This project links to the PRRS Host Genetics Consortium (PHGC) initiative by testing if the results obtained in the nursery pig model apply to reproductive PRRS.

Phenotypic data associated with multiple aspects of host immune response in pregnant gilts and foetuses will be analysed. Purebred Landrace gilts, synchronized and bred homospermically to York boars, are infected with type 2 PRRSV (NVSL 97-7895) at gestation day 85 (n=113) or are sham-inoculated (CTRL; n=19). Clinical signs are monitored daily. Blood, collected on 0, 2, 6, 19 days post inoculation (dpi), provides samples for measurement of PRRSV RNA concentration using a strain-specific in-house qPCR, white blood cell counts, and lymphocyte subset typing (T-helper, CTL, B-cells, gamma-delta cells, NK cells, myeloid cells) using flow cytometry. Gene networks regulating the *in vitro* responsiveness of peripheral blood mononuclear cells (PBMC) to homologous PRRSV and phorbol myristate acetate/ionomycin (PMA/I) will be assessed. Sera and the supernatants of PRRSV and PMA/I stimulated PBMCs will be tested for innate, T helper 1 (Th1) and Th2 cytokine levels by fluorescent microsphere immunoassay (FMIA).

Following humane euthanasia at 21 dpi, gilts and foetuses are dissected. The position, preservation status and body weight of each foetus is recorded. Serum and multiple tissues are collected enabling measurement of PRRSV RNA concentration in gilt tissues (uterus, lung, tonsil, tracheobroncheal and reproductive LN), amniotic fluid, fetal sera and thymus. Alveolar macrophages harvested from gilt bronchoalveolar lavage fluid are tested to determine variability in functional responses and kinase activities associated with PRRSV infection (kinases have central roles in virtually all cellular behaviour, including immune defence). Pertinent tissues will be examined histopathologically to assess mechanisms of fetal death and variability in host pathologic responses. Gilts, sires and foetuses will be genotyped with the Illumina Porcine SNP60 BeadChip, enabling genome wide association studies. Bayesian genomic selection models will be developed to evaluate associations among phenotypic and genotypic data using imputed or measured 60K data from gilts and foetuses (n=1400).

Funding for this project is provided by Genome Canada and Genome Prairie. We wish to thank the many people assisting with this project, including barn, animal care and necropsy staff, students, and administrative personnel.

# 7
# Progress of the PRRS Host Genetics Consortium: variation in gene and protein expression in response to PRRSV infection

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the most important infectious disease threatening pig production worldwide. The PRRS Host Genetics Consortium (PHGC) was established to probe the role of host genetics in resistance to PRRSV infection and related growth effects. The PHGC uses a nursery pig model; to date, 13 groups of 200 commercial crossbred pigs have been infected with PRRSV and followed for 42 days post infection (dpi). A major goal of the PHGC is to collect extensive samples for deep phenotypic analyses; to that end blood serum and Tempus (RNA) samples were collected at 9 timepoints, body weights recorded weekly, and tonsils stored for viral persistence studies. Genomic DNA was genotyped with the PorcineSNP60 chip for genome wide association studies (see Boddicker abstract). Results affirmed that all pigs become PRRSV infected with peak viremia from 4-21 dpi. Bivariate statistical analyses of viral load and weight data have identified PHGC pigs in different high/low virus/weight categories. Sera and RNA from pigs in selected high/low categories are now being analyzed for immune and genetic factors involved in viral replication and recovery from infection. Data will be presented on speed and levels of gene expression based on Pigoligoarray, RNA-seq and quantitative PCR analyses and of immune proteins using fluorescent multiplex immunoassays. Combined with the genetic data these analyses should identify biomarkers that distinguish PRRS resistant/maximal growth pigs from PRRS susceptible/reduced growth pigs. PHGC funding: US National Pork Board, USDA ARS and NIFA, NRSP8 Swine Genome and Bioinformatics Coordinators, Genome Alberta/ALMA, Genome Canada, and private companies.

# 8
# Molecular breeding of PRRSV for novel vaccine development

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Molecular breeding via DNA shuffling can direct the evolution of viruses with desired traits. We demonstrated rapid attenuation of PRRSV by DNA shuffling of the viral envelope genes from multiple strains. The GP5 and the GP5-M genes of different PRRSV strains were molecularly bred by DNA shuffling, and cloned into the backbone of a DNA-launched PRRSV VR2385 infectious clone. Two representative chimeric viruses, DS722 with shuffled GP5 genes and DS5M3 with shuffled GP5-M genes, were rescued. The two chimeric viruses replicated at lower levels and formed smaller plaques *in vitro*, and have significant reductions in viral RNA loads in sera and lungs, and in gross and microscopic lung lesions in pigs. Pigs vaccinated with the chimeric virus DS722 still induced protection against PRRSV challenge at a level similar to that of its parental VR2385 virus. Additionally, we also bred the PRRSV GP3 genes of genetically different PRRSV strains in an attempt to improve its heterologous cross-neutralizing ability. Eight GP3-shuffled chimeric viruses were recued and characterized. The chimeras had similar levels of replication both *in vitro* and *in vivo*, compared to the backbone parental virus, indicating that the GP3 shuffling did not impair the replication capability of the chimeras. The chimera GP3TS22 induced a significantly higher level of cross-neutralizing antibodies in pigs against a heterologous strain PRRSV FL-12. The results indicated that DNA shuffling of PRRSV structural genes can attenuate the virus and produce a chimeric strain with improved cross-neutralizing activity against a heterologous strain. Therefore, the findings have important implications future vaccine development.

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# 9
# Alphavirus replicon-based PRRSV vaccine development

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Alphavirus-derived replicon RNA particles (RP) are propagation-defective, single-cycle RNA vaccine vectors that are capable of eliciting potent humoral and cellular immunity to a variety of antigens. In addition, RP induce robust systemic innate immune responses. Recently, the RP platform system, derived from Venezuelan equine encephalitis virus (strain, TC-83), was granted USDA regulatory approval for use in veterinary vaccines. This represents the first ever licensing of the RP technology, which has demonstrated efficacy and safety in a wide range of disease and animal models.

The RP platform has been used to express various PRRSV proteins as vaccine antigens, and several candidate vaccines have been evaluated in challenge models. The RP PRRS vaccines induced specific humoral and cellular immune responses, and significantly reduced viremia and viral load. Additionally, pigs treated with an RP influenza vaccine 24 hours prior to PRRSV challenge had significantly reduced viremia and viral load. The use of RP allows for differentiation of vaccinated from infected animals, which is an important consideration for future PRRSV control efforts.

These results demonstrate that the RP platform is a potent tool for PRRSV research and vaccine development.

# 10
# Regional PRRS elimination

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The first regional project in United States aimed at controlling PRRS virus spread started in 2002 in eastern Rice Cy, Minnesota. Some progress was made but in retrospect, the region did not have the ideal attributes to determine whether a voluntary, regional, coordinated, PRRS control & elimination program was feasible. A second regional project started in 2004 in Stevens County in west central Minnesota and the project has been a resounding success. Approximately 90% of the producers in the county have participated and the prevalence of PRRS has decreased from approximately 50% of sites to less than 5% known sites having PRRS. The project has expanded twice and now includes the northern half of Minnesota. Today, we count approximately 20 regional projects around United States with the goal of controlling or eliminating PRRS virus. This is a phenomenal expansion and Canada has also initiated several projects in Ontario, Quebec and Alberta as have regions in Mexico, Netherlands and Japan.

The overarching goal of each project varies. Most projects have the goal of regional **control** whereby "*PRRS incidence, prevalence, morbidity or mortality is reduced to a locally acceptable level as a result of deliberate efforts. Continued intervention measures are required to maintain the reduction*." A regional control program will entail testing herds, surveillance, sharing information & acting accordingly. A few projects have the goal of regional **elimination** whereby "PRRS is *reduced to zero incidence in a defined geographical area as a result of deliberate efforts. This requires continued measures to prevent re-establishment of virus transmission*."

The projects vary in their progress and challenges. Seven regional projects have participated in the USDA funded PRRS CAP project and as such, share reports on their progress (PRRS.Org). The AASV PRRS task force has been integrally involved in the progress and its first effort led by Drs. Derald Holtkamp, Dale Polson and Montse Torremorell was the sow herd classification guideline (2010 JSHAP). Its second project was initiated at the 2010 committee meeting and is led by Dr. Jim Lowe. This is the development of a standard operating protocol for managing sow herds in an effort to manage PRRS virus and potentially move herds from being positive and unstable to negative. Additional challenges have been identified and working groups formed to address the following issues: (1) Develop a program to monitor prevalence and incidence of PRRS virus infection, (2) Develop guidelines for managing confidentiality and the risk of disclosure, (3) Develop guidelines for eliminating PRRS virus from farrow to finish farms, (4) Develop oral fluid sampling guidelines, (5) Developing guidelines for implementing common reports, including mapping Legends for Regions, (6) Uniform guidelines for risk based surveillance & geostatistical mapping, and (7) Develop guidelines for a certifying system for regions undergoing control / elimination projects. Each of these working groups reports progress to the AASV PRRS task force and ultimately to the AASV membership.

# 11
# Ten years of advancements in PRRSV immunity

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Vaccination is the principal means used to control and treat porcine reproductive and respiratory syndrome virus (PRRSV) infection. An array of PRRS vaccine products is available in various regions of the world. However, despite extensive efforts, little progress has been made to improve efficacy since the first introduction of a live, attenuated vaccine in 1994 in the USA. 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 in vivo challenge 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. In addition to these substantial barriers to new PRRSV vaccine development, there are enormous gaps in our understanding of porcine immunological mechanisms and processes that provide immunity to PRRSV infection and memory responses for long-term protection. In the last 10 years, PRRS CAP and NPB funded a variety of PRRS immunology and vaccinology studies to address these issues.

# 12
# Scaling and sustaining effective large-scale PRRS control– it takes a (committed) village, and a (full) toolbox

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This is not is a discussion about the past, present and future of PRRS vaccines. Instead, it is a discussion about the past, present and future of PRRS control and, ultimately, elimination. To be sure, vaccines should be considered an essential part of achieving the most effective and sustained PRRS control, just as is the case with other vaccines for contributing to the most effective control of other swine disease agents.

Many institutions and companies continue working on PRRS vaccine advancements, as are we. For two decades now, Boehringer Ingelheim Ventmedica Inc. (BIVI) has invested millions of dollars and tens-of-thousands of hours, and has collaborated extensively with the best researchers and institutions in the world to research and evaluate the newest and best vaccine technologies. BIVI continues to invest heavily and collaborate broadly with clear purpose: To develop and provide to the pork industry with new PRRS vaccines with significant advancements in performance and protection.

Yet in spite of these major investments, research hours, collaborations and technologic advancements, it remains a reality that no one has yet developed anything that improves on the original modified-live vaccine that first became available in the United States back in 1994. Since the introduction of the first MLV vaccine we have seen other modified live commercial and killed autogenous and commercial vaccines introduced – none of which have, by any scientific standard, proven to offer better performance.

The perfect (DIVA) vaccine may be out there – we continue to believe it is, and believe that it is well worth pursuing. But we also clearly understand that we cannot simply wait for its arrival as the "ultimate answer" for PRRS control. The BIVI Swine Team continues to invest, collaborate and aggressively pursue major advancements in controlling and eliminating PRRS. We're committed to leveraging every available effective tool to its maximum, as well as collaborating with all others in the "committed village" who, like ourselves, are driven to control and eliminate PRRS across the pork production industry.

Controlling (and, ultimately, eliminating) PRRS is clearly the consensus industry objective at every level – pig, pen, barn, site, flow, system, area, region and, yes, even globally. Vaccine plays an important role in achieving that objective at every level. However, in this discussion we are not going to focus on vaccine, but we are going to discuss vaccine in its relevant context – as one of several necessary, valuable and complimentary tools available today which, when used in concert, are highly effective for dealing with PRRS and achieving our consensus objective at every level. Since 2003 the BIVI Swine Team has been actively developing capabilities and collaborations for industry-wide application of PRRS Area-Regional Control and Elimination (ARCE). It is our belief that this approach offers the greatest opportunity for the effective and sustainable control (and eventual elimination) of PRRS in the USA and across North America.

# 13
# Disrupting s*ialoadhesin* and *CD163* to create pigs resistant to PRRSV infectivity

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Porcine reproductive and respiratory syndrome virus (PRRSv) is thought to bind to sialoadhesin as the primary receptor on the surface of alveolar macrophages. This binding enables vesicular internalization of the virus. After viral transit into endosomes, CD163 facilitates viral disassembly and release of the viral genome into the cell. Since PRRSv has such a negative economic impact on the swine industry our goal was to genetically engineer pigs that would be resistant to PRRSv infection. These genetic modifications would be useful for more fully understanding the mode of infection and, if permitted to enter the food supply, could reduce the economic impact of PRRSv on the world's pork supply. Our first modification created heterozygous *SIGLEC1* knockout boars by disrupting part of exon 1 and all of exons 2 and 3 in fetal-derived fibroblast cells followed by somatic cell nuclear transfer. These founder boars were mated to wildtype gilts, and male and female F1 $SIGLEC^{+/-}$ were produced. Some of these F1 females have been mated to the F1 males and are currently pregnant. If $SIGLEC^{-/-}$ animals are produced, our goal is to challenge them with PRRSv and determine if they show resistance to infection. Our second goal is to modify the N-terminal scavenger receptor cysteine-rich (SRCR) domain 5 of *CD163*. Previous reports have shown that disruption or replacement of SRCR domain 5 of *CD163* in cultured cells results in loss of PRRSv infectivity. For the *CD163* modification, exon 7, which encodes SRCR domain 5, is being modified to mimic human exon 10 which encodes SRCR domain 8 of the CD163-like 1 (*CD163L1*) gene. A targeting vector containing the mutated *CD163* is being used to transfect fetal-derived fibroblast cells. Once candidate colonies are identified, then somatic cell nuclear transfer will be used to create the pigs with this modification. Once animals with the homozygous domain swap of *CD163* are created, then a PRRSv challenge study will be conducted. It is anticipated that either mutation (*CD163* or *SIGLEC1*) will restrict infectivity. If this is not the case, then the combination of both *SIGLEC1* disruption and the modification of pig SRCR domain 5 of *CD163* will be tested. While researchers can use these pig models to characterize the mechanics of PRRSv infectivity, these genetic modifications may have a direct role in improving production agriculture.

# 14
# The role of host genetics in bringing the pig closer to the vaccine

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PRRSV employs an array of host evasion strategies that subvert innate and adaptive immune responses. One important strategy involves the capacity of the virus to generate a high degree of genetic diversity. The development of the next generation of PRRS vaccines is dependent on understanding its unique biology. A new tool for studying the immunological components of anti-PRRSV immunity is the availability of a pig that lacks B and T cells. The pig with severe combined immunodeficiency, or SCID pig, creates the opportunity to characterize the contributions of innate and adaptive immunity in the control of PRRSV replication (see Waide et al., abstract no. 59). Previous work (Boddicker et al, J Anim Sci. 90:1733-1746) identified a marker in SSC 4 linked to decreased virus load and increased weight gain during PRRSV infection. The marker locates to a family of interferon inducible proteins linked to innate immunity. More recent work focuses on the analysis of markers in SSC 1 (Boddicker et al., abstract no. 63). Genomic markers related to adaptive immunity during PRRSV infection have not been characterized. As a first step, Trible et al. (abstract no. 57) describe the PRRSV neutralizing antibody responses in several hundred experimentally infected pigs. Neutralization was performed using the homologous virus, as well as three viruses located on different branches of an ORF5 phylogenetic tree. The results show that pigs can be placed into distinct groups, including a small number of pigs that possess "broad" neutralizing activity. As reported in another abstract (Hess et al., abstract no. 70), virus neutralizing activity possesses a heritable component. The presence of a broad neutralizing response suggests the existence of conserved neutralizing epitopes within the PRRSV proteome. The study of PRRS immunity within large populations will identify unique protective responses that can be incorporated into the design of the next generation of vaccines, a means to bring the vaccine closer to the pig. The results from genome-wide association studies and marker-assisted selection for innate and adaptive immune responses will bring the pig closer to the vaccine.

# 15
# Ten years of research in structural function of PRRSV proteins

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The PRRSV genome is a plus-sense RNA of 15 kb in size containing at least 11 open reading frames (ORFs). ORF1a and ORF1b are translated directly from the viral genome by the -1 ribosomal frameshifting mechanism and two large polyproteins are made. These polyproteins are subsequently processed into 14 non-structural proteins. During replication, 6 different subgenomic RNA species are transcribed from the viral genome, and from these RNAs, 6 structural proteins corresponding to ORF2 through ORF7 are translated. During the past decade, some of the ORFs have appeared to be bicistronic. ORF2b is a small internal ORF residing within ORF2, from which the small membrane (E) protein is translated. The E protein may function as an ion channel protein to facilitate the uncoating process during virus entry. A small ORF within ORF5, termed ORF5a, has also been identified and the ORF5a protein has been determined as a membrane-associated structural protein with an unknown function. Recently, an unusual -2 ribosomal frameshifting mechanism has been determined to utilize an alternative ORF that overlaps the nsp2-coding region of ORF1a in the +1 frame. This ORF is translated to make the transframe fusion (nsp2TF) protein of 883 amino acids. Extensive studies have been conducted to elucidate the function of nsps' and it appears that some of the nsps' function as viral antagonists to modulate host innate immunity. Nsp1a degrades the CREB-binding protein in the nucleus, while nsp1b blocks the nuclear translocation of ISGF3, thus both proteins resulting in the suppression of type I interferon (IFN) response. Nsp2 is a viral protease containing the ovarian tumor domain-containing deubiquitinase motif. Nsp2 deconjugates ubiquitine and ISG15 from cellular targets to evade innate immune responses. Nsp11 is a viral endoribonuclease and appears to degrade mRNA for the mitochondrially located adaptor protein MAVS, resulting in the suppression of IFN induction. The recent findings highlight that PRRSV has evolved to take the general plasticity of RNA virus genome and to codes for multiple proteins to take selective advantages to evade the rigorous host innate immune surveillance.

# 16
# Basics of PRRSV epidemiology - I. Transmission

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The basics of PRRSV transmission are well-described:

1. Infected animals shed virus in saliva (Wills et al. 1997a), nasal secretions (Christianson et al. 1993), urine (Wills et al. 1997a), semen (Christopher-Hennings et al., 1995), and feces (Christianson et al. 1993). Pregnant susceptible females inoculated in late gestation shed virus in mammary secretions (Wagstrom et al. 2001).
2. Pigs are susceptible to PRRSV by several routes of exposure, including intranasal, intramuscular, oral (Magar et al. 1995; Magar and Larochelle 2004), vaginal (Yaeger et al. 1993), and airborne (Kristensen et al. 2004). Pigs are not equally susceptible to PRRSV by all routes of exposure and are most susceptible via intramuscular (Yoon et al., 1999) and (at least some isolates) aerosol exposure (Cutler et al., 2011).
3 Indirect transmission of PRRSV to susceptible swine can occur by a number of routes (reviewed by Cho and Dee 2006), including contact with contaminated fomites and via the use of contaminated needles. Once in the environment, PRRSV is relatively fragile and readily inactivated by heat and drying, but can remain infectious for an extended time under specific conditions of temperature, moisture (Linhares et al., 2012), and pH.
4. PRRSV is transmitted from viremic dams transplacentally to fetuses, resulting in fetal death or in birth of infected pigs, some of which may appear clinically normal (Terpstra et al. 1991).
5. PRRSV produces a chronic, persistent infection in pigs, i.e., virus replication occurs in clinically inapparent carrier animals for several months. This is extremely well-documented (Allende et al. 2000; Benfield et al. 2000; Horter et al. 2002; Wills et al. 1997b; Zimmerman et al. 1992). Persistence is undoubtedly the single most significant epidemiological feature of PRRSV infection and the reason why PRRSV elimination is so difficult.
6. Once infected, PRRSV tends to circulate within a herd indefinitely. Endemicity is driven by a combination of persistent PRRSV infection in carrier animals and the continual availability of susceptible animals that enter the herd through birth, purchase, or loss of protective immunity.

What is the limitation of the information given above? The majority of the work was performed using a very small number of PRRSV isolates because of an underlying assumption that PRRSV kinetics would be relatively uniform. Working against this assumption is the fact that PRRSV is increasingly recognized as remarkably genetically diverse (Stadejek et al., 2008). From this perspective, is "uniformity in viral kinetics" a valid assumption? This was most sharply drawn into question when Cutler et al. (2011) reported a median infectious dose (ID50) of $<1 \times 10^{1}$ for isolate MN184 by aerosol exposure whereas Herman et al. (2005), working with the same aerosol equipment and laboratory procedures, estimated an aerosol exposure ID50 of $1 \times 10^{3.1}$ $TCID_{50}$ for isolate VR-2332 (Herman et al., 2005). This ~100-fold difference in infectivity is important. Among other things, it implies that the behavior of PRRSV in populations could differ markedly among isolates. In the field, it could explain why practitioners observe that some PRRSV isolates are easier to eliminate from herds than others. Uniformity may be a safe assumption for some PRRSV characteristics, e.g., environmental stability, but clearly does not seem to be the case for others. Which characteristics and how they vary among isolates has never been explored.

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# 17
# Summary of the PRRS control measures utilized by breeding herds in North America participating in the PADRAP database (2006-2011)

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## INTRODUCTION
The Production Animal Disease Risk Assessment Program (PADRAP) database is a resource that enables identification and tracking of key attributes of swine breeding herds that relate to individual herd risk of experiencing PRRSV outbreaks. The purpose of this investigation was to summarize the frequency with which several key health management practices were used among herds participating in PADRAP. A specific goal was to understand the prevalence of use of modified live-virus vaccine and methods for live virus exposure as part of herd level PRRS control programs.

