

2011 International PRRS Symposium

Final Program

**Chicago Marriott
Downtown Magnificent Mile
Chicago, Illinois**

December 2-3, 2011

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Dr. David Benfield (travel fellowship)

Dr. Joan K. Lunney (travel fellowship)

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The 2011 International PRRS Symposium Program has been approved for 12 hours of continuing education (CE) credit in jurisdictions which recognize AAVSB RACE approval for veterinarians and veterinary technicians.

Confirmation forms will be available at the PRRS Symposium Registration Desk; signed CE forms must be submitted at the registration desk before the end of the Symposium.

Keynote presentations: 60/50 minute total with a 50/40 minute oral presentation and a 10 minute interactive discussion.

Oral presentations: 20 minutes total with a 15 minute oral presentation and 5 minutes interactive discussion

David A. Benfield Student Travel Fellowship

David A. Benfield received his BS and MS degrees from Purdue University and a PhD from the University of Missouri-Columbia. He has a distinguished 22 years in research related to virus diseases of food animals. In 1990, he was the co-discoverer of the cause of “mystery swine disease” or porcine reproductive and respiratory syndrome virus (PRRSV). He has remained a role model and mentor to many of those who are currently in the PRRS field. He is a member of the American Society for Virology, American Association of Veterinary Laboratory Diagnosticians, and Honorary Diplomat of the College of Veterinary Microbiologists, American Association of Swine Veterinarians and the American Association for the Advancement of Science. Currently, he is the Associate Director of the Ohio Agricultural Research and Development Center, The Ohio State University and a Professor in the Food Animal Health Research Program in the College of Veterinary Medicine. It is his generous donation that initiated this fellowship program. It is his hope that these fellowships provide students with the experience of attending the International PRRSV Symposium to present their work on PRRS.

2011 David A. Benfield Student Travel Fellowship Recipients

Caiwei Chen

Chinese Academy of Sciences

Li Gao

China Agricultural University

Jun Hou

China Agricultural University

Sophie Morgan

AHVLA Weybridge, University of Surrey, UK

Hein Min Tun

The University of Hong Kong

Fu Yi

China Agricultural University

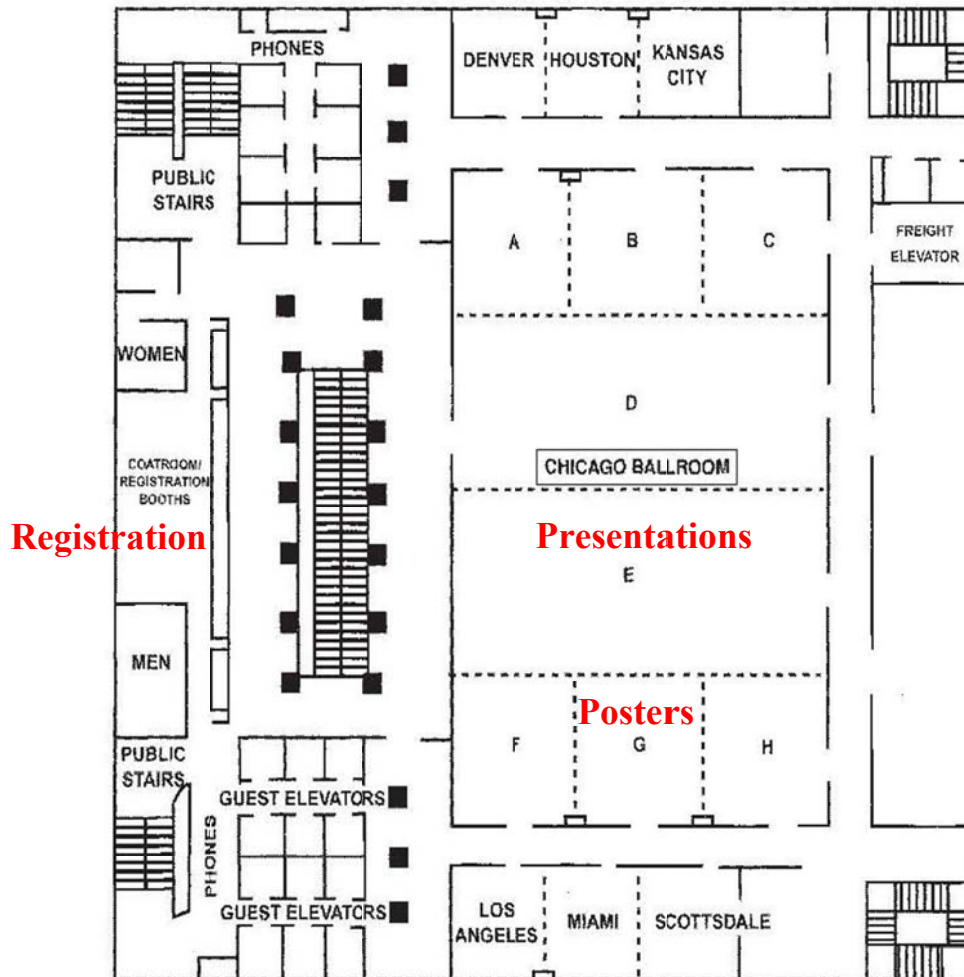
Chicago Marriott Downtown Magnificent Mile

540 North Michigan Avenue,
(Driveway Entrance on 541 North Rush Street)
Chicago, Illinois 60611 USA
Phone: 1-312-836-0100; Fax: 1-312-836-6139

Friday, December 2nd

14:00-17:30 Symposium Registration (5th floor registration booth)

**Chicago Marriott Downtown Magnificent Mile
5th Floor Meeting Rooms**



Friday, December 2nd

14:00-17:30 Symposium Registration (5th floor registration booth)

Pre-Symposium Meetings-Open to public

NC-229 2011 Business and Scientific Meeting, 1:00pm-2:45pm

NC229 Business Meeting:

- 1:00 **XJ Meng**, opening remarks
- 1:05 **Update of NC229**
David Benfield, Administrative Advisor
- 1:15 **Election of new NC-229 officer**
- 1:20 **Update from USDA-NIFA Funding**
Peter Johnson and Margo Holland, USDA NIFA

NC229 Scientific Meeting:

“What works & what questions still need to be answered to obtain PRRSV elimination and control?”

- 1:30 **European perspective**
Hans Nauwynck, Laboratory of Virology, Faculty of Veterinary Medicine,
Ghent University, Salisburylaan, Merelbeke, Belgium
- 1:55 **Canadian perspective**
Leigh Rosengren, Canadian Swine Veterinarian Association PRRSV Area
Regional Control and Eradication Coordinator, Midale, Saskatchewan
- 2:20 **US perspective**
Scott Dee, Director of Research, Pipestone Veterinary Clinic, Pipestone, MN
- 2:45 **Jane Christopher-Hennings**, closing comments

Novel Vaccine and Vaccination Strategy Workshop **(Sponsored by USDA PRRS CAP2. Director, Bob Rowland)**

3:00 pm to 4:45 pm, December 2, 2011, Chicago Downtown Marriott
Workshop Co-chairs: X.J. Meng and Bob Rowland

- 3:00 **Luis Enjuanes**, PhD, Professor, Centro Nacional de Biotecnología, CSIC, Campus
Universidad Autónoma de Madrid, Spain.
Novel vaccines against SARS-Coronaviruses
- 3:30 **Chris Roberts**, PhD, Associate Professor, Center for Molecular Medicine and Infectious
Diseases, Virginia Tech, Blacksburg, VA.
**Co-presentation of bioactive membrane-bound immunomodulators on viral
vaccines to enhance vaccine immunogenicity and efficacy: a novel vaccine
platform targeting enveloped viruses**
- 4:00 **XiaoPing Zhu**, PhD, Associate Professor, University of Maryland, College Park, MD.
**Efficient mucosal vaccination against viral infections through neonatal Fc
receptor (FcRn)**
- 4:30 **Q/A and Panel Discussions**

2011 International PRRS Symposium

14:00-17:30 Symposium Registration (5th floor registration booth)

Friday, December 2nd

2011 International PRRS Symposium Opening Session

Co-Chairs: X.J. Meng and Lisa Becton

17:00 Welcome. X. J. Meng, Chair – 2011 International PRRS Symposium

17:10 Keynote Presentation: Dr. Christopher Walker, The Research Institute at
Nationwide Children's Hospital, Columbus, Ohio
Immunity and control of chronic hepatitis-C virus infection

18:15 Reception and cash bar
Poster Session until 20:00

Saturday, December 3th

Session I: Molecular Biology

Moderators: Eric Vaughn & Carl Gagnon

8:00 Keynote Presentation: Dr. Asit Pattnaik, University of Nebraska
**Porcine reproductive and respiratory syndrome virus proteins in infection
and immunity**

8:40 ISG15 and PRRSV nsp2 OTU domain mediated deISGylation function.
Ying Fang, South Dakota State University, Brookings, SD.

**9:00 Lipid-anchored membrane association of the PRRS virus GP4 glycoprotein and
co-localization with CD163 in lipid rafts.**
Dongwan Yoo, University of Illinois at Urbana-Champaign.

**9:20 Comparative microRNAs and messenger RNAs expression analyses in PRRSV-
infected MARC-145 cells and in lung tissues of pigs experimentally infected
with PRRSV.**
Yaowei Huang, Virginia Polytechnic Institute & State University, Blacksburg, VA.

9:40 Coffee break and Poster session

Session II: Vaccine & Immunity

Moderators: Cinta Prieto & Roman Pogranichniy

- 10:40 **Different cytokine patterns in ex vivo stimulated PBMC are related to the PRRSV isolate.**
Luca Ferrari, University of Parma, Italy.
- 11:00 **Antiviral regulation underlying the activation status of porcine monocytic innate immune cells.**
Yongming Sang, Kansas State University, Manhattan, KS.
- 11:20 **Attenuation of a virulent North American PRRS virus isolate on CD163-expressing cell lines, and demonstration of efficacy against a heterologous challenge.**
Jay Calvert, Pfizer Animal Health, Kalamazoo, MI.
- 11:40 **PRRS Host Genome Consortium database: Development of a system of data storage and sharing for a multi-organizational group.**
Eric Fritz, Iowa State University, Ames, IA.
- 12:00 ***Lunch buffet in the poster area***

Session III: Virus-Host Interaction

Moderators: Yanjin Zhang & Andrea Wilson

- 13:00 **The recombination between two strains of PRRSV in vivo results in the generation of recombinant virus with higher pathogenicity.**
Hanchun Yang, China Agricultural University, Beijing, China.
- 13:20 **Comparison of immune transcriptome response following infection with PRRSV, PCV2 and SIV.**
Laura Miller, National Animal Disease Center-USDA-ARS, Ames, IA.
- 13:40 **Interaction of PRRSV genotype I and II with plasmacytoid dendritic cells.**
Artur Summerfield, Institute for Virology and Immunoprophylaxis, Mittelhausern, Switzerland.
- 14:00 **Non-structural proteins 1 α and 1 β down modulate tumor necrosis factor- α expression after porcine reproductive and respiratory syndrome virus infection.**
Sakthivel Subramanian, University of Nebraska-Lincoln.
- 14:20 **Characterization of Asian highly pathogenic strains of Type 2 PRRSV.**
Kay Faaberg, National Animal Disease Center-USDA-ARS, Ames, IA.
- 14:40 ***Break (refreshments in the poster area)***
Removal of posters at end of break

Session IV: Epidemiology, Heterogeneity, and Evolution

Moderators: William Laegreid & Fred Leung

- 15:10 **Association between PRRSV ORF5 genetic distance and differences in space, time, ownership, and animal sources among commercial pig herds.**
Thomas Rosendal, University of Guelph, Canada.
- 15:30 **Detection of PRRSV antibodies in oral fluid specimens using a commercial PRRSV serum antibody ELISA.**
Apisit Kittawornrat, Iowa State University, Ames, IA.
- 15:50 **Novel PRRSV ORF5a protein is not immunoprotective but drives GP5 Glycosylation.**
Sally Robinson, University of Minnesota, Roseville, MN.
- 16:10 **A high resolution characterization of spatial dissemination of Type 2 PRRSV in North America.**
Mang Shi, The University of Hong Kong.
- 16:30 **Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on U.S. pork producers.**
Derald Holtkamp, Iowa State University, Ames, IA.
- 16:50 **Closing Remarks,**
Jane Christopher-Hennings, Scientific Program Chair
- 17:00 **Adjourn**

ABSTRACTS

2011 IPRRSS Keynote Speakers

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4	The regulatory role of the zinc finger motif of the PRRSV Nsp1 α protein for IFN modulation. <i>M. Han*, Y. Du, C. Song, D. Yoo, Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA.</i>	p. 22
5	High level protein expression of a synthetic reproductive and respiratory syndrome virus nucleocapsid gene optimized in E. Coli host. <i>M. Achacha*, A. Khyar, A. Bensari, Arivac Inc, St-Hyacinthe, Québec, Canada.</i>	p. 23
6	Differential gene expression and functional analysis of RNA from blood of PRRSV infected PHGC pigs. <i>Maria Arceo^{1*}, Catherine W. Ernst¹, Joan K. Lunney², Nancy E. Raney¹, Tinghua Huang³, Christopher K. Tuggle³, R.R.R. Rowland⁴, Juan P. Steibel⁵, ¹Department of Animal Science, Michigan State University, East Lansing 48824, MI, USA, ²USDA, ARS, BARC, ANRI, APDL, Beltsville 20705-2350, MD, USA, ³Department of Animal Science, Iowa State University, Ames, IA 50011, US, ⁴Dept. Diagnostic Medicine and Pathobiology, Kansas State Univ., Manhattan, KS, ⁵Department of Fisheries and Wildlife, Michigan State University, East Lansing 48824, MI, USA.</i>	p. 24
7	Lipid-anchored membrane association of the PRRS virus GP4 glycoprotein and co-localization with CD163 in lipid rafts. <i>D. Yoo^{1,*}, Y. Du^{1,2}, A. Pattnaik³, C. Song¹. ¹Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL; ²Institute of Animal Science and Veterinary Medicine, Jinan, China; ³Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE.</i>	p. 25
8	Establishment of a stable cell line expressing non-structural protein 11 of PRRSV. <i>Dong Li, Yan Sun, Cheng Song, Dongwan Yoo. Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA.</i>	p. 26
9	Real time PCR detection of the porcine respiratory and reproductive syndrome (PRRSV) genome using a single primer set. <i>R. Sina, V. Lazar, R. Pogranichniy. Department of Comparative Pathobiology, Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN.</i>	p. 27
10	Investigation of model systems for the study of recombination in PRRSV. <i>Ranjni Chand*, Yu Wang, Raymond R.R. Rowland. Department of Diagnostic Medicine & Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.</i>	p. 28

11	Comparative microRNAs and messenger RNAs expression analyses in PRRSV-infected MARC-145 cells and in lung tissues of pigs experimentally infected with PRRSV. <i>Y.W. Huang^{1*}, A. Krishnan², B.A. Dryman¹, Y.Y. Ni¹, T. Opriessnig³, X.J. Meng.¹</i> ¹ College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, ² Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA; ³ Iowa State University, Ames, IA.	p. 29
12	Pathway-based approach revealed candidate genes significantly associated with genetic resistance to PRRSV infection in pigs. <i>Z. Jiang^{1*}, L.F. Zhang¹, J.J. Michal¹, J.K. Lunney², R.R. Rowland.³</i> ¹ Department of Animal Sciences, Washington State University, Pullman, WA, ² USDA, ARS, BARC, ANRI, APDL, Beltsville, MD, ³ Department of Diagnostic Medicine & Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.	p. 30

Vaccine and Immunity

13	Evaluation of cytokines and immunomodulatory hormones in pigs vaccinated against PRRSV and naturally exposed to a heterologous field isolate. <i>L. Ferrari*, P. Borghetti, M. Morganti, P. Martelli,</i> Department of Animal Health, Faculty of Veterinary Medicine, University of Parma, Italy.	p. 31
14	Immune reactivity is associated with clinical protection upon vaccination with a modified-live PRRSV-1 vaccine and subsequent exposure to natural infection by a field strain. <i>L. Ferrari*, P. Borghetti, M. Morganti, P. Martelli.</i> Department of Animal Health, Faculty of Veterinary Medicine, University of Parma, Italy.	p. 32
15	Different cytokine patterns in ex vivo stimulated PBMC are related to the PRRSV isolate. <i>L. Ferrari*, P. Borghetti, E. De Angelis, M. Morganti, R. Saleri, P. Martelli.</i> Department of Animal Health, Faculty of Veterinary Medicine, University of Parma, Italy.	p. 33
16	Adjuvants for live PRRS vaccines. <i>Robert Parker^{1*}, K. Nechvatalova², L. Dupuis¹, G. Ionkoff¹, F. Bertrand¹, M. Blahutkova², D. Biryuchenkov³, S. Kukushkin³, T. Baybikov³, V. Borisov³, M. Faldyna.²</i> ¹ SEPPIC Inc. 30, Two Bridges Road, suite 210 Fairfield, New Jersey 07004-1530. ² Veterinary Research Institute 621 00 Brno, Czech Republic. ³ FGI "Federal Centre for Animal Health" (FGI "ARRIAH"), Yur'evets, 600901 Vladimir, Russia.	p. 34
17	Inhibition of porcine reproductive and respiratory syndrome virus (PRRSV) replication by extracts from an eatable mushroom. <i>Li Gao^{1, 2}, Wen-hai Feng^{1, 2}, Hexiang Wang.^{1, 2}</i> ¹ State key laboratories for Agro-Biotechnology, ² Department of Microbiology and Immunology, College of Biology, China Agricultural University, Beijing 100193.	p. 35
18	Antiviral regulation underlying the activation status of porcine monocytic innate immune cells. <i>Y. Sang^{1*}, R.R.R. Rowland², F. Blecha¹.</i> Departments of ¹ Anatomy and Physiology, and ² Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.	p. 36
19	Comparison of host immune responses to homologous and heterologous porcine reproductive and respiratory syndrome virus (PRRSV) challenge. <i>X. Li^{1*}, N. Chen¹, L. Pappan¹, B. Triple², M. Kerrigan², A. Beck², Y. Li¹, D. Hesse², F. Blecha¹, J.C. Nietfeld², R. Rowland², J. Shi¹.</i> ¹ Dept. of Anatomy and Physiology, ² Dept. of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.	p. 37