## MATERIAL AND METHODS
Data from the PADRAP database were obtained from PADRAP consisting of the initial or most recent herd survey as of the cutoff date of September 2011. A total of 1290 herds were included. Data were analyzed to assess the relationship between herd demographics as well as seven key management practices. Demographic variables included location (i.e. country, limited to USA, Canada and Mexico), type of production (genetic vs. commercial), stages of production (farrow-to-wean, farrow-to-feeder and farrow-to-finish), and herd size. Additionally, a distinction was made between PRRS virus status of farms, which was categorized within PADRAP as naïve, negative, positive, or unknown for the demographic summary. The management practices evaluated included use of MLV, serum or other methods to immunize the herd or replacement females, use of naïve replacement females, use of naïve or negative semen and type of semen source.

## RESULTS
From 1290 participating herds, 839 were from the USA, 292 from Canada and 150 from Mexico. Commercial production herds accounted for 1,029 herds and genetic-related production accounted for 257 herds. The majority of herds were positive (787 or 61%) for PRRSV infection, whereas 366 herds were naïve, 120 were negative and 16 were of unknown status. Herd size was categorized in increments of 500 sows, with 19.2% of herds < 500 sows, and 21.2% of herds at 501-1000 sows, 12.1% from 1001-1500 sows, 30.4% from 1501-3000, 9.7% from 3001-5000 and 7.4% > 5001. Modified live-virus vaccine was used in 36.7% of non-naïve herds reporting its use. Use of serum as method for herd exposure was identified in 22.5% of non-naïve herds. Use of “PRRS positive materials”, “live pigs” or “serum” in acclimation process was identified in 17.8%, 31.5% and 21.8% of non-naïve herds, respectively. Serum was used on a “routine basis” in only 4.6% of the non-naïve herds. Other management practices included use of naïve or negative gilt replacements in 49.3% of non-naïve herds, use of naïve/negative semen in 74.8% of non-naïve herds and use of semen from off-site, producer controlled boar studs in 39.5% of non-naïve herds.

## DISCUSSION and CONCLUSION
Use of the PADRAP database and the extensive survey questions developed to determine herd risk levels can be a useful tool to demonstrate differences among herds. Understanding the differences based on location, type of production and size, offers the industry ability to develop more thorough herd PRRSV control plans. In many areas, herds of varying status and sizes co-exist and may impact each other in regional control programs. Furthermore, data from the PADRAP database can also be helpful in understanding trends over time on PRRSV control methods.

# 18
# An evaluation of the long-term effect of air filtration on the occurrence of new PRRSV infections in large breeding herds in swine-dense regions

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Airborne transmission of porcine reproductive and respiratory syndrome virus (PRRSV) is a risk factor for the infection of susceptible populations. Therefore, a long-term sustainability study of air filtration as a means to reduce this risk was conducted. Participating herds (n = 38) were organized into 4 independent cohorts and the effect of air filtration on the occurrence of new PRRSV infections was analyzed at 3 different levels from September 2008 to January 2012 including the likelihood of infection in contemporary filtered and non-filtered herds, the likelihood of infection before and after implementation of filtration and the time to failure in filtered and non-filtered herds. Results indicated that new PRRSV infections in filtered breeding herds were significantly lower than in contemporary non-filtered control herds ($P < 0.01$), the odds for a new PRRSV infection in breeding herds before filtration was 7.97 times higher than the odds after filtration was initiated ($P < 0.01$) and the median time to new PRRSV infections in filtered breeding herds of 30 months was significantly longer than the 11 months observed in non-filtered herds ($P < 0.01$). In conclusion, across all 3 levels of analysis, the long-term effect of air filtration on reducing the occurrence of new PRRSV infections in the study population was demonstrated.

# 19
# Pen-side molecular diagnosis of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus 2 (PCV2) – a pilot project in Alberta, Canada

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**Objective:** The early and rapid detection of swine pathogens is critical for effective biosecurity and disease management. There is a pressing need to provide veterinarians with simple and sensitive assays that can be used in the field. Reliable testing with minimal turn-around times improves disease management within herds and can help to prevent the spread of disease between herds.

Highly sensitive and specific molecular diagnostic assays such as quantitative real-time RT-PCR (qRT-PCR) are available, but these require expensive and fragile equipment and are not suitable for on-farm use. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) has been developed as a pen-side tool for PRRSV and PCV2 diagnosis, but it shows variable sensitivity and only limited repeatability.

With the novel hydrogel post technology, which integrates easy sample processing and qRT-PCR capability in a small portable device, we will create robust PRRSV and PCV2 assays suitable for use under field conditions.

**Methods:** The Domino diagnostic platform uses an array of colloidal hydrogel posts on a disposable plastic chip. Each post is a separate reaction vessel that contains all the reagents required for nucleic acid extraction, PCR and melting curve analysis, including enzymes and specific primers.

Raw samples are loaded directly onto the chip; prior nucleic acid extraction is not required. The same chip can run multiple diagnostic tests in parallel using the same sample.

The test is carried out in a portable detection device, which contains a heating element, a laser and a CCD-based detector for real-time PCR and melting curve analysis with maximum specificity. It has originally been developed for point-of-care molecular diagnostics in human medicine, where it is successfully used to detect polyomaviruses, herpesviruses and malaria in complex biological matrices including whole blood, urine and saliva.

**Outlook:** We will create prototype Domino chips specific for PRRSV and PCV2. The chips will be validated using field samples whose disease status has been defined by an accredited veterinary diagnostic laboratory.

This is a pilot project to test the technical feasibility of pen-side assays for PCV2 and PRRSV that can be used in local and regional control and eradication schemes.

The project is funded by the Alberta Livestock and Meat Agency.

# 20
# Sequence diversity of PRRS virus in the Czech Republic

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Genetic diversity among PRRS virus strains is of great importance for diagnostics of the disease and vaccine development. Higher genetic variability among European strains of PRRSV was observed than was originally expected. The aim of this work was to characterize the genetic diversity of Czech PRRSV strains by ORF 5 gene sequencing and compare it with heterogeneity of strains circulating in Europe.

PRRS virus sequence heterogeneity was estimated on the collection of virus strains detected in conventional pig farms in the Czech Republic in the period from 2006 till 2012. Viral RNA was extracted from clinical field samples using a commercial kit and immediately used for RT-PCR with specific primers. Seventeen virus strains were collected and their ORF 5 gene was sequenced. Obtained sequences were analyzed and compared with PRRS sequences from GenBank.

All analyzed virus stains clustered into two major groups. One cluster showed similarity to strains originating from Denmark, Poland and Italy. The second cluster was highly similar to several strains isolated in Spain as well as to some vaccine strains. As vaccines originating from Spanish strains are widely used in the Czech Republic, the second group may likely represent detected vaccine strains. No North American strains were detected in the monitored period.

Results of our survey show rather limited PRRS heterogeneity in ORF 5 gene, further analysis will be focused on ORF 7 and nonstructural genes of the virus.

# 21
# Detection of PRRSV antibody in oral fluid specimens from individual boars using a commercial PRRSV serum antibody ELISA

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Oral fluid specimens are used in human medicine for detection of a variety of infectious agents, hormones, and drugs. Oral fluid samples are of interest in swine medicine because they are easily collected, yet highly efficacious for the surveillance of PRRSV and other pathogens using PCR-based assays (Kittawornrat et al., 2010; Ramirez et al., 2012). Recent work showed that a commercial PRRSV serum antibody ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA) could be adapted to detect PRRSV antibody in oral fluid specimens (Kittawornrat et al., 2012). The object of this study was to describe the kinetics of the ELISA detectable anti-PRRSV IgG response in oral fluid collected from individually-housed boars. The study was conducted in 72 boars ranging from 6 months to 3.6 years in age. Boars were under the ownership of PIC North America (Hendersonville, TN, USA) and housing, study procedures, and protocols were approved and supervised by the PIC USA Health Assurance and Welfare department. Boars were assigned to three trials (I, II, III). Boars (n = 24) in Trial I were intramuscularly (IM) inoculated with 2 ml of a modified live virus (MLV) vaccine (RespPRRS®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA). Boars (n = 24) in Trial II were IM inoculated with 2 ml of a Type 1 PRRSV field isolate. Boars (n = 24) in Trial III were IM inoculated with 2 ml of a PRRSV Type 2 isolate (MN-184). Boars were monitored for 21 days post inoculation (DPI). Oral fluid samples were collected daily using 5/8" 3-strand 100% cotton rope. Serum samples were collected from all boars on DPI -7, 0, 7, 14, 21 and from 4 randomly selected boars on DPIs 3, 5, 10, and 17. Thereafter, serum and oral fluid were assayed for PRRSV antibody using the ELISA protocol appropriate for each sample type (serum or oral fluid). Individual boar oral fluid samples were ELISA positive from DPI 8 to DPI 21. Overall, 96% of the results were in agreement, i.e., 145 oral fluid samples and 150 serum samples were ELISA positive. These data support previous reports on the detection of anti-PRRSV antibody by ELISA in oral fluid and suggest that this approach could be used for disease surveillance in commercial breeding swine populations.

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# 22
# Ring test evaluation for the detection of PRRSV antibody in oral fluid specimens using a commercial PRRSV serum antibody ELISA

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A commercial PRRS serum antibody ELISA (IDEXX Laboratories, Inc., Westbrook ME USA) was recently adapted to detect anti-PRRSV antibody in oral fluid specimens (Kittawornrat et al., 2012). Based on testing of field and experimental samples, diagnostic sensitivity and specificity was estimated at 94.7% (95% CI: 92.4, 96.5) and 100% (95% CI: 99.0, 100.0), respectively, at a sample-to-positive (S/P) cutoff of ≥ 0.40. The purpose of this study was to evaluate the reproducibility and repeatability of the PRRS oral fluid ELISA in a ring test (check test) format. A total of 263 oral fluid samples were collected, completely randomized, and sent for testing in 12 collaborating diagnostic laboratories. In addition to the set of oral fluid samples, each laboratory received the materials required for conducting the test: ELISA plates (HerdChek® PRRS X3 ELISA, lot #40959-W721), reagents, positive and negative controls, pre-diluted conjugate antibody, and a copy of the standard operating procedure for the PRRS oral fluid IgG ELISA. The laboratories tested the samples and returned the results for analysis. Assay results were analyzed as S/P ratios, with S/P ratios ≥ 0.40 considered positive. Variation in S/P results increased as the concentration of antibody in the sample increased. Overall, this had little impact on categorical results. That is, among the 263 samples tested by the 12 laboratories, 132 samples tested positive in all laboratories; 124 samples tested negative in all laboratories, and 7 samples had discordant results. With the exception of sample #7, a discordant result was reported in each case by only one of the 12 laboratories. Discordant results for sample #7 were reported at 3 laboratories, but this may be explained by the fact that all results for sample #7 clustered close to the 0.40 cutoff. The ring test results showed that the PRRS oral fluid IgG ELISA was highly reproducible across laboratories. These results support the routine use of this test in laboratories providing diagnostic service to pig producers. Thus, herd monitoring based on oral fluid sampling could be one part of a PRRSV control and/or elimination program. Further, the successful adaptation of one assay to the oral fluid matrix suggests that this approach could provide the basis for monitoring specific health and welfare indicators in commercial swine herds using a "pig friendly" approach.

Reference:

1. Kittawornrat, A et al.: 2012. Detection of PRRSV antibodies in oral fluid specimens using a commercial PRRSV serum antibody ELISA. JVDI 24(2):262-269

# 23
# Developing a work plan and toolbox for regional control and elimination of PRRS virus

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PRRS Virus continues to cause major disease challenges and severe economic impact in the swine industry in North America. In 2008, the USDA funded PRRS CAP in the United States to develop strategies for control and elimination of PRRS in the United States. Despite regional success stories many regions in the U.S., even while involved in control and elimination projects, continue to have problems with regional spread and reinfection of herds that have eliminated the virus.

Holtkamp et al. proposed guidelines for classification of herd status in 2011, with the intention to facilitate communication and aid with regional control and elimination. As we move forward in our attempts to control and eliminate the virus from regions, a classification of the status of regions based on prevalence and incidence could also be useful in communicating within and between regions, and aid in control and elimination projects.

In their paper, Lowe et al. outlined standard management strategies for the herd control and elimination of PRRS, using the above outlined classification system. By looking at current scientific knowledge, along with effective management techniques, a standardized table of guidelines was established to allow herds to control and / or eliminate PRRS.

This article is an attempt to do on a regional basis what the above authors have done at the herd level. It will attempt to develop Regional PRRS Status categories into which regions can be classified, and develop regional biosecurity practices and control tools which can be used by the defined regions to control and eliminate PPRRS. To accomplish this, the existing scientific knowledge on transmission and control of PRRS will be used. After reviewing existing knowledge, a set of best management practices or management plans can be developed for each regional classification. Through use of these management plans, regions will be better equipped to control and potentially eliminate PRRS. Through this process, the authors hope to provide a "toolbox", from which regional leaders can easily access information to undertake and be successful in regional control and elimination of the PRRS Virus.

# 24
# Deep sequencing of PRRSV isolates: rapid and large-scale characterization of viral genomes

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a single stranded, positive sense RNA virus with a genome size of approximately 15 kb. Much of the genetic characterization or viral genotyping of PRRSV isolates is limited to one or two viral genes only (ORF5 and/or ORF7) for a number of reasons, for example: (1) characterizing one or two ORFs is sufficient for diagnostics; (2) genome characterization is laborious because traditional (Sanger) sequencing yields only a single sequence of ~800-1000 bases per reaction; (3) large-scale genome characterization is time-consuming and costly. Collectively, this hinders the study of PRRSV genomic evolution at different levels (host, regional, and global). We demonstrate here the use of 454 technology to rapidly sequence PRRSV genomic nucleic acid from different sources (cell culture and swine tissue), genotypes (type 1 and type 2), and genome structure (non-deletion vs. deletion variants). Samples (n=16) were multiplexed to bring down cost per genome sequence. Assembly of sample specific reads resulted in a single contig in almost all instances (15 out of 16). Average genome coverage was 96.7% with reference to prototype isolates (Lelystad virus for type 1 and ATCC VR2332 for type 2). Average sequence depth was 405 reads per nucleotide position. This high sequence depth allowed characterization of variants from quasispecies that occurred at frequencies even lower than 1%. In summary, next generation sequencing technology offers unparalleled opportunity to quickly and efficiently characterize near complete length PRRSV genomes in an economical manner. This allows experiments to be designed with considerations to viral genomic evolution rather than those with limited insights from select viral genes only.

# 25
# Deletion polymorphism of NSP2 protein of HP-PRRSVs in China

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Highly pathogenic PRRSV (HP-PRRSV) with 30-amino-acid discontinuous deletion (481 and 534-562) in NSP2 protein was epidemic in China since 2006. Here, we investigated HP-PRRSV in the clinical samples from several provinces, and compared the NSP2 of PRRSV strains which were isolated in mainland China from 1996 to 2010. Besides of the 2 deletions (481, 534-562), other 6 different types of enlarged deletions in NSP2 was found in the present study. This report showed the NSP2 of HP-PRRSV were enlarged deletions with different polymorphism. The result will contribute to understand the evolution trend of HP-PRRSV in China.

# 26
# Development of a new IDEXX ELISA for the detection of PRRS antibodies in swine oral fluids

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Testing of swine oral provides a convenient and cost-effective tool for disease surveillance in commercial pig herds. Recently, Kittawornrat *et al.* (J Vet Diagn Invest. 2012 Mar;24(2):262-9.) described detection of antibodies to PRRS in oral fluids adapted using an overnight sample incubation format adaptation of the HerdChek® PRRS X3 ELISA, (IDEXX Laboratories, Inc.). In this study, we describe the new IDEXX PRRS oral fluids ELISA for same-day detection of PRRS antibodies in swine oral fluids. An S/P $\geq$ 0.4 is considered a positive result. A comparison between the new protocol and the standard overnight protocol (SOP) using a set of reference standards consisting of pooled oral fluids from vaccinated pigs indicated 100% agreement between the two tests, with average S/P values 1.4 to 1.5-fold higher than SOP. Moreover, analysis of a temporal series of paired oral fluids and serum collected from individual boars vaccinated or experimentally infected with type I or type II PRRSV for up to 21 days post-infection (DPI), indicated little difference between the days to detection of anti-PRRSV antibodies in serum as in oral fluids, as well as a similar ability to detect both strains. the new IDEXX PRRS Oral Fluids ELISA had a percentage agreement of 98.7% with the PRRS X3 results in paired serum. Finally, evaluation 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 indicated that the new test detects PRRS antibodies in ≥96% of all collection events in pens of at least 20% prevalence. Taken together, these results describe a new sensitive test for anti-PRRS antibody detection aimed to support the emerging use oral fluids for frequent surveillance of pig herds.

# 27
# The current situation and some researches of PRRS in Vietnam

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Porcine reproductive and respiratory syndrome (PRRS) caused by RNA PRRS virus, belong to family Arteriviridae. It caused serious economic loss for farmers in Vietnam. PRRS was first found in Vietnam in 1997 on pigs, which were imported from USA, with serum samples were tested. Until 2007, PRRS occured in many provices such as Hai Duong, Hung Yen, Bac Ninh, Bac Giang, Thai Binh.... and then PRRS outbreaks spreaded to Central and South provinces. The number of PRRS infected pigs died was 254.242. PRRS have been causing severe economic loss for the farmers since 2007 to now. In 2012, PRRS outbreaks happened quickly, has occured in 11 province of Vietnam and the first outbreak was in Lao Cai province. We collected samples from pigs showing typical clinical signs of PRRS infected pigs in Hoa Binh, Quang Ninh, Lao Cai, Bac Ninh provice....The clinical signs and gross findings were recorded during autopsy. RT-PCR and immunohistochemistry method were used to diagnose pigs infected with PRRS virus. The samples, which were positive with PRRSV, were selected for isolation of PRRSV by using Marc 145 cells. The biological and molecular characteristics of PRRS isolates were researched. PRRSV isolated in Vietnam were closed to each other and Chinese vaccine strains in phylogenetic trees. The homology of nucleotide sequence among Vietnamese isolates were from 93% to 98%.

# 28
# Probability of detecting PRRSV infection using pen-based swine oral fluid specimens as a function of within-pen prevalence

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**Introduction** Pen-based oral fluid sampling allows for the collection of samples that represent a large number of animals. For interpretation of results, however, performance of oral fluid assays (antibody- and nucleic acid-based) in populations of low disease prevalence must be established. Therefore, the objective of this study was to determine the probability of detecting PRRSV infection in pen-based oral fluid samples from pens of known PRRSV prevalence.

**Materials and Methods** In one commercial swine barn, 25 pens were randomly assigned to 1 of 5 levels of PRRSV prevalence (0%, 4%, 12%, 20%, or 36%). PRRSV prevalence was established by placing a fixed number (0, 1, 3, 5 or 9) of pigs 14 days post-vaccination with a MLV PRRSV vaccine in pens such that the combination of negatives and positives in each pen totaled 25 pigs. In total, 6 oral fluid samples were collected from each of the 25 pens (n = 150).

To confirm individual pig PRRSV status, serum samples from the PRRSV-negative pigs (n = 535) and the PRRSV vaccinated pigs (n = 90) were tested for PRRSV antibodies and PRRSV RNA. The 150 pen-based oral fluid samples were assayed for PRRSV antibody and PRRSV RNA at 6 laboratories.

**Results and Conclusions** The overall mean (% positive) among all laboratories is as follows:

| Assay | Prevalence | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0%** | **4%** | **8%** | **12%** | **20%** | **32%** | **36%** |
| PRRSV RT-PCR | 0.7% | 15% | 77% | 80% | 64% | NA | 90% |
| PRRSV ELISA | 0.3% | 15% | 0% | 63% | 85% | 93% | 91% |

Overall, this data supports the use of pen-based oral fluid sampling and testing by either ELISA or PCR for PRRSV surveillance in commercial pig populations.

# 29
# Effect of sample collection material on the detection of PRRSV in oral fluid

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**Introduction** Reports in human diagnostic medicine suggest that the material used to collect oral fluid samples can affect the detection of diagnostic targets, including hormones and antibody.1,5 The objective of the present study was to determine if the sampling material used to obtain pen-based swine oral fluid specimens affected the results of PRRSV antibody ELISA or RT-PCR testing.

**Materials and Methods** Oral fluid samples were collected from 104 pens on 2 commercial swine farms. Three oral fluid samples2,3,4 were collected in succession from each pen using ropes made either of cotton (C), hemp (H) or nylon (N). To account for the possible effect of collection order on assay results, samples were collected from pens in one of three sampling sequences: C-N-H, N-H-C, or H-C-N. Thereafter, oral fluid samples were assayed for PRRSV antibody3 and PRRSV RT-PCR. The effect of sampling material and collection order on the ELISA sample-to-positive (S/P) ratio was analyzed by ANOVA. Qualitative (positive or negative) results of PRRSV RT-PCR testing were analyzed for differences in sampling material and collection order using logistic regression.

**Results and Conclusions** Analysis of the results found sampling material affected diagnostic results, but cotton rope provided the best overall diagnostic performance. Therefore, oral fluid samples should be collected using cotton-based materials.

**Acknowledgments**
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# 30
# A pilot study to determine risk of PRRS infection from 3 years of retrospective data as a means of predicting future outbreaks in a large sample of US sow herds

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**Introduction.** Porcine Reproductive and Respiratory Syndrome virus (PRRSv) continues to cost the United States swine industry $664 million annually. In the fall and winter of 2011we began a collaborative effort between multiple swine production companies to describe the incidence and prevalence of PRRS infections in a sample of the US sow herd.