20	Analysis of two genomic regions shown to be associated with response to experimental infection with PRRS virus in piglets. <i>E.H. Waide^{1*}, N. Boddicker¹, B. Rowland², J.K. Lunney³, J.C.M. Dekkers.¹</i> ¹ Department of Animal Science, Iowa State University, Ames IA; ² College of Veterinary Medicine, Kansas State University, Manhattan, KS; ³ USDA, ARS, BARC, Beltsville MD	p. 38
21	Synthetic peptides containing B- and T-cell epitopes of porcine reproductive and respiratory syndrome virus (PRRSV) with Gp96N355 as adjuvant provoked humoral and cellular immunity. <i>Caiwei Chen*, Jing Li, Yuhai Bi, Lei Sun, Wenjun Liu.</i> Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China.	p. 39
22	Simultaneous detection of antibodies against PRRSV Nsp7 and nucleocapsid protein in swine oral fluid and sera using a fluorescence microsphere immunoassay. <i>R. J. Langenhorst¹, S. Lawson¹, A. Kittawornrat², J. Zimmerman², Z. Sun¹, Y. Li¹, J. Christopher-Hennings¹, E. A. Nelson¹, Y. Fang.^{1*}</i> ¹ Department of Veterinary and Biomedical Science, South Dakota State University, Brookings, SD 57007, USA; ² Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.	p. 40
24	Differences in cytokine expression in sera from pigs infected with Type 1 and 2 PRRSV using a multiplex fluorescent microsphere immune assay. <i>K.P.C. Araujo^{1*}, S. Abrams¹, A. Kittawornrat², J. Zimmerman², Y. Fang³, J.K. Lunney.¹</i> ¹ USDA, ARS, Animal Parasitic Diseases Laboratory, ANRI, Beltsville, MD; ² Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; ³ Department of Veterinary Science, South Dakota State University, Brookings, SD.	p. 41
25	Swine Toolkit progress for the US Veterinary Immune Reagent Network. <i>JK Lunney^{1*}, P. Boyd¹, A. Crossman¹, J. LaBresh², L. Kakach², Y. Sullivan², B. Wagner³, H. Dawson⁴, D. Tompkins⁴, E. Hudgens⁴, C. Baldwin.⁴</i> ¹ APDL, BARC, ARS, USDA, Beltsville, MD; ² Kingfisher Biotech, St. Paul, MN; ³ Cornell University, Ithaca NY; ⁴ DGIL, BARC, ARS, USDA, Beltsville, MD; ⁵ University of Massachusetts, Amherst MA.	p. 42
26	Intranasal delivery of an adjuvanted modified live porcine reproductive and respiratory syndrome virus vaccine reduces the ROS induced lung pathology. <i>Basavaraj Binjawadagi¹, Varun Dwivedi¹, Cordelia Manickam¹, Jordi B. Torrelles², Renukaradhya J. Gourapura.¹</i> ¹ Food Animal Health Research Program, Ohio Agricultural Research and Development Center, Department of Veterinary Preventive Medicine, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, United States. ² Center for Microbial Interface Biology, Division of Infectious Diseases, Department of Internal Medicine, The Ohio State University, 460 West 12th Avenue, Columbus, Ohio 43210, United States.	p. 43
27	Effects of area and regional control programs for porcine reproductive and respiratory syndrome virus on the production performance of swine. <i>C. Pollard^{1*}, N. Garbes¹, B. Payne², L. Batista.²</i> ¹ Bethany Swine Health Services, Sycamore, IL, ² Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO.	p. 44
28	Interleukin-1beta expression by a recombinant PRRSV. <i>Steven Lawson¹, Yanhua Li¹, John Patton², Robert J. Langenhorst¹, Zhi Sun¹, Zhiyong Jiang¹, Jane Christopher-Hennings¹, Eric A. Nelson¹, David Knudsen¹, Ying Fang¹, Kyeong-Ok Chang.^{2*}</i> ¹ Department of Veterinary and Biomedical Science, South Dakota State University, Brookings, SD 57007, USA; ² Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS 66506, USA.	p. 45
29	The highly pathogenic porcine reproductive and respiratory syndrome virus GP5 B epitope is not a major neutralizing epitope. <i>Chao-Liang Leng, Tong-Qing An, Jia-Zeng Chen, Da-Qing Gong, Jin-Mei Peng, Yong-Qian Yang, Jiang Wu, Juan-Juan Guo, Deng-Yun Li, Yi Zhang, Zhen-Xiang Meng, Yu-Quan Wu, Zhi-Jun Tian, Guang-Zhi Tong.</i> Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China.	p. 46

30	Attenuation of a virulent North American PRRS virus isolate on CD163-expressing cell lines, and demonstration of efficacy against a heterologous challenge. <i>J.G. Calvert^{1*}, M.L. Keith¹, L.P. Taylor¹, D.S. Pearce¹, D.E. Slade¹, S. Rai¹, S.W. Newport¹, R.G. Ankenbauer.¹</i> ¹ Pfizer Animal Health, Veterinary Medicines R&D, Kalamazoo, MI.	p. 47
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33	Developing a broadly protective porcine reproductive and respiratory syndrome vaccine. <i>K. Hemnani^{1*}, J. Trujillo¹, T. Opriessnig², G. Tobin^{1,4}, R. Messel³, P. L. Nara.^{1,4}</i> ¹ Center for Advanced Host Defenses, Immunobiotics and Translational Comparative Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa. ² Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa. ³ Immunobiotics Iowa (IBI2), Ames, Iowa. ⁴ Biological Mimetics Inc., Frederick, MD.	p. 50
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36	Replicon particle administration prior to challenge reduces PRRSV viremia. <i>M. Mogler^{1,4*}, R. Vander Veen^{1,3}, K. Kamrud^{1,2}, D.L. Harris.^{1,2,5}</i> ¹ Harrisvaccines, Inc d/b/a Sirrah Bios, Departments of ² Animal Science, ³ Immunobiology, ⁴ VMPM and ⁵ VDPAM, Iowa State University, Ames, IA	p. 53
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39	PRRS modified live virus vaccine as a tool to decrease viral shedding from an infected growing pig population. <i>Daniel Linhares¹, Jean Paul Cano², Thomas Wetzell², Joel Nerem³, Montserrat Torremorell¹, Scott Dee³.</i> ¹ Veterinary Population Medicine, University of Minnesota, Saint Paul, MN. ² Boehringer Ingelheim Vetmedica Inc, St Joseph, MO. ³ Pipestone Veterinary Clinic, Pipestone, MN.	p. 56

40	Effectiveness of herd exposure methods to produce PRRSV-negative piglets from infected breeding herds. <i>Linhares D^{1*}, Cano JP², Torremorell M¹, Morrison R.¹ ¹CVM, University of Minnesota, Saint Paul-MN; ²Boehringer Ingelheim Vetmedica.</i>	p. 57
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42	Use of avian immunoglobulins to control a PRRS outbreak and its impact in productive parameters. <i>W. González*, E. Lucio, J. Munguía, D. García. Investigación Aplicada S.A. de C.V., Tehuacán, Puebla, México.</i>	p. 59
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45	Different immunobiological features of two genetically distinct type 2 PRRS Viruses. <i>A. Khatun¹, E.-J. Choi², C.-H. Lee², D. Sun³, K.J. Yoon³, W.-I. Kim¹. ¹College of Veterinary Medicine, Chonbuk National University, Jeonju, Korea. ²Department of Virology, Animal Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea. ³College of Veterinary Medicine, Iowa State University, Ames, IA, USA.</i>	p. 62
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Virus-Host Interaction

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2

Porcine Reproductive and Respiratory Syndrome Virus Proteins in Infection and Immunity

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Porcine reproductive and respiratory syndrome (PRRS) is considered to be globally the most significant infectious disease of swine. The causative agent of the disease, the PRRS virus (PRRSV), has now been found endemic in most pork-producing countries. A recent economic study revealed that annual losses to the swine industry due to PRRSV infection in the USA alone are over US\$660 million. The economic impact of PRRSV, the sudden emergence of new PRRSV strains of unprecedented virulence in China and S. E. Asia, and the current inability to effectively control the disease emphasize the importance of more detailed understanding of the biology and immunopathogenic mechanisms of the virus to identify specific and unique targets for intervention and control. Although more than 20 years have elapsed since the discovery of PRRSV, much remain unknown about the virus and the host's response to infection. Pigs infected with PRRSV do not elicit strong neutralizing antibody response. While the mechanisms of protective immunity against PRRSV are not fully understood, studies indicate that neutralizing antibodies are important. Furthermore, the pronounced diversity that exists among the various subtypes and isolates representing the two major genotypes of the virus, the European (Type I) and the North American (Type II), adds significant barrier for development of efficacious and broadly protective vaccines. Our laboratory has been studying the structural and nonstructural proteins of PRRSV in an attempt to understand the role of these proteins in the biology of the virus and in innate and adaptive immune response in vivo and in vitro. Recent studies have revealed that the viral GP5, the major envelope glycoprotein and the GP3, one of the minor envelope glycoproteins of PRRSV employ "glycan shielding" mechanism not only to escape neutralization by the host's antibody response but also diminish the neutralizing antibody response by the host. This may, in part, explain the meager neutralizing response observed in PRRSV-infected animals. Another contributing factor to this poor adaptive immune response may be the inadequate innate immune response observed in infected animals and in vitro. We and others have noted that several nonstructural proteins of PRRSV actively suppress innate immune signaling mechanisms, resulting in downregulation of interferon and TNF production. Genetic manipulation of the viral genomes to generate "designer viruses" with altered ability to modulate host's innate and adaptive immune response pathways may provide a rational approach for development of safer and efficacious vaccines to combat PRRS. The focus of the presentation will be on examining the role of specific viral structural and nonstructural proteins that contribute to our understanding of biology and immunopathogenesis of the virus.

3

**ISG15 and PRRSV nsp2 OTU domain mediated
deISGylation function**

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Interferon-stimulated gene 15 (ISG15) is an ubiquitin-like protein. Its expression is stimulated by type I interferon (IFN- α/β) or induced by viral or bacterial infection. ISG15-conjugated proteins encompass diverse cellular pathways, particularly in the regulation of antiviral innate immune responses. In this study, we demonstrated that *in vitro* over-expression of ISG15 in MARC-145 cells significantly reduced the PRRSV titer by 20 fold at 24 hours post-infection. This effect is further confirmed by using small interfering RNAs directed against ISG15-conjugating enzymes. Interferon-induced antiviral activity against PRRSV is significantly alleviated by inhibiting ISG15 conjugation. Among various PRRSV nonstructural proteins, nsp2 was identified as a potential antagonist to ISG15 conjugation activity. *In vitro* deISGylation assay result showed that the N-terminal OTU domain of nsp2 has deconjugating activity towards ISGylated products. To determine whether the nsp2 deISGylation function could be ablated from the virus, a series of deletions and mutations in nsp2 region were constructed. A 23-amino acid (aa402-424 of pp1a) deletion completely abolished the de-ISGylation function of nsp2. However, no viable recombinant virus was recovered. A 19 amino acid deletion (aa402-420) in combination with a single amino acid mutation (S465A) partially relieved the nsp2 de-ISGylation function, and a viable recombinant virus (vSD01-08/d19+1) was obtained. In comparing to the wild-type virus, the vSD01-08/d19+1 recombinant virus is attenuated in growth in cell culture and is more sensitive to ISG15 over-expression. Taken together, results from this study demonstrate that ISG15 conjugation plays an important role against PRRSV infection, and modifying certain regions of nsp2 could reduce the deISGylation ability of the virus.

4

The regulatory role of the zinc finger motif of the PRRSV Nsp1 α protein for IFN modulation

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Non-structural protein (Nsp) 1 α of porcine reproductive and respiratory syndrome virus (PRRSV) is an IFN- β antagonist manipulating the RIG-1 signaling pathway. Nsp1 α inhibits the I κ B α phosphorylation and blocks the NF- κ B nuclear translocation, resulting in the suppression of IFN- β production during infection. To study the structure function relationship of Nsp1 α , eight progressive deletion mutants were made and examined for their ability for IFN induction after poly[I:C] stimulation in a reporter assay. Nsp1 α - Δ 1-40, Nsp1 α - Δ 20-60, and Nsp1 α - Δ 40-80 exhibited high induction of IFN- β activity and showed that the N-terminal one third of Nsp1 α contains a crucial element for IFN- β down-regulation. Nsp1 α contains two zinc finger motifs, ZF1 and ZF2, to tetrahedrally coordinate zinc ions using C8, C10, C25 and C28 for ZF1 and C70, C76, H146 and M180 for ZF2. To study the importance of zinc finger motifs for IFN- β modulation, eight mutations were individually introduced as below to destroy the motifs: C8S, C10S, C25S, C28S, C70S, C76S, H146Y and M180I. All but C28S suppressed IFN- β induction through both the IRF3 and NF- κ B pathways. To avoid a possibility of incomplete destruction of the motifs, double mutations were introduced to each of ZF1 and ZF2. The mutants impairing ZF1 did not suppress the IFN- β production while all mutants disrupting ZF2 retained the IFN- β suppressive activity suggesting that ZF1 is the element important for suppression of IFN- β production. To further confirm the importance of ZF1, triple- and quadruple-mutations were introduced to ZF1 and ZF2 to construct C8S+C70S+C76S, C8S+C70S+H146Y, C8S+C70S+M180I, C8S+C76S+H146Y, C8S+C25S+C70S, C8S+C25S+C76S, C8S+C25S+H146Y, C8S+C25S+M180I, C8S+C25S+C70S+C76S, C8S+C25S+C70S+H146Y, C8S+C25S+C70S+M180I, and C8S+C25S+C76S+H146Y, and their ability for IFN modulation was examined. The mutants with double mutations at ZF1 reversed the IFN- β inhibition, and the results were consistent with the findings from deletions and double mutations studies. Taken together, our data show that ZF1 in the N-terminal region of Nsp1 α participates in the IFN regulation during PRRSV infection.

5

High level protein expression of a synthetic reproductive and respiratory syndrome virus nucleocapsid gene optimized in *E. coli* host

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The diagnostic of porcine reproductive and respiratory syndrome virus (PRRSV) infection is currently performed by the use of an enzyme-linked immunosorbent assay, IFA test and PCR. These methods are time-consuming and require specialized equipment operated by trained technicians. A rapid immunochromatographic strip assay (ICSA) was developed by using recombinant PRRSV nucleocapsid protein as major capture antigen in detecting specific antibodies. The recombinant nucleocapsid protein is usually expressed in *E. coli* in low levels and with more instability compared to fusion partner counterparts.

The purpose of this study was to improve the stability and the amount of recombinant nucleocapsid protein production in *E. coli* that might be used in immunodetection assays for specific detection of PRRSV antibody in swine sera such as simple strip assay (based on a chromatographic and immunogold system) by changing the codon usage of the PRRSV ORF7 in *E. coli* according to an *E. coli* codon usage analyzer Software.

The wild-type PRRSV ORF 7 was amplified from viral RNA by RT-PCR. However, the synthetic ORF7 was amplified by primer extension-overlap PCR to generate a mutated gene in which 58 silent mutations were introduced into the new sequence corresponding to 47 % codon changes and 16 % nt point mutations. The wild-type and synthetic genes were cloned into pGEX 4T1 plasmid and subsequently verified by sequence analysis. Recombinant protein expressions from both plasmids were compared by SDS-PAGE, Western blot and ELISA analyses. Results indicated that the N protein expressed from the synthetic gene was highly improved compared to the wild-type gene.

The significance of these results will be discussed.

6

Differential gene expression and functional analysis of RNA from blood of PRRSV infected PHGC pigs

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The objective of this study was to assess differential expression of individual genes, as well as to discover networks and pathways enriched for those genes, in pigs showing a range of response to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) experimental infection. Healthy pigs from the Pig Host Genetics Consortium (PHGC) initiative were inoculated with a known isolate of PRRSV (NVSL 97-7985). RNA was prepared from blood Tempus tube samples collected at 0, 4, 7, 11, 14, 28 and 42 days post-infection (dpi). Statistical analyses assigned pigs from each PHGC trial into four phenotypic groups. These were defined according to the pigs' serum viral level and weight gain: 1) high viral level (Hv)-high growth (Hg), 2) Hv-low growth (Lg), 3) low viral level (Lv)-Hg and 4) LvLg. RNA from 3 pigs per group (all from PHGC trial 1) was hybridized to the 20K 70-mer oligonucleotide Pigoligoarray following a blocked reference design with time 0 of each individual animal as a reference sample. The linear mixed model used to analyze the data included the fixed effects of dye, time, phenotypic group (combination of viral level and weight gain), the interaction of time with group, and random effects of array and biological replicate (pig). The distribution of random effects was assumed to be Gaussian. A two-stage testing procedure was used to assess significance of growth and viral level group status over time. Expression levels for a total of 491 genes showed significant viral level-growth interaction for all time-points. Due to the numbers of DE genes, only early time-points (4, 7, 14 dpi) were further evaluated by enrichment analysis with Ingenuity Pathways Analysis software (Ingenuity® Systems, www.ingenuity.com). For comparisons of viral level, 308 genes were DE with 16 significant gene networks ($p \leq 0.0001$). For comparisons of growth level, 367 genes were DE with 17 significant gene networks. Significant pathways ($FDR \leq 5\%$) related to immune response were associated with DE genes and networks for 4 and 7 dpi. A few of the prominent DE genes ($p \leq 0.05$) were ADIPOQ, MERTK, PYCARD and GZMA. The more significant biological functions identified ($FDR \leq 5\%$) were those related to cell death, cellular function, maintenance and compromise, and inflammatory disease. For the growth comparison at 4 dpi in Lv animals, the antigen presentation pathway was over-represented ($FDR \leq 5\%$) in the list of DE genes ($p \leq 0.001$), which included CALR, CD74, HLA-DRA, and HLA-DRB1. Comparison of viral level within Hg pigs at 7 dpi identified GZMA ($p \leq 0.03$), a gene in the Granzyme A signaling pathway that plays a role in apoptosis induced by T-lymphocytes. ADIPOQ and MERTK (reported to be involved in trafficking of lymphocytes) were down-regulated in HvHg versus LvHg pig RNA at 4 dpi. At 7 dpi GZMA and PYCARD were down-regulated for HvHg compared to LvHg pig RNAs, whereas they were overexpressed in HvLg versus LvLg pigs. Confirmatory qPCR work is planned to explore these DE genes and their roles in PRRS control. This work was supported by the National Pork Board and USDA NIFA AFRI grant #2010-65205-20433.

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Lipid-anchored membrane association of the PRRS virus GP4 glycoprotein and co-localization with CD163 in lipid rafts

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The porcine reproductive and respiratory syndrome virus (PRRSV) glycoprotein 4 (GP4) resembles a typical type I membrane protein in its structure but lacks a hydrophilic tail at the C-terminus suggesting that GP4 may be a glycosylphosphatidylinositol (GPI)-modified lipid-anchored membrane protein. Using the human decay-accelerating factor (DAF; CD55), a known GPI-anchored protein, chimeric constructs were made to substitute the GPI-anchor domain of DAF with the putative lipid-anchor domain of GP4, and their membrane association and sensitivity to lipase were determined in cells. The DAF-GP4 fusion protein was transported to the plasma membrane and was cleaved by phosphatidylinositol-specific phospholipase C (PI-PLC) digestion, indicating that the C-terminal domain of GP4 functions as a GPI anchor. Mutational analysis of residues flanking the presumptive GPI modification site and characterization of the mutant viruses generated from infectious cDNA clones show that the viability and growth characteristics of the mutant viruses correlated with the ability of the GP4 to associate with the membranes. The residues T158 (ω -2, where ω is the GPI attachment site at E160), P159 (ω -1), and M162 (ω +2) of GP4 were determined to be important for virus replication, with M162 of particular importance for virus infectivity. The complete removal of the peptide-anchor domain of GP4 resulted in the loss of virus infectivity. Remarkably, GP4 was found to co-localize with CD163 in the lipid rafts on the plasma membrane. Since CD163 has been reported as a cellular receptor for PRRSV, our data implicates an important role of lipid rafts during entry of the virus.

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**Establishment of a stable cell line expressing
non-structural protein 11 of PRRSV**

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Porcine reproductive and respiratory syndrome virus (PRRSV) non-structural protein 11 (Nsp11) has been reported as a type I Interferon antagonist, but how it modulates the interferon production is still unknown. In an attempt to investigate the molecular action of Nsp11, a stable cell line expressing PRRSV Nsp11 was constructed using a retroviral gene transfer system. The Nsp11 coding sequence was cloned from the FL12 North American strain of PRRSV and inserted into the retroviral expressing vector pLNCX2. The plasmid was then co-transfected with pVSV-G into the pantropic packaging cell line GP2-293 to produce a packaged infectious virus containing the Nsp11 gene. MARC-145 cells were infected with the Nsp11-gene containing virus in the presence of Geneticin and cell colonies resistant to the drug were selected and amplified. The Nsp11 gene integration and expression was confirmed by PCR, RT-PCR, and immunoprecipitation. When these cells were examined for IFN induction by poly(I:C) stimulation, the interferon promoter activity was inhibited. Our results indicate that the newly established MARC-145 cells constitutively express the functional Nsp11 protein. This cell line may be a useful tool to study the function of Nsp11.

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Real time PCR detection of the Porcine Respiratory and Reproductive Syndrome (PRRSV) genome using a single primer set

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has presented a challenge in veterinary science for over a decade. Two types of PRRSV have been described as type 1 and 2 with multiple subtypes which are circulating in the pig population. Early detection of the virus is crucial to the pork industry; however, the PRRSV genome varies to a large degree across different strains which make the early diagnostics of the virus more difficult.

A relatively new method has been implemented allowing the detection of a wide variety of PRRSV strains by utilizing multiple primer sets and real time PCR technique. The efficiency and cost associated with testing is an important aspect for diagnostic laboratories. We have utilized a single primer set designed from the conserved region of the PRRSV genome using a real time PCR detection method to establish a more cost effective alternative. All the cases submitted to the Animal Disease Diagnostic Laboratory (ADDL) and identified positive for PRRSV by the PRRSV kit from Tetracore[®] during the 2010-2011 fiscal year were analyzed. The diversity of the PRRSV genome among the submitted cases was determined by phylogenetic analysis ranging around 40% difference from European strains (type 1) to North American (type 2). In this study all the cases which were previously determined as positive by the Tetracore[®] method were identified as positive using a single primer set designed from the PRRSV conserved region by real time PCR.

In summary, we have demonstrated that by using a single primer set in real time PCR, the PRRSV genome was detected across a wide diversity of the viral genome and produced comparable threshold cycle (CT) values to a similar assay available from Tetracore[®].

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Investigation of model systems for the study of recombination in PRRSV

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Sources of genetic variation in PRRSV include substitutions, insertions-deletions and recombination. The overall goal of this research is to develop quantitative empirical models that can be used to study recombination. The first step in investigating recombination is to determine if cells can be infected by two different PRRS viruses. Two identical viruses were constructed which contained GFP or RFP cloned into nsp2 or expressed as a subgenomic fragment. Different concentrations of two viruses: sg-RFPv (red fluorescent protein) and sg-GFPv (green fluorescent protein) were used to co-infect MARC-145 cells or porcine alveolar macrophages (PAMs). Confocal microscopy identified red and green dual-fluorescent MARC and PAM cells. Flow cytometry showed that dual-infected MARC cells constituted approximately 17% of the infected population. Two models were developed to investigate recombination. The first involved the construction of a non-fluorescent virus that contained a mutated non-fluorescent GFP gene (sg-nfGFPv). The nfGFP gene possessed a single point mutation in the tripeptide fluorophore domain that resulted in loss of fluorescence. A stable green fluorescent MARC cell line was created that expressed the wild-type GFP gene. nfGFPv was used to infect the GFP-MARC cells. The presence of recombination within the GFP gene region would result in fluorescence being restored to the non-fluorescent virus. The GFP MARC cells could be productively infected; however, several experiments failed to yield a green virus. One possibility for the negative result was the inability of the viral genome to recombine with a messenger (positive polarity) RNA. Therefore, we developed a second approach to closely mimic recombination that would occur during co-infection of cells with two different PRRS viruses. We constructed a defective sg-GFPv (def-sg-GFPv) virus that lacked ORFs 2-6. Transfection of MARC and 293T cells with def-sg-GFPv plasmid resulted in green fluorescence. Transfected cells also stained positive for the N protein, confirming that the N subgenomic region was intact and properly expressed. Furthermore, the transfected cells showed characteristic PRRSV CPE; i.e., cell rounding, detachment and death. To study recombination, 293T cells were co-transfected with different ratios of sg-nfGFPv and def-sg-GFPv plasmids. The supernatant virus was recovered after three days and placed on MARC cells. Even though MARC cells were productively infected, all virus-positive cells were negative for infection by a green virus. Various modifications to this model approach are being tested to determine the conditions necessary for recombination.

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**Comparative microRNAs and messenger RNAs expression analyses
in PRRSV-infected MARC-145 cells and in lung tissues of pigs
experimentally infected with PRRSV**

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The interplay between PRRSV and its host is a complex process that involves extensive changes of cellular gene expressions and protein interactions. Cellular transcriptome and proteome analyses of PRRSV infection have provided useful information. In this study, we focused on a group of important cellular factors, microRNAs (miRNAs), which are a class of small noncoding RNAs that bind to targeted mRNAs and regulate many biological and pathologic processes. Increasing evidences indicated that cellular miRNAs play critical roles in interactions between host and pathogenic viruses such as SARS-coronavirus and influenza virus.

To determine whether cellular miRNAs play a role in host response to PRRSV infection, we first performed a global profiling of both cellular miRNA and mRNA using miRNA (multiple species) and exon (human) expression microarrays (Affymetrix) in MARC-145 cells infected with type 1 (SD01-08) or type 2 (VR2385) strains of PRRSV at the early and late stages of infection. The results showed that the expressions of approximately 240 miRNAs were significantly altered (up-regulated or down-regulated) upon infection by PRRSV type 1 or 2 (114 for type 1, and 82 for type 2), and at least 15 specific miRNAs (such as miR-23a and miR-205) were shared by both PRRSV types. In addition, approximately 4,500 genes showed differential expression upon infection by either virus type (1,739 for type 1, and 3,636 for type 2; $p < 0.05$).

Subsequently, we conducted a global human/bovine/porcine miRNA and porcine gene expression microarray (Agilent) analyses using a pool of lung homogenates of 10 SPF pigs at 14 days post-infection with PRRSV. Compared to the negative control, PRRSV infection resulted in significant changes of the expression level (>2 -fold) of 17 miRNAs ($p < 0.05$) such as let-7e and 3,713 mRNAs ($p < 0.01$) including genes involved in host innate and adaptive immune responses. Since only a limited number of porcine miRNAs is available in the public miRNA database, we further produced two small RNA libraries from the PRRSV-infected and negative control samples to discover unknown differentially-expressed porcine miRNAs via Illumina deep sequencing. Subsequently, we uncovered 270 unique miRNAs that were differentially up- (151 miRNAs) or down-regulated (119 miRNAs) in response to PRRSV infection. A number of miRNAs were correlated between the miRNA microarray and the RNA sequencing data. The deep sequencing data also uncovered 1,892 novel putative porcine miRNAs that do not align to any known *Sus scrofa* miRNAs. Based on the known and predicted miRNA targets, we have correlated inverse regulation between miRNAs and putative target genes to build a miRNA-gene network that could underlie the observed regulatory patterns. For example, a highly up-regulated novel miRNA, PC-5p-18316_49, was found to regulate 1,767 putative mRNA transcripts.

In summary, we revealed that, for the first time, particular host miRNAs are capable of regulating cellular gene expression during PRRSV infection. Understanding the PRRSV-host miRNAs interaction provides new insight into the role of miRNAs in PRRSV pathogenesis. This study is supported in part by grants from USDA-AFRI (USDA-NIFA 2011-67015-30165) and USDA PRRS-CAP2 (USDA-NIFA 2008-55620-19132).

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Pathway-based approach revealed candidate genes significantly associated with genetic resistance to PRRSV infection in pigs

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Porcine reproductive and respiratory syndrome (PRRS), caused by a single-stranded RNA virus (PRRSV), is the most economically important disease in pigs worldwide. Studies have shown that PRRSV is able to mutate, thus causing challenges in effective vaccine development. Furthermore, once infected, the virus can evade the pig's immune system, causing a delay in protective immunity for several weeks. As evidence has shown that genetic variation exists in host response to the PRRSV infection, identification of desirable genetic markers, or interactions responsible for their genetic variation, appears to be a viable option for the pig industry to consider to combat this disease. In particular, the PRRS research community has suggested that more studies that focus on the possible pathways of PRRS resistance are needed so that powerful control methods can be found to ease the disease burden and thus increase animal welfare and production profitability. In the present study, we compiled a list of genes based on the public database that are differentially expressed in pulmonary alveolar macrophages at different stages post infection with PRRSV and then selected a total of 22 candidate genes involved in three pathways for association analysis. Among them, 132 mutations (mainly single nucleotide polymorphisms, SNPs) were discovered in 19 genes and 82 of these SNPs were genotyped on 616 samples from 3 trials provided by the PRRS host genetics consortium. Statistical analysis revealed four genes that are significantly associated with virus load at different days post infection. In particular, three genes also significantly affect pig growth. More interestingly, one of these genes is located on SSC4 where a major QTL for virus load and growth was detected using the GWAS (genome wide association study) approach. Therefore, our current research would provide two major end-products to the pig industry: a marker panel for genetic selection and potential molecular targets for new drug design, thus helping the industry to efficiently control PRRS disease. This work was supported by the National Pork Board, USDA ARS and PRRS CAP, USDA NIFA Award 2008-55620-19132.

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Evaluation of cytokines and immunomodulatory hormones in pigs vaccinated against PRRSV and naturally exposed to a heterologous field isolate

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Several *ex vivo* and *in vitro* observations support the hypothesis that PRRSV infection of permissive immune cells, such as monocytes/macrophages and dendritic cells, does not prime efficient recognition of viral determinants, which leads to a consequent down-regulation of inflammatory cytokines and weak innate immune responses; this compromises the onset and development of antigen-specific adaptive immune responses. Furthermore, although the exposure to the virus can induce protective/sterilizing immunity upon re-exposure to a homologous virus under field conditions, previously infected recovered pigs can become re-infected and clinically affected when exposed to a heterologous PRRSV isolate (1, 2, 3). Since both cytokines and hormones represent important mediators to cope with external stimuli such as viral infections and since the impairment of their bi-directional communication can be co-responsible for more severe clinical outcomes of the disease, the changes of major cytokines and neuroendocrine hormones in PRRSV-vaccinated and unvaccinated pigs naturally infected by a PRRSV heterologous field strain were evaluated. Thirty 4-week-old conventional pigs from a PRRSV-free and *M. hyopneumoniae*-free farm were divided into 3 groups (IM, ID, C) and housed in an isolation barn (site-2 unit). After 1 week of acclimatization (5 weeks of age), pigs of the IM (n = 10) and ID (n = 10) groups were vaccinated with Porcilis® PRRS (Merck AH) (10^{4.5} TCID₅₀ strain DV) intramuscularly (2 ml) and intradermally (0.2 ml), respectively. Intradermal vaccination was performed by using a needle-less vaccinator (IDAL®). Pigs from group C (n = 10) were not vaccinated and served as controls. Forty-five days post-vaccination (PV), the IM, ID and C groups were moved to a site-3 unit, which housed a conventional, continuous-flow herd, to be naturally exposed to field pathogens. ORF5 sequencing revealed that the field isolate responsible for natural infection was 84% ORF5 identical to the DV vaccine strain. During the post-exposure period (PE), blood samples were collected on day 0 (45 days PV), 7, 14, 21, 28 and 34 PE for the quantification of pro-inflammatory/pro-immune (TNF- α , IL-1 β , MCP-1, IL-6, IFN- γ) and anti-inflammatory/immunomodulatory (IL-10) cytokine gene expression in PBMC by PCR and immunomodulatory hormone (GH, cortisol) plasma levels by ELISA/RIA. All vaccinated pigs showed an increase of pro-inflammatory (IL-1 β , IL-6, MCP-1, TNF- α) and pro-immune (IFN- γ) cytokine gene expression compared to controls and a prompt increase of GH levels that could be consistently associated with pro-inflammatory cytokines in sustaining innate immunity. Furthermore, the detection of higher cortisol levels after PRRSV exposure (7 days PE) in vaccinated animals indicates that the hypothalamus-pituitary-adrenal (HPA) axis response was further stimulated. Differently, unvaccinated pigs showed an early down-regulation of cortisol and basal levels of GH in plasma, in parallel with basal or low levels of pro-inflammatory and pro-immune cytokines. In addition, in the control group, TNF- α , IL-6 and MCP-1 increased in the late phase of natural infection together with IL-10 and IFN- γ . The concomitant trends of pro-inflammatory and anti-inflammatory cytokines together with the cortisol level demonstrate that PRRSV vaccination using the modified-live vaccine virus DV promotes the early expression of immune responsiveness markers in PBMC of PRRSV-vaccinated pigs and sustains a more efficient control of inflammation in the late phase of infection with a heterologous PRRSV isolate. On the contrary, unvaccinated animals subsequently infected showed an inefficient and delayed immune response; long-term maintenance of higher TNF- α , IL-6 and IL-10 levels and deregulation of cortisol production could represent markers of uncontrolled inflammatory damage and could functionally explain the severity of clinical signs. Both aspects paralleled with clinical protection induced by vaccination with Porcilis® PRRS (Merck AH) (4).

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Immune reactivity is associated with clinical protection upon vaccination with a modified-live PRRSV-1 vaccine and subsequent exposure to natural infection by a field strain

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Because of the presence of various PRRSV isolates in the field characterized by genetic divergence, an important issue to be addressed regards both viral and host immune factors that influence the efficacy of PRRSV vaccines against close-related or distant strains and the clinical outcome under experimental and field conditions (1, 2, 3). The present study was aimed at evaluating the degree of clinical protection in conventional pigs vaccinated against PRRS and naturally exposed to/infected by a heterologous (Italian cluster) virulent PRRSV strain under field conditions. Thirty PRRSV-seronegative pigs, aged 4 weeks, were allocated to one of 3 groups (IM, ID, C). After acclimatization, at 5 weeks of age, pigs of groups IM (n = 10 pigs) and ID (n = 10 pigs) were vaccinated intramuscularly and intradermally, respectively, with a modified-live PRRSV vaccine (Porcilis® PRRS - Merck AH). Pigs of group C (n = 10 pigs) were kept as unvaccinated controls. On post-vaccination (PV) day 0, 7, 14, 28 and 45, blood samples were collected for detection of vaccine virus titres (qPCR) and serological response (ELISA antibodies), identification and quantification of lymphocyte subsets (flow cytometry), and frequencies of PRRSV-specific IFN- γ secreting cells (SC) (ELISpot assay). At 45 days PV, pigs were moved to a site-3 conventional finishing herd with a history of respiratory disease caused by PRRSV to be exposed to natural challenge. The PRRSV field isolate that naturally infected the experimental pigs was demonstrated to share 84% ORF5 identity with the Porcilis® PRRS vaccine virus (DV strain). At 0 (exposure day = 45 days PV), 4, 7, 11, 14, 19, 21, 28, and 34 days post-exposure (PE) blood samples were collected for detection and quantification of PRRSV titres and PRRSV-specific antibodies, as well as of lymphocyte subsets and PRRSV-specific IFN- γ SC. During the PE period, pigs were monitored daily for clinical signs. The clinical signs (measured by an overall score) were reduced by 68% and 72% respectively in the IM- and ID-vaccinated pigs compared to controls. Also the average daily weight gain (ADWG) was positively influenced by vaccination. Daily weight gains were 510 ± 77 , 515 ± 59 and 346 ± 105 g in IM, ID and control groups, respectively. Clinical protection induced by vaccination with Porcilis® PRRS was associated with marked activation of the cellular immune response. The highest frequencies of PRRSV-specific IFN- γ SC at 21-34 days PE were concomitant and associated with changes of natural killer (NK) cells, γ/δ T lymphocytes and cytotoxic T lymphocytes (CTL) in the blood. Despite not particularly high levels of IFN- γ SC were observed after vaccination, cellular reactivity, evaluated by identification of responsiveness categories and responder animals upon cut-off definition (30 IFN- γ SC/ 10^6 PBMC), was maintained and further triggered during natural infection, testifying sustained antigen recall/memory response of the cellular compartment. In this field study, clinical protection induced by this EU-modified live virus vaccine based on the DV strain against natural exposure to a genetically divergent PRRSV strain (Italian cluster) was demonstrated by statistically significant reduction of clinical signs in terms of incidence, duration and severity as well as a more efficient cell-mediated immune response in vaccinated pigs as compared to unvaccinated controls. The results suggest that the different severity of the disease observed between groups may also depend on the intensity and progression of the innate and virus-specific immune response over time. Furthermore, intradermal vaccination using a needle-less (IDAL®) device proved to be more efficacious than traditional intramuscular delivery in terms of immunological responses and clinical protection.