**Materials and Methods.** The database currently contains 285 sow farms, 10 systems, approximately 971,000 sows (estimated 17% of the US sow herd) in 13 states. Weekly PRRS status (AASV classification) for each farm is reported from July 2009. Using July 1 as the start of the observation period for each year, an annual cumulative incidence and prevalence is calculated and reported at the aggregated and system specific level. Cumulative incidence is also reported by beginning PRRSv status. An exponentially weighted moving average (EWMA) curve from July 2009 through the end of June 2012 is calculated. This method uses the number of weekly PRRS infections observed during the summer months as a means of defining an outbreak threshold. Each week the observed number of PRRS infections is weighted for previous weeks and a EWMA 'smoothed' value is calculated. An epidemic begins when this value exceeds the threshold and concludes when it falls below. Using a subset of 161 sow farms, we are beginning to identify locations of all pig sites within a three mile radius of each farm. Using the concept of spatial kernels and reproduction ratios we have developed a preliminary risk score for each site.

**Results.** New infections increase gradually in September, followed by a dramatic increase at the end of October, then plateau in late February. The pattern is repeated over all three years of observation. At the end of June 2012, 40% of the herds in the project reported a new infection. Positive stable, vaccinated herds had the highest incidence, and the percentage of PRRS negative farms remained fairly stable over time. The EWMA suggests the epidemic begins approximately mid-October and subsides approximately mid-March. These data also suggest a mini epidemic in the spring lasting only a few weeks.

**Discussion.** This is the first effort to quantify the incidence of PRRS virus in a large sample of US sow herds. Due to the voluntary nature of the study, this cohort may not be representative of the US sow industry. That said, preliminary results are strikingly consistent across three years of data and among systems. These data present several questions for investigation including understanding what factors lead to the high incidence every October and spring and why vaccinated herds have the highest incidence of new PRRSv infections.

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# 31
# Circulating genotypes of porcine respiratory and reproductive syndrome viruses in southern China: re-emerging lineage 3 and genomic recombination with vaccine variants

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Porcine reproductive and respiratory syndrome virus (PRRSV) was first reported in China since the end of 1995 and several variants were further reported in subsequence years, causing huge economic losses to Chinese swine industry. In 2006, a country-wide outbreak of porcine high fever syndrome, caused by a highly pathogenic PRRSV (HP-PRRSV) emerged in China affecting more than 20 million pigs. To date, three lineages (lineage 3, lineage 5.1 and lineage 8.7) of PRRSV were reported in China based on our global genotyping for type 2 PRRSV. Among these three lineages, only lineage 8.7 was the most significant variant where infected pigs showing severe clinical symptoms. Much investigation to this economically important lineage (lineage 8.7) caused lesser attention on other lineages in China during recent years. Based on our viral isolation in Guangdong Province of Southern China during 2009 and 2011, the majority of isolates collected were grouped as HP-PRRSV cluster. Moreover, circulating vaccine-associated lineage (lineage 5.1) and re-emerging lineage 3 isolates were also found. Lineage 3 was previously reported in China, Hong Kong and Taiwan, but there were no reports for this lineage after 2005 in China. Here, we present the re-emerging of this lineage 3 viruses in southern China in 2010 and their evolutionary story. Additionally, we had identified inter-lineage genomic recombination between MLV vaccine strain and re-emerging lineage 3 virus in a farm practicing MLV vaccination. Although modified live virus (MLV) vaccines provide solid protection against PRRSV infection, the safety of MLV vaccines has been questioned since the detection of vaccine revertants which caused productive problems similar to those of wild-type PRRSV. Based on our experimental study, no differences in virulence between the recombinant variant and wild-type lineage 3 were observed. However, further investigations on genomic recombination among PRRSV variants in China are needed.

# 32
# Seroprevalence of porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza (SIV), and porcine circovirus 2 (PCV2) in feral Hawaiian swine

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Little is known about the prevalence of PRRSV, PCV2, and SIV in feral swine in the state of Hawaii. The overall goal of this project was to apply a multiplex serological test for the detection of IgG and IgM antibodies to PRRSV N protein (N), PCV2 capsid protein (CP), and SIV nucleoprotein (SIV NP). Serum samples were collected from 345 feral swine in the counties of Hawaii and Honolulu over a four year period (2007-2010). Target proteins were expressed in E. coli and coated onto Luminex beads, using standard protocols. Antibodies to the three antigens were present in the population. Overall, PCV2-specific antibody was most prevalent with 61% of the samples testing positive for PCV2-specific IgG. SIV and PRRSV N IgG antibodies were detected at much lower rates of 7% and 3.5%, respectively. Approximately 3% of samples were positive for the presence of PCV2-specific IgM. Five of the PCV2 IgM-positive samples were further subjected to PCR amplification for the detection of genomic DNA. Products were obtained from three of the samples. Sequencing showed the presence of PCV2b-like viruses. The results illustrate the utility of multiplex serological assays for disease surveillance in feral populations.

# 33
# Development of model systems for the study of recombination in porcine reproductive and respiratory syndrome virus (PRRSV)

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Sources of genetic variation in PRRSV include substitutions, insertions-deletions and recombination. The goal of this research is to develop in-vitro models to study recombination. The first step is to determine if cells can be infected by two different PRRS viruses. MARC-145 cells were co-infected with two identical viruses which contained GFP or RFP and confocal microscopy identified dual-fluorescent cells. Flow cytometry estimated a 17% rate of co-infection. The model system developed to study recombination is a two-step process of co-transfection of HEK cells followed by infection of MARC-145 cells. Two infectious clones were constructed. The first was a non-fluorescent virus containing a mutated EGFP gene (pP129-EGFP-97C) and the other was a 'defective virus' (pP129-EGFP-d(2-6)) containing wild-type EGFP gene but lacking ORFs 2 to 6. Recombination events in the mutated EGFP region resulted in restoration of fluorescence. Emerging recombined viruses were detected as green fluorescent plaques in MARC-145 cells. The estimated recombination frequency was approximately 0.1%. This in-vitro system was used to determine if recombination events occur in sequences that lack significant homology. For this purpose another defective construct (pP129-GFP-d(2-6)) was used for co-transfection with P129-EGFP-97C. Recombination was not detected in this experiment implying the need for greater than 83% homology for recombination to occur. Further, the system was applied to determine if P129-EGFP-97C virus can recombine with mRNA derived from a separate plasmid instead of a virus genome. A stable HEK cell line that expressed wild-type EGFP was created and transfected with pP129-EGFP-97C. MARC cells were productively infected but failed to detect a green recombined virus. This suggests that co-localization of infecting viruses within specialized replication complexes is critical for recombination to occur. The in-vitro system developed in this study is being applied to further investigate the molecular mechanism of recombination in PRRS virus as a model for other RNA viruses.

# 34
# Understanding patterns of change in permissiveness of porcine alveolar macrophages to PRRSV over time and underlying factors

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PRRSV infection studies have shown that the viraemia levels of infected pigs start to decline long before the onset of adaptive immune response processes. One of the suggested mechanisms responsible for this observed decline is that the permissiveness of porcine alveolar macrophages (PAMs), i.e. the primary target cells of PRRSV, may reduce over time. Given that the PAM receptors CD163 and CD169 have previously been established as a necessary condition for productive infection with PRRSV, it could be hypothesized that changes in PAM permissiveness are mirrored by structural changes of PAMs with respect to CD163 and CD169.

An in-vitro infection study was carried out to assess (i) whether and how PAM permissiveness to PRRSV changes over (incubation) time, (ii) whether expression levels of CD163 and CD169 at the cell surface are reliable markers for (changes in) PAM permissiveness to PRRSV. PAMs from naive pigs were pre-incubated for 0, 1, 2, 4, 6, 8 and 9 days prior to PRRSV (UK strain Humberside-2) infection. Eighteen hours post-infection, cells were either dually stained for viral antigen and CD163, or for CD169. The proportion of PRRSV permissive cells and / or cells expressing CD163 or CD169 on the cell surface was established via flow cytometry. PAM numbers and CD163 / CD169 -positive cells were compared with those of a mock control. The experiment was repeated three times, with each independent trial consisting of PAMs extracted from 3, 2 and 3 pigs, respectively. Statistical analysis was carried out using *proc mixed* in SAS with class (infected / control), trial, incubation time and their interactions as fixed effects and pig as random effect.

PAM permissiveness to PRRSV changed significantly over time in all three trials, although the patterns of change differed significantly between the trials. The proportions of cells expressing CD163 or CD169 on the cell surface declined over time in all 3 trials, and there was no indication that infection decreased the number of viable cells or altered expression of either receptor (i.e. no significant class effect). The analysis revealed that the observed changes in PAM permissiveness to PRRSV could not be explained by changes in the proportions of cells classified as CD163 or CD169 positive. Surprisingly, a high proportion of PAMs (up to 65%) not expressing CD163 on the cell surface were found to be permissive, and this proportion of cells was found to increase over time. Thus, two alternative hypotheses emerged from this study: (i) Cells classified as CD163 negative in the flow cytometry may express CD163 in the early endosomes, where virus uncoating takes place, and (ii) an unknown alternative factor plays a vital role in PAM permissiveness to PRRSV. Both hypotheses are currently explored using a process-based mathematical model. The combined results of the described experiment and the mathematical model provide important insight about the factors involved in altering PAM permissiveness to PRRSV, which could be exploited for the development of vaccines or treatment.

# 35
# Can the robustness related condition Osteochondrosis be assessed on live pigs using computer tomography and is the trait heritable?

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Several studies have previously suggested that osteochondrosis (OC) is an important cause of lameness and leg weakness in pigs. OC is a disturbance in the process of enchondral ossification affecting individuals at early growth in several different species, and the disturbance is caused by ischemia. Necrosis of the epiphyseal growth cartilage is the first structural changes of OC and can cause abnormalities of cartilage and thereafter bone lesions. Formation of detached fragments [osteochondrosis dissecans (OCD)], fissures, or subchondral bone cysts can appear at predilection sites. OC has traditionally been assessed either by macroscopically scoring the cartilage or by means of sectioning the bone and scoring the slabs. The aim of this study was to evaluate if images from routine Computer Tomography (CT) of live boars could be utilized for assessment of OC. Annually, Norsvin CT-scans 3500 purebred live boars. Feed intake and growth (FIRE) are also part of the phenotypic measures on the boars, and DNA is extracted for genotyping with the porcine 60K SNP chip (Illumina Inc, CA, USA). In this study, 1449 Norsvin Landrace boars were assessed for signs of OC. The distal ends of Humerus and Femur, both medial and lateral condyles were assessed, giving eight phenotypes for each animal. The scores (0 to 5) were assigned based on visible changes in the subchondral bone. In addition, the assessments were added up to a "total score" (OCT). The results reveal that most boars (~90 %) have changes in the subchondral bone tissue (OCT >0). However, the vast majority of boars (~99 %) have no signs of OCD (joint specific score 3 or less). Medial condyle of femur was most affected with only ~30 % being scored as normal (score 0). Lateral condyles of humerus were little affected with ~97 % of the animals being scored as normal. The variation of the heritabilities for OC between the anatomical locations was present, ranging from $h^2_{\text{humerus right medial}}$=0.21 to $h^2_{\text{humerus left lateral}}$=0.06. OCT gave the highest estimated heritability ($h^2$=0.31). The genetic correlations between OC at the different scoring positions varied from $r_{\text{ghumerus right medial, humerus left medial}}$=0.96 to non-significant correlations between humerus left lateral and humerus left medial. The genetic correlations between the total score of OC and OC scores at all eight positions were high and favorable. We therefore conclude that the total score OC is the preferred trait for implementation in the breeding goal. Phenotypes from FIRE were used to calculate genetic correlations between growth in different periods and OCT. Results indicate that animals having genetically potential for high early growth are more susceptible for OC. As part of the project, the boars are in process of beeing genotyped with the porcine 60K SNP chip. Genome wide association studies will be performed in order to identify markers/genes affecting OC and other traits related to longevity. Genotypes of relevant markers/genes and implementation of genomic selection will be used in the breeding program to intensify the genetic improvement of traits related to longevity and robustness.

# 36
# Next generation sequencing of the porcine reproductive and respiratory syndrome virus genome

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**AIM:** 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 Ion Torrent™ PGM sequencing technology, sequencing results can be generated in a relatively short period of time. Using the latest bioinformatics techniques, the sequence information can be compared and contrasted to determine strain similarity based on different ORFs and potentially determine new regions of interest for robust assay development.

**METHODS:** 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 genome sequences. PRRSV genome libraries are then prepared and amplified to capture all representative portions of the genome. Finally, these libraries are attached to Ion Sphere Particles that are loaded onto a sequencing chip and placed on the Ion Torrent PGM for sequence generation. Bioinformaticians then analyze the data based on the researchers needs.

**CONCLUSION:** Although this is an ongoing project, initial results are promising. Contigs obtained from de novo assembly of sequence reads span over 98% of the PRRSV genome based on comparison to a reference sequence. Less than 20% 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.

# 38
# Developed RT-PCR technique for the diagnosis of porcine reproductive and respiratory syndrome virus (PRRSV) in Mexico

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## Objective
The present study is based on the correct design of specific primers for the detection of American and European strains of PRRS virus in Mexico, specifically, the two commonly studied ORF's (ORF5 and ORF7).

## Material and Methods
The samples were obtained from fourteen states of Mexico, positive samples were determined through the RT-PCR test, using 4 pairs of specific primers designed for American lineage (ORF5, ORF7) and a nested PCR for ORF5 and ORF7 of European lineage. The samples used were: 40 lungs, 16 lung and lymph node, 9 nasal swabs, 2 lungs and trachea, 1 nasal turbinate, 1 semen. The primer designs were made with the program ClonManager 7.0 with the lineup of 200+GenBank sequences and then evaluated with the programs Primer and BLAST. RNA extraction was performed using the Gibco Life Tech., 1996 technique. The PCR technique was conducted with the RT-PCR One Step Invitrogen ® kit. The temperatures used for amplification of the primers were: a cycle of 50°C for 30 min.; a cycle of 95°C/15 min.; 40 cycles of 94°C/30 sec., 54°C/ 60 sec. (ORF 5) or 58°C/60 (ORF7), 72°C for 60 sec., and a cycle of 72°C/10 min. and the result was kept at 4°C indefinitely. The amplification products were obtained and analyzed with Kodak Gel Logic 100 imagining system program.

## Results
From the total (69) samples, 47 (68.12%) were found positive. At least one positive sample was found from the 14 states sampled. With the primers targeting ORF7 more positive samples were detected (34/69) followed by the nested ORF5 (28/69), the ORF5 of American lineage detected only 5 positive samples. Regarding the presence of European virus in Mexico, our study has not detected any positive sample. From the 41 lung samples processed, 23 were positive to the PRRSv (57%), from macerated lung and lymphoid tissues 75% positive (12/15 samples), 100% of nasal swabs were positive (9/9) and 1 sample of tracheal tissue was positive (1/2). The only sample of nasal turbinate and semen were both positive.

## Conclusions
The RT-PCR targeting ORF7 gave more positive samples, followed by the nested RT-PCR, suggesting that the laboratory diagnosis of PRRS virus should be focused to the detection of ORF7 rather than ORF5. The use of RT-PCR technique allowed determining the positive samples to PRRS virus regardless of the type specimen. We conclude that the use of a single pair of primers would create false-negative results. The deepening of the analysis of the virus identified by sequencing is essential to know the sites of the genome with nucleotide variation. However, when we obtained amplicons of different sizes it shows the variation of this virus and suggests the presence of endemic strains in our country. Heterogeneity is considered to be a major obstacle for the effective prevention and control of PRRS. The high degree of genetic and antigenic diversity among field isolates underlines the complexity of the control and eradication of the disease and the development of future vaccines.

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# 39
# Whole genome association study for lactation feed efficiency in Yorkshire sows selected for residual feed intake during finishing

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Over the past decades, genetic selection and management changes have resulted in sows with increased litter size and high milk production. These biological changes have resulted in higher energy requirements and greater mobilization of body reserves during lactation, leading to a prolonged negative energy balance. To counteract this, feed intake of sows during lactation must be increased. However, as a result of selection of leaner pigs with an improved feed conversion ratio during finishing, appetite and feed intake capacity of sows shows a declining trend. Thus, efforts are needed to increase feed efficiency during lactation, i.e. increase milk output per unit of energy obtained from feed intake and body reserves. Genetic improvement of sows for lactation efficiency is, however, hampered by the ability to collect accurate feed intake and body composition data during lactation. Thus, the objective of this study was to conduct a genome wide association study (GWAS) to identify SNPs / chromosomal regions associated with sow lactation efficiency. The Illumina porcine 60k SNP chip was used to genotype 512 purebred Yorkshire sows from the ISU Residual Feed Intake (RFI) lines, which were divergently selected for high and low RFI during finishing. After quality control, 48,521 SNPs were used for analysis. Traits included lactation feed intake (FI), RFI, estimated maintenance requirements (MR), energy balance (EB) and lactation efficiency (LE), along with sow body weight (BWW), fat mass (FMW), and protein mass (PMW) at the time of weaning. Lactation efficiency and energy utilization of sows and piglets was calculated based on on-farm measurements of sow body weight, back fat and loin muscle area before farrowing and at the time of weaning, sow feed intake during lactation, and piglets weights at birth, death and weaning. The GWAS was implemented separately for each trait using method Bayes B of GENSEL software, with genetic variances and proportions of SNPs with non-zero effects estimated using Bayes C. The fixed effects of line (high and low RFI), generation (7 levels), and parity (2 levels) were included in the model. The proportion of phenotypic variance explained by markers was 0.12 for LE, 0.28 for FI, 0.09 for RFI and EB, 0.49 for MR, 0.57 for BWW, 0.51 for FMW and 0.43 for PMW. These estimates were comparable to pedigree-based estimates of heritability. Although there were no regions that explained a large proportion of variance for LE or RFI, several informative regions were identified for traits such as PMW that are components of LE. The proportion of variance explained by the most important regions varied widely by trait. E.g., for PMW, six 1 Mb windows (86 SNPs) together explained ~20% of genetic variance and for MR seven windows (166 SNPs) explained ~ 12%. Across the genome, for all traits analyzed, more than 80 1 Mb windows explained at least 1% of the genetic variance. Some regions on SSC 8 and SSC18 were associated with multiple traits. Nearly all important regions differed between parties but were little affected by removing line as a fixed effect. Overall, this GWAS revealed several genomic locations and markers associated with sow lactation feed efficiency and associated traits, which can provide a road map for future research and application. This research was funded by the Iowa Pork Producers Association. Partial funding for DMT was by Genome Alberta and Genesus Inc.

# 40
# Dissection of cis-acting elements in 5′ untranslated region of porcine reproductive and respiratory syndrome virus

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It is believed that the genomic 5′ untranslated region (UTR) of Arterivirus plays crucial roles in viral genomic replication, subgenomic mRNA transcription and protein translation, yet the structure and function still remain largely unknown. In this study, we conducted serial nucleotide truncation, ranging from 1 to 190 nucleotides, to the 5′ UTR of the porcine reproductive and respiratory syndrome virus (PRRSV) infectious full-length cDNA clone pAPRRS. *In vitro* synthetic RNAs were transfected into MARC-145 cells for further genetic and virologic analysis. Our results demonstrated that the first three nucleotides of PRRSV 5′ UTR were dispensable for virus viability, which however was repaired with foreign sequences. In order to assess if the primary sequence or structural element play more important regulatory roles, the CMV promoter-driven 5′ UTR truncation mutant cDNA clones were directly transfected into the BHK-21 cell lines. We found that PRRSV tolerated the first 16 nucleotides sequence alteration of 5′ UTR without losing virus viability. However, these revertant viruses contained a range of non-templated with unknown origin exogenous nucleotides in the repaired 5′ end. Further analyses revealed that the 5′ proximal stem-loop 1 (SL1) in the highly structured 5′ UTR was invariably required for virus infectivity. Taken together, we conclude that authentic 5′- proximal primary sequence is nonessential, but the resultant structural elements are probably indispensable for PRRSV infectivity.

The 5′ untranslated region (UTR) is believed to be vital for the replication of porcine reproductive and respiratory syndrome virus (PRRSV), yet its functional mechanism remains largely unknown. In this study, to define the cis-acting elements for viral replication and infectivity, the 5′ UTR of type 1 PRRSV was used to replace its counterpart in the type 2 infectious full-length cDNA clone pAPRRS. The chimeric cDNA clone pTLV8 was constructed and used for further genetic and virologic analysis. The chimeric virus vTLV8 could be rescued, and it had similar virologic properties to vAPRRS, including plaque morphology, growth kinetics and subgenomic mRNA (sg mRNA) transcription. Taken together, the results present here demonstrate that the 5′ UTR of type 1 PRRSV dose not affect the growth rate of type 2 PRRSV in vitro. The 5′ UTR of type 2 PRRSV can be functionally replaced by its counterpart from type 1.

This is the first report that the type 1 5′ UTR could play regulatory function in type 2 genomic backbone and fully functional. In addition, we did the related research to explore the origin of 5′ UTR 5′ terminal exogenous sequences, results showed that the insertion sequences which used for repair was template independent.