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Different cytokine patterns in *ex vivo* stimulated PBMC are related to the PRRSV isolate

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Modified live virus (MLV) vaccines seem to be effective in sustaining complete or partial protection respectively upon homologous and heterologous challenge. The degree of protection seems to be more related to the ability of a strain to stimulate some immune elements rather than with the degree of homology (1, 3, 4).

The present study aims at characterizing the immune response in vaccinated and unvaccinated pigs naturally exposed to PRRSV, especially with regards to cytokine patterns in PBMC upon *ex vivo* stimulation with different PRRSV isolates. Twenty-one PRRSV naïve pigs, aged 4 weeks, were assigned to 3 groups: group IM (n = 7) and ID (n = 7) were vaccinated with Porcilis® PRRS (10^{4.5} TCID₅₀) intramuscularly (2 ml) and intradermally (0.2 ml) by the I.D.A.L.® needle-less vaccinator in adjuvant (Diluvac Forte®) respectively; group C (n = 7) was inoculated with the adjuvant alone (unvaccinated controls). During the post-vaccination (PV) period pigs were kept in an isolation barn (0-35 days PV) and then moved to a conventional PRRSV-positive herd to be naturally exposed to/infected by the resident PRRS virus. Blood samples were collected on day 0 (vaccination), 21, 35 (end of the isolation period) and on day 70 PV (35 days post-exposure, PE). Cellular immunity as cytokine production was measured in terms of frequencies of IFN-γ secreting cells (SC) and per cell IFN-γ productivity by ELISpot assay, PRRSV-specific IFNγ+ immune subsets by surface (CD8) - intracellular (IFN-γ) staining/flow cytometry as well as IL-10/TNF-α secretion by ELISA and expression by RT-qPCR in PBMC after *in vitro* stimulation for 48 h with the vaccine virus (strain DV) and two Italian virulent field isolates (BS/114/S and BS/55) + PMA/ionomycin (PMA/I; last 24 h) (2). Serum PRRSV levels of exposed/infected pigs were quantified by qPCR. Humoral immunity was measured as anti-PRRSV antibodies by ELISA. An increase of IFNγ+, CD8+IFNγ+ and CD8-IFNγ+ cells was detected in all groups after *in vivo* PRRSV natural infection: stimulation with the PRRS vaccine or field isolates highlighted slight increases of IFNγ+, IFNγ+^{high} and CD8+IFNγ+ in vaccinated pigs at 35 days PV. Marked stimulation of CD8+IFNγ+ cells was observed after PRRSV natural exposure in both IM and ID groups upon stimulation with the vaccine and BS/114/S strain. Lower or no stimulation was observed in the control group. The frequencies of IFN-γ SC slightly increased in vaccinated pigs in the PV period. Higher IFN-γ SC were detected in ID pigs upon stimulation with the vaccine strain at 35 days PV and with the BS/114/S strain at 21 days PV. No cellular response was observed in control animals after vaccination. However, after PRRSV exposure, especially the vaccinated groups showed an increase of IFN-γ SC after recall with the vaccine and BS/114/S strain. After exposure, ID pigs exhibited a much higher frequency of IFN-γ SC compared to both the IM and control group when stimulating cells with the vaccine and the BS/114/S strain. PBMC stimulated with the BS/55 strain after exposure did not show different responsiveness between groups. Comparable per cell IFN-γ productivity (IFN-γ secreted by single cell) was observed at each time point in pigs of all groups. Antibody levels in IM pigs did not significantly change after vaccination whereas ID animals showed an increasing trend. After PRRSV exposure, all animals tested positive at ELISA and showed a strong anti-PRRSV antibody response, however higher in vaccinated groups compared to controls. No clear association between viral titres upon infection and cellular or humoral immune responses were observed despite some lower titres paralleled with higher IFN-γ ELISpot responses. The results highlight that PRRSV-vaccinated animals mounted an IFN-γ response associated with cross-reactive CD8+IFNγ+ cytotoxic cells that can be promptly triggered upon infection although vaccination did not stimulate per se high levels of IFN-γ producing cells. The extent of the immune response did not appear to be related to the genetic similarity between the vaccines and PRRSV isolates used for *ex vivo* antigen recall. Furthermore, data on IL-10 and TNF-α by ELISA and RT-qPCR highlighted a different response to different virus strains used for *in vitro* PBMC stimulation.

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Adjuvants for live PRRS vaccines

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Introduction

A large number of live vaccines are used in pig farming activity. Such vaccines are based on attenuated or modified live pathogen having some infectious properties but lacking their pathogenic properties. Live vaccines are usually not added of adjuvant. Our aim is to improve the efficacy of live vaccines by adding adjuvants in the formula. Our findings demonstrate that in injectable PRRS model we did enhance the efficacy of a marketed live vaccine by adding an adjuvant formulation allowing reducing the antigen content while maintaining partially the vaccination efficacy.

Experimental vaccines: All experimental vaccine formulations were formulated by simple dilution of the saline buffer in the adjuvants under a gentle stirring. Various Adjuvants technologies were studied: Polymeric adjuvant (Montanide™ Gel 01), Water in oil in Water (W/O/W: Montanide™ ISA 201 VG) and nanoparticles based formula (Montanide™ IMS 1313 N VG). Montanide™ Gel was used at 10% with 100% (4.3 log TCD₅₀/ml virus titer) or 50% of the antigenic load. A commercial not adjuvanted vaccine was tested as a positive control while a non-vaccinated group was used a negative control. PRRS virus seronegative. Pigs weighting 10 to 15 kg were vaccinated with 2ml intramuscularly in the neck at day 0. Safety was followed by body temperature assessment after vaccination and injection site dissection at the end of the trial. Groups of 10 pigs were used. Efficacy was followed by antigen specific antibodies detection and by a challenge procedure (on day 30). After challenge the clinical signs (body temperature) presented by all animals were followed and at the end, the bacterial over-infections in lungs scored using Halbur et al., 1995 method.

Results and discussion

Formulation demonstrated an absence of general and local reaction: even when dissecting the injection site, no local reactions were found in the muscles. The antibody titers were detected by antigen specific ELISA and no differences could be observed between adjuvanted and non adjuvanted formulation containing 100% of antigen. The formulation with adjuvant but 50% of antigen induced a reduced immune response in terms of antibody. Protection to challenge using the lung scoring was significantly superior for the adjuvanted formulation containing 100% of antigen compared to other groups. The vaccine commercially available but without adjuvant induced a similar protection as the formulation containing 50% of antigen and the adjuvant.

Conclusion

We demonstrated that adding a relevant adjuvant in live vaccine injected in pig improved the vaccine efficacy facing an infectious challenge. Furthermore, the use of such polymeric adjuvant could improve the protection conferred by a vaccination using 50% of the antigenic load to an equivalent level compared to commercial formulation containing a full antigen dose. This opens a door to modification of the vaccine formulation allowing a better control of the vaccine safety and improved efficacy.

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Inhibition of porcine reproductive and respiratory syndrome virus (PRRSV) replication by extracts from an eatable mushroom

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Porcine reproductive and respiratory syndrome (PRRS) is the most severe viral disease in pigs, causing great economic losses worldwide each year. Even though vaccines against PRRSV are available, they fail to provide sustainable disease control. The medicinal use of mushrooms has a very long tradition in the China, and many mushrooms are reported to have antiviral activity. In this study, we first report that the extract of one eatable mushroom has a strong anti-PRRS virus activity. The saline water extracts could significantly inhibit PRRSV replication both in PAM and Marc-145 cells at a dose-dependent manner. Further studies showed that the extracts could not only directly inhibit virus infection ability, but also inhibit PRRSV entry, RNA and protein expression, and cell to cell spreading, whereas it did not block PRRSV binding to cells and the releasing of virus particles, suggesting the extracts could inhibit multiple steps of PRRSV infection in vitro. Most importantly, we showed that intra-muscle and oral injection of the extract at the time of PRRSV infection not only could reduce the virus load in pigs, but also reduced the death rate of pigs infected with highly pathogenic PRRSV strain. Further studies need to be done to find out which element (or elements) in the extract is critical for the anti-PRRSV activity.

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Antiviral regulation underlying the activation status of porcine monocyctic innate immune cells

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Specific objective: Analogous to the well-established Th1 and Th2 paradigm in T cells, macrophages, as well as monocytes and dendritic cells (DCs), exist as classical M1 and alternative M2 statuses. M1 and M2 macrophages have been linked to regulation of inflammation, tissue repair, T- and B-cell proliferation and antimicrobial activity. Recent studies have shown that virus infection skewed monocyctic cell polarization, and that activation statuses of the monocyctic cells are critical for antiviral immunity. Unfortunately, there are no studies on the activation status of porcine monocyctic cells or how cell activation status modulates antiviral immunity. This is a significant omission because many porcine viruses are monocyctotropic including, porcine reproductive and respiratory syndrome virus (PRRSV). PRRSV is ideal for deciphering how monocyctic cell activation statuses modulate antiviral immunity, because it directly infects subsets of macrophages and DCs and subverts overall immune responses. Our goal is to integrate activation statuses with antiviral responses in these cells and to functionally modulate them for a prototypic cellular adjuvant/vaccine to potentiate antiviral immunity. The objectives of this study are to induce/categorize the activation status of porcine monocyctic cells and to determine the permissiveness and cytokine profiles following PRRSV infection in monocyctic cells at different activation statuses.

Methods: We used primary inductive mediators to polarize and induce conventional subtypes of activation statuses (M1, M2a, M2b and M2c) and antiviral states in porcine monocyctic cells. The cell activation statuses were determined by cytokine secretion using a SearchLight chemiluminescent multiplex assay (Aushon BioSystems, Billerica, MA). The expression of some status-specific gene markers and the PRRSV-permissive cells were quantified with fluorescence cell counting using flow cytometry.

Results and conclusions: Porcine monocyctic cells could be skewed to various activation statuses, similar to M1 and M2 described in mice and humans. We showed that porcine monocytes, macrophages and DCs respond to various mediators and produce cytokines corresponding to individual phenotypes. For example, LPS/IFN- γ -induced M1 macrophages showed up-regulated production of proinflammatory cytokines (including IL-1, IL-6 and IL-8); and, in contrast, IL-4/IL-10-induced M2 cells showed down-regulation of these cytokines. Interestingly, macrophages treated with type I IFN to induce antiviral states have cytokine production patterns compatible to the activation paradigm, namely IFN- α treated macrophages are similar to IFN- γ -M1 cells and IFN- β treated macrophages are similar to IL-10-M2c cells with regard to the increase or decrease of cytokines. A second set of experiments showed that cells at different activation statuses (including antiviral states) react differently to PRRSV infection in cytokine production and have altered permissiveness to the viral infection. In summary, we have determined the activation status of porcine monocyctic cells and the relationship of these statuses with PRRSV infection. These findings suggest that integration of activation status with antiviral responses in porcine monocyctic innate immune cells may allow functional modulation, thus facilitating the development a prototypic cellular adjuvant/vaccines for potentiating antiviral immunity.

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Comparison of host immune responses to homologous and heterologous porcine reproductive and respiratory syndrome virus (PRRSV) challenge

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Porcine reproductive and respiratory syndrome (PRRS) is a high-consequence animal disease worldwide. The objective of this study was to identify immune responses that are predictive of protection against heterologous PRRSV challenge. Using MLV IngelVac PRRSV vaccine, its parental strain VR2332, and K-LI (a Kansas isolate of PRRSV), we compared immune responses induced by a single vaccination and by experimental infection of two different PRRSV isolates. Four groups (5 pigs/group) of pigs were utilized in this study in which groups 1 & 2 were vaccinated with MLV PRRSV and groups 3 & 4 were not vaccinated. Twenty eight days post vaccination (DPV), pigs in groups 1 & 3 were challenged with VR2332, and groups 2 & 4 were challenged with K-LI. All pigs were euthanized 14 days post challenge (DPC). PRRSV was identified by RT-PCR in serum samples from vaccinated pigs 7 DPV. Quantitative PCR analysis showed that PRRSV was detected in serum samples of groups 2, 3, & 4 pigs but not in group 1 pigs 7 DPC. Lung pathology score of group 1 pigs was significantly lower than that of groups 2, 3, & 4 pigs 14 DPC, confirming the vaccination-induced homologous protection. Although PRRSV-specific antibody in the serum can be detected 7 DPV, serum neutralizing antibody against VR2332 was detected only after 28 DPV. Serum neutralizing antibody against K-LI was detected only in group 2 pigs 14 DPC. ELISpot assay indicated that VR2332 is a stronger inducer of IFN- γ -secreting PBMCs than K-LI in all pigs, and group 2 pigs possessed more PRRSV-specific IFN- γ -secreting PBMCs. This study indicates that heterologous challenge induces a higher level of cellular immune response against PRRSV in vaccinated pigs, but only serum neutralizing antibody titer is associated with PRRSV vaccination-induced protection against homologous and heterologous challenge.

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Analysis of two genomic regions shown to be associated with response to experimental infection with PRRS virus in piglets

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Vaccines for the prevention of PRRSV infection have generally been only partially effective. Therefore, establishing alternative methods to control the most economically devastating disease in the swine industry is of great importance. The objective of this study was to evaluate genomic data obtained from the PRRS Host Genetics Consortium (PHGC) PRRS-CAP project, which aims to identify genetic host components of viral resistance, tolerance, or susceptibility to PRRSV. Three trials of ~200 barrows, approximately 3 weeks of age, were infected with PRRS virus isolate NVSL97-7985. Pigs were Landrace by Large White cross barrows from a single genetic source. Weekly piglet weights, measured up to 42 days post infection (dpi), were used to evaluate weight gain (WG). Viremia was measured from blood samples collected over the same time period. Viral load (VL) was quantified as area under the curve of log transformed viral qRT-PCR levels in serum at 0, 4, 7, 11, 14, and 21 dpi. Piglets were genotyped using the Illumina Porcine SNP60 Beadchip. A genome wide association study of VL, using Bayesian methods implemented in GenSel, revealed regions on *Sus Scrofa* chromosome 4 (SSC4) and SSCX that explained a considerable amount of variation; the region on SSC4 was also found to affect WG. ASREML software was used to further explore the impact of both regions, including their interaction. The model used to assess the effects of SNP genotype and the interaction between SNP genotypes in the two regions included the interaction of trial and parity of dam as a fixed factor and litter, animal, and pen within trial as random effects. One SNP from each region that explained a substantial proportion of the region's variance were fitted as fixed factors, along with their interaction. Frequencies of the favorable alleles were low, at 16.1 and 13.0% for the SSC4 and the SSCX SNP. Both SNP were shown to significantly affect VL ($P < 0.005$) and WG ($P < 0.02$), but the interaction between SNP was not significant ($P > 0.2$). The favorable effect on SSC4 appeared to be dominant, with the favorable allele estimated to decrease VL by 5.0 units, which represents 0.66 phenotypic standard deviations (sd), and increase WG by 2.5 kg, 0.57 sd. The favorable SSCX allele was estimated to decrease VL by 6.3 units or 0.84 sd, and increase WG by 2.3 kg or 0.53 sd. A brief investigation of the two regions revealed several suitable candidate genes. These results show that it may be possible to select animals for resistance or tolerance to the PRRS virus. Analysis of additional trials and phenotypes, and a more thorough investigation into the biology of these SNP regions could further substantiate these findings. This work was supported by the National Pork Board, PRRS CAP, USDA ARS and NIFA Award 2008-55620-19132, funding from the NRSP-8 swine and bioinformatics coordination projects, and by the breeding companies involved in the PHGC that provided pigs. EW is a Fellow supported by USDA NIFA National Needs grant # 2010-38420-20328.

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Synthetic peptides containing B- and T-cell epitopes of porcine reproductive and respiratory syndrome virus (PRRSV) with Gp96N355 as adjuvant provoked humoral and cellular immunity

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Current evidence suggests that either inactivated or attenuated vaccine only provided a limited protection against PRRSV infection. In the past decades, the synthetic peptide approach has attracted great attention for vaccine development. A growing number of B- and T- cell-epitope (BCE and TCE) of PRRSV has been identified and closely related to the clearance of PRRSV infection. In the present study, two BCE (Gp4-B59, Gp5-B37) and seven TCE (Gp4-T7, Gp4-T170, Gp5-T117, Gp5-T149, N-T49, N-T63, N-T104) of PRRSV and a Pan DR T-helper cell epitope were synthesized and mixed with N-terminal (22-355aa) of gp96 (Gp96N355) as adjuvant, and the humoral and cell mediated immunity elicited by these peptides were evaluated in mice and piglets, respectively. In the mouse model, ELISA and serum-virus neutralization assay showed a high level of ELISA-antibody and neutralizing antibody (NA) induced by the BCE peptides, while Gp96N355 could increase both for about 3-fold. Furthermore, lymphocyte proliferation and IFN- γ /ELISPOT assays demonstrated that each TCE peptide could induce significantly peptide-specific cellular immune responses in the splenocytes of vaccinated mice. In the piglet model, no NA was detected even with a low detectable ELISA-antibody. However, virus-specific and TCE peptide-specific lymphocyte proliferative responses were detectable. More importantly, we found that Gp96N355 could improve the production of IL-12 and TNF- α , decrease IL-4 and IL-10 in the serum of peptides-vaccinated piglets, showed a predominant Th1 type of immune response. These evidences may provide important information for the development of PRRSV epitope-based synthetic peptide vaccines and Gp96 may be used as an attractive immunomodulator in swine.

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Simultaneous detection of antibodies against PRRSV nsp7 and nucleocapsid protein in swine oral fluid and sera using a fluorescence microsphere immunoassay

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For effective disease surveillance, rapid and sensitive assays are needed to detect antibodies against PRRS virus (PRRSV) infection. In this study, we developed a multiplexed fluorescent microsphere immunoassay (FMIA) 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 FMIA achieved greater than 92% sensitivity and 91% specificity. In serum samples (n = 1639), the FMIA 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 revolutionize 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.

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Differences in cytokine expression in sera from pigs infected with Type 1 and 2 PRRSV using a multiplex fluorescent microsphere immune assay

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the most important infectious disease threat to swine production worldwide. Cytokines are small secreted proteins that mediate and regulate innate and adaptive immune responses. After PRRSV infection, most pigs exhibit weak early innate and delayed adaptive cytokine responses; this is associated with clinical disease, growth losses and viral persistence. Recent studies have highlighted the potential utility of oral fluid samples as an alternative to sera to assess anti-viral responses (Kittawornrat et al. Virus Res. 154: 170, 2010). We proposed to 1) determine whether serum cytokine protein levels in response to PRRSV were correlated with virus load in serum; and 2) verify if the oral fluid samples represent a good source to study the cytokine responses to PRRSV in pigs. Samples collected on 0, 7, 14 and 21 days post-inoculation (DPI) from 24 boars inoculated IM with PRRSV type 2 (North American) isolate (MN-184) and 24 with Type 1 (European) isolate (D09-012131) were analyzed using our cytokine fluorescent microsphere immune assay (FMIA) with a Bio-Rad Bio-Plex 100 instrument. Boar sera and oral fluid samples from that study were tested simultaneously for 8 cytokines [interleukin-1b (IL-1b), IL-4, IL-8, IL-10, IL-12, interferon-a (IFNa), IFNg and chemokine CCL2] with our FMIA. Pigs from each infection were grouped based on low (LV) or high (HV) virus load using the sum of virus load quantified by qRT-PCR. Pigs that had high level of IL-1b, IL-10 and IL-12 in serum pre infection, whether infected with type 1 or type 2 PRRSV, retained high expression for these cytokines throughout the time course of infection independent of viral load. For Type 1 PRRSV sera from HV pigs, more IL-1b, IFNa, and IL-10 were expressed, while sera from LV pigs contained more IL-12 at DPI 0. For the Type 2 isolate sera from the LV pigs expressed more IL-1b, IL-8, and IL-12, IFNa was continued to express longer than that of HV group. For Type 2 PRRS HV pigs expressed more CCL2, HV and LV had similar values for IL-10. In conclusion, we observed differences between innate cytokines expressed in pig sera from LV pigs with Type 1 and 2 PRRS; the innate cytokines were higher in LV group sera for Type 2 while in Type 1 the HV group had higher expression of innate cytokines. When we analyzed the oral fluids up to 21 DPI, our FMIA was not sensitive enough to detect any of the cytokines, indicating low levels of cytokine expression in oral fluids during the early stage of PRRSV infection. More than just viral load data will be required to predict whether cytokines could be used as immune markers to identify PRRSV resistant or susceptible pigs. Ongoing work is directed to using the cytokine FMIA for testing sera and oral fluids from PRRS Host Genetics Consortium samples, which were collected from pigs with type 2 PRRSV infection followed to 42 DPI. This research was supported by a CNPq postdoctoral fellowship (200602/2010-1) to KPCA and a National Pork Board grant #09-244.

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Swine Toolkit progress for the US Veterinary Immune Reagent Network

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The US Veterinary Immune Reagent Network (US VIRN, www.vetimm.org) was established to address the lack of immunological reagents specific for veterinary species. Efforts are targeted at swine, ruminants, poultry, equine and aquaculture species. Our goal is to produce reagents that function in ELISA, Luminex assays, ELISPOT and flow cytometric applications. In the last year recombinant chemokines (CCL3L1, CCL4 CCL5 and CCL20) and cytokines [interleukin-6 (IL-6) and IL-22] were expressed in *Pichia*, purified and all but IL-22 shown to be bioactive using chemotaxis, upregulation of marker expression or cell stimulation assays. We have also cloned, expressed and proven bioactivity of swine immunoregulatory cytokines, IL-17A and IL-17F. Hybridoma fusions for monoclonal antibodies (mAb) to CCL3L1, IL-6, IL-13, IL-17A, interferon-alpha (IFN α) and IFN β are underway at Univ. Massachusetts. A sensitive fluorescent microsphere, Luminex bead, immunoassay for CCL2 was developed with US VIRN produced mAb and included in the 8-plex swine cytokine assay. At Cornell Univ. a fusion protein expression system was used to generate material for immunizations for swine T cell receptors, TCR $\alpha\beta$; hybridoma fusions are continuing. Additional fusions will target IFNAR, CD19, and NK cell markers, NKp36 (NCR3) and NKp44 (NCR2). The US VIRN website www.vetimm.org has a progress update for swine as are all bioassay methods and gene sequences. Since many swine cytokine and CD reagents are available commercially the website includes a listing of those reagents and their sources. Products developed in this proposal are available to collaborators and have been made commercially available through Kingfisher Biotech, Inc. <http://www.kingfisherbiotech.com/>. This project was funded by USDA NIFA proposal #2006-35204-16880, renewal #2010-65121-20649, US DHS IAA #HSHQDC10X00021 and USDA ARS funds.

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Intranasal delivery of an adjuvanted modified live porcine reproductive and respiratory syndrome virus vaccine reduces the ROS induced lung pathology

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Reactive oxygen species (ROS) are produced predominantly by phagocytic cells in response to microbial infections. ROS when produced at optimal levels have potent antimicrobial properties. However, excess production of ROS induces apoptosis/necrosis of infected as well as bystander cells resulting in inflammatory pathology. Previously, we showed that vaccination of pigs with a modified live porcine reproductive and respiratory syndrome virus vaccine (PRRS-MLV) administered intranasally with a potent mucosal adjuvant *Mycobacterium tuberculosis* whole cell lysate (*Mtb* WCL) induces protective immune response against PRRS viral challenge. In this study, bronchoalveolar lavage fluid cells and peripheral blood mononuclear cells harvested from pigs vaccinated and challenged with PRRS virus were quantified for the levels of ROS production using colorimetric and flow cytometric analyses. Our results indicated that in vaccinated pigs (PRRS-MLV+*Mtb* WCL) levels of ROS were significantly less compared to unvaccinated PRRS virus challenged pigs. In control unvaccinated but PRRS virus challenged pigs the enhanced ROS production was associated with increased inflammatory lung pathology. In conclusion, our results suggested that intranasal vaccination using PRRS-MLV along with a potent mucosal adjuvant protects pigs against both homologous and virulent heterologous PRRS virus induced ROS mediated lung pathology. This project is supported by National Pork Board and USDA-NIFA PRRS CAP2 award to RJG.

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Effects of area and regional control programs for porcine reproductive and respiratory syndrome virus on the production performance of swine

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Porcine Reproductive and Respiratory Syndrome virus (PRRSv) costs the United States' swine industry \$560 million annually. Most of that loss, valued at greater than \$10 per pig finished, is attributed to production losses due to PRRSv circulation in the commercial wean to market pig. A PRRSv Area and Regional Control (ARC) project was initiated in the Northeast Illinois (NE IL) region in January of 2010. Control procedures including McRebel protocols, vaccination of both sows and piglets with Ingelvac® PRRS MLV, and improved internal and external biosecurity started in January of 2010 and were fully implemented by April of the same year on all sow farms in the area. The goal of this project was first controlling, and then eliminating PRRSv from the defined area. Success would also be expected to improve growing pig performance of the area, resulting in increased financial gains to the swine producer. Therefore, the objective of this study is to present the production improvements obtained by the successful establishment and accomplishment of an ARC project in the NE IL region. A dataset (Bethany Swine Health Services) representing 400,000 pigs originating from 12 independent sow farms was analyzed to determine the effects of ARC implementation on performance in the downstream flow. Historical diagnostic data showed that most pigs in the area are exposed to PRRSv during or prior to the nursery phase. Therefore, nursery comparisons were examined for gains and mortalities by month over a two year time period (2009-2010). Mean ADG for the entire dataset between 2009 and 2010 showed a minimal increase of 17 g/d (376 to 393 g/d). However, between March and December 2010, ADG increased 34 g/d (381 to 415 g/d). Piglets originating from Herd Category 1 (HC-1) sow farms showed an even more drastic improvement of 83 g/d (314 to 397 g/d) between March and December 2010. The entire dataset showed a decrease in mortality of 0.22%. HC-1 farms showed a decrease in mortality of 4.38% during the same time period in 2010. While these improvements are small, the decrease in nutrient value and increase in toxins of corn utilized in the diet in 2010 led to decreased performance in all pigs. In 2010, when the ARC project began, 80% (n= 10) of the sow farms were PRRSv positive, with 50% (n=6) being unstable (HC-1); today only 17% (n=2) are unstable (HC-2). Therefore, implementing ARC programs is beneficial to all producers in an area due to a decreased risk of external and lateral PRRSv infections. Producers that undertake a control and elimination plan as a part of an ARC project also take on associated costs (diagnostics, vaccine, biosecurity). The dataset shows there is a return on those investments by ways of improved performance. Considering that an improvement of 50 g/d in ADG equates to additional revenue of \$1.36/pig and an improvement of 0.1% mortality represents an additional \$0.08/pig; producers in this area retained an additional \$0.64/pig totaling \$256,000 in increased revenue in 2010 over 2009. So, in conclusion, this dataset shows that there are tangible performance benefits not only for the individual producer, but for the area as a whole when undertaking an ARC project.

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Interleukin-1beta expression by a recombinant PRRSV

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The cytokine interleukin 1 beta (IL-1 β) is a potent inflammatory mediator in response to infection, and can be used as an immunological adjuvant. In this study, we constructed a recombinant porcine reproductive and respiratory syndrome virus (vP129/swIL1 β) expressing swine IL-1 β from the separate subgenomic mRNA inserted between the ORF1b and ORF2 genome region. MARC-145 cells infected with vP129/swIL1 β secreted 1947 pg of IL1 β per 2×10^5 cells at 36 hours post-infection. *In vitro* growth kinetics analysis in MARC-145 cells showed that the vP129/swIL1 β virus had a similar replication rate as that of parental virus. We further performed *in vivo* characterization of the vP129/swIL1 β virus in a nursery pig disease model. The vP129/swIL1 β infected pigs did not show visible clinical signs, while respiratory distress and lethargy were evident in pigs infected with the parental virus. The expression of various cytokines from peripheral blood mononuclear cells measured by fluorescent microsphere immunoassay showed that IL-1 β , IL4 and IFN γ expression levels were up-regulated in pigs infected with vP129/swIL1 β at 7 and 14 days post-infection. However, no detectable level of IL-1 β was found in serum samples from pigs infected with either vP129/swIL1 β or parental virus. In summary, this study demonstrated a recombinant PRRSV as a useful tool to study the role of different cytokines in disease progression and immune responses, which represents a new strategy for future therapeutic application and vaccine development.

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The highly pathogenic porcine reproductive and respiratory syndrome virus GP5 B epitope is not a major neutralizing epitope

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In 2006, highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) caused great economic losses emerged in China and continues to be a threat for the pig industry. The B epitope (37SHL/FQLIYNL45) of GP5 was considered to be a major linear neutralizing epitope in PRRSV classical strains. Compared with classical PRRSV, however, one amino acid mutation (L/F39→I39) was found in the B epitope of HP-PRRSV. To study the ability of the B epitope of HP-PRRSV to induce neutralizing antibody (NA) in vitro and in vivo, rabbit antisera against B epitope with and without the mutation and pig hyperimmune sera with high titer of NAs against HP-PRRSV were prepared. Immunofluorescence assays (IFA) showed that the two rabbit antisera both had reactivity to classical PRRSV CH-1a and HP-PRRSV HuN4 with no observable difference in titer. However, antisera did not have neutralizing activity against classical PRRSV CH-1a and HP-PRRSV HuN4. No correlation was observed between the levels of anti-B epitope peptide antibodies and NAs in pig hyperimmune sera were detected by indirect ELISA and virus neutralization, respectively. Based on these findings, we conclude that the B epitope of HP-PRRSV is not a major neutralizing epitope of HP-PRRSV GP5.

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Attenuation of a virulent North American PRRS virus isolate on CD163-expressing cell lines, and demonstration of efficacy against a heterologous challenge

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The discovery of the CD163 PRRSV receptor in 2004 has permitted the development of PRRS-permissive cell lines from various species. Attenuation of virulent field viruses on these cell lines has the potential to yield new modified live vaccine viruses that differ from existing monkey-cell attenuated vaccines. In one such example, the virulent genotype 2 US strain P129 was isolated directly from serum on a recombinant CD163-expressing pig kidney cell line. Following adaptation/attenuation for 24 passages, this virus was evaluated in a small study in which it showed satisfactory safety and efficacy against a heterologous challenge in a young pig respiratory model. Beginning at passage 31 the virus was adapted to a more manufacturing-friendly CD163-expressing cell line, derived from the baby hamster kidney cell line BHK21. With additional rounds of passaging, viral titers increased. At passage 57 the vaccine virus was evaluated for efficacy in a dose-titration study. Five groups of 24 pigs were mock-vaccinated or vaccinated with various doses of the P129 vaccine virus (targeting 1.5, 2.5, 3.5, and 4.5 log₁₀ TCID₅₀/2 mL dose) at approximately three weeks of age and challenged with virus NADC20 at approximately seven weeks of age. Following vaccination, all pigs in all vaccinated groups seroconverted by ELISA. After challenge, vaccinated pigs had minimal clinical signs, and the three groups receiving higher vaccine doses were significantly ($p \leq 0.0132$) heavier than the unvaccinated control group. Lung lesions were assessed at necropsy (10 days post-challenge), and were significantly ($p \leq 0.0233$) reduced in all vaccinated groups. This study illustrates the utility of recombinant CD163-expressing cell lines in the generation of modified live PRRS vaccines. The animal use protocol was approved by the site Animal Care and Use Committee prior to initiation of the study.

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The porcine antibody repertoire and its response to PRRSV infection

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Antibody responses are critical to effective immunity to viral infections. Thus, extensive efforts have been directed to characterize the antibody and neutralizing antibody responses to PRRSV infection, in the hope of elucidating key insights into protective and cross-protective immunity. Despite these efforts, the role of antibody responses in PRRSV immune protection remains poorly understood. To address this, we characterized the expressed immunoglobulin repertoires in healthy and JA142 PRRSV-infected pigs using amplicon-based 454 high-throughput sequencing. Bioinformatic analysis of approximately 450 thousand reads revealed preferential usage of CDR3s of specific lengths in the infected pool. These PRRSV-specific CDR3 lengths corresponded with unique sequences that accounted for between 11 and 35 percent of all transcripts in their respective CDR3 size class. Furthermore, these same sequences were rare (~0.1%) in the uninfected pool. Diversity analysis estimated the size of the porcine heavy chain immunoglobulin repertoire to be approximately 3.5×10^5 , an estimate similar to that reported in humans, suggesting that the swine antigen-binding repertoire is similarly complex, despite the apparent lack of diversity in the porcine heavy chain variable gene (IGHV) framework regions. As a consequence of their repertoires being dominated by a small number of sequences, pigs infected with PRRSV showed a decrease in their total repertoire diversity. Furthermore, PRRSV-specific IGHV gene segment usage was dominated by IGHV4/IGHV10, suggesting a possible immunogenetic component of PRRSV immunity. We expect the results of this research to open the door to development of therapeutic reagents to treat acute PRRS, genetic testing for PRRS resistance, and a mechanistic understanding of cross-protective immunity.

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Induction of regulatory T cells by porcine reproductive and respiratory syndrome virus infection

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The aim of the present study was to determine the phenotype of regulatory T cells (Tregs) induced by PRRSV infection. In a first experiment, six nine weeks-old pigs were intranasally infected with PRRSV and serum and blood samples were taken at 0, 7, 14, 21 and 28 days post-infection (dpi). Viral load, frequency of CD4⁺CD25⁺Foxp3⁺, CD4⁺CD8⁺CD25⁺Foxp3⁺ and CD8⁺CD25⁺Foxp3⁺ T cells were quantified, as well as IL-10⁺ and TGF-β⁺ cells. The frequency of Tregs was also quantified in mediastinal lymph nodes (MLN) and tonsils from four-weeks old infected (n=20) and uninfected (n=15) pigs. Tissue samples from four infected and two uninfected pigs were taken at 0, 3, 7, 11, 18 and 24 dpi, at each time and analyzed for Tregs. The results showed that the frequency of CD8⁺CD25⁺Foxp3⁺ population remained stable during the time of infection. However, CD4⁺CD25⁺Foxp3⁺ show a little increase at 28 dpi (p<0.05), while CD4⁺CD8⁺CD25⁺Foxp3⁺ cells increased two-fold at 14 dpi and three-fold at 28 dpi (p<0.05). CD4⁺CD8⁺CD25⁺Foxp3⁺ Treg from MLN and tonsils were detected at 18 dpi. An analysis of IL-10- and TGF-β-producing cells indicate that in response to PRRSV, a moderate number of CD4⁺CD8⁺Foxp3^{low} and CD4⁺CD8⁺Foxp3^{high} are IL-10⁺ cells, contrary to Foxp3⁻ cells, which did not produce IL-10. TGF-β was only observed in the CD4⁺CD8⁺Foxp3^{high} population after PRRSV stimulation, whereas stimulation with PHA provoke that CD4⁺CD8⁺Foxp3^{neg}, CD4⁺CD8⁺Foxp3^{neg} and CD4⁺CD8⁺Foxp3^{low} populations produce TGF-β. In conclusion, these results demonstrate that PRRSV infection increases the frequency of regulatory T cells with the phenotype CD4⁺CD8⁺CD25⁺Foxp3^{high} and that these cells produce TGF-β and IL-10. These data could explain the immunopathology induced by PRRSV.