# 41
# Identification of virulence determinants of highly pathogenic porcine reproductive and respiratory virus HuN4 strain

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To investigate the molecular mechanisms of the different virulence between highly pathogenic porcine reproductive and respiratory virus (HP-PRRSV) HuN4-F5 strain and its attenuated vaccine strain HuN4-F112, we generated six full length infectious cDNA clones with interchange of ORF1a, ORF1b, or ORF2-7 region, between the two viruses, named rHuN4-F5-ORF1a, rHuN4-F5-ORF1b, rHuN4-F5-ORF2-7(genetic backbone of virulent HP-PRRSV, HuN4-F5 with ORF1a, ORF1b or ORF2-7 from attenuated HP-PRRSV, HuN4-F112) and rHuN4-F112-ORF1a, rHun4-F112-ORF1b, rHun4-F112-ORF2-7 (genetic backbone of HuN4-F112 with ORF1a, ORF1b or ORF2-7 from HuN4-F5) , respectively. The piglets were infected with chimeric viruses rHuN4-F5 and rHuN4-F112, and the sera from each pig were collected. The virus titer in sera from pigs infected with the chimeric viruses was significantly lower than that from parental virus at 3, 5, 7 and14 dpi. But pigs infected with rHuN4-F5-ORF2-7 showed significantly lower titer than that of rHuN4-F5 at 7 and 14 dpi. There was no significantly difference among pigs infected with chimeric viruses except rHuN4-F112-ORF1a at 7 dpi. The level of IL-1 in response to rHuN4-F5 was significantly increased and all the chimeric viruses were not significantly changed. The levels of IL-6 in response to rHuN4-F5-ORF2-7 infection were significantly higher than that infected with the other chimeric viruses or rHuN4-F112 at both 3 and 5 dpi. At 3, 5, 7 and 14 dpi, higher levels of IL-10 were induced by the infection of rHuN4-F5-ORF2-7 or rHuN4-F5 than that induced by the other virus. And at 3, 5, 7 and 14 dpi the infection of rHuN4-F5-ORF1b triggered much higher amount of IL-10 than that infected by the other viruses except rHuN4-F5-ORF2-7 and rHuN4-F5. The rescued viruses with a common backbone of rHuN4-F5 stimulated higher amount of IL-10 than rescued viruses with a backbone of rHuN4-F112, except rHuN4-F5-ORF1a and rHuN4-F112-ORF1a. The infection of all the viruses also resulted in the secretion of IFN-γ with a moderate level. Taken together, in this study we concluded that: the regions encoding for Non-structure proteins were important for the virus infection, especially in the production of viremia and the induction of IL-6 and IL-10. ORF1a plays a very important role in viremia production, IL-6 and IL-10 induction. ORF1b was involved in the production of IL-6 and IL-10. Therefore, this study set up a foundation for further elucidation of the diversity between HP-PRRSV and avirulent PRRSV in genetic level.

# 42
# Suppression of type I interferon induction and signal transduction by the porcine reproductive and respiratory syndrome virus non-structural protein (nsp) 1-beta subunit

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Activation of type I interferon (IFN-alpha/beta) production is essential for cellular resistance to virus infection, but during infection with porcine reproductive and respiratory syndrome virus (PRRSV) only a low level of IFN is induced and the disease in pigs is often associated with viral persistence. Non-structural protein (nsp) 1 of the virus has been reported as an IFN antagonist, and in the present study the nsp1-beta subunit was examined for IFN modulation. Nsp1-beta was localized to both the cytoplasm and nucleus of the cell and inhibited IFN induction by targeting the IRF3- and NF-kB-dependent IFN production pathways. The nsp1-beta subunit-mediated IFN suppression was consistently weaker than that of nsp1, the non-cleaved precursor of nsp1-alpha and nsp1-beta The inhibitory activity was not limited to the IFN production pathway, but also observed in the IFN-induced Janus kinase (JAK)-signal transducers and activators of transcription (STAT) signaling pathway responsible for IFN-dependent ISRE expression.

# 43
# Applying benchmarking in porcine reproductive and respiratory syndrome area regional control and elimination projects

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Benchmarking is an ongoing process used by many industries seeking continuous improvement. Benchmark is a point of reference for a measurement and serves as a standard by which others may be measured or judged. In quality based science, benchmarking is the process of comparing the current project, methods or processes with the best practices and using this information to drive improvement of overall company performance. It focuses on how to improve any given business process by exploiting best practices rather than merely measuring the best performance. This abstract describes benchmarking implementation model in porcine reproductive and respiratory syndrome (PRRS) Area Regional Control an elimination (ARC&E) projects. Currently, there are several ARC projects lead by veterinarians, producers and researches all around the globe. Most of them follow a standard structure: **I. Feasibility Study, II. Pig related site id. III. Region characterization, IV. Design control strategies, V. Execution & Monitoring**. Benchmarking among these ARC projects is possible by applying DIAMR model. (**D**etermine current best practices; **I**dentify best practices; **A**nalyze best practices; **M**odel best practices; **R**epeat the cycle). Benchmarking in PRRSv ARC represents an opportuntiy to boost current projects for improvement learning from best projects in class.

Table 1. Model DIAMR to apply Benchmarking to PRRS ARC projects.

| D | I | A | M | R |
|---|---|---|---|---|
| +Select what we want to improve within the 5 phases<br>+Identify critical success factors from several ARC successfully implemented<br>+Select specific measurement of success according to the goals and expectations | +Evaluate the measurement of success for the current project and get a baseline<br>+Determine 1 or 2 state of the art projects to benchmark with<br>+Ask for interaction and a formal benchmarking to experts with profound knowledge on the selected projects. | + Collect critical information and data for success in ARC projects<br>+Evaluate and compare current practices with the selected projects<br>+Note potential improvements areas at each ARC phase considering current conditions | +Drive improvement changes to advance performance levels<br>+Communicate these initiatives and extend the measurement of success goals within the working group<br>+Incorporate this information in ARC project's newsletter<br>+Share results with benchmarking partner(s) | +Repeat the process until achieving desired goals<br>+ As the need forimproveme nt changes include new leading ARC projects |

# 44
# Pilot project to develop a novel methodology for the detection of airborne porcine reproductive and respiratory syndrome virus during a twenty-four hour period

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Porcine reproductive and respiratory syndrome virus (PRRSv) is known to be spread via aerosol. Aerosol collectors have been used for ≤30 minute periods and with saline solution (SS) as the sampling solution. The objectives of this pilot study where: 1. To determine if lactated Ringer's solution (LRS) could be an alternative to SS, and 2. To confirm the aerosol collector could run for a 24 h period. On day 0, fifty milliliters (mL) of SS and LRS were placed in two 50mL sterile containers. Each solution was spiked with 0.5mL of Ingelvac® PRRS MLV vaccine (Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO). In order to detect the presence of PRRSv, a 12.0 mL aliquot was removed from each container at 1 and 30 minutes, and 6 and 24 hours post-spiking. Each 12.0mL aliquot was subdivided in three 4.0mL aliquots, placed into sterile tubes, and stored at three different temperatures: 19°, 3° and -18°C (frost-free freezer). In order to confirm if the aerosol collector (Midwest MicroTek, Brookings, SD, USA), could be used for a 24 hour period, the collector was left running for five (n=5) consecutive 24 hours periods at 4800 RPM. A 1000mL LRS intravenous (IV) fluid bag was placed next to the collector and an IV drip set (60 drops per ml) was installed, allowing the IV line to drip into one of the collector's container holes. The IV line was allowed to drip at a rate of one drop every 4-5 seconds (12-15ml/hr). All samples were tested in triplicate for the presence of PRRSv nucleic acid by reverse transcriptase polymerase chain reaction (rt-PCR) and were reported in cycle threshold (Ct) values. As shown in table 1, there was no numerical difference in Ct values at the various sampling times and storage temperatures. Due to small sample size no statistical inference should be made. Aerosol collectors were able to run for 24 continuous hours with a LRS IV drip during 5 days with no effect on functionality or damage to the motor. The results of this study present a novel methodology for the detection of airborne PRRSv for ≥30 minutes. Implementation of this methodology would allow for practical and longer sampling periods to monitor the the presence of PRRSv in the air.

**Table 1. PRRSv RT PCR results (Ct) from PRRS MLV spiked saline solution (SS) and lactated Ringer's solution (LRS)**

| | Storage Temperature ($^0$C) | | | | | |
|---|---|---|---|---|---|---|
| Sampling time | 19° | | 3° | | -18° | |
| | SS | LRS | SS | LRS | SS | LRS |
| 1 min | 25.5 | 26.3 | 25.2 | 26.1 | 26.4 | 27.4 |
| 30 min | 25.4 | 26.4 | 25.4 | 26.1 | 25.8 | 26.8 |
| 6 h | 25.8 | 26.8 | 25.3 | 26.3 | 27.4 | 27.5 |
| 24 h | 25.5 | 26.6 | 25.4 | 26.4 | 27.3 | 27.2 |

# 45
# Nanoparticle-based adjuvanted inactivated porcine reproductive and respiratory syndrome virus vaccine elicits cross-protective immunity in pigs

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Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease of pigs, caused by PRRS virus (PRRSV), incurring estimated $664 million losses annually to the US pork industry. Routinely used modified live PRRSV vaccine is implicated in transmission of mutated vaccine virus to susceptible pigs. Unfortunately, currently available killed PRRSV vaccine elicits poor immunity. Therefore, our goal was to develop a safe and protective killed PRRSV vaccine. We adopted two strategies: firstly, entrapped the killed PRRSV vaccine in PLGA [poly(lactide co- glycolide)] nanoparticles (Nano-PRRSV vaccine); and secondly, co-administered the vaccine with a potent adjuvant, *Mycobacterium tuberculosis* whole cell lysate (*M. tb* WCL) that we identified earlier. To analyze the cross-protective efficacy of the vaccine, pigs were co-administered with killed PRRSV vaccine (VR2332 strain) and *M. tb* WCL, in the form of entrapped (Nano-PRRSV vaccine) or unentrapped in nanoparticles, intranasally, and then challenged with a virulent heterologous PRRSV (MN 184 strain). Our results indicated that, Nano-PRRSV vaccine co-administered with unentrapped *M. tb* WCL elicited cross-protective immunity, indicated by the following immune correlates detected at both the lungs and blood: (1) increased levels of PRRSV specific IgG and IgA antibody titers with enhanced antibody avidity; (2) balanced Th1 and Th2 type responses; (3) upregulated heterologous and heterogenotypic PRRSV specific neutralizing antibody titers; (4) Increased secretion of Th1 cytokines (IL-12 and IFN-γ) with enhanced frequency of IFN-γ secreting cells in the lungs and reduced immunosuppressive cytokines (IL-10 and TGF-β); and (5) complete clearance of replicating virus from the lungs and blood. In conclusion, our study for the first time demonstrated that adjuvanted Nano-PRRSV vaccine elicits cross-protective immunity against PRRS in pigs. This project was supported by National Pork Board, USDA PRRSV CAP2, and OARDC OSU to RJG.

# 46
# Regional PRRS control within a high pig density area: the Southeast Iowa experience

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The annual loss associated with PRRS in the US swine industry has been recently estimated at USD-$664 million. The elimination of PRRS virus (PRRSv) from breeding herds through load, homogenize and close protocols is effective and repeatable; however, transmission between farms occurs all too often, making it very difficult to meet and maintain health and productivity expectations. An alternative is to implement cooperative and coordinated disease control efforts among producers and veterinarians working together with their neighbors and business partners in order to significantly reduce the risk of re-infection in those herds. The objectives of this project are: 1) To reduce the incidence and prevalence of PRRS in the region. 2) To minimize the severity and duration of PRRS episodes, and 3) To establish a structure for disease surveillance and communication in the region. As part of this voluntary project, producers sign participation agreements to allow information sharing and minimize the concern of legal actions among neighbors. Participants are granted access to the project website by the project coordinator. Every other week, veterinarians communicate any change in PRRSv infection status during the previous 2 weeks to the coordinator. A prevalence summary table, a list of herds that changed status and the maps are updated in a bi-weekly bulletin that is sent to all participants. Quarterly meetings are held to discuss needs and actions as well as to evaluate the progress of the initiative considering change in prevalence, number of new cases, outbreaks identified by veterinarians and genetic diversity. The primary control area of this project is mostly occupying Washington County, located in Southeast Iowa. A peripheral control area includes parts of the 6 surrounding counties and 3 more counties to the Northeast. Washington County is the sixth most hog dense county in the state of Iowa and twelfth in the US. A significant proportion of the pigs introduced to the region are from diverse pig sources. By September 2012, 27 breeding herds, 63 nursery sites and 309 finishing sites have enrolled in the project. Most are family owned farms averaging 2700 pigs per site. About 21% of the herds have been reported as PRRSv stable or negative and the infection status of 1% of herds remains unknown. The regional control plan for this project is based on the use of modified-live virus vaccination in breeding and grow-finish herds, air filtration in specific sow farms and comprehensive biosecurity programs to systematically mitigate the impact of PRRSv and reduce transmission between herds. The monitoring/surveillance protocol allows for early detection of PRRSv infection (oral fluids, PCR, sequence analysis) and facilitates continuous proactive communication and coordinated pig flow or transit modifications.

# 47
# Identification and characterization of a novel PRRSV nsp2 deletion mutant

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The present study aimed to determine the full-length nucleotide sequence of a Korean type 2 PRRSV nsp2 deletion mutant, designated CA-2. The full-length genome of KNU-07 was found to be 15,018 nucleotides in length excluding the poly(A) tail, which was 393-nucleotide shorter than that of the type 2 NA prototype strain VR-2332 due to the notable large 393-bp (131-aa) deletion within the nonstructural protein 2 (nsp2) gene. The CA-2 genome consisted of a 189-nucleotide 5' untranslated region (UTR), a 14,677-nucleotide protein-coding region, and a 151-nucleotide 3' UTR. Full-length sequence analysis of the CA-2 genome with the 20 fully sequenced PRRSV genomes revealed sequence divergence ranging from 13.7% (the Korean CA-1 strain) to 42.2% (the Lelystad strain). To evaluate the *in vitro* immunity of CA-2, we sought to explore alteration of inflammatory cytokine and chemokine expression in PAM-pCD163 cells infected with CA-2 by using quantitative real-time RT-PCR. Cytokine genes including interferons (IFN-β, γ, and λ), IL-7, IL-8, IL-15, and TNF-α, and chemokines such as AMCF-1 and MCP-2 were found to be significantly elevated in CA-2 virus-infected PAM cells. In addition, antiviral genes including ISG-15 and ISG-54 were up-regulated in PAM cells upon CA-2 infection. However, expression of the anti-inflammatory cytokine IL-10 was dramatically reduced during the course of CA-2 infection in PAM cells. Animal studies to determine pathogenicity of this nsp2 deletion mutant are currently in progress and results of *in vivo* assessment will be discussed.

# 48
# Globin reduction in porcine whole blood for improving sensitivity and accuracy of transcriptome analysis for host response to PRRSV infection

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RNA sequencing technology has created unprecedented possibilities for deciphering genetic mechanisms. Its application towards elucidating the transcriptome changes after viral infection will provide major insights to help improve pig health and disease resistance. The abundance of globin transcripts, however, impedes the ability to detect less abundant transcripts when whole blood samples are examined. Our preliminary studies determined that globin depletion methods effective for human whole blood do not work for pig blood samples. The objective of our study was to develop and evaluate a porcine specific globin reduction protocol using globin (HBA and HBB) oligonucleotides and RNA-seq. Total RNA was prepared from blood samples collected using PAXgene (n = 6) and Tempus (n = 17) tubes. RNA quality pre/post treatment was checked using Agilent Bioanalyzer. RNA-seq was performed using Illumina HiScan SQ system. On average, 46.9 and 35.6 million reads were generated and 36.1 and 27.7 million reads were uniquely mapped in pre versus post globin reduction treated samples, respectively. The uniquely mapped reads of HBA and HBB tags comprised up to 55 % of the pre-treatment reads; this was reduced to 0.1 - 2.5 % post globin RNA reduction. For validation, qPCR results confirmed that HBA and HBB mRNAs were efficiently depleted (97.2 % and 96 %, respectively). In addition, 1030 genes on average were newly identified after globin reduction; these were involved in differential biological and toxicological functions, and canonical immune pathways. In summary, our porcine specific globin reduction protocol minimizes the number of reads of globin RNA and provides increased accuracy and reproducibility of transcriptome analysis. Based on these results we will now be able to unravel the complexities of host response to Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infection. Plans are underway to use the Tempus tube blood samples from the PRRS Host Genetics Consortium to reveal immune pathways enhanced in pigs which are genetically more resistant to PRRSV infection. This work was supported by grants from Genome Alberta and Genome Canada.

# 49
# Development of a fluorescent microsphere immunoassay for detection of antibodies against PRRSV using oral fluid samples as an alternative to serum-based assays

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For effective disease surveillance, rapid and sensitive assays are needed to detect antibodies developed in response to PRRS virus (PRRSV) infection. In this study, we developed a multiplexed fluorescent microsphere immunoassay for detection of PRRSV specific antibodies in oral fluid and serum samples. Recombinant nucleocapsid protein (N) and nonstructural protein 7 (nsp7) from both PRRSV genotypes (Type I and Type II) were used as antigen and covalently coupled to Luminex fluorescent microspheres. Based on an evaluation of 488 oral fluid samples with known serostatus, the oral fluid-based FMIAs achieved greater than 92% sensitivity and 91% specificity. In serum samples (n = 1639), the FMIAs reached greater than 98% sensitivity and 95% specificity. The assay was further employed to investigate the kinetics of antibody response in infected pigs. In oral fluid, N protein was more sensitive for the detection of early infection (7 and 14 dpi), but nsp7 detected higher level and longer duration of antibody response after 28 days post infection. In serum, the antibodies specific to nsp7 and N proteins were detected as early as 7 days post infection, and the responses lasted more than 202 days. This study provides a framework from which a more robust assay could be developed to profile the immune response to multiple PRRSV antigens in a single test. The development of oral fluid-based diagnostic tests will change the way we survey for diseases in swine herds and improve our ability to cheaply and efficiently track PRRSV infections in both population and individual animals.

# 50
# Development of sandwich immunochromatographic dipstick tests for the rapid field diagnosis of porcine respiratory disease complex

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The dipstick tests were developed based on the high binding capacity of a specific pathogen antigen to swine antibodies, and the conjugation of swine immunoglobulin with colloidal gold nanoparticles as a color probe. Using nucleocapsid proteins (SIV, PCV2) and nsp7 (PRRSV) as antigens, dipstick assays were developed based on testing standard positive and negative control sera as well as a panel of field serum samples, and the diagnostic specificity and sensitivity were compared to the classical enzyme-linked immunosorbent assay (ELISA) or hemagglutination inhibition (HI) test. Based on the evaluation of 456 serum samples of known serostatus from pigs experimentally infected with either type I or type II PRRSV, the PRRSV dipstick assay showed an overall sensitivity and specificity of 95% and 96% respectively, in comparison with the IDEXX HerdCheck X3 ELISA. The inter-rater agreement (kappa value) between dipstick test and ELSA was 0.965. The SIV dipstick test achieved a sensitivity of 96% and a specificity of 99% using 177 field serum samples previously tested in HI assay, and the kappa value between dipstick and HI was 0.953. For PCV2 antibody detection, results from nucleocapsid protein-based dipstick test and ELIA were compared using 135 samples from experimentally infected animals. The sensitivity and specificity of PCV2 dipstick test were 88% and 80%, respectively. These assays present advantages of simplicity, rapidity and cost-effectiveness, and the test is field deployable with a user-friendly format, which can be performed on-site in a swine farm by untrained personnel; therefore, it is very suitable for large-scale field application on porcine respiratory disease surveillance and epidemiology studies.

# 51
# Adjuvants selection for an improved PRRSV Vaccine

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Current vaccines fail to provide complete protection against Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) for several reasons, including the inability to overcome the suppression of the innate immune responses, and in particular the expression of interferon alpha (IFN-a). We hypothesize that adjuvants can overcome this suppression and help to stimulate the immune response to the vaccine. To achieve this goal we are using a recently developed adjuvant platform consisting of three individual components, namely innate defense regulator peptides (IDR), Toll-Like Receptor (TLR) ligands and polyphosphazenes (PCEP).

The first step is to optimize of the adjuvant formulation *in vitro* and to identify synergy between the individual components and their interactions with antigen. To this end, adjuvants are tested in porcine dendritic cells (DCs) and Porcine Alveolar Macrophages (PAMs) alone or in combination for their ability to stimulate the expression of pro-inflammatory cytokines. The VR-2385 North American strain was used to stimulate PAMs or DCs.

Using RT-qPCR analysis we confirmed the mRNA expression of TNF, IL-8, CCL2 and IFN-b by Poly(I:C) stimulation on PAMs. We also found that IDR LL37 (human origin) and 1002 (synthetic) have the capacity to decrease the Poly(I:C)-induced TNF and IL-8 mRNA expression but not CCL2 and IFNb. Thus, by acting synergistically these adjuvants may prevent excessive inflammation, boost IFN production, and possibly facilitate monocyte recruitment. As the virus itself inhibits expression of IFN-a, the adjuvant might help to overcome this deficiency.

Using Flow cytometry, we demonstrated that LL37 inhibited apoptosis in PAMs and moDCs. IDR peptide 1002 displayed a similar effect but to a lesser extent. In addition, we were able to show that IDR LL37 was able to inhibit virus infection on PAMs.

Thus, we were able to demonstrate that the described combination of adjuvants may help to overcome the immune suppression by the PRRSV virus and will help to stimulate an effective immune response.

The next steps are to test the adjuvant formulation with a vaccination/challenge experiment *in vivo* and to analyze the immune cells like PAMs and pDC, their infection status and their capacity to stimulate an anti PRRSV immune response. Work with new strains and heterologous challenge will follow to validate our improved PRRSV vaccine.

# 52
# Impact of the use of specific avian immunoglobulins (ImmunoPRRS®) to control PRRS in a multisite farm in Northern Mexico

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**Introduction.** Porcine Reproductive and Respiratory Syndrome (PRRS) is considered the most significant infectious disease in swine with the most economically significant effects worldwide. Previous studies have shown that the administration of specific immunoglobulins against PRRSv (ImmunoPRRS®) to gestating sows affords full protection against the agent, a phenomenon evidenced by the fact that no affected piglets were produced after treatment, suggesting egg derived immunoglobulins are capable of blocking the infection.

**Material and methods.** The farm is located in orthern Mexico at 27 04 51 and 109 26 43 W, with an altitude of 33 masl and 1000 sows in production. During 2010 an unstable behavior was observed along with abortions, thus an immunoglobulin based program against PRRS was established by administering a dose of 5mL of the product ImmunoPRRS® to the breeding herd and repeating the dose 15 days later. Afterwards, the same program was applied each 4 months to sows at the 10th and 12th weeks of gestation and also to the replacements 15 days prior to their introduction to the farm and on the day of introduction.

**Results**. Comparative results of the serological analysis (ELISA), before and after the application of the product show how antibody levels against PRRS decrease after administering the egg derived immunoglobulins (ImmunoPRRS ®), reaching levels under the test cut point (Chart 1).

| DATE | S/P (ELISA) |
|---|---|
| 06/JUL/2010 | 0.786 |
| 29/OCT/2010 | 0.877 |
| 14/JAN/2011 | 0.918 |
| 21/APR/2011 | 0.341 |
| 12/JUL/2011 | 0.317 |
| 29/OCT/2011 | 0.007 |
| 18/JAN/2012 | 0.265 |
| 09/APR/2012 | 0.327 |
| 30/JUL/2012 | 0.245 |

**Chart 1: Dynamic S/P levels**

| PRODUCTIVITY SUMMARY | | | | |
|---|---|---|---|---|
| | **2009** | **2010** | **2011** | **2012*** |
| **Births** | 2,011 | 2,054 | 2,174 | 2,391 |
| **LBP** | 20,571 | 22,810 | 25,466 | 28,692 |
| **Avg. Born** | 10.23 | 11.11 | 11.71 | 12.00 |
| **Weaned Piglets** | 16,929 | 18,932 | 22,002 | 26,683 |
| **%Mat. mortality** | 17.70% | 17.00% | 13.60% | 7.0% |

* Values projected with data up to August

**Chart 2: Productive parameters**

Along with the decrease of S/P levels, the increasing birth rate shows a tendency to increase the numbers of live born piglets further (Chart 2). The sanitary control program favored the absence of viremic piglets, as corroborated by PCR results at the third week and also increased productivity of site 1 and 2 as compared to results from previous years.

**Discussion and conclusions**. Productive parameters show a clear stability and improvement after beginning the egg derived immunoglobulins program (ImmunoPRRS®). This can be observed by the increase in farrowing rates, live born piglets (LBP) and birth averages, as well as weaned piglets (WP) with their corresponding rates and finally an important decrease in mortality at the maternity stage.

# 53
# A methodical approach to PRRS management, a success story of production improvements

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Porcine Reproductive and Respiratory syndrome Virus (PRRSv) continues to be a major pathogen that impacts the swine industry. The PRRS virus is known to have a wide range of impact on breeding herd and growing pig performance. A 26,000 sow system had been affected by outbreaks of PRRSv infections throughout several years, and traditional strategies of gilt isolation, virus inoculations and herd closures achieved unacceptable levels of success for managing disease and production performance. The objective of this project was to improve the reproductive and growing pig performance, through strategic use of PRRS modified live vaccine (MLV), herd closure, and flow management. The duration of this project was from January 2009 through May of 2011 in two phases. The system had 26,000 sows housed in 12 sow farms, 70,000 pigs in two nursery flows, 64,000 finishing pigs in three finishers; 16,000 pigs in two wean to finish farms, gilt development and isolation. The rest of the pigs were finished at another location. In addition, these animals were positive to Mycoplasma, PRRS and SIV. Phase 1 occurred the first 18 months post-vaccination and Phase 2 was the following 10 months. The project protocols were: 1] Sow herd closure for approximately 170 days; 2] Whole herd vaccination with Ingelvac® PRRS MLV (Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO) 4 weeks after closures, and again 30 days later, followed by quarterly sow herd vaccinations; 3] Vaccination of suckling pigs at approximately 15 days of age followed by a second dose of vaccine three weeks later during the nursery stage; 4] Flow management: managing rooms all in/all out, and when possible entire barns emptied, washed and disinfected; 5] Cessation of intentional exposure to wild-type PRRS viruses. Production performance was monitored using Statistical Process Control methodology (SPC). Significant improvements were achieved in both reproductive and post-weaning performance. Results are described in Table 1 below. A significant improvement in reproductive and growing pig performance was achieved by a well defined process that included the strategic use of Ingelvac PRRS MLV.

Table 1. Performance before and after PRRS control process implimented

| **Production Data** | **Baseline** | **Phase 1** | **Achieved difference** | **Phase II** | **Achieved difference** |
|---|---|---|---|---|---|
| Pigs weaned/sow farrowed | 9.2 | 9.66 | +0.44* | 10.03 | +0.83* |
| Nursery ADG, lb/d | 0.763 | 0.930 | 0.170* | 0.988 | 0.225* |
| Nursery mortality, % | 5.62 | 3.39* | -2.226* | 2.124 | -3.50* |
| Finisher ADG, lb/d | 1.64 | 1.70 | +0.06* | 1.74 | +0.10* |
| Finisher mortality, % | 2.96 | 2.21 | -0.75* | 2.22 | -0.74* |

*Significant change in process behaviour

# 54
# Growth performance improvement and mortality reductions derived from a PRRS large-scale control project in the U.S.

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PRRSv has demonstrated to have an economical impact per pig up to $ 28.3 U.S dollars in finisher phase. Modified-live virus vaccine represents a viable option to minimize the negative impact of PRRS in growing pigs. The objective of this project was to determine if strategic use of Ingelvac® PRRS MLV (Boehringer Ingelheim Vetmedica, Inc. St. Joseph MO) could improve growing pig performance and reduce mortality compared to the previous 24 months of production data in a large-scale pig production system. The project was performed in a 70,000 sow multisite production system. Breeding herds, nurseries and finishers were endemically infected with PRRSv type 1 (EU) and type 2 (NA). The primary interventions were herd closure (130 days) and systematic breeding herd mass vaccination with Ingelvac PRRS MLV (2 ml), followed by quarterly sow mass vaccination, and ongoing pig Ingelvac PRRS MLV vaccination (2 ml) at weaning. Project duration was 15 months. In growing pigs, system-wide average daily gain (ADG) and mortality percentage were compared in a before-after analysis, running 2-sample t-test for ADG and 2-sample proportion test for % mortality (MINITAB 16.1). In the nursery phase, a total of 703 closeout groups (3,656,862 pigs) were included in the before period, and 328 closeout groups (1,463,539 pigs) were included in the after period. In the finisher phase, 489 closeout groups (2,659,631 pigs) were included in the before period and 188 closeout groups (1,006,072 pigs) were included in the after period. Monthly diagnostic monitoring in piglets at each breeding herd as well as nursery and finisher pigs (hospital pens) was implemented to assess PRRSv circulation dynamics and system-wide wild type (WT) presences (using ORF-5 sequencing) proportion during the project. Before and after vaccination results are shown in Table 1.This large scale PRRS control project was successfully implemented for 15 months. A significant increase in ADG ($P<0.01$) and significant decrease in mortality ($P<0.01$) was detected in both nursery and finisher pigs when compared against the previous 24 months closeouts. In addition, a reduction of PRRS wild type virus proportion in the whole system was observed. PRRS MLV pig vaccination and modified herd closure were the primary tools utilized to achieve an improvement in ADG and reductions in mortality even in the presence of both PRRSv type 1 and type 2 in the system.

**Table 1**. Analysis of system-wide closeout gain and mortality before and after vaccination

| | **Vaccination** | |
|---|---|---|
| **Parameter** | **Before** | **After** |
| **Nursery** | | |
| ADG, lbs | $0.905\pm0.12^{a}$ | $0.975\pm0.07^{b}$ |
| Mortality, % | $3.19\pm0.008^{a}$ | $2.45\pm0.008^{b}$ |
| **Finisher** | | |
| ADG, lbs | $1.704\pm0.01^{a}$ | $1.825\pm0.06^{b}$ |
| Mortality, % | $5.56\pm0.009^{a}$ | $3.65\pm0.003^{b}$ |
| System-wide WT-PRRS virus | $100\%^{a}$ | $49.6\%^{b}$ |

Rows with different superscripts differ at $P<0.01$

# 55
# Development of a differentiable virus through a spontaneous deletion in nsp2 region associated with cell-adaptation of porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is notorious for genetic, antigenic and pathogenic heterogeneity. As a consequence, high pathogenic PRRSV (HP-PRRSV) has been emerging and resulting in tremendous economic loss of swine industry. In this study, a Chinese HP-PRRSV JX143 isolate was serially passaged in MARC-145 cells up to 100 times. We found that the phenotypic changes involving the cell-adaptation process of PRRSV JX143 were characterized by higher titer, faster growth kinetics, and larger plaque size as the passage number increasing compared with parental virus. Piglets inoculated with JXM100 virus did not show high fever in body temperature and clinical signs throughout the experiment and the average body weight of piglets in JXM100 infected group were similar to that of the control groups, suggesting that the virulence of the JX143 isolate to piglets was decreased greatly at passage 100 (JXM100). The JXM100-infected group could resist the lethal challenge of JX143 and did not show any changes in body temperature nor clinical signs and gained weight as well as that of control, suggesting that the JXM100 could protect piglets from lethal challenge and might be a candidate vaccine against the HP-PRRSV. Genome-wide analysis revealed that JXM100 strain contains a continuous 264 nucleotide (88 amino acids) deletion in the nsp2 region and 75 nucleotides mutation randomly across the genome. These nucleotide changes that arose during MARC-145 passage of the HP-PRRSV JX143 virus provide a potential molecular basis for the observed cell-adapted phenotype in MARC-145 cells and attenuated phenotype *in vivo*. The study also showed that pigs inoculated with JXM100 with 88 aa deletion (del88) in nsp2 elicited a strong antibody response against the N protein but did not develop antibody against the del88, whereas a strong reactivity was observed in the sera obtained from piglets infected with JX143 using the same del88-based ELISA. It is suggested that del88 can use as the genetic marker to differentiate JXM100 serologically from JX143. Finally, we developed an infectious cDNA clone of JXM100, which provide platform for further dissection of molecular basis of HP-PRRSV as well as rational design of DIVA vaccine.

# 56
# Construction and identification of a recombinant PRRSV expressing immunodominant of VP1 gene of type O foot-and-mouth disease virus

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Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), is an acute transmissible disease of pigs characterized by reproductive disorders in sows, and respiratory distress and mortality in piglets. PRRSV consists of two genotypes, North American type and European type represented by VR2332 and LV, respectively. The disease can be spread horizontally and vertically in swine herds and causes great economic losses to swine industry. In 2006, a "high fever disease" emerged in Chinese swine herds characterized by high morbidity and mortality in all age pigs, the causative agent of the disease was later identified as a variant of PRRSV (also called highly pathogenic PRRSV). At present, an attenuated vaccine against the highly pathogenic PRRSV has been developed in China, but when vaccination of pigs together with this vaccine and other vaccines (such as attenuated foot and mouth disease vaccine), it may cause immune failure of other vaccines as PRRSV could induce immunosuppression in pigs. To avoid the interference, developing of a combined vaccine by using PRRS vaccine virus as vector should be significantly practical.

In the study, based on an infectious molecular clone of highly pathogenic PRRSV derived vaccine strain HuN4-F112, one infectious cDNA clones deletions in nsp2 region was constructed and named as rHuN4-F112-Δ508-532, respectively. Using rHuN4-F112- Δ508-532 as vector, a synthesized fragment representing 421-480nt（141-160aa）and 598-639nt（200-213aa）of VP1 gene of foot and mouth disease virus (FMDV) was inserted into the deleted site of nsp2 region. The two discontinued sequences contain multiple neutralizing antigenic epitopes of FMDV. The recombinant cDNA was in vitro transcripted followed by trancfection of BHK-21 cells, 36 h later, supernatant of the cell culture was transferred to monolayer of MARC-145 cells for recovery of the recombinant virus. CPE was developed visible after a couple of passages in MARC-145, and the rescued virus (designated as rPRRSV-F112-O/VP1ep) was identified by *Mlu I* digestion, sequence determination and immunofluorescence assay. At meanwhile, expression of inserted FMDV epitopes was detected by indirect immunofluorescence assay with polyclonal antibodies to VP1 protein of FMDV. The titer of the rescued recombinant PRRSV was similar to its direct parental virus rHuN4-F112-Δ508-532, but higher than rHuN4-F112.

# 57
# Characterizing the Antibody Response Following Experimental PRRSV Infection in a Large Population of Pigs

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In general, the neutralizing antibody (nAb) response following PRRSV infection is weak and delayed. Furthermore, nAb is primarily directed against the homologous virus used for infection. The purpose of this study is to evaluate the total and nAb response in a large population of experimentally infected pigs. Based studies of immune response to HIV, we predicted that a subpopulation of pigs exists that are capable of producing broadly neutralizing antibody (bnAb). Serum samples from an ongoing study evaluating the role of host genetics in resistance-susceptibility to PRRSV infection were used for this study. Samples were obtained from 689 pigs challenged at about 4 weeks of age with NVSL 97-7895 (482 pigs) or a contemporary isolate, KS06 (207 pigs), which is still circulating in the field. Blood and other samples were collected regularly for phenotypic data. Virus neutralizing activity was measured at 42 days after infection. Neutralizing assays were performed using 4 viruses, KS-06, NVSL 97-7895, 2867, and VR-2332, which are well separated within a phylogenetic tree. Isolates had a difference ranging from 5-11% at the nucleotide level of ORF5. Based on the results of neutralization assays, samples were placed into the following categories: Group 1, no neutralizing activity (14.4%); Group 2), neutralizing activity against only the homologous virus (50.5%; homologous nAb); Group 3, neutralizing activity against the homologous virus and one or two additional isolates (29.6%; heterologous nAb); or 4) neutralizing activity against all four isolates (5.5%; broadly nAb). There was no apparent correlation between total anti-PRRSV antibody (measured against the N protein) and the amount of neutralizing activity. As reported in another abstract (Hess et al.), virus neutralizing activity has a heritable component. There is also an inverse relationship between virus load and homologous neutralizing titer, indicating a role for nAb in the control of virus replication. The identification of pigs that possesses a “broadly” neutralizing antibody response suggests the existence of conserved neutralizing epitopes. The genetic basis for a bnAb response creates the opportunity to develop pig lines that are tailored to respond optimally to current or future PRRS vaccines.

# 58
# Antibody Immune Masking, a new platform to develop a broadly protective porcine reproductive and respiratory syndrome virus (PRRSV) vaccine

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Porcine Reproductive and Respiratory syndrome virus (PRRSV) has become a major health issue for swine industry worldwide, with an estimate loss of $640 million/year in US alone. Current vaccines provide limited protection especially against minor and divergent strains (up to 40% variability). Major challenges in the development of a broadly protective vaccine besides high mutation rate are multiple host evading properties of the virus, amongst them is the property of certain epitopes and other domains on the pathogen to be immunodominant and non-protective by focusing the stimulation of antibodies, innate and cell-mediated responses on non-critical or strain-restricted targets on the virus. This leads to a non-protective immune response, away from the recognition of conserved subdominant more broadly protective epitopes. We hypothesize that a proprietarily prepared autogenous PRRSV vaccine derived from the technology of Antibody immune Masking (AIM) can refocus the immune response from these immunodominant epitopes to broadly protective subdominant epitopes. Here we present the efficacy of two challenge models in reducing viremia, PRRSV associated pathology in primary organ of PRRSV replication and reduction of infection. In the preliminary study, pigs receiving the AIM vaccine and later challenged with a heterologous virus developed anti-virus antibody response, towards both the homologous and heterologous strains, a week earlier as compared to the control groups receiving the virus alone and also had greatly reduced lung lesions. The most recent trial demonstrated that AIM vaccination was 33% effective in prevention of infection from a heterologous PRRSV virus. AIM vaccinated pigs that were not protected from infection had significant reduction in peak viremia post challenge as compared to the autogenous vaccines ($p=0.0034$) and unvaccinated controls ($p=0.0001$). AIM vaccinated pigs also had a significant reduction in pulmonary viral loads as compared to unvaccinated controls ($p=0.0126$) that was reflective of reduced pulmonary pathology in vaccinated groups as compared to the non-vaccinated controls. Future goals would be to translate the vaccine to field and elucidate the mechanism of vaccine protection.

# 59
# Bone marrow allotransplantation rescues severe combined immunodeficiency phenotype in pigs

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Severe combined immunodeficiency (SCID) is a primary immune deficiency that occurs naturally in humans, dogs, and horses, and has been introduced by transgenics into mice and recently into pigs. We recently identified the first natural occurrence of SCID in pigs. Routine necropsy of four piglets that died soon after weaning revealed underdeveloped lymph organs, few T- and B-cells, and low levels of antibodies. These piglets were from related parents of the ISU low residual feed intake selection line, which descends from commercial purebred Yorkshire pigs. Repeat matings of the two sires and four dams have produced 6 additional litters with confirmed SCID piglets in an approximately 20-25% frequency. Affected piglets appear healthy while on the sow but deteriorate quickly after weaning, succumbing to opportunistic infections. To identify the genomic region associated with this defect, 20 affected piglets, 50 unaffected littermates, the 6 parents, and 96 normal ancestors were genotyped using the Illumina Porcine SNP60 Beadchip. The dfam option of PLINK was used to identify a 5.6 Mb region that is associated with the threshold disease trait ($P = 2.7x10^{-7}$). Haplotype phasing analyses using Phase (2.1.1) identified two distinct haplotypes that are associated with the defect. Single SNP testing in the region confirmed the BeadChip data and co-segregation of this region and the SCID phenotype. The region contains a very strong candidate gene based on comparative information from other species. Sequencing is underway to identify the causative mutation. Pre-suckling serum antibody levels, lymphocyte numbers from complete blood counts (CBC), and single SNP testing were used to rapidly identify nine affected piglets from four additional litters. Bone marrow transplantation was used to treat affected piglets, in order to obtain genetically affected pigs of reproductive age for more effective production of SCID piglets for biomedical research. A fully MHC matched bone marrow donor of the opposite sex bone marrow donor was identified for each affected recipient; marrow was harvested and transferred into each affected piglet through an ear vein. Blood was drawn biweekly post transfer to assess engraftment. CBC data showed an increase in lymphocyte and total white blood cell numbers over a two-month period. Flow cytometry showed increases in both B- and T-cell populations. Five of the nine bone marrow recipients developed graft versus host disease and were euthanized. Two pigs from the first two transplant litters are currently eight months of age and two pigs from the most recent two litters are four months of age. EHW is supported by USDA NIFA National Needs grant #2010-38420-20328; project is supported by funds from the ISU Research Foundation and from the Office of the Vice President for Research and Economic Development.

# 60
# Recombinant highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) expressing GFP is highly attenuated: Implications for vaccine development

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In May 2006, highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) attacked the swine industry of China, with the characteristics of high fever, high morbidity and mortality in pigs of all ages. Several conventional live attenuated HP-PRRS virus vaccines have been generated by continuous blind cell passages. However, this kind of vaccines has a disadvantage that they cannot be distinguished from naturally infected field virus strains by serological assays. Reverse genetics system provides a powerful tool to rationally develop vaccines with marker. Here, a recombinant HP-PRRSV expressing TRS6-drived GFP (RvHV-GFP) was constructed by inserting a copy of the transcription regulatory sequence for ORF6 (TRS6) between ORF1b and ORF2a. Intramolecular homologous RNA recombination was identified not only between the copy of TRS6 and the authentic TRS6 but also between the inserted GFP gene and ORF4/5. Thus, structural protein-encoding genes deleted non-viable viruses were continuously produced upon virus replication. The recombinant RvHV-GFP virus replicated slower than both wild-type HV and rescued HV (RvHV) virus and the different was significant. Moreover, RvHV-GFP also caused less cell death and milder cytopathic effect (CPE) in vitro. Most importantly, pigs infected with RvHV-GFP survived with none or mild syndromes such as lower rectal temperature, lower virus titers both in serum and tissues and scarcely pathologic changes, whereas all pigs infected with HV or RvHV (3 pigs per group) died before 12 days post infection. Furthermore, vaccination with RvHV-GFP could fully protect pigs challenged with both homologous and heterologous HP-PRRSV strains. Our data implicates the possible development of novel live attenuated marker virus vaccines against arterivirus and coronavirus based on intramolecular homologous RNA recombination.

# 61
# Development of a new vaccine based on a PRRSV replicon

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Currently used modified live vaccines (MLV) for PRRSV are far from ideal. First, it provides full protection against PRRSV strains that are antigenically close to the vaccine virus, but only partially protective against heterologous PRRSV strains. Second, there is a risk of the reversion of the vaccine virus to virulence. Third, vaccinated pigs become viremic and shed the virus into the environment. Fourth, current modified live vaccine lack a DIVA potential (possibility to differentiate infected and vaccinated pigs). Therefore, a new better vaccine is urgently needed for the swine industry.

In this study, we used a DNA-launched infectious clone of a highly virulent PRRSV strain VR2385. A propagation-defective viral genome was generated by deleting the ORF2 region of the genome that encodes the GP2 and E proteins that are essential for virus spread. The self-replicating but propagation-defective PRRSV was generated by injection of the plasmid DNA containing the deletion mutant genomic clone into the pig muscle. To evaluate immunogenicity and protective efficacy of this vaccine candidate, PRRSV-negative 3 weeks-old piglets were divided into three groups comprising eight animals each. Group 1 pigs were vaccinated twice at a 3 week interval intramuscularly with the vaccine candidate (replicon); group 2, as a negative control, received pCI-neo DNA and group 3 had a commercial MLV. Three weeks after the second immunization, all pigs were intranasally challenged with PRRSV VR2385. Rectal temperatures were measured every other day after challenge. Blood samples were collected for virus and viral RNA isolation and serology. The pigs were euthanized on day 14 post-challenge, and bronchoalveolar lavage fluids (BALF) were collected for virus isolation.