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Developing a broadly protective Porcine Reproductive and Respiratory Syndrome vaccine

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The need to develop a more broadly effective vaccine against Porcine Reproductive Respiratory Disease virus (PRRS) is considered to be one of the most important goals for the pork animal health industry. PRRSV is genetically diverse and is the most economically significant pathogen of swine worldwide. Currently licensed vaccines offer limited protection against the virus due to multiple host evading properties. The major mechanisms appear to be stimulation of antibodies that are directed at non-protective immunodominant epitopes. These antibodies then consume the host immune responses preventing recognition of more conserved protective antigenic determinants. Our hypothesis is that a proprietary autogenous PRRS vaccine derived from the new technology of Antibody Immune Masking (AIM) can be developed to divert the immune system towards more protective epitopes producing a more broadly protective immunity (immune refocusing). Here we detail the results of a recent immunization/heterologous challenge trial and determine the efficacy of our AIM PRRSV vaccine.

This trial demonstrates that AIM vaccination was 33 % effective in prevention of infection from a heterologous PRRSV virus. Pigs receiving the autologous vaccine had 100% infection rates. AIM vaccinated pigs that were not protected from infection had significant and marked reduction in viremia 7 days post challenge as compared to the autogenous vaccines ($p=0.0034$) and unvaccinated controls ($p=0.0001$). AIM vaccines also had a significant reduction in pulmonary viral loads as compared to unvaccinated controls ($p=0.0126$). Reduced viral loads were reflective of reduced pulmonary pathology in vaccinated groups as compared to the non-vaccinated control groups.

The major organ for PRRSV replication is the lung, and respiratory transmission is the primary route, therefore it is likely that AIM vaccination may also significantly reduce transmission rates of PRRSV in addition to immediate reduction of infectivity due to the 33% vaccine efficacy. These data are promising as we design field clinical trials for rapid translation of this vaccine to the industry and perform experiments to determine the immunological mechanism of this vaccine's efficacy. Determination of the mechanism of immunological protection afforded by this first generation AIM vaccine and the biological derived from these early studies will then be employed for the development of a non-replicating molecularly immune refocused PRRSV vaccine with DIVA (differentiation of Infected vs. vaccinated animals) capabilities.

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Evaluation of Airborne Shedding of Ingelvac PRRS Modified Live Virus Vaccine from Growing Pigs raised under Commercial Conditions

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Current Status of the Problem:

Herds have been very successful at PRRS eradication to generate negative pigs. This has resulted in a source of negative pigs. These negative pigs that are at risk populations are being vaccinated with Ingelvac PRRS MLV vaccine. Vaccinating these groups has shown to improve performance in the face of field virus infections^{1,2,3}. There is still concern with the use of Ingelvac PRRS MLV that it will shed and spread to other herds.

Project Objectives:

Identify the amount and duration of aerosol shedding of Ingelvac PRRS MLV inside the barn in standard commercial setting, outside the barn directly outside the pit fan and 1 mile down wind of the barn.

Procedures to achieve Objectives:

1. 3200 PRRS negative pigs were housed in a tunnel ventilated double-sided wean-to-finish barn and split in 2 rooms with separated air spaces and entries.
2. 30 sentinel pigs per room were identified and blood samples tested periodically by PRRS PCR and ELISA. Six oral fluid samples were also collected per room from the pens where the sentinel pigs were located and tested by PCR.
3. Air samples were tested for Quantitative PRRS PCR
4. Pigs in room A were vaccinated with Ingelvac PRRS MLV labeled dose at 4 weeks of age Pigs in room B were not vaccinated. The Danish entry system was implemented to prevent transmission of MLV to non-vaccinated controls in room B.
5. Liquid cyclonic collectors were used to collect 10 daily air samples for 28 days.

Results:

Viremia was detected from 2 to 50 days in vaccinated pigs. PRRS virus was detected in oral fluids from 2 to 77 days post-vaccination. No PRRS virus RNA or ELISA antibody were detected in serum or oral fluids in the non-vaccinated pigs performed from 0 to 112 days post-vaccination.

Table 1. PRRS virus detection in air samples

Days post-vaccination	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Sample 1 / 1 Mile away																														
Sample 2 / 1 Mile away																														
Sample 3 / Pit Fan 1																														
Sample 4 / Pit Fan 1																														
Sample 5 / Pit Fan 2																														
Sample 6 / Pit Fan 2																														
Sample 7 / Pen 7																														
Sample 8 / Pen 9																														
Sample 9 / Pen 10																														
Sample 10 / Pen 12																														

White boxes represent PCR positive air samples with the quantity of PRRSv expressed in logs

Discussion:

Vaccine virus was found in air samples at all 3 collection sites. At 1 mile down wind only 1.7% of the time, outside of the fan it was only more frequent at 5.8% of the time and in the room itself was 5% of the time. The frame of the shedding was short with the last positive air sample by day 14. The concentration of virus was relatively low in all samples identified at less than 4 logs of virus. The concentrations were low enough that they could not be sequenced. The non-vaccinated control room remained negative the entire time up to markets

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Differential regulation of mucosal immune responses at various mucosal tissues in pigs infected with PRRSV strain VR2332

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Porcine Reproductive and Respiratory Syndrome has been devastating the swine industry economically for the past two decades. The RNA virus which primarily infects the alveolar macrophages of pigs occurs as two major prototype strains, VR2332 and the Lelystad virus. The VR2332 is the strain commonly occurring in North America and also forms the basis of the attenuated live vaccine available commercially. This study was conducted to understand the pathogenesis of VR2332 and its modulation of immune correlates at mucosal tissues. Pigs were infected with VR2332 virus intranasally and euthanized at post infection days (PID) 15, 30 and 60. Gross and microscopic examination of the lungs of infected pigs showed pneumonic lesions at PID 15 and 30, whereas virus titers remained high until PID60 in the lungs. Similarly, viremia was maintained in all the infected pigs until PID 60. The virus neutralizing antibodies started to appear from PID 7 and was maintained at low titers. Re-stimulatory cytokine analysis of immune cells detected a moderately increased Th1 cytokines (IFN- γ , IL-12) secretion and immunoregulatory (IL-10) and proinflammatory (IL-6) cytokines were high until PID 60. In serum, IFN- γ secretion was detected at PID35, while IL-10, IL-6, and TGF- β were maintained high throughout the study. Flow cytometric analysis of mononuclear cells from different sites (lungs, blood, tonsils, tracheobronchial and iliac lymph nodes) identified varying trends for total T Lymphocytes, cytotoxic T lymphocytes, memory cells, natural killer cells, and T regulatory cells in each tissue, which indicated overall immunopathogenesis of the virus. This information may further pave the way in formulation of preventive and therapeutic measures against PRRSV. This project was supported by National Pork Board, USDA-NIFA PRRS CAP2, and OARDC The Ohio State University to RJG.

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Replicon particle administration prior to challenge reduces PRRSV viremia

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Alphavirus-derived replicon particles (RP) are single-cycle, propagation-defective viral vectors. Numerous studies in a wide range of species have demonstrated the safety and efficacy of RP as a vaccine platform. In addition to vaccine antigen expression, RP have been used to deliver cytokines, such as Interleukin-12 and Type I interferon. Other investigators have demonstrated that RP enhance innate immune responses following administration.

Harrisvaccines, Inc. is developing veterinary vaccines utilizing RP technology. One vaccine candidate under development expresses the hemagglutinin gene from swine influenza virus H3N2 (H3-RP). The H3-RP was chosen to examine the possible anti-PRRSV effects of RP vaccines using a young pig challenge model.

Three-week-old pigs were obtained from a commercial herd historically free of PRRSV and swine influenza virus. Pigs were randomized into groups of six animals, and housed together in BSL-2 animal facilities. At approximately four weeks of age, pigs were injected with either H3-RP or vaccine diluent (placebo). Challenge occurred 24 hours post-treatment via intranasal inoculation with virulent PRRSV. Pigs were monitored for clinical signs and bled periodically for 21 days post-challenge. Serum samples were assayed for PRRS viral RNA by quantitative real-time RT-PCR, and for anti-PRRSV antibodies by commercial ELISA.

All animals had detectable viral RNA in serum by three days post-challenge, indicating successful challenge. Compared to placebo, animals treated with H3-RP had significantly lower qPCR titers at 10, 14, 17, and 21 days post-challenge. Area under the curve (AUC) analysis of qPCR titers also showed a statistically significant reduction in the H3-RP group compared to placebo. ELISA data were used to calculate group mean S/P ratios for each sample date. Compared to placebo, the H3-RP group had significantly reduced mean S/P ratios at 7, 10, 14, 17, and 21 days post-challenge.

These results suggest that the non-specific immune response stimulated by H3-RP vaccination is effective at reducing PRRSV viremia in a young pig challenge model. Further work is underway to elucidate the possible mechanisms of this observed effect. Additional data will be presented at the meeting.

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The use of nucleocapsid N and nsp7 proteins in PRRS genotype 1 virus diagnostics

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The aim of this work was to express recombinant structural N and nonstructural Nsp7 proteins of Czech field strain (genotype I) of PRRS virus and to compare their diagnostic sensitivity and specificity. Another goal was to analyze the use of Nsp7 antigen for the differentiation of post-infection and post-vaccination antibodies in pigs vaccinated with an inactivated vaccine.

ORF 7 and Nsp7 genes were cloned into pDest17 vector. The expression of N and Nsp7 proteins was tested in different *Escherichia coli* strains. Resulting recombinant proteins were purified by IMAC using a polyhistidine tag under denaturing conditions from the insoluble fraction of bacterial lysate. Purified N and Nsp7 proteins were applied as antigens in indirect ELISA tests. Serological reactivity of both proteins was assessed on a panel of 274 swine sera separated to three groups: 1) 44 sera from non-vaccinated herds free of PRRS infection for calculation the cut-off value, 2) 44 PRRS-negative pigs vaccinated by the inactivated vaccine and 3) 186 serum samples from PRRS positive farms for a comparison of both ELISA tests.

Both antigens proved to be suitable for serological detection of PRRS specific antibodies, showing diagnostic specificity of 95.6 %, and sensitivity of 90.5 % for N protein ELISA test and 85.7 % specificity and 97.2 % sensitivity for Nsp7 ELISA test. Nsp7 antigen proved to be suitable for differentiation of post-infection and post-vaccination antibodies. Sera from PRRS free herds vaccinated by inactivated vaccine were compared with N and Nsp7 based ELISA tests. Although 100 % of sera gave positive results in N protein based ELISA test, these sera were tested negative with Nsp7 antigen.

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PRRS Host Genome Consortium Database: Development of a system of data storage and sharing for a multi-organizational group

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The PRRS Host Genome Consortium (PHGC) is a group created from multiple labs focused on genetic resistance to PRRS infection in swine. As a result there was a need for a method to easily store and share data across the multiple lab and commercial groups, which are located across multiple institutions in the US and Canada. The solution to this issue was to develop a centrally located database where all the data produced by the groups could be stored. A website, <http://www.animalgenome.org/lunney/index.php>, has been developed as a frontend to the database to allow access via the world wide web and allow users without the knowledge of databases or server languages to access the database and obtain needed information. The database was originally designed based upon data produced by the “Big Pig” project led by Zimmerman and Rowland. This data included phenotypic oriented data, such as weights, viral levels in serum, antibody responses, serum cytokine levels, qPCR based gene expression, and genotypic information such as pedigree information and Swine Leukocyte Antigen alleles. More recently the database has been expanded to include data from genotyping (60K SNP chip), new phenotypic information such as Pigoligoarray day, and results produced from statistical analysis of the data, e.g., assignment of high/low groups. All data is available to PHGC members in the secure, password protected database access to which is open to PHGC members who have signed a Cooperative Research and Development Agreement (CRADA) Material Transfer Agreement (MTA) organized by BARC. Recently, a system that utilizes the PHGC website and database to share presentation and publication information amongst the consortium has been developed. Further development includes a system to track samples between the different labs, development of ways to store newly produced data, including data from genome sequencing, pipelines to streamline data processing and sharing among PHGC members, development of a gene centric database to store new, non-pig centric data, and development of a method to allow for selective data access. The PHGC Database is under continual development in order to facilitate data storage and sharing on a large scale between consortium members. This work was supported by the National Pork Board, USDA ARS and the NRSP-8 swine and bioinformatics coordination projects.

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PRRS modified live virus vaccine as a tool to decrease viral shedding from an infected growing pig population

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Introduction. There are ongoing efforts to eliminate porcine reproductive and respiratory syndrome virus (PRRSv) from regions in North America. A major challenge for the regional control and elimination programs is the re-infection of swine herds due to PRRSv area spread. The purpose of this study was to measure the effect of a PRRS modified live virus (MLV) vaccine on wild-type virus shedding in a previously infected growing pig population raised under field conditions.

Materials and methods. The study was conducted in a wean to finish research barn composed of two side-by-side rooms of 1,000 pigs each. One hundred pigs in each room were inoculated with a wild-type PRRSv (restriction fragment length polymorphism pattern 1-18-2) isolate. Rooms had separate pit and air spaces and strictly validated biosecurity protocols were utilized to avoid transmission of PRRSv between rooms. Treatments consisted of a challenged-vaccinated room and a challenged-unvaccinated control group. At 8 and 36 days post inoculation (dpi), all pigs in the challenged-vaccinated group received Ingelvac ® PRRS ATP vaccine (Boehringer Ingelheim Vetmedica Inc). Pigs of the challenged-control group were sham-inoculated with saline at the same days. One aerosol sample per day was collected from each room 6 days per week from 0 to 118 dpi and samples were tested for PRRSv-RNA using qPCR assay. Blood and oral fluid samples were collected at 0, 8, 36, 70, 96 and 118 dpi for PRRSv PCR and ELISA. Additionally, tonsil-scraping samples were collected from both groups at 70, 96 and 118 dpi and tested by PCR.

Results. There was no significant difference in the PRRSv infection dynamics measured as duration and magnitude of viremia and seroconversion between groups. However, there was a significant difference in PRRSv shedding between groups. PRRSv was detected for 31 days in aerosol samples from the challenged-control group and 17 days in the challenged-vaccinated group ($p = 0.0004$). PRRSv was last detected in the challenged-control group on day 70 while the last PRRSv detection in the challenged-vaccinated group occurred on day 45 ($p = 0.003$). At 36 dpi, 6/6 oral fluid samples in the challenged-control group tested PCR positive and 2/6 tested positive in the challenged-vaccinated group ($p = 0.03$).

Conclusions. Under the conditions of this study, the use of MLV vaccine led to a reduction in PRRSv shedding frequency and duration from the infected pig population. The cumulative PRRSv-RNA detected in the air samples was significantly lower in the challenged-vaccinated population.

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Effectiveness of herd exposure methods to produce PRRSv-negative piglets from infected breeding herds

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This study compared the effectiveness of administering PRRS modified live virus vaccines to that of wild-type resident virus when used as part of a herd closure/stabilization program. Eligible farrow-to-wean breeding herds that got infected with PRRSv and adopted herd stabilization programs to eliminate PRRSv were enrolled in the study. Time to produce PRRSv-negative pigs at weaning (TTNP) was compared from herds that used modified live virus¹ to herds that used the wild-type resident virus². TTNP was defined as obtaining 90 days of consecutive monthly PCR-negative results from prewean pigs. Preliminary descriptive analysis indicates that PRRSv shedding in farms going through herd closure is intermittent, indicating that PRRSv-monitoring must be done systematically over time. A total of 33 herds have been enrolled in the study at this time. From 20 farms with ongoing PRRSv monitoring, 12, 2 and 2 farms had respectively 1, 2 and 3 month of PRRSv PCR-negative results followed by PCR-positive results. In the farms with 2 or 3 months of PCR-negative results followed by PCR-positive results (n=4), phylogenetic comparison of the PRRSv isolates suggested that no new virus was introduced in those farms. As of the writing of this abstract, 4 farms achieved TTNP. In the modified live virus group the TTNP periods for two farms were of 12.3 and 33.5 weeks and for the wild-type virus group, 27.1 and 29.9 weeks.

The enrollment of herds using modified live vaccine (n=6) as the exposure method has been limited by the fact that most of the contacted herds using that strategy do not extend the closure period for the minimal of 180 days required by the study.

¹ PRRSv Ingelvac® ATP or PRRSv Ingelvac® MLV, Boehringer Ingelheim Vetmedica, Inc

² Live resident (wild-type) virus inoculation (Batista et al., JSHAP 2002; 10(4):147-150)

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Nanoparticles entrapped killed PRRSV vaccine reduces PRRSV viremia in both homologous and heterologous PRRSV challenged pigs

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Porcine reproductive and respiratory syndrome remains as the leading cause of economic burden to US swine producers in spite of 20 years of research efforts to control the disease. Intranasal vaccination of killed PRRSV antigens marginally reduced the virus titer in blood to homologous (VR2332) as well as heterologous (MN184) viral challenge. To potentiate the effect of killed PRRSV vaccine (killed vaccine), poly (lactide-co-glycolide) (PLGA) - nanoparticles were prepared to encapsulate PRRSV killed antigens (Nano-PRRSV). Nano-PRRSV received pigs showed reduction in viremia on post-challenge day 8 and complete viral clearance by day 15 to both VR2332 and MN184 PRRSV challenge. Immunohistochemistry analysis showed more PRRSV antigens in the lungs of challenged pigs of unvaccinated and killed vaccine inoculated compared to Nano-PRRSV inoculated pigs. Hematoxylin & Eosin staining of the lung sections revealed severe infiltration of mononuclear cells in unvaccinated and killed-vaccine inoculated compared to Nano-PRRSV received virus challenged pigs. Immune responses based on the frequency of various cell populations (Natural Killer cells, T-helper, T-cytotoxic, Gamma-delta T cells and T-regulatory cells) at mucosal and systemic sites and the cytokine secretions, namely IFN- α (Innate), IL-12 & IFN- γ (Th1), and IL-6 (pro-inflammatory) in lungs, serum, and by re-stimulated immune cells supported our results. In addition, PRRSV specific IgA and IgG antibodies and virus neutralizing antibody titers were also detected in nasal wash, lung lysate and serum. Overall, our results suggested that intranasally administered PLGA-nanoparticles-PRRSV-Killed vaccine is capable of inducing protective immunity to PRRSV. This project is supported by National Pork Board to RJG.