Before PRRSV challenge, only pigs vaccinated with MLV developed antibody response measured by ELISA, and none of the pigs developed PRRSV neutralizing antibodies in serum. The MLV vaccinated pigs also showed high PRRSV-specific INF-γ response, whereas replicon-vaccinated pigs showed low but detectable INF-γ response. After challenge, all pigs were positive for PRRSV-specific ELISA antibody, and replicon-vaccinated pigs elicited higher titers than control pigs. In addition, all pigs showed similar PRRSV-specific serum neutralizing titers on day 14 post challenge. Also, on day 14 post challenge, all MLV-vaccinated pigs were PRRSV-negative based on virus isolation from serum and BALF, whereas 25% sera and 50% BALF of replicon-vaccinated pigs were PRRSV-positive. Control pigs showed virus in 50% serum and 85% BALF samples. To further evaluate quantitative levels of PRRSV replication in challenged pigs, RNA genome copy numbers in serum samples at 6 and 14 days post challenge were determined by real-time RT-PCR. The RNA copy numbers in sera from control group were significantly higher than from other groups. Rectal temperatures were significantly decreased in the MLV-vaccinated pigs on day 2, 6 and 10 post-challenge in comparison to other groups.

Vaccination with the PRRSV replicon increased numbers of IFN-γ producing cells and decreased viremia in blood and BALF of the PRRSV-challenged pigs. Thus, the vaccine is partially protective and is a potential vaccine candidate for the future with further improvement.

# 62
# Longitudinal analysis of genetic variation in ORF2-6 in pigs experimentally infected with porcine reproductive and respiratory syndrome virus

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The effectiveness of vaccines against porcine reproductive and respiratory syndrome virus (PRRSV) is hampered by the high error rate and genetic diversity of PRRSV, which are thought to play an important role in virus immune evasion. Neutralization escape is the proposed mechanism for the emergence of PRRS in vaccinated herds; however, it has been difficult to demonstrate a causative relationship between specific mutations and immune escape. In the present study, we examined genetic diversity in samples available through the PRRSV Host Genetics Consortium, in which over 1500 pigs experimentally infected with PRRSV were followed through 42 days post-infection (dpi). In approximately 30% of the pigs, the virus was cleared by 21 dpi, but rebounded by 42 dpi, indicating potential escape from host immune control. Serum samples were obtained from seven pigs, including two that successfully cleared the virus (cleared), two that maintained high levels of viremia throughout the trial (persistent), and three that initially cleared the virus then rebounded by day 41 (rebound). Viral RNA was isolated from the PRRSV NVSL97-7985 inoculum, from day 7 serum, and from late day sera from persistent and rebound pigs. cDNA was synthesized and ORF2-6, which codes for GP2-5 and M, were amplified, cloned, and 10-30 clones were sequenced from each pig/day post infection. Viral populations within pigs and the inoculum were compared by multiple sequence alignment (ClustalW). Comparisons between the inoculum and day 7 virus populations identified six sites with a change in dominant amino acid in at least one pig. Comparisons between day 7 and late day populations from persistent and rebound pigs identified twelve sites with a change in predominant amino acid, with the number of dominant amino acid changes ranging from one to eight within individual pigs. The dominant amino acid changes were observed in GP2, GP3, GP4, GP5a and GP5, as well as in overlapping reading frames; however, only one of these changes occurred in an identified B-cell epitope. Phylogenetic analysis (RAxML) of the entire GP2-M region within single pigs revealed that late day virus populations often formed a distinct clade, especially in rebound pigs, indicating divergence of the late day virus from inoculum and day 7 virus populations. Simulations were used to check if the changes in allele frequencies were greater than predicted by genetic drift under a neutral model. Assuming the effective population size never dropped below 100 during the course of infection, evidence of selection was found in six of seven pigs at day 7 and three of five pigs later in infection. Selected sites tended to be shared among all pigs at day 7, but were unique for each pig later in infection. Together, these results suggest that some mutations in ORF2-6 of PRRSV arise from selective pressure, and may contribute to immune escape *in vivo*.

# 63
# Region on *Sus scrofa* chromosome 1 associated with viremia in pigs infected with porcine reproductive and respiratory syndrome virus

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Host genetics has been proven to be associated with resistance/susceptibility to Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infection. Specifically, a region on *Sus scrofa* chromosome (SSC) 4 harbors a large quantitative trait locus (QTL) that is associated with decreased virus load and increased weight gain. The objective of the current study was to explore other genomic regions associated with resistance/susceptibility to PRRSV infection using data from the PRRS Host Genetics Consortium (PHGC) PRRS-CAP project by conducting a genome-wide association analysis. Eight groups of ~200 commercial crossbred pigs from 5 breeding companies were experimentally infected between 18 and 28 days of age with virus isolate NVSL 97-7985. The first 3 trials were from the same company and cross. Breeds represented in the crosses included Large White, Landrace, Yorkshire, Duroc, and Pietrain. Blood samples and body weights were collected up to 42 days post infection (dpi). Pigs were genotyped for over 60,000 single nucleotide polymorphisms (S P s) across the genome with Illumina's Porcine 60k Beadchip. Whole genome analyses focused on viral load (VL = area under the curve for log-transformed RT-PCR based serum virus from 0-21 dpi) and weight gain (WG = gain from 0-42 dpi). Viral load data was only available for the first 7 trials. A 1 Mb region that was associated with VL was identified on SSC1. This region explained only 0.24% of the genetic variance in trials 1-3 and 0.04% in trials 4-7. Jointly, this region explained 1.5% across trials 1-7. For WG, the SSC1 region explained less than 0.04% in trials 1-3 and 1-8. This region included 21 SNPs across trials. Two important candidate genes lie within 2 Mb of this region: DBC1 (deleted in bladder cancer 1) and TLR4 (toll-like receptor 4). Gene DBC1 has been reported as a tumor suppressor gene and may partially regulate apoptosis. Gene TLR4 is a cell receptor that recognizes pathogenic invasion in different cell types, including macrophages, which is where PRRSV replicates. Previous QTL mapping work has also identified a QTL Complement 3 concentration (C3c) in this region. The C3c QTL is associated with assisting antibodies to clear pathogens from the host. Further haplotype and SNP analyses are underway. This work was supported by PRRS CAP, USDA NIFA Award 2008-55620-19132, NRSP-8 Swine Genome and Bioinformatics projects, the National Pork Board and breeding companies of the PRRS Host Genetics Consortium.

# 64
# Comparison of the pathogenesis of Asian highly-pathogenic PRRSV isolates to US isolates and their ability to cause secondary bacterial infection in swine

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The appearance of highly-pathogenic porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Asia necessitates investigation into the clinical repercussions of these viruses if the strains were to appear in the US. Epidemiologic data from Asian outbreaks suggest that disease severity was associated with both the PRRSV isolates from these cases and secondary bacterial infections. Previous reports have indicated that US isolates of PRRSV predispose to secondary bacterial infections as well, but outbreaks like the ones described in Asia have not been reported in the US. The objectives of this research were to compare the pathogenesis of Asian and US PRRSV isolates of varying virulence with regard to their ability to cause disease and predispose to secondary bacterial infections in swine. The experiment consisted of 10 groups of 9-10 pigs each. At 6 weeks of age, half the groups were inoculated with a bacterial cocktail of *Streptococcus suis*, *Haemophilus parasuis*, and *Actinobacillus suis* and 1 week later 4 bacterial colonized groups and 4 non-bacterial colonized groups were inoculated with 1 of 2 Asian HP-PRRSV strains (JXwn06 or SRV07) or 1 of 2 US PRRSV strains (SDSU73 or VR2332). The pigs infected with JXwn06 were clinically the most severely affected (based on clinical signs, febrile response, and weight gain) while the pigs infected with SRV07 and SDSU73 were moderately affected, and pigs infected with VR2332 showed minimal clinical signs. One pig coinfected with JXwn06 and bacteria became moribund and was euthanized. An increase in the levels of proinflammatory cytokines in the sera occurred, in general, around day 6-8 post viral infection with the magnitude of increase generally correlating with the severity of clinical disease. The highest viral titers were detected in pigs challenged with JXwn06. *A. suis* and/or *H. parasuis* was cultured from the lungs of 3/9 pigs from groups challenged with the bacteria alone, VR2332/bacteria, and SDSU73/bacteria, and from 6/9 pigs challenged with SVR07/bacteria and JXwn06/bacteria, respectively. These bacteria were not isolated from the non-challenged control pigs or pigs challenged with virus alone. Lesions consistent with bacterial pneumonia, including abscesses, were seen in the groups coinfected with PRRSV and bacteria. The levels of proinflammatory cytokines in the serum were often lower for pigs coinfected with virus and bacteria compared to pigs only infected with PRRSV. There was a range of virulence among the PRRSV isolates and differences in their ability to predispose to secondary bacterial infection.

# 65
# Lung lesions and dynamic distribution of porcine reproductive and respiratory syndrome virus(PRRSV) and porcine circovirus Type 2 (PCV2) in sera and tissues after PCV2 vaccination in field pigs

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**Specific objective:** Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) are the main causes of porcine respiratory disease complex (PRDC) which is one of the most important disease in Taiwan pig farms. The Boehringer Ingelheim CircoFLEX ® was the only PCV2 vaccine marketed in Taiwan when this study was conducted a year ago. The purpose of this study was to evaluate its influence on lung lesions and distribution of PRRSV and PCV2 in sera and tissues after PCV2 vaccination in the field pigs.

**Methods:** Three hundred 4-week-old pigs were divided into vaccinated and control groups, with 150 pigs each in three pens respectively in the same house. The pigs in the vaccinated group were intramuscularly injected with one dose of BI CircoFLEX® vaccine and the pigs in the control group with one ml of saline. Twenty pigs in each group were randomly selected and tagged. Among them, four pigs in each group were humanely euthanized at days 0, 7, 21, 35 and 49 after vaccination. The lung lesions were macroscopically examined, scored and collected. The sera, lymph nodes and spleens were also collected at same time to quantify the amounts of PRRSV RNAs by reat-time PCR. ANOVA analysis was performed to compare significant difference between groups.

**Results and conclusions:** The results showed that the scores of lung lesions in vaccinated pigs are clearly lower than those in the control pigs, with significant difference at day 21. It was also found that the pigs in the vaccinated group had lower quantities of PRRSV RNAs than those in control group, as significant difference seen in lung, lymph node and spleen at day 49 ($p<0.05$). Taken together, the usage of BI CircoFLEX® could reduce the lung lesions and tissues' PRRSV RNAs in vaccinated pigs and so that could provide certain protection from PRRSV infection.

# 66
# Analysis of mutations within variable regions of the PRRSV genome in pigs at 42 days after infection

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Genetic variation in both structural and non-structural genes is a key factor in the capacity of PRRSV to maintain persistence within an animal, and within a production system. However, the exact mechanisms of how genetic variation contributes to persistence remain unclear. As part of a study to understand the role of host genetics in disease resistance, we identified pigs that were still viremic at 42 days after PRRSV infection. We took advantage of this subpopulation to identify mutations that appeared in circulating viruses at 42 days after infection. The focus was on sequencing four regions of the genome that show sequence hypervariability. Specific and universal primers were designed for four hypervariable regions within nsp1β, nsp2, ORF3 and ORF5. Total R A was extracted from 100 μL sera using TRIzol and cD A generated by reverse transcription using random hexamer primers. The first round of PCR was performed using sequence-specific primers. A second amplification was performed using 454 adaptor multiplex identifier (MID) primers. For unidirectional sequencing, MID were included on only the forward primers. PCR products were then subjected to 454 sequencing. Mutations were identified in all regions, including distinct differences between pigs and at different frequencies within virus populations. Frequencies ranged between 12.3% and 100%. The same $D_{85}E$ substitution (frequencies: 99.9-100%) in GP3 in all pigs was identified. In addition, we also found some mutations identical in at least in two pigs, for instance, a $Q_{44}R$ mutation (38.7-83.1%) in nsp1β, a $L_{469}S$ mutation (86.1-86.2%) in nsp2, a $T_{30}A$ mutation (28.3-98.9%) within a N-glycosylation site in GP3, a $A_{27}V$ mutation (40.3-70.6%) in GP5, and a $N_{32}S$ mutation (53.6-59.3%) in GP5. The $N_{32}S$ mutation created an additional N-glycosylation site. This study illustrates the usefulness of deep sequencing for the analysis of PRRSV qausispecies. Only one mutation, $D_{85}E$, was common to all pigs.

# 67
# Modulation of the immune response in bone marrow derived dendritic cells by the porcine reproductive and respiratory syndrome virus

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Introduction:

The porcine reproductive and respiratory syndrome virus (PRRSV) has a tropism for pulmonary alveolar macrophages (PAMs) and may also infect specific dendritic cell (DCs) subsets in pigs. Modulation of the innate and adaptive immune response by PRRSV results in the establishment of persistent infection in herds, making it the most economically significant disease in the swine industry today. PRRSV mechanisms of immunomodulation, which confer variable pathogenicity amongst strains, are highly unknown.

Results:

Infection by PRRSV is restricted to cells that express CD163, which is needed for viral entry into the cytoplasm. Because monocytes and PAMs constitutively express CD163, it is highly plausible the monocyte derived DCs (MODCs) express the molecule as well. Unfortunately, differentiated MODC populations are often contaminated with monocytes and macrophages. As a result, models demonstrating infection of MODCs aren't entirely reliable. Other models for PRRSV infection of DCs include the blood DC (BDC) and bone marrow derived DC (BMDC) populations. Our lab has performed comparisons of PRRSV infection of the three different DC populations using flow cytometry, q-RT-PCR, and Northern blots. Results have shown that BMDCs are the most appropriate model to demonstrate DC infection. Furthermore, we have launched an investigation into the role that non-structural protein 2 (nsp2) of the virus plays in immunomodulation. A unique feature shared amongst highly pathogenic strains of PRRSV is a major deletion in the nsp2 gene. We have established a model using Adenovectors to express nsp2 from three different strains of PRRSV in order to efficiently transduce DCs and PAMs. We will determine the immunomodulatory capabilities of nsp2 in order to further understand viral pathogenicity.

Conclusions:

Dendritic cells are the professional antigen presenting cells of the immune system and provide a direct link between the innate and adaptive immune response. Establishing that BMDCs are the most appropriate model to demonstrate PRRSV infection of DCs has allowed us to launch an investigation into the immunomodulatory mechanisms of nsp2, which is responsible for increased virulence.

# 68
# A new framework for quantifying pigs' responses to PRRSV infection for genetic improvement programs

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PRRS not only constitutes a major threat to pig health but also has detrimental impacts on growth and reproductive performance. Simultaneous improvement of disease resistance and reduction of the impact of PRRSV infection on performance would therefore constitute a desirable goal for genetic improvement programs. Accumulating evidence suggests that there may be substantial variation between pigs in their resistance and tolerance to PRRSV infection. However, defining and quantifying resistance and tolerance phenotypes for genetic analyses is not straightforward because the effects of these traits are usually confounded and their (co-) expression can vary drastically throughout the time course of infection.

We propose a new quantitative framework for obtaining individual phenotypes that capture the dynamic interactive effects of host resistance and tolerance on health and performance. This framework is based on individual performance–health trajectories obtained from plotting pairwise individual measurements of performance and health traits (e.g. growth and virus load) taken at different stages of the infection. Like viraemia and growth profiles, these trajectories are a visual representation of the impact of PRRSV on the host over time. However, as outlined previously for human diseases, these trajectories reveal a wealth of additional information about a host's response to infectious pathogens at different stages of the infection that cannot be detected from infection or growth profiles alone. This information can be exploited to devise treatment strategies. For example, inspection of these trajectories allows classification of hosts according to specific types of response or impact, and informs on which stages of the infection to focus on in future genetic, immunological or intervention studies. Future breeding goals may aim to achieve specific health-performance trajectories. In order to implement this information into genetic improvement programs, quantitative phenotypes that fully specify the individual trajectories will need to be derived using tools available from mathematical dynamical systems theory. Current studies are underway to implement these concepts for genetic improvement of pigs' responses to PRRSV infections.

# 69
# Different patterns of suppressed IFN-β transcription and reduced IRF3 phosphorylation in poly(I:C)-treated MARC-145 cells infected with PRRSV

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes huge economic loss to the swine industry. The virus can escape innate immune surveillance probably by suppressing interferon signaling pathways. However, the definite mechanisms by which PRRSV inhibits production of IFN-β are poorly understood and there are contradictory results regarding the role of PRRSV in suppressing IFN regulatory factor 3 (IRF3)-mediated responses. This study attempted to examine the time-course profiles of IFN-β transcription, IFN-promoter activity and IRF3 phorphorylation in poly(I:C)ptreated and PRRSV-infected MARC-145 cells using quantitative PCR, luciferase reporter assay, Western-blotting and confocal imaging.

There was no stimulation of IFN-β promoter activity during the course of PRRSV infection up to 60 hs even though PRRSV RNA increased exponentially from 8 to 24 hpi with a plateau reached thereafter. Similarly, there was virtually no significant IFN-β expression in virus infected cells throughout the infection period, which was in sharp contrast with marked increase of IFN-β transcript in poly (I:C) stimulated cells. PRRSV infection suppressed poly(I:C)-induced IFN-β gene expression. Such inhibition was time-dependent with PRRSV infection, being significant at 12 hpi and more pronounced with the progression of viral infection up to 60 hs. The IFN promoter based luciferase activity was not significantly decreased until 48 hpi in PRRSV-infected and poly(I:C)-treated cells. There was apparent gap of at least 36 hs between suppressed IFN-β transcription and reduced luciferase activity. IRF3 phosphorylation did not occur in cells infected with PRRSV only, but did occur in poly(I:C)-stimulated and PRRSV infected cells. PRRSV infection significantly suppressed poly(I:C)-induced IRF3 phosphorylation between 48 and 60 hpi. The quantity of pIRF3 was less in poly(I:C)-stimulated and PRRSV-infected cell nuclei than that in poly(I:C) stimulated control. Confocal co-localization also provided evidence that PRRSV partially abolished pIRF3 nuclear translocation.

In conclusion, the significant time gap between suppressed IFN-β expression and IRF-3 nuclear translocation/IFN-β promoter activity suggests that IRF3 pathway does not appear to have a major role in PRRSV suppressed IFN-β expression in poly(I:C)-activated MARC-145 cells in the early period of infection. PRRSV seems to deploy a yet to be identified mechanism to disarm the type I IFN responses in the cell line used in early infection.

# 70
# Factors influencing neutralizing antibody response to experimental infection of piglets with porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome (PRRS) is a devastating disease that has plagued the pork industry for nearly three decades. The influence that this disease has on the industry has prompted much research on the interaction between the PRRS virus (PRRSv) and the host's immune system. The aim of the PRRS Host Genetics Consortium is to identify genomic markers and pathways associated with host response to PRRSv infection. A marked characteristic of PRRS is a delayed and unusually weak neutralizing antibody (NAb) response to PRRSv; however, studies have shown that the level and breadth of the NAb response contributes to the host's ability to fight infection. Thus, strategies to control PRRS should include methods to enhance the NAb response to infection. The aim of the research reported herein was to examine host genetic differences in NAb response and elucidate the factors that influence NAb response. Samples and data collected on 464 Large White-Landrace piglets, all infected with PRRSv strain NVSL 97-7895, were used. Viremia, evaluated by quantitative PCR, increased and peaked for all pigs at 7-14 days post infection (dpi) and thereafter declined. Viral load was defined as area under the curve of log viremia from 0-21 dpi. In about 30% of pigs, virus rebound was observed, defined as at least a one Log increase in viremia after the virus had started to clear. Serum samples collected at 42 dpi were assayed for NAb to the homologous NVSL 97-7895 using the following assay: 200 TCID50 of the virus was incubated with 1:2 dilutions of serum then transferred to tissue culture plates containing confluent MARC-145 cells; PRRSv cytopathic effects were assayed 4 days later. The inverse of the highest serum dilution without cytopathic effects was recorded as the NAb titer and ranged from <8 to >1024. For statistical analyses, titers were converted to an adjusted $Log_2$ scale ranging from 0 (<8) to 8 (>1024). Analyses were performed using an animal model in ASReml in order to utilize pedigree information to establish the presence of a heritable component to NAb response. The NAb response to NVSL 97-7895 was found to be a lowly heritable trait ($h^2$=5.7± 9.7%). Systematic and environmental factors influenced NAb response (plate explained 12.1±4.2% of variance, pen 1.1±2.1%, and dam 2.1±5.9%). Other variables were fitted as fixed effects in the model. A one standard deviation increase in viral load was associated with a 0.34±0.09 decrease in $log_2$ NAb titer ($p< 0.001$). Virus rebound was associated with a 0.81±0.19 increase in $log_2$ NAb titer ($p<0.001$). Viral load and virus rebound were uncorrelated (p=0.3). Interestingly, the genotype of a SNP on chromosome 4 (WUR10000125) that was previously found to be associated with viral load in these data was not correlated with NAb response ($p= 0.84$). These data demonstrate the presence of a possible heritable genetic component to NAb response to PRRSv that independently either influences or is influenced by both level of viremia immediately following infection and virus rebound following initial clearance. This work was supported by PRRS CAP, USDA NIFA Award 2008-55620-19132, NRSP-8 Swine Genome and Bioinformatics projects, the National Pork Board and breeding companies of the PRRS Host Genetics Consortium.

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# Quantitative analysis of viremia profiles of pigs experimentally infected with PRRS virus

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Viremia profiles of PRRSV challenged pigs reflect disease progression and severity in the host, and impact on the subsequent control measures required. Inspection of serum viremia profiles of 964 pigs experimentally infected with PRRSV revealed that there were three broad categories of PRRS viral profile: clearance, persistence and reactivation [data provided by the PRRS Host Genetics Consortium (PHGC)]. The first category of viral profile is when the virus is cleared within 4-6 weeks post infection (pi), the second category is when the virus persists at a high level after 6 weeks pi and the third category is when the virus is initially cleared but there is a rebound in viremia levels after 4 weeks pi. Virus reactivation profiles consist of two distinct phases: the initial phase from 0-21days pi and a secondary phase containing the rebound from 21days pi onwards. These viraemia profiles further indicate that there may be large variation between hosts in their response to a PRRSV challenge.