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Use of avian immunoglobulins to control a PRRS outbreak and its impact in productive parameters

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INTRODUCTION

Porcine Respiratory and Reproductive Syndrome (PRRS), produces important economic losses to areas of high pig density. The most common of these problems arise in gestating sows and piglets; in sows it can cause anorexia, fever, reproductive failures such as constant oestrous delays, repetitions, abortions, litters of weak born piglets; increasing perinatal mortality. The objective of this study was to determine the impact of the application of avian immunoglobulins specific against PRRS virus during an outbreak and to compare productive parameters before and after establishing the program.

MATERIAL AND METHODS

The farm is located in Northern Mexico at 27° 04' 51" N, 109° 26' 43" W, at 33 masl with extreme weather throughout the year. It is a farm with 1500 sows in production with recurrent PRRS outbreaks, between March and April 2010, the most important outbreak was observed, with 70 recorded abortions; at this point the avian immunoglobulins specific against PRRS were applied to all breeding swine on a 5 mL dose, a second dose was administered 15 days later. Afterwards applications to the breeding swine were made every 4 months with their second dose 15 days later; gestating sows were given the product at the 10th and 12th week of gestation and replacements were administered a dose 14 days before introducing them to the farm and on the day of introduction to the farm.

RESULTS

The comparative results of the serological analyses performed before and after treatment with the product showed that antibody levels against PRRS decrease after the application of the immunoglobulins, some reaching levels under the cut-point of the test. Along with antibody decreases, the number of abortions shows a clear low trend, during the months of May and June there were only 3 and 4 abortions respectively. The low S/P levels and the decrease in abortion numbers have shown an improvement in productive parameters of site 1 compared to previous years and as time progresses and with the program still in place the parameters also improve in sites 2 and 3 of the farm.

DISCUSSION

The production parameters show stability after the immunoglobulin program was started. Some of the parameters improved were the number of births, live born piglets, average born piglets, weaned piglets, average weaned piglets and percentage of mortality in nursing. These results confirm what has been found in other studies performed by Investigación Aplicada, S.A. de C.V.

CONCLUSION

The placement of an immunoglobulins based program on a PRRS outbreak is capable of diminishing S/P levels and improve productive parameters.

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Effects of the use of specific avian immunoglobulins to control PRRS on the productive parameters of site 1

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INTRODUCTION

The reproductive form of PRRS is usually produced during the acute phase of the disease and may affect a variable percentage of animals, up to 50%. The objective of the study was to determine the effect of the use of avian immunoglobulins, specific against PRRS virus on productive parameters of site 1.

MATERIAL AND METHODS

Data of the productive performance of 2009, 2010 up to June 2011 was obtained from a farm located in Northern Mexico. The farm is PRRS positive and has periodic outbreaks; in March 2010 an immunoglobulins based program was established aiming to control PRRS in the breeding swine, replacements and before birth. The data was analyzed comparatively using descriptive statistics. The only change was the application of avian immunoglobulins specific against PRRS.

RESULTS

The year to compare was 2009 when the farm was not under a control program with immunoglobulins, the program was established March 2010.

There was a 2.1% improvement in nursing sows from 2009 to 2010 even though the first semester of 2010 had the most important PRRS outbreak recorded in this study, by 2011 it is expected to have an 8.1% improvement over the values obtained in 2009. The number of born piglets has also increased by 15.88% between 2009 data and expected projections for 2011; born piglets per litter improved 7.4% and total born piglets improved substantially, 25.62% comparing 2009 data against 2011. Total dead born piglets decreased 40% between 2009 and 2011, total mummies decreased 70% comparing the same years; the number of mummies per litter also decreased significantly.

DISCUSSION

Even though efforts have been made to improve biosecurity measures, these have not prevented infections on their own; nevertheless it was proven that the use of avian immunoglobulins specific against PRRSv improve productive parameters and thus increase the profitability of companies that place it as a preventative and corrections method that is strengthened by sanitary and biosecurity measures.

CONCLUSION

The use of avian immunoglobulins against PRRSv is a fundamental tool to stabilize farms with PRRS problems that substantially improves production parameters of site 1 that will over time benefit sites 2 and 3.

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Use of avian immunoglobulins specific against PRRS virus in “cluster” of farms in northern Mexico

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INTRODUCTION

Porcine Reproductive and Respiratory Syndrome is one of the most important problems in the swine industry. Certain characteristics of the virus: viremia (30 days) and prolonged persistence (135 days) as well as not knowing every factors that produce sterilizing immunity and zone dispersion, make control in production areas difficult. Due to the above, the current approach on PRRSv is to control it and subsequently eradicate the disease regionally. The objective of this study was to evaluate the effect of the use of avian immunoglobulins specific for PRRS control in a “cluster” of farms in Northern Mexico.

MATERIAL AND METHODS

10 farms in Northern Mexico were selected for this study, they have an average of 1500 production sows. The program began by application of the immunoglobulins to all breeding swine with a second dose 15 days later, this operation is repeated every 4 months; two applications to gestating sows followed at the 70 and 85 days of gestation and to replacements 15 days before and on the day of their introduction to the farm. Biosecurity measures, cleaning and disinfection of facilities were reinforced, also a flow deviation was performed 12 weeks after the program began since PRRSv negative piglets were first found, thus avoiding contact between populations with different sanitary status. The protection level of sows is monitored every 6 weeks by means of ELISA tests (IDEXX, USA) and Real-time Polymerase Chain Reaction (PCR). 5 sows with different numbers in births were selected and identified with earrings in order to monitor them throughout the study. Additionally, piglets were sampled at 8, 10 and 12 weeks after the program started in order to corroborate negative piglets production by means of real-time PCR. The data obtained from serologic tests and productive parameters recorded during the immunoglobulin based program will be compared to the farm historic records.

RESULTS

The information gathered corresponds to 6 months after the program began and is currently being analyzed; a preliminary analysis of the data shows a substantial improvement of the productive parameters during the program, S/P levels in sows have a low trend and even though some sows are positive to ELISA there is no clinical evidence of the infection and real-time PCR results have been negative, all farms have initiated production of negative piglets corroborated by real-time PCR and productive parameters of site 2 have been improving and will likely improve site 3 results in the short-medium term.

DISCUSSION

The use of control strategies applicable on a regional level is one of the most viable options to control PRRSv, in this case the use of immunoglobulins provided stability in antibody levels of the breeding swine and favored production of negative piglets, which along with a strict biosecurity program are kept in that status, thus turning porcine production companies into more profitable entities.

CONCLUSIONS

The use of avian immunoglobulins as a strategy to control PRRSv on a regional level proved to be an adequate tool for the stability and improvement of the productive parameters in farms.

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Different immunobiological features of two genetically distinct type 2 PRRS Viruses

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Although it has been generally accepted that porcine reproductive and respiratory syndrome virus (PRRSV) induces weak and delayed protective immunity after infection, it is still unclear that the same immunological concept can be applicable to all PRRS viruses because huge genetic variation exists even in the same genotypes of PRRSV (type 1 or type 2). In the current study, two genetically distinct type 2 PRRS viruses (A and B) which showed approximately 90% nucleotide homology based on ORF5 sequences were characterized based on both *in vivo* and *in vitro* assessments to determine the immunobiological features of the viruses. For *in vitro* assessment, porcine alveolar macrophage (PAM) collected from 3-week old PRRSV-free pigs were infected with each of the two viruses at 1×10^3 TCID₅₀/ml then supernatants and cells were collected separately at 0, 1, 3, 6, 9, 12, 24, 36, and 48 hrs post infection to determine the relative expression level of IL-1, IL-10, TNF- α , and INF- α/β by RT-PCR and/or ELISA. In addition, five PRRSV-free pigs were inoculated with either of A or B virus for *in vivo* assessment. At 6 week post inoculation, the pigs were crossly inoculated with each of the viruses to assess the level of cross protection between the viruses. Serum and whole blood samples were collected before virus inoculation and every week thereafter until 8 weeks post inoculation. All of the pigs were euthanized at 8 weeks for pathological evaluation. The serum samples were analyzed for the levels of viremia, nucleocapsid-specific antibody and virus neutralizing antibody. PBMC was also prepared from the whole blood samples and cultured with each virus for 24 hrs to determine the expression level of IL-1, IL-4, IL-10, IL-12, and IFN- γ . Based on the *in vivo* and *in vitro* assessments, the two viruses showed different patterns of cytokine expression in PAM and different level of virulence and immune responses in pigs after infection. B virus induced a significantly higher level of neutralizing antibody and protection parameters as compared to A virus whereas B virus induced a significantly lower level of IL-10 expression than A virus. These results indicate that genetically distinct PRRS viruses could have different immunobiological features and defined immunobiological features could be used to classify various PRRS. The genes responsible for the different immunobiological feature between the two viruses need to be determined in the future.

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Strategies for reducing the economic impact during an outbreak of PRRS in a pig farm 1.000 bellies in western Mexico

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Objective: To use the tools with which Mexico has in PRRS control and reduce the economic impact of this outbreak could generate.

Material and methods:

In a pig farm located 1.000 belly in western Mexico, where it is located about 40% of Mexico's swine inventory. In late October, 2010 (Week 44) initiated an outbreak of PRRS characteristic with abortions (7%), stillborn pigs (+12%), reduction of piglets born alive, premature birth, agalactia in females and high mortality of piglets in motherhood.

Two weeks after the start of the outbreak (week 46), the whole herd vaccinated with **Modified Live Virus Ingelvac PRRS (PRRSMLV)** with the intention of accelerating pollution and raise the status herd immunity against PRRS. Two weeks later (week 48) was applied to all females **ImmunoPRRS 5ml (avian immunoglobulins Against PRRSV)** in order to limit viral circulation.

Results.

From week 49 there were no abortions, stillbirths and maternal mortality has improved, but from week 1 of 2011 saw an improvement in litter size, live births, fertility among others.

Week 1 of 2011 saw an improvement in litter size, live births, fertility, among others.												
	OUTBREAK				PRRS MLV		INMUNOPRRS					
Week	42	43	44	45	46	47	48	49	50	51	52	1
Fertility	88	84	80	70	70	72	75	80	80	84	84	84
Partos	51	50	50	51	45	43	43	44	48	50	50	50
Total Born piglets	530	520	518	529	468	447	445	450	487	510	540	560
born alive piglets	497	476	478	466	407	382	376	390	433	456	496	515
Avg born alive	9.74	9.52	9.56	9.13	9.05	8.89	8.74	8.87	9.03	9.13	9.91	10.29
% mummies	4%	6%	5%	7%	9%	10%	11%	9%	7%	7%	5%	5%
% stillborns	2%	3%	2%	5%	4%	5%	5%	4%	4%	4%	3%	3%
Mortality in maternity	7%	6%	7%	9%	12%	12%	15%	13%	10%	8%	6%	6%
Total weaned	462	450	445	424	358	336	320	339	390	420	466	486
Weaned/sow/farrow	9.06	8.99	8.89	8.31	7.96	7.82	7.43	7.71	8.13	8.40	9.32	9.73

Conclusions:

Stop this case the use of PRRSV MLV vaccine during the outbreak with the application of ImmunoPRRS (avian immunoglobulins Against PRRSV) two weeks after vaccination to halt virus circulation, with resulting improvement in production parameters. There will be an economic assessment of the value of having a natural outbreak of about 16 weeks vs. the results obtained in this field.

In January the introduction of new females to the herd is quarantined after serving a 12-week-adaptation, fulfilling a vaccination schedule according to the region where we are. They have so far had two entries in the current herd females and females is 1500 and there has been no outbreak of PRRS repeated.

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Porcine Reproductive and Respiratory Syndrome virus (PRRSV) activating the production of interleukin-10 in macrophages is dependent on P38 MAPK pathway

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Previous studies demonstrated that IL-10 levels are enhanced in pigs infected with Porcine Reproductive and Respiratory Syndrome virus. Exposure of Bone marrow-derived DC, monocyte-derived mature DC, and porcine alveolar macrophage to PRRSV results in an increased secretion of IL-10. However, the exact molecular mechanism by which PRRSV increases the induction of IL-10 during infection remains less well defined. In this study, we report the effects of PRRSV infection on bone marrow-derived macrophages (BMDM) and monocyte-derived macrophages (MDM). Our results showed that PRRSV efficiently activated the production of IL-10 both in bone marrow-derived macrophages and monocyte-derived macrophages; and this effect is dependent on the activation of p38 MAPK pathway, not by the activation of ERK MAPK signal transduction pathways. Then, we examined which PRRSV proteins are involved in the up-regulation of IL-10 expression. Among all the PRRSV proteins tested, the gene encoding GP5 (ORF5) with 6xHistidin was able to induce the IL-10 expression. In addition, GP5-mediated IL-10 induction was abolished by inhibitors of p38 MAPK, suggesting that GP5-induced IL-10 expression was also regulated by p38 MAPK. We further showed that the first 119 amino acids constituted a region that mediates IL-10 expression as demonstrated by quantitative RT-PCR using GP5 deletion mutants. The study provides some insights into the mechanisms by which PRRSV induces IL-10 production.

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Role of Phosphatidylinositol-3-Kinase (PI3K)-dependent Akt Pathway in Porcine Reproductive and Respiratory Syndrome virus (PRRSV) replication

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We have previously reported that treatment of cells with a phosphatidylinositol 3-kinase (PI3K) specific inhibitor, LY294002, prior to porcine reproductive and respiratory syndrome (PRRSV) infection reduced virus replication in porcine monocyte-derived dendritic cells. Here, we further investigate which step of virus replication process is actually affected by PI3K inhibition. We focus on three major steps of virus replication process, which include viral gene transcription, viral protein synthesis, virus budding and release. Our results suggested that PI3K inhibition has reduced viral gene transcription by approximately 1.5-fold. Additionally, viral protein synthesis was dramatically reduced by PI3K inhibition as determined by Western blot analysis. We have also observed that PI3K inhibition slightly enhanced the apoptosis caused by PRRSV and facilitated the release of virus particles from infected cells. Overall, our data suggest that PI3K/Akt pathway plays important role in viral gene transcription, viral protein synthesis and efficient virus particle release for PRRSV.

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Porcine Reproductive and Respiratory Syndrome virus nucleocapsid protein induces IL-15 through the NF- κ B signal pathway

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PRRSV infection could interact with host innate immune systems by regulating the production of proinflammatory cytokines. The IL-15 is a major cytokine in the innate immune response which displays many immunoregulation activities. In this study, we showed that IL-15 was up-regulated in the porcine alveolar macrophages (PAMs), monocyte-derived macrophages (MDM), and monocyte-derived DCs after PRRSV infection both on mRNA and protein levels. Induction of IL-15 requires the PKC and NF- κ B signal pathways. To further investigate the mechanisms of IL-15 induction by PRRSV, the porcine IL-15 promoter pGL3 report vector was first cloned and constructed in our lab. Using Realtime-PCR and Dual-luciferase assays, we showed that the PRRSV nucleocapsid (N) protein could induce IL-15 production, and the induction of IL-15 was NF- κ B pathway-dependent. Furthermore, mutational analysis of pIL-15 promoter demonstrated that the NF- κ B element was essential for up-regulation of IL-15 by PRRSV N protein. We concluded that PRRSV N protein could induce IL-15 production by stimulating NF- κ B signal pathway for interacting with porcine IL-15 promoter to up-regulate IL-15.

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The recombination between two strains of PRRSV *in vivo* results in the generation of recombinant virus with higher pathogenicity

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The recombination is an important genetic mechanism for porcine reproductive and respiratory syndrome virus (PRRSV). In this study, we carried out experimental co-infection of two strains of PRRSV in pigs and cloned the viruses from sera of the co-infected animals by plaque assay, then analyzed the recombination events between the two viruses during the co-infection by sequencing the ORF5, ORF3 and Nsp2 genes of each cloned virus and using SimPlot and Genetic Algorithm for Recombination Detection (GARD) analysis, and finally investigated the growth ability *in vitro*, genomic characteristics and the pathogenicity of the recombinant viruses. The objective of our study is to provide scientific evidence for understanding the molecular mechanisms contributing to the variation and pathogenesis of PRRSV. Totally, 133 recombinant viruses out of the 352 plaque viruses were acquired from four of five infected pigs during days 7 to 21 pi upon co-infection of JXwn06-81c and HB-1/3.9c. The intragenic recombination and intergenic fragment exchange of the ORF5, ORF3 and Nsp2 genes between the two viruses exhibited different patterns. Of the three genes, Nsp2 gene showed more complicated recombination situation. Further analyses revealed that the growth ability *in vitro* of two recombinant viruses was higher than the parental viruses. The pathogenicity of the recombinant viruses 14-1-3 and 10-3-2 were analyzed and compared with that of the parental viruses. The results showed that the pigs inoculated with the recombinant viruses displayed higher average rectal temperatures, viremia and scoring of clinical symptom and histopathological lesions compared to the pigs infected with the parental viruses, indicating that the recombinant viruses exhibited enhanced pathogenicity. Our data indicate that the co-infection with two strains of PRRSV with higher homology results in the emergence of recombinant virus in pigs, suggesting that extensive use of attenuated vaccine undoubtedly contributes to the increased diversity of PRRSV in field, and suggest that the recombination among different strains of PRRSV results in the generation of novel virus strain with higher pathogenicity. Therefore, the concerns associated with the potential risk caused by extensive use of attenuated vaccine of PRRSV should be emphasized.

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Comparison of immune transcriptome response following infection with PRRSV, PCV2 and SIV

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a major pathogen of swine. Here we identified and compared gene expression changes in tracheobronchial lymph nodes (TBLN) following viral infection using Digital Gene Expression Tag Profiling (DGETP). Pigs were infected with 1×10^5 cell culture infectious dose 50% per pig of PRRSV (strain SDSU73), porcine circovirus type 2 (PCV2; group 2), or swine influenza virus (SIV; A/SW/OH/511445/2007). Pigs were allotted to one of 4 treatment groups: sham inoculated control, PRRSV-challenge, PCV2-challenge, or SIV-challenge. On 0 dpi pigs received an intranasal challenge with 2 ml of either sham or virus inoculum. Control pigs were sham inoculated with tissue culture supernatant. Five pigs from each group were euthanized and necropsied on 1, 3, 6, and 14 dpi. Body temperatures of pigs euthanized on 14 dpi were recorded daily. At necropsy, lungs were scored for gross lesions. TBLN were homogenized and aliquots used for RNA extraction or immunophenotyped by flow cytometry analysis and cytokine expression. Total RNA was pooled for each group and timepoint to make 16 libraries, for analysis by DGETP using the whole-genome expression analysis platform (Illumina Technologies). Data underwent image analysis, base calling, and standard filtering to generate a list of sequence tags and counts. Multidimensional statistical tests and clustering analysis identified significant changes in tag abundance. PRRSV infection reduced the unique tag sequences (i.e., transcriptome diversity) in the TBLN transcriptome at 1, 3, 6 and 14 dpi to 55.7%, 69.3%, 56.4% and 55.5% of control TBLN. PCV2 infection increased the unique tag sequences to 95.0%, 111.6%, 148.7% and 106.8% of controls whereas, SIV induced a more modest reduction in unique tag sequences at 87.1%, 67.6%, 100.7% and 70.9% of the respective control transcriptome at 1, 3, 6 and 14 dpi. Tags were annotated with available transcript information from 7804 swine RefSeq sequences and 240420 HarvardGI Accessions. GenBank and Refseq virus sequences were used to determine and subtract viral tag counts. For select genes of interest, transcript changes were validated by real-time RT-PCR, and transcripts with changes in abundance were mapped to known metabolic, signaling and other pathways/networks. Comparative studies will define the unique aspects of a PRRSV infection and this insight into how PRRSV causes disease may aid development of more efficacious vaccines.

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Immunohistochemical characterization of type II pneumocyte proliferation after PRRSV (Type I) challenge

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The aim of the study was to characterize immunohistochemically the lung lesions after a challenge with a recently isolated PRRSV field strain in growing pigs. Methods: 9-week-old PRRSV negative pigs were challenged with 2.2×10^5 TCID₅₀ of a Type 1, subtype 1 virulent PRRSV field isolate. Negative control pigs were inoculated with virus free cell culture supernatant. Animals were euthanized on 10 DPI (n=7) and 21 DPI (n=5). Lung lesions were compared to age matched pigs of the non-infected control group.

In the first phase of the study lung lesions were evaluated on routine HE stained slides. The microscopic evaluation of the lung lesions was performed as a blinded analysis and the lesions were scored based on the following criteria: (1) pneumocyte hypertrophy and hyperplasia, (2) septal mononuclear infiltration, (3) intraalveolar necrotic debris, (4) intraalveolar inflammatory cell accumulation and (5) perivascular inflammatory cell accumulation.

For further characterization of the lung lesions, immunohistochemical stainings were performed using anti-cytokeratin, anti-Ki67, anti-TTF-1 (Thyroid Transcription Factor-1) and anti-myeloid receptor (MAC387) antibodies to identify alveolar epithelial cells, proliferating cells, type II pneumocytes, and macrophages, respectively. In case of Ki67, TTF-1 and MAC387 the labelled cells were counted in 50 non-overlapping and consecutively selected high magnification fields of 0.20 mm². SPSS software was applied to carry out statistical analyses.

The evaluation of the immunohistochemical stainings revealed that humanized anti TTF-1 antibodies can successfully identify type II pneumocytes in porcine lung tissues. Marked proliferation of these cells was confirmed by a significant ($p < 0.05$) increase of TTF-1 positive cells in acute cases compared to the lungs of control pigs. Upregulation of Ki67 and MAC387 positive cells was also observed, however due to the relative low number of the sample animals and high values of standard deviation, the increase of these values were found not to be statistically significant. Cytokeratin labelling marked the type I, and type II pneumocytes as well as bronchial epithelial cells, however this staining was not suitable for cell counting purposes.

When the routine histological scores were compared to the number of immunohistochemically positive cells, Ki67 cell counts were found to show positive correlation ($p < 0.05$) with the overall severity of the lesions.

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Pathogenicity and immune response in the lung of pigs experimentally infected with diverse genotype I PRRSV strains, including a pathogenic subtype 3 strain

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The evolution and diversification of porcine reproductive and respiratory syndrome viruses (PRRSV), particularly within genotype 1, is known to affect the clinical outcome of infection and poses significant problems in producing efficacious and broadly protective vaccines. The aim of this study is to characterise the *in vivo* response to a diverse range of genotype I PRRSV viruses.

Animals were inoculated with the prototype type I strain, Lelystad virus (LV), a recent British field strain (215-06), both subtype 1 viruses, or a recently isolated subtype 3 strain from Belarus (SU1-bel). Clinical scores and temperatures were monitored daily, post-mortems were performed at 3, 7 and 36 days post-infection (dpi), when lung gross pathology was scored and broncho-alveolar lavage fluid (BALF) was collected. The phenotype of immune cell populations within the BALF was determined by flow cytometry using an SWC1, SWC3 and SWC8 stain for B and myeloid cells and CD3, CD4 and CD8 stain for T lymphocytes and natural killer cells. Virus load in the BALF was determined using quantitative real-time PCR.

Animals infected with the SU1-bel strain developed a fever and had significantly higher clinical scores compared with the other infected groups which developed sub-clinical disease. At post-mortem, lungs from these pigs were found to have higher gross pathology scores at both 3 and 7 dpi. Two animals had to be euthanized at 14 dpi, all other animals recovered well with temperatures falling around day 10 and clinical signs disappearing at 16 dpi, gross pathology was absent at the final post-mortems at 36 dpi. Virus load was higher in the lungs at 3 and 7 dpi for SU1-bel pigs compared to the 215-06 infected group, although surprisingly they were similar to pigs infected with LV. At day 7, there was an influx of both myeloid and lymphoid cells into the lungs of infected pigs, which was most striking in the SU1-bel group. SU1-bel infected pigs presented higher numbers of CD8^{hi} T cells and mature neutrophils compared with the others. Interestingly, only the LV and 215-06 infected groups displayed elevated numbers alveolar macrophages, with SU1 infected pigs having numbers comparable to that of uninfected controls.

This study describes the characterisation of the response to another pathogenic subtype 3 strain, distinct from Lena, highlighting the importance of this subtype and its potential impact on pig health in Europe. A clear difference in the populations of immune cells within the BALF of these animals compared with animals infected with other strains indicates a potential cause of the increased pathology. Experiments are on-going to test the hypotheses that (1) infiltration by neutrophils, potent activators of inflammation and tissue damage, may explain the increased gross pathology seen following SU1 infection, and (2) the influx of cytotoxic lymphocytes into the lung following infection may represent an important virus control mechanism.

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Identification of host microRNAs associated with PRRSV infection using an *in vitro* model

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MicroRNAs (miRNA) are small non-coding RNAs and are associated with gene regulation at the post-transcriptional level. MiRNAs possess multiple functions, including modulation of the immune system. Studies have found that viral infections induce changes in the expression of miRNAs of infected host cells. The objective of this study was to fully characterize miRNA expression changes induced by a PRRSV infection *in vitro*. Swine alveolar macrophages (SAMs) from three seven-week old pigs were either infected with PRRSV isolate VR-2332, m.o.i. 10, or were mock infected. Total RNA was isolated at 0 hpi, 12 hpi, 24 hpi, and 48 hpi and small RNA populations were enriched. These small RNA populations were then used to generate 21 libraries for deep sequencing using an Illumina GAIIX platform. Each library was given a unique tag for sequencing in a single lane. For each library, high quality sequences with an overall quality score of ≥ 15 were sorted and identical sequences were grouped using the Galaxy sequencing analysis suite (<http://main.g2.bx.psu.edu/>). Sequences were then searched against miRBase (the miRNA database; www.mirbase.org) and Rfam (database of RNA families; <http://rfam.sanger.ac.uk/>), using the DSAP small RNA sequence analysis pipeline (<http://dsap.life.nthu.edu.tw/>) to determine sequence reads representing known miRNAs and other types of small RNA, such as tRNA. In the case of potential miRNAs, their sequences were compared to the porcine genome, using NCBI (<http://blast.ncbi.nlm.nih.gov/>) and Ensembl (<http://www.ensembl.org/index.html>), to determine their identity and genomic locations. The secondary structures of the identified sequences and their flanking regions were determined using the mFold algorithm (<http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form>). Overall the total number of high quality sequencing reads ranged from 499,848 to 790,003 per library. At least 200 known miRNAs were identified in each library. The expression of multiple miRNAs was affected by PRRSV infection. Among the identified miRNAs, miR-30a-3p was represented by a higher number of sequence reads in the small RNA libraries generated from mock infected macrophages at 24 hpi and 48 hpi than from VR-2332 infected macrophages. Target prediction analysis suggests that miR-30a-3p is involved in the regulation of the response to dsRNA and GTPase activity, as well as leukocyte differentiation and activation. MiR-331-5p was represented by a higher number of reads from infected macrophages at 24 hpi compared to the mock infected controls. MiR-331-5p is likely involved in the regulation of cell-cell signaling and cell surface receptor-linked signaling transduction. More sequencing reads represented miR-99a from libraries produced from mock infected SAMs than from libraries produced from PRRSV infected SAMs at 12 hpi. MiR-99a is predicted to target multiple genes associated with phosphatase and kinase activity. These results suggest the PRRSV infection induces changes in the expression of host miRNAs and that these miRNAs are involved in the regulation of multiple host cell processes.

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**Characterization of Asian highly pathogenic strains of
type 2 PRRSV**

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Highly pathogenic PRRSV (HP-PRRSV) isolates of Type 2 PRRSV appeared in China in 2006 and were genetically similar to 2007 isolates from Vietnam. Virus was rescued (rJXwn06) from an imported infectious cDNA clone of a 2006 Chinese strain. Purified RNA from the Vietnamese isolate prepared by the National Veterinary Services Laboratories-USDA-APHIS was used to produce an infectious clone and rescue virus (rSRV07). Our goal was to examine the viruses in vitro and to compare and contrast the ability of the viruses to replicate in U.S. swine in the absence of other biological factors present in Asia. We also compared the genomes of these HP-PRRSV strains.

Multistep growth kinetics in MARC-145 cells revealed that at 24 hr (peak titer), rJXwn06 (1.75×10^7 plaque forming units (PFU)/ml) replicated ~3-fold higher than both rSRV07 and VR-2332 (5.5×10^6 PFU/ml), but rSRV07 attained similar titers to JXwn06 at later times. Both Asian strains had similar plaque morphologies to VR-2332, but were both 1.5X larger. These data revealed that the Asian strains had an increased ability to cause cytopathic effect in MARC-145 cells when compared to strain VR-2332, but that rJXwn06 replicated to higher titers than rSRV07. Northern blot analysis showed that the Asian strains were fairly devoid of defective RNAs, but they had similar levels of all nested subgenomic (sg) RNA when compared to each other. However, some sgRNAs were less abundant when compared to the full-length viral RNA.

We examined the consequences of intranasal low (2 ml of 10^3 TCID₅₀/ml) and high dose (2 ml of 10^6 TCID₅₀/ml) inoculation of Chinese strain rJXwn06 and Vietnamese strain rSRV07, as well as strain VR-2332. VR-2332-infected pigs showed mild clinical signs of PRRSV infection. However, both low and high dose rJXwn06 inoculants suffered severe morbidity and mortality, as noted previously, whereas those inoculated with SRV-07 suffered severe morbidity, but no mortality. The results suggested a difference between the clinical diseases caused by the two Asian isolates. The relative clinical results parallel the ability of the three viruses to replicate in swine. Both low and high dose rJXwn06-infected swine (5.1×10^6 TCID₅₀/ml) had 20-fold higher viral peak loads than both doses of swine infected with rSRV07 (2.6×10^5 TCID₅₀/ml) and 700-fold higher loads than VR-2332-infected swine (7.4×10^3 TCID₅₀/ml).

Genomic alignment and comparison between the three PRRSV strains indicated that both Asian HP-PRRSV genomes had 89.1% nucleotide identity to that of VR-2332 and 99.4% identity to each other. The nucleotide differences seen between the two Asian HP-PRRSV strains were spread throughout the genome, but they both retained the identical bipartite deletion in nsp2 when compared to VR-2332. Eighteen amino acid changes in ORF1 were seen, primarily in nsp1 β , nsp2 and nsp7. One to two amino acid changes were also predicted in GP2, E, and GP3-5, and five nonconservative changes were seen in N. Our results suggest that Asian strains vary in their ability to replicate in vitro as well in the animal, and mirror clinical disease seen in U.S. swine. Furthermore, there was no virus challenge dosage effect on the clinical outcome seen in Chinese strain rJXwn06 infected pigs. The difference in disease outcome between rJXwn06 and rSRV07 may be studied by site-directed mutagenesis of the two viral strains to determine the regions of the genome responsible for differences in highly virulent Asian PRRSV strains.

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Validation of a major quantitative trait locus associated with host response to experimental infection with PRRS virus

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Vaccination is commonly used in the prevention of many livestock diseases. An alternative method to improve animal health is through genetic improvement of the host. The objective of this study was to validate the genetic basis of host response to PRRS virus that had been discovered in earlier trials using data from the PRRS Host Genetics Consortium PRRS-CAP project. The five PHGC trials analyzed each had approximately 200 commercial crossbred pigs that were infected between 18 and 28 days of age with virus isolate NVSL 97-7985. The first three trials consisted of pigs from a cross between commercial Large White (LW) and Landrace (LR) lines. Trials 4 and 5 consisted of a Duroc sire line crossed with either a LW/LR/Yorkshire (Y) composite (4) or LR/Y dam lines (5). Blood samples and body weights were collected up to 42 days post infection (dpi). Pigs were genotyped with the Illumina 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). Across trials, heritability estimates using pedigree information were 23 and 30% for VL and WG, with maternal effects accounting for 17 and 10%, respectively. Phenotypic and genetic correlations between VL and WG were -0.31 and -0.38. Associations with single-nucleotide polymorphisms (SNPs) were identified using Bayes-B of GenSel software. Previous analysis of the first three trials identified a 33 SNP region (~1 Mb) on chromosome 4 (Porcine sequence build 10) that explained 15.7% of the genetic variance for VL and 11.2% for WG. This region was supported in the analysis of all five trials, explaining 22.6% of the genetic variance for VL and 19.2% for WG. Estimates from the analysis of the first three trials were used to predict the genomic breeding values (GEBV) of the pigs in trials 4 and 5. The correlations between the GEBV predicted for the 33 SNP region using results from trials 1-3 and phenotypes of pigs in trials 4 and 5 were 0.24 for VL and WG. The favorable effect of this region appeared to be dominant, with the favorable allele estimated to decrease VL by 5 units (0.66 phenotypic sd) and increase WG by 2.5 kg (0.63 phenotypic sd). The favorable allele had an effect regardless of whether it came from the sire or dam breeds involved in the crosses. The frequency of the favorable allele ranged from 0.09 to 0.21 across trials. In conclusion, the 1 Mb region on SSC4 explains a substantial proportion of the genetic variance in response to experimental challenge with a specific strain of the virus. The effect of this region has been identified in unrelated populations and does not appear to be breed specific. This work was supported by the PRRS CAP, USDA NIFA Award 2008-55620-19132, the NRSP-8 Swine Genome and Bioinformatics coordination projects, the National Pork Board and the breeding companies involved in the PRRS Host Genetics Consortium that provided pigs.

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Porcine Reproductive and Respiratory Syndrome virus inhibits signaling of type I IFNs by blocking ISGF3 nuclear translocation

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Type I interferons (IFNs), IFN- α/β , play an important role in innate immunity against viral infections by inducing antiviral responses. Porcine reproductive and respiratory syndrome virus (PRRSV) inhibits the synthesis of type I interferons (IFNs) and interferes with IFN signaling pathway. Suppression of innate immunity is believed to be an important contributing factor to the PRRSV modulation of host immune responses. The objective of this study was to examine the mechanism of PRRSV inhibition of IFN-activated signaling pathway. After IFN treatment, both the signal transducer and activator of transcription (STAT), STAT1 and STAT2, undergo phosphorylation, form heterotrimers with IRF9, and translocate to the nucleus to activate the expression of interferon-stimulated genes. In PRRSV-infected cells, IFN-induced phosphorylation of both STAT1 and STAT2, and their heterodimer formation were not affected. However, majority of the STAT1/STAT2/IRF9 heterotrimers (ISGF3) remained in the cytoplasm of PRRSV-infected cells, which indicates that the nuclear translocation of the ISGF3 was blocked. Overexpression of PRRSV NSP1 β inhibited nuclear translocation of STAT1, which suggests that NSP1 β is the viral protein inhibiting the IFN signaling. Furthermore, NSP1 β interferes with the interaction of STAT1 and nuclear import factor karyoperin- α 1. This result indicates that NSP1 β blocks ISGF3 nuclear translocation via interfering with its interaction with nuclear import factor. Further study is being undertaken to elucidate the exact mechanism for the interference of IFN signaling.

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Effect of deoxynivalenol (DON) mycotoxin on porcine reproductive respiratory syndrome virus (PRRSV) *in vitro*

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Deoxynivalenol (DON) is a mycotoxin produced by *Fusarium spp.* DON should be treated as an important food safety issue because it is a very common contaminant of grains. Among monogastric farm animals, swine are the most susceptible to DON because it markedly reduces feed intake and decreases weight gain, even at low feed contamination. Investigations of host resistance, cell-mediated immune response and humoral immunity indicate that DON is both immunostimulatory and immunosuppressive depending on dose frequency and duration of exposure as well as type of functional immune assay.

The objective of this study was to investigate the *in vitro* effect of the DON mycotoxin on cytopathic effect and replication of PRRS virus in the permissive cell line MARC-145.

Non infected cells and cells infected with North American IAF-Klop PRRSV strain at 0.5 MOI, were treated with increasing concentration of DON mycotoxin (0, 70, 140, 280, 560, 1200 ng/ml). Cell survival was evaluated by determining the number of viable cells with a tetrazolium compound (CellTiter, Promega) after 48 and 72hrs of infection. Virus titration was performed after 0, 24, 48 and 72 hrs of infection. Finally, cytokines mRNA expression was evaluated, after 24 and 48 hrs of infection, by qPCR.

DON significantly affects the survival of non-infected cells at the concentration of 560 ng/ml or higher, but had no impact on cytokine mRNA expression of these cells. DON significantly increased the survival of cells infected with PRRS virus, at lower concentration, without affecting virus replication. However, high concentration of DON blocks virus replication, presumably by affecting cell survival. In PRRSV infected cells, low concentration of DON decreased cell expression of type-I interferon and TNF- α mRNA, but high concentration of DON increased mRNA expression of interferon- α but decreased mRNA expression of interferon- β and TNF