The primary objective of the current study was to provide a suitable quantitative description that captured the full range of PRRS viremia profiles. For this purpose a method by which profiles could be classified was defined and thresholds for reactivation and persistence were calibrated to the PHGC data used. Initial visual inspection of PRRS viremia profiles indicated that the non-reactivated viral profiles and the primary phase of reactivated profiles could be captured by both the rapid increase to an asymptotic peak and the gradual exponential decline of the Woods curve: a gamma-type function often used to empirically describe lactation curves in dairy cattle. Further, in order to capture the biphasic reactivation profiles, the Woods function was extended to result in a bimodal function. The uni-modal profiles were analysed using a mixed models framework applied to a linearised Woods function, thus allowing the investigation of random subject-specific effects that represent the natural heterogeneity of the population and various fixed effects. However, to improve the bimodal reactivation profiles a Bayesian framework was adopted: we used an adaptive population-based Markov chain Monte Carlo method to fit the bi-phasic model to the viral profiles of the individual pigs in this study. Model fits were compared using the Akaike information criterion (AIC) and inspection of the standardised residuals.

The threshold of reactivation calibrated to this dataset on the $\log_{10}$ scale was z=1.5 for the secondary phase of the profile (i.e. viremia increased by 1.5 units), and persistence was defined as $\log_{10}$(viremia) $\geq$1 at 40-42 days pi. It was observed that 31% (n=300) of the pigs in this study experienced virus reactivation and 6% (n=38) of the non-reactivation profiles were classed as persistent. Under a mixed models framework the best fitting model was one in which individual variation was expressed in all three model parameters. There were no significant systematic differences between trials. Reactivation could not be predicted from the parameters derived from the primary phase. Bayesian inference confirmed that the uni-modal and bimodal Woods functions provided an adequate fit to all types of profile, but suggested that the exponential decline of the Woods function was too fast. This is the first study to quantitatively address the viral reactivation process. Parameter estimates derived from this study can be integrated into subsequent quantitative genetic and genomic analyses, and they can be used to validate process based dynamic mathematical models of PRRS viremia.

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# Phenotypic responses of type 2 PRRSV in pregnant gilts: fetal preservation and virus levels

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**Objective**

A large scale project investigating the genomic and phenotypic predictors of PRRS resistance in pregnant gilts is underway at the University of Saskatchewan. One of the objectives of this project is to evaluate the susceptibility of gilts by assessing fetal preservation and viral levels in late term fetuses. Since the experiment is still ongoing, preliminary results from 6 out of 12 groups are presented.

**Materials and Methods**

Fifty-six PRRS-naïve high-health purebred Landrace gilts were synchronized and bred to Yorkshire boars. At gestation day 85 ±1, 49 gilts were inoculated with a Type 2 PRRSV, 50% IM and 50% IN ($10^5$ $TCID_{50}$ total dose). Seven gilts were similarly sham inoculated (control). All gilts were monitored daily. On 21 dpi, dams and their litters were humanely euthanized and fetal preservation scored (Table 1). PRRSV RNA levels in fetal thymus were quantified by in-house RT-PCR and categorized as: 0=not detected, 1=positive not quantifiable, 2=quantifiable, below mean, and 3=quantifiable, above mean. Mummified fetuses, considered dead prior to inoculation, were excluded from analysis.

Table 1: Criteria for scoring fetal preservation in PRRS infected gilts 21 dpi (gestation day ~105)

| Preservation status | Appearance external surfaces | Appearance internal organs |
|---|---|---|
| Viable (**VIA**) | Normal, white with visible hair | Normal |
| Meconium stained (**MEC**) | Alive at termination but covered with inspissated, brownish amniotic fluid | Normal |
| Decomposed (**DEC**) | Dead, generally white, < 50% of surface discolored | Liquefied, friable, adhered |
| Autolysed (**AUT**) | Dead, > 50% of surface discolored | Liquefied, friable, adhered |
| Mummified (**MUM**) | Small, dehydrated, inspissated remains: less than 20 cm crown rump length | |

**Results and discussion**

One infected gilt died (11 dpi) and one aborted (20 dpi); results from these gilts are not included. Apart from a reduced feed intake and rise in rectal temperature in some individual gilts, no other clinical signs were noted. No PRRSV RNA was detected in fetal thymus of control gilts. About half of the fetuses were VIA at termination (Table 2). MEC fetuses were observed only in infected gilts suggesting a PRRS-related pathologic process. Fetal preservation status was positively related to thymic viral loads ($P$<0.001; GLLAMM proportional odds model). Given a 1 unit increase in thymic viral load (scored 0.3), a fetus was 1.6 times more likely to be elevated to the next preservation category. Fetal preservation and PRRS virus levels in fetal thymus were highly variable among litters suggesting differences in susceptibility exist.

Table 2: Fetal preservation and viral load in fetal thymus of infected gilts

| **BY FETUS** | | | | | **BY LITTER** | | |
|---|---|---|---|---|---|---|---|
| ***Fetal preservation*** | ***% VIA*** | ***% MEC*** | ***% DEC*** | ***% AUT*** | ***Live/dead status*** | ***% live*** | ***% dead*** |
| Control (n=84) | 98.8 | 0 | 0 | 1.2 | Control (n=7) | 98.70 | 1.30 |
| Infected (n=624) | 50.3 | 8.5 | 8.3 | 32.9 | Infected (n=47) | 59.92 | 40.08 |
| ***Viral load (infected)*** | ***VIA*** | ***MEC*** | ***DEC*** | ***AUT*** | ***Fetal viral load (n=624)*** | ***live*** | ***dead*** |
| % negative | 47.8 | 5.7 | 3.9 | 7.8 | % negative | 41.69 | 7.03 |
| % PNQ | 15.9 | 5.7 | 0.0 | 15.2 | % PNQ | 14.44 | 12.11 |
| % positive | 36.3 | 88.7 | 96.2 | 77.0 | % positive | 43.87 | 80.86 |
| Mean (SD) of positive $log_{10}$ copies/mg | 5.9 (1.8) | 6.9 (1.1) | 5.7 (1.1) | 4.3 (1.0) | Mean (SD) of positive $log_{10}$ copies/mg | 6.2 (1.7) | 4.7 (1.2) |

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# Phenotypic responses of type 2 PRRSV in pregnant gilts: temporal changes in viremia and WBC

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**Objective**

A large scale project investigating the genomic and phenotypic predictors of PRRS resistance in pregnant gilts is underway at the University of Saskatchewan. One of the objectives of this project is to evaluate temporal relationships in white blood cells (WBC), PBMC subsets and PRRS RNA levels in serum following infection. Since the experiment is still ongoing, preliminary results from 6 out of 12 groups are presented.

**Materials and Methods**

Fifty-six PRRS-naïve high-health purebred Landrace gilts were synchronized and bred to Yorkshire boars. At gestation day 85 (±1), 49 gilts were inoculated with a Type 2 PRRSV strain, 50% IM and 50% IN ($10^5$ $TCID_{50}$ total dose). Seven gilts were similarly sham inoculated (control). All gilts were monitored daily. Blood was collected on 0, 2, 6 and 19 dpi into plain and heparinized tubes. PRRS RNA was quantified in sera using an in-house RT-qPCR. Automated WBC counts (Cell-Dyn 3500, Abbott Diagnostics) and manual differential counts were performed (300 cells total) on heparinized blood. PBMC isolated from whole blood were stained with fluorochrome-conjugated, monoclonal antibodies to define cell subsets using flow cytometry.

**Results**

One PRRS-infected gilt aborted (20 dpi) and 1 gilt died (11 dpi). All cell subsets decreased by 2 dpi in PRRS infected gilts (Table 1). All except B-lymphocytes began to recover by 6 dpi. PRRS RNA concentration in serum peaked at 6 dpi (D2 = 2.9 ±0.6, D6 = 4.2 ±0.8 $log_{10}$ copies/µl). At 21dpi, 17% (8/48) of animals had no detectable PRRS RNA in serum, 42% (20/48) had detectable but non-quantifiable levels, and 42% (20/48) had quantifiable levels (mean 1.34 ±0.33 $log_{10}$ copies/µl).

Table 1. Mean (SD) cell counts in 49 gilts inoculated with type 2 PRRS virus at 85 days of gestation

| **Cells** | | 0 dpi | | 2 dpi | | | 6 dpi | | | 19 dpi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **($x10^9$/L)** | Group | mean | SD | mean | SD | % chg | mean | SD | % chg | mean | SD | % chg |
| WBC | control | 9.5 | 1.5 | 11.2 | 3.0 | 119 | 10.5 | 2.5 | 111 | 9.6 | 2.2 | 102 |
| | PRRS | 11.2 | 1.9 | 6.2 | 1.8 | 55 | 8.5 | 1.7 | 75 | 11.2 | 3.0 | 99 |
| T-lymphocyte | control | 4.8 | 1.1 | 5.4 | 1.2 | 112 | 4.9 | 1.2 | 101 | 5.0 | 1.2 | 103 |
| ($CD3^+$) | PRRS | 5.4 | 1.2 | 2.3 | 0.9 | 42 | 4.5 | 1.2 | 84 | 4.7 | 1.4 | 87 |
| B-lymphocyte | control | 1.1 | 0.2 | 1.1 | 0.3 | 109 | 1.1 | 0.3 | 100 | 1.1 | 0.4 | 105 |
| ($CD79a^+$) | PRRS | 1.3 | 0.5 | 0.7 | 0.3 | 57 | 0.6 | 0.3 | 49 | 1.0 | 0.4 | 81 |
| NK cells | control | 0.1 | 0.1 | 0.1 | 0.05 | 99 | 0.1 | 0.1 | 130 | 0.1 | 0.1 | 136 |
| ($CD3^-CD8a^+$) | PRRS | 0.2 | 0.1 | 0.05 | 0.04 | 23 | 0.1 | 0.1 | 62 | 0.2 | 0.1 | 93 |
| Myeloid cells | control | 0.8 | 0.2 | 0.8 | 0.3 | 104 | 0.7 | 0.2 | 85 | 0.7 | 0.2 | 82 |
| ($CD172a^+$) | PRRS | 0.8 | 0.2 | 0.5 | 0.2 | 64 | 0.6 | 0.2 | 84 | 0.8 | 0.4 | 106 |

Legend: % change (%chg) = no. cells at D2, 6 or 19 divided by no. cells at D0

**Conclusions and Discussion**

PRRS infection in pregnant gilts results in an acute profound transient pan-lymphopenia associated with a prominent viremia. The leukocyte responses however, varied considerably between individual gilts. Virus levels in serum peaked within 1 week; some animals are able to clear the virus by 21 dpi, while others are still viremic. Understanding how these changes correlate with clinical signs is underway.

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# Pathogenesis of HP-PRRSV in gnotobiotic pigs

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Introduction: Porcine high fever disease (PHFD) was first described in China as an acute onset of high morbidity and mortality in growing pigs. Although a number of bacterial and viral pathogens were isolated from these cases, porcine reproductive and respiratory syndrome virus (PRRSV) was consistently detected leading to the assumption that PRRSV was the cause. PHFD was reproduced with virus derived from an infectious clone of the PHFD JX143 PRRSV isolate resulting in this lineage of virus being called highly pathogenic PRRSV (HP-PRRSV). In previous studies, virus rescued from a full-length clone of a 2006 Chinese HP-PRRSV (JXwn06) induced severe disease in conventionally-raised pigs leading to 100% mortality in 4-week-old pigs. In contrast, pigs given VR-2332 challenge virus were much less affected with a mild fever and no mortality. A variety of respiratory bacterial pathogens and opportunists were isolated from the HP-PRRSV infected group. Based on clinical signs, lesions, and bacterial loads in previous studies, we hypothesized HP-PRRSV has a potent immunomodulating capacity that negatively affects the pig's homeostasis allowing the onset of secondary bacterial disease which contributes significantly to the clinical disease. A study using germ-free pigs housed in sterile isolators was conducted to evaluate the pathogenic potential of HP-PRRSV in the absence of bacteria.

Methods: Germ-free pigs were surgically derived and housed in sterile isolators. At 15 days-of-age 12 pigs were inoculated with about 1000 CCID50 virus derived from the JXwn06 HP-PRRSV isolate, and 5 pigs were left as controls. Pigs were scheduled to be bled on 0, 4, 7, 11, and 14 days-post-challenge (dpc). Periodic rectal swabs collected from the pigs were used to monitor bacterial-free status. At necropsy, tissues were collected for histopathology. Virus load was determined by testing ante-mortem plasma samples and the bronchoalveolar lavage collected at necropsy for virus titer.

Results: Control pigs remained normal throughout the study. Most virus-challenged pigs began developing anorexia, fever, and listlessness 2 dpc; the clinical signs were variable and intermittent for the next 2-3 days as the pigs became more affected. By 6 dpc some pigs were severely affected and a seizure was observed in 2 pigs. Several pigs had developed a dark stool and one pig passed frank blood. A pig was euthanized the evening of 6 dpc and within 24 hours 7 more pigs were either found dead or were euthanized. The remaining 4 pigs had succumbed by 12 dpc. Gross lesions in most pigs consisted of a mild pneumonia and petechial hemorrhages to varying degrees in the cortex of the kidney. In addition, about half of the pigs had a hemorrhagic enteritis and swollen kidneys. Cutaneous petechial hemorrhages were noted in two pigs. Histopathology results are pending. One isolator containing 4 pigs became contaminated with bacteria, although the GI tract became colonized in each pig, no sepsis was found (bacterial identification pending). Virus loads were similar to previous conventional pig studies.

Conclusion: The objective of this pig study was to evaluate the pathogenicity of the JXwn06 HP-PRRSV in the absence of bacteria. The virus induced a fatal disease in the gnotobiotic pig model beginning about 6 dpc. Interestingly, this is about the time when the onset of mortality is observed in the conventional pig model. Moreover, the clinical disease in the one contaminated isolator was similar to what was seen in the other 2 isolators suggesting the bacterial infection did not potentiate the disease. Additional studies will be necessary to understand the putative synergy between HP-PRRSV and bacteria.

# 75
# A recombinant porcine reproductive and respiratory syndrome virus expressing Gaussia luciferase gene

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We generated a recombinant porcine reproductive and respiratory syndrome virus (PRRSV) that expresses the Gaussia luciferase, designated as vSD-Gluc. The Gaussia luciferase gene was inserted into the nsp1beta and nsp2 junction of the viral genome, with its N- and C-terminus flanking a protein auto-cleavage cassette encoding the oligopeptides of foot-and-mouth disease virus (FMDV) 2A motif and PRRSV nsp11/12 cleavage site. The in vitro growth characterization demonstrated that vSD-Gluc remains the similar growth properties of the wild-type virus. Stability of the recombinant virus was followed for 10 serial passages in MARC-145 cells. Sequence analysis on the passage 10 of the vSD-Gluc showed that the Gaussia luciferase gene and auto-cleavage cassette coding region remained intact in the virus genome. The potential application of the vSD-Gluc in the screening of anti-viral inhibitors was explored. When tested using IFN-α, the luciferase expression was highly corrected with virus titer reduction. Furthermore, ribavirin was identified as PRRSV replication inhibitor at a low millimolar concentration (0.2 mM). The information obtained in this study will be valuable for the development of anti-viral inhibitors. The luciferase reporter gene could also be effectively used to follow virus replication and gene expression in both cell culture system and in vivo animal studies.

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# MiR-26a inhibits the replication of porcine reproductive and respiratory syndrome virus (PRRSV)

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Porcine reproductive and respiratory syndrome (PRRS) has caused large economic losses in swine industry in recent years. However, current antiviral strategy could not effectively prevent and control this disease. Consequently, there is a need to develop a new antiviral strategy. MicroRNAs play critical roles in intricate host-pathogen interaction networks, but the involvement of miRNAs during PRRS virus (PRRSV) infection is unknown. In this research, eleven microRNAs were expressed by the psiSTRIKE$^{TM}$ vectors and the inhibition against PRRSV was determined in MARC-145 cell. It was found that pretreatment with miR-26a could significantly inhibit the viral replication up to 120 hours post infection in a dose-dependent manner with evidence of alleviating CPE, reducing viral titer in supernatant, inhibiting viral RNA and protein synthesis in Marc-145 cells infected either with PRRSV VR2332 strains or with the highly pathogenic PRRSV 7-1 strain. Additionally, the expression of miR-26a by instant transfected plasmid at 8 hours post PRRSV infection could still efficacious inhibits PRRSV replication. Our study is the first attempt to suppress PRRSV replication in MARC-145 cells through vector based cellular miR-26a, which indicated that cellular miR-26a might be applied to be a new promising anti-PRRSV strategy.

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# Changes in circulating and thymic lymphocyte populations following infection with strains of North American or Highly Pathogenic PRRSV

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Recently, a highly pathogenic (HP) PRRSV strain has emerged in Asia, which causes severe clinical disease and mortality. Since its emergence, work has focused on characterizing the virus and host response following infection to determine the mechanism of enhanced virulence. PRRSV infection has been shown to cause a decrease in circulating T cell populations, lymphadenopathy and thymic atrophy; however, the relationship between these features in relation to HP-PRRSV has not been evaluated. Groups of pigs were challenged with one of two different North American isolates (VR-2332 or SDSU73) or one of two different HP-PRRSV isolates (SRV07 or JXwn06) for this study. Circulating T cell populations were enumerated on 1-4, 6, 8 and 10 days post-infection (dpi) using a newly developed flow cytometric based assay with whole blood. T cell populations in the thymus and lymph node were evaluated on dpi 4 and 10. Regardless of the challenge strain, there was a significant decrease in the number of circulating $CD3^+$ T-cells, including $CD4^+$ and $CD8^+$ subsets, following infection. The sharpest decline occurred between dpi 1 to 2 in VR-2332, SDSU73 and JXwn06 groups and between dpi 2 to 4 for SRV07 group. There was not a significant difference in the lowest number of circulating T cells between the SDSU73, SRV07 or JXwn06 groups. VR2332, SDSU73 and JXwn06 groups were viremic by dpi 1 while pigs challenged with SRV07 displayed a gradual increase in serum virus titers. The JXwn06 group had the greatest amount of virus in the sera beyond dpi 2; however, the number of circulating T cells was similar between SDSU73 and JXwn06 groups. Thus, serum virus titers alone do not explain the decrease in circulating T-cells. There was a significant increase in the number of dead T cells (CD4, CD8 and CD4/CD8 double-positive) in the thymus of all PRRSV infected pigs, though SDSU73 and JXwn06 pigs were the most affected. There was not a significant increase in serum cortisol levels in any of the pigs. Taken together, there is a rapid decrease in the number of circulating T-cells following PRRSV infection, but, this effect does not appear to correlate to virulence, serum virus titers nor serum cortisol levels.

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# Swine tracheobronchial lymph node mRNA responses in swine infected with a highly pathogenic strain of porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a major pathogen of swine worldwide. Emergence in 2006 of a novel highly pathogenic PRRSV (HP-PRRSV) isolate in China warranted a comparative investigation into the host transcriptome response in tracheobronchial lymph nodes (TBLN) 14 days post-infection with HP-PRRSV rJXwn06, strain VR-2332 or sham inocula. RNA from each was prepared for next-generation sequencing. Amplified library constructs were directly sequenced and a list of sequence transcripts and counts was generated using an RNAseq analysis pipeline to determine differential gene expression. Transcripts were annotated and relative abundance was calculated based upon the number of times a given transcript was represented in the library. Major changes in transcript abundance occurred in response to infection with both PRRSV strains, each with over 630 differentially expressed transcripts. The largest increase in transcript level for either virus versus sham-inoculated controls were three serum amyloid A2 acute-phase isoforms. However, the degree of up or down-regulation of transcripts following infection with HP-PRRSV rJXwn06 was greater than transcript changes observed with US PRRSV VR-2332. Also, of 632 significantly altered transcripts within the HP-PRRSV rJXwn06 library 55 were upregulated and 69 were downregulated more than 3 fold, whilst in the US PRRSV VR2332 library only 4 transcripts were upregulated and 116 were downregulated more than 3 fold. The magnitude of differentially expressed gene profiles detected in HP-PRRSV rJXwn06 infected pigs as compared to VR-2332 infected pigs was consistent with the increased pathogenicity of the HP-PRRSV *in vivo*.

# 79
# Preliminary study of effect of interfering NMHCII-A on PRRSV induced cell apoptosis

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Cell apoptosis caused by virus infection is not only an antiviral defense mechanism, but also a strategy for virus escaping immune response and/or developing persistent infection. In mitochondrial pathway and death receptor pathway of cell apoptosis, the composition of Bcl-2 family members, especially the ratio of Bcl-2/Bax, is "molecular switch" which starts the cell apoptosis. Nonmuscle myosin heavy chain II-A (NMHC II-A) has been identified in our laboratory as a co-receptor or mediator for PRRSV infection of cells. To determine the function of NMHC II-A in PRRSV induced apoptosis, Marc-145 cells transfected with artificial microRNAs (amiNMHC II-A) to knock down NMHC II-A expression were infected with PRRSV strain SD16 at 0.01MOI for different time and the levels of Bcl-2, Bax and NMHC II-A mRNA were detected by SYBR Green I real-time PCR method. β-actin was served as an internal reference. The results showed that (1) Compared with the Marc-145 cells ( P5 ) transfected with negative control plasmid, the level of NMHC II-A was knocked down about 50% in the Marc-145 cells ( P4 ) transfected with amiNMHC II-A expression plasmid, Bcl-2 and Bax mRNA expression levels were increased but Bcl-2/Bax relative ratio was no significant changes before the virus infection. (2) At different times after PRRSV inoculation, Bcl2/ Bax relative ratios of these cells decreased slightly after PRRSV absorption for 1h at 37℃, increased from 3h to 6h after PRRSV inoculation and then decreased again after 12h of infection. These results suggested that the cells were in condition of suppression of the apoptosis at the beginning of PRRSV infection which favors the PRRSV replication. However, cell apoptosis was increased at late stage of PRRSV inoculation. Transfection of interfering plasmid targeting NMHC II-A had no effect on PRRSV induced cell apoptosis.

# 80
# Development and validation of swine oral fluid based porcine reproductive and respiratory syndrome virus neutralizing assay: a potential diagnostic tool for PRRS herd immunity

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Porcine reproductive and respiratory syndrome (PRRS) is an economically devastating disease of pigs worldwide. PRRS virus neutralizing (VN) antibodies are important in providing protection against the disease. To evaluate PRRSV herd immunity using individual serum samples in a statistically valid manner requires collecting blood samples from an unacceptably high number of pigs. In other viral diseases, e.g., rhinovirus, HIV, CMV, and others, VN antibodies were shown to be present in oral fluid samples. Therefore, determination of PRRS VN antibody titers in oral fluid samples could potentially serve as an indicator of PRRS herd immunity. The first step in addressing this question was the VN assay, itself. We standardized a PRRSV VN assay using oral fluid samples collected over a period of three months from swine herds vaccinated with MLV-PRRS. The data showed PRRSV V antibody titer ≥1:8 in oral fluids was PRRSV-specific. Thereafter, for validation purposes, we used 104 pen-based oral fluid samples (25 pigs per pen) and serum (5 pigs per pen) from two commercial wean-to-finish barns (A and B) in the Midwest USA. All the 47 oral fluid samples from Barn A, a site with a history of PRRSV infection, were positive for virus specific antibodies by ELISA, and 34 out of 47 oral fluids were positive for vrial RNA by qRT-PCR. Among the 47 oral fluid samples, 8 were VN antibody negative (4/8 virus negative) and 39 positive (9/39 virus negative), with positive VN antibody titers ranging from 1:8 to 1:32. In addition, all the 235 serum samples from Barn A were positive for virus-specific antibodies, with the average VN antibody titer of 1:10 and 1:20 in pigs from pens with oral fluid VN antibody negative and positive, respectively. In contrast, all the 57 pens oral fluid and 284 sera belong to Barn B, with no PRRSV infection history, were negative for virus, virus-specific antibody, and VN antibody titers. In conclusion, we have standardized and validated an oral fluid-based PRRS VN antibody assay. This assay has the potential to monitor PRRS herd immunity in infected and/or vaccinated herds. This project was supported by USDA-NIFA PRRS CAP2 and OARDC, The Ohio State University to RJG.

# 81
# M1 polarized macrophages are resistant to genotype 1 but not to highly pathogenic genotype 2 PRRSV

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**Objective**
The polarization into M1 and M2 macrophage (MØ) is essential to understand MØ function. Consequently, the aim of this study was to determine the impact of IFN-γ (M1), IL-4 (M2) and IFN-β activation of MØ on the susceptibility to genotype 1 and 2 PRRSV strains varying in virulence.

**Methods**
Peripheral blood mononuclear cells were isolated from SPF pig blood by Ficoll density gradient and $CD172a^+$ monocytes were purified by MACS. MØ were differentiated by culture during 72h in DMEM+GlutaMAX cell medium with 10% porcine serum and polarization was induced for another 24h by addition of IFN-γ, IL-4 or INF-β. MØ were infected with genotype 1 (LVP23, 3267, 2982 and Olot/91) and genotype 2 (American VR 2332 and JA1182, and Chinese SY0608) PRRSV strains at MOI 0,1 during 20 h. Nucleocapsid expression and viral titers were determined. Kruskal-Wallis and Mann-Whitney-U tests were used for statistical analysis (SPSS-15 software).

**Results**
Undifferentiated and M2 MØ were highly susceptible to all PRRSV isolates. In contrast, M1 and IFN-β activated MØ were resistant to genotype 1 PRRSV but not to genotype 2 PRRSV strains. Highly pathogenic PRRSV isolates showed the highest level of infection, also in undifferentiated and M2 MØ ($p<0,05$). This was seen at the level of nucleocapsid expression, viral titers and virus-induced cell death.

**Conclusions**
Using IFN-γ and IF -β stimulated MØ it is possible to discriminate between PRRSV varying in virulence. This indicates that highly pathogenic PRRSV strains are more efficient at escaping the intrinsic antiviral effects induced by type I and II IFNs. Our in vitro model will help to identify viral genetic elements responsible for virulence. Our results, also suggest that monocyte-derived MØ could be used as a PRRSV infection model instead of alveolar MØ, avoiding the killing of pigs.

# 82
# Nonstructural protein 2 of porcine reproductive and respiratory syndrome virus inhibits the antiviral function of interferon-stimulated gene 15

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Type I interferon (alpha/beta interferon [IFN-α/β]) stimulates the expression of interferon-stimulated gene 15 (*ISG15*), which encodes a ubiquitin-like protein, ISG15. Free ISG15 and ISG15 conjugates function in diverse cellular pathways, particularly regulation of antiviral innate immune responses. In this study, we demonstrate that ISG15 over expression inhibits porcine reproductive and respiratory syndrome virus (PRRSV) replication in cell culture and that the antiviral activity of interferon is reduced by inhibition of ISG15 conjugation. PRRSV nonstructural protein 2 (nsp2) was previously identified as a potential antagonist of ISG15 production and conjugation. The protein contains a papain-like protease domain (PLP2) that plays a crucial role in the proteolytic cleavage of the PRRSV replicase polyproteins. PLP2 was also proposed to belong to the ovarian tumor domain containing superfamily of deubiquitinating enzymes (DUBs), which is capable of inhibiting ISG15 production and counteractingISG15 conjugation to cellular proteins. To determine whether this immune antagonist function could be selectively inactivated, we engineered a panel of mutants with deletions and/or mutations at the N-terminal border of the nsp2 PLP2-DUBdomain. A 23-amino-acid deletion (amino acids 402 to 424 of the ORF1a-encoded protein) largely abolished the inhibitory effectof nsp2 on ISG15 production and conjugation, but no viable recombinant virus was recovered. A 19-amino-acid deletion (amino acids 402 to 420), in combination with a downstream point mutation (S465A), partially relieved the ISG15 antagonist function and yielded a viable recombinant virus. Taken together, our data demonstrate that ISG15 and ISGylation play an important role in the response to PRRSV infection and that nsp2 is a key factor in counteracting the antiviral function of ISG15.

# 83
# GSK-3 is required for porcine reproductive and respiratory syndrome virus replication in MARC-145 cells

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Porcine reproductive and respiratory syndrome virus (PRRSV) poses significant threat to the swine production worldwide. Currently, no therapeutic options are available. In this report, we demonstrated that the treatment of MARC-145 cells with glycogen synthase kinase 3 (GSK-3) inhibitor X significantly reduced PRRSV replication. It indicated that the activity of GSK-3 was essential for the virus infection and this molecule exhibited a potential target for the treatment of PRRSV infection.

In this study, MARC-145 cells pretreated with GSK-3 inhibitor X at various concentrations as indicated were infected with HuN4-F112 (an attenuated strain of highly pathogenic porcine reproductive and respiratory syndrome virus HuN4) at various multiplicity of infection (MOI) at 24 h postinfection (h p.i) the progeny virus was titrated with TCID50 assay. To select the mutant virus which was conferred the drug resistance, GSK-3 inhibitor X was used to suppress the virus replication in MARC-145 for 14 passages. After fourteen passages, the virus titer increased and restored to level of the control without pharmaceutical treatment. Then complete genome of the selected mutant virus which tolerated to the GSK-3 inhibitor X was sequenced, and several mutations were found.

The chemical significantly diminished the virus infectivity with a does-dependent manner. With a concentration of both 10 and 20μM, it could efficiently inhibit the virus replication when infected with less than 10 MOI. While, when infected with a higher MOI of 10, the inhibitor of 10 μM showed miners inhibitory effect.

Alignment of the complete genome sequence of the parental virus to the mutant virus with GSK-3 inhibitor X resistance indicated that there are fifteen nucleotides changes. Six of them led to non-synonymous mutation, one was found in both Nsp12 and GP3, and two in Nsp2 GP4, respectively.

Here, our study indicated that GSK-3 was essential for the PRRSV infection, and it may serve as a potential target for the therapeutic and prophylactic reagent development. The complete genome sequence analysis revealed that some amino acids in Nsp2, Nsp12, GP3 and GP4 were essential for the interaction with GSK-3 individually or coordinately, which was required for the virus replication.

# 84
# A novel porcine reproductive and respiratory syndrome virus vector system that stably expresses enhanced green fluorescent protein as a separate transcription unit

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Insertion of enhanced green fluorescent protein (EGFP) encoding-gene into virus genes has provided a valuable tool for porcine reproductive and respiratory syndrome virus (PRRSV) research. Several data have proven the possibility to generate viable PRRSV with EGFP gene fused with the different genes. This study aimed to develop a PRRSV replicon expressing EGFP reporter that would provide a fast in vitro system to analyze functional roles of PRRSV in viral replication. Here, we report rescue of a recombinant PRRSV carrying an EGFP reporter gene as a separate transcription unit. Two unique restriction sites (*Asis* I and *Mlu* I) and a copy of the transcription regulatory sequence for ORF6 (TRS6) were inserted between the N protein and 3`-UTR, yielding a general purpose expression vector. A viable recombinant of the PRRSV, vPRRSV-Ch-1R-EGFP, was obtained, which contained the EGFP insert at the site specifying the junction between the N protein and 3`-UTR. The vPRRSV-Ch-1R-EGFP could be launched by transfection of cells with a DNA launch plasmid pBAC-Ch-1R$^{FL}$-EGFP. So far, vPRRSV-Ch-1R-EGFP showed growth characteristics similar to those of the wild-type virus and was found to maintain the insert stably for at least ten passages. Live-cell imaging was used to follow virus spread in real time. In conclusion, the EGFP-expressing PRRSV will be a useful tool not only to monitor virus spread and screen for antiviral compounds, but also to investigate the biology of inclusion body formation.

# 85
# Enhancing sow health through genomic selection

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Recent swine genomics results have demonstrated that specific chromosomal regions impact nursery pig response to experimental PRRS virus challenge. One would expect these results to extend to natural outbreaks of PRRS in a production system. Gilts and sows can face many health challenges in commercial production systems. Understanding the impact of genomics on the response of gilts and sows to these challenges can provide tools to select for improved sow health.

This project combines a novel gilt acclimation and health/productivity assessment strategy with genetic information to evaluate the impact of swine genetics on gilt and sow health in commercial herds. A target of 4,000 high-health replacement gilts in approximately 35 lower-health commercial herds will be used. Blood will be collected on gilts prior to entry, 30 days post-entry and at parities 1 and 2. Herd records will be used to evaluate gilt health and productivity. Gilts will be screened for exposure to PRRS, circovirus, influenza, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*. Genomic analyses will evaluate the relationships of genetic markers with health and productivity outcomes. Possible early-life indicators of sow health and productivity based on relationships of gilt acclimation with sow productivity traits will also be evaluated. Collected samples and data will also be a valuable resource for future studies.

By fall 2012, approximately 2,000 gilts will be sampled, and serologic analysis begun. Approximately 0.5% of gilts experienced adverse health that required removal from the herd within 30 days of arrival. Additionally, use was made of natural PRRS outbreaks to collect sow performance, genomic information and PRRS status on nearly 600 sows and 75 gilts.

Project funding from CSHB and PigGen Canada is used for this project and this has been highly leveraged through participation in swine genomics research sponsored by Genome Canada and Genome Alberta.

# 86
# MiRNAomics of PAMs infected with highly virulent and low virulent PRRSVs

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MicroRNAs (miRNAs) repress the expression levels of genes by binding to mRNA transcripts, acting as master regulators of cellular processes. Cellular miRNAs also play critical roles in virus-host interactions, such as influenza A virus, hepatitis C virus, and retrovirus families. In contrast, no biological and molecular information has been reported on the potential role of cellular miRNAs in the lifecycle of PRRSV.

In this report, we hypothesize that elucidating the miRNA expression signatures induced by H-PRRSV and N-PRRSV infections could reveal strain specific miRNA fingerprints, shedding important insights into the potential role of cellular miRNAs in host-PRRSV interactions. PRRSV preferentially infects and replicates in pulmonary alveolar macrophages (PAMs), using an Illumina deep sequencing platform, we profiled cellular miRNA expression in PAM infected with H- and N-PRRSV at multiple time-points during the viral lifecycle, including global mRNA gene expression profiling.

By integrating the deregulated miRNAs and deregulated mRNAs (inversely correlated miRNA targets), miRNA-mRNA interactome networks were constructed. The functional relationship between miR10a and its potential target identified by the miRNA-mRNA inverse correlation interactome network was validated.

Using gene ontology (GO) and pathway enrichment analyses, we identified the key GO and cellular pathways associated with the differentially expressed miRNAs and predicted inversely correlated miRNA targets during PRRSV infection, including immune response, phagosome, autophagy, Lysosome, autolysis, apoptosis, cell cycle. Our results also identify novel potential exosomal miRNA biomarkers associated with H-PRRSV and N-PRRSV-host infection.

We used Illumina deep sequencing analysis to identify miR10a and miR10b as significantly deregulated upon PRRSV infection. Inhibition of miR10a and miR10b in PAM cells significantly increased transcription level of PRRSV N gene, while over-expression of miR10a and miR10b decreased virus N protein expression and decreased virus titer. Simultaneously, over-expression of miR10a and miR10b increased production of IL-1β. Further study showed that over-expression of miR10a inhibited expression of SRP14 and reduced the luciferase activity of SRP14 3'UTR. These results implicate miR10a and miR10b may inhibit PRRSV replication.

# 87
# Differential host cell gene expression and regulation of cell cycle progression by nsp11 of PRRS virus

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Non-structural protein (nsp) 11 of PRRS virus is a vial endoribonuclease that is essential for infectivity with an unknown mechanism. To examine the cellular gene expression profiles regulated by nsp11, MARC-nsp11 cells were constructed by retrovirus-mediated gene transfer to express the nsp11 gene of PRRSV, and an RNA microarray was conducted in these cells. In MARC-nsp11 cells, the IFN-beta, IRF3 (interferon regulatory factor 3), and nuclear factor (NF)-kappaB luciferase reporter activities were suppressed compared to those of parental MARC-145 cells, suggesting that nsp11 may be an IFN antagonist and MARC-nsp11 cells retained the biological function of nsp11. Differential cellular transcription profiles regulated by nsp11 were then examined using Affymatrix exon chips representing 28,536 human gene transcripts since MARC-145 cells are non-human primate origin. After statistical analyses, 66 cellular genes were shown to be up-regulated and 104 genes were down-regulated. These genes were further examined and grouped into five major cellular pathways according to their functional relations. The flow cytometry analysis of MARC-nsp11 cells showed that the nsp11 expression caused the delay of cell cycle progression, and the BrdU staining for cellular DNA in replicating cells indicated slower cell cycle through the S phase. Our study shows that the PRRSV nsp11 protein plays a role to modulate the host cell cycle progression and provides insights into specific cellular responses to nsp11 during infection.

# 88
# Regulatory role of the PRRS virus nsp1-alpha zinc finger motif for type I IFN modulation

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Non-structural protein (nsp) 1 of PRRS virus has been shown to be a viral antagonist for type I interferon (IFN) production, and in cells expressing nsp1, CREB-binding protein (CBP) is degraded. Nsp1 is autoprocessed into nsp1-alpha and nsp1-beta subunits, and in the present study we show that the nsp1-alpha subunit was responsible for CBP degradation. The nsp1-alpha subunit contains three distinct functional motifs; a papain-like cysteine protease (PCP) motif, an N-terminal zinc finger motif (ZF1), and a C-terminal zinc finger motif (ZF2). To study the structure function relationship of nsp1-alpha and IFN antagonism, theses motifs were individually mutated and examined for their ability for IFN suppression. The mutations that destroyed the cysteine protease activities (C76S, H146Y, and C76S/H146Y) maintained the IFN suppressive activity, indicating that the cysteine protease activity did not participate in the IFN suppression. The mutations of C70, C76, H146, and/or M180, which coordinate the ZF2 motif, also did not change the IFN suppression, showing that ZF2 was not involved in the IFN down-regulation. The mutations of C8, C10, C25, and/or C28 for the ZF1 motif appeared to impair the IFN suppression, indicating that ZF1 at the N-terminal region was the essential element for IFN suppression by nsp1-alpha. The wild-type nsp1-alpha subunit was normally localized in the both nucleus and cytoplasm, but the ZF1 mutants that lost the IFN suppressive activity were found to remain in the cytoplasm. Consistent with their cytoplasmic localization of the ZF1 mutants, CBP was not degraded by these mutants. Our results indicate that the ZF1 motif of Nsp1-alpha plays an important role for IFN regulation during PRRSV infection, and further demonstrate that the CBP degradation is likely the key mechanism for IFN suppression mediated by nsp1-alpha subunit of PRRS virus.

# 89
# Role of dsRNA-dependent Protein Kinase R (PKR) and Eukaryotic Translation Initiation Factor 2-alpha (eIF2α) in porcine reproductive and respiratory syndrome virus (PRRSV) replication

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Porcine reproductive and respiratory syndrome virus (PRRSV) belongs to the Arteriviridae family and encompasses a single-stranded positive-sense RNA genome. As an obligate intracellular parasite, PRRSV depends on the host's translation machinery for its protein synthesis. A key regulator of the translation process is the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α). When phosphorylated, eIF2α inhibits the initiation of translation by preventing eIF2 from delivering the initiator Met-tRNA to the 40S ribosomal subunit. During viral infections, double-stranded RNA-dependent protein kinase R (PKR) is known to phosphorylate eIF2α following activation by double-stranded RNA. The status of PKR activation with regards to phosphorylation of eIF2α has yet to be determined in PRRSV-infected cells. We sought to reveal the roles of PKR and its substrate, eIF2α, in relation to PRRSV replication. Results showed that PRRSV induced the phosphorylation of PKR early in infection. Similarly, the transcription of GADD34, a regulatory subunit of the protein phosphatase 1 complex (PP1c), was also upregulated early in infection. PRRSV induced the phosphorylation of eIF2α during late infection in a time-dependent manner. It is likely that GADD34 is responsible for the dephosphorylation of eIF2α mediated by activated PKR to facilitate viral protein synthesis during early infection. We have indeed observed that phosphorylation of eIF2α correlated with a reduced rate of viral protein synthesis, indicating viral protein synthesis depends on eIF2α. The appearance of cytopathic effects was strongly correlated with eIF2α phosphorylation, suggesting a possible role of eIF2α phosphorylation in PRRSV-induced apoptosis. Furthermore, inhibiting the phosphorylation of PKR with the PKR-specific inhibitor, 2-aminopurine (2-AP), did not affect the phosphorylation status of eIF2α, indicating that another eIF2α kinase is responsible for the phosphorylation eIF2α in PRRSV-infected MARC-145 cells. Although PRRSV protein synthesis was not affected by 2-AP treatment, PRRSV titers were reduced by approximately 1 log, suggesting that PKR activation may contribute to PRRSV replication in an eIF2α-independent manner. Overall, the results obtained in this study reveal that PRRSV activates the phosphorylation of PKR while limiting the phosphorylation of eIF2α during early infection to promote viral protein synthesis. PRRSV induced the phosphorylation of eIF2α during later infection by undefined kinases, which may contribute to the observed cell death and virus release from infected cells. The results will aid in future research regarding control of the host translation machinery with respect to maintaining viral replication.

# 90
# Involvement of non-muscle myosin heavy chain II-A in PRRSV entry into permissive cells

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A monoclonal anti-idiotypic antibody (designated Mab2-5G2) was generated against anti-PRRSV antibodies and characterized as an internal image Ab2, which mimics GP antigen and binds Marc-145 cells and porcine alveolar macrophages. Mab2-5G2 was used to purify a 230Kda soluble protein from these cells using immunoprecipitation and separated by SDS-PAGE. Based on the amino acid sequence by MS, the 230 KDa protein was identified as nonmuscle myosin heavy chain II-A (NMHC II-A) with the functional region located in its carboxyl terminus. A 930bp gene (named PRA) at carboxyl terminus of NMHC II-A was amplified and cloned into eukaryotic expression vector. In cells transfected with PRA, the PRRSV infection was decreased by 50%. The recombinant PRA protein was produced which not only bound with Marc-145 cells but blocked PRRSV infection of Marc-145 cells. Six truncated PRA proteins were then expressed to define the binding site of NMHC II-A by Mab2-5G2. Using ELISA and Western blotting, the binding site was identified within N1669-C1720 of NMHC II-A. One of the truncated protein, named PR3 (targeting N1811-C1893), bound with Marc-145 cells and blocked PRRSV infection. Seven peptides within PRA region were synthesized and their polyclonal antibodies were prepared. Three peptides and their polyclonal antibodies blocked PRRSV infection. By using RNA interference to knockdown NMHC II-A expression, the binding of PRRSV to the cells was not interfered, but the PRRSV internalization and replication were markedly decreased. These results indicated that NMHC II-A identified by Mab2-5G2 that functionally mimics GP5 is involed in PRRSV entry into cells by direct interaction with PRRSV or in association with other cellular receptor proteins.

# Meetings of Interest

The **2012 Conference of Research Workers in Animal Diseases (CRWAD)** Meeting will be held December 2-4, 2012, at the Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois.
For more information, go to: http://www.cvmbs.colostate.edu/mip/crwad/

The 44th annual meeting of the **American Association of Swine Veterinarians** (AASV) is scheduled for March 2-5, 2013 in San Diego, California, USA.
For more information, go to: http://www.aasv.org/annmtg/

**European Federation of Animal Science** 64th Annual Meeting will be held August 26-30 in Nantes, France. For more information, go to: www.eaap.org

The 10th annual **International Veterinary Immunology Symposium** will be held in Milano, Italy on August 28-September 1, 2013. For more information, go to: http://www.ivis2013.org/

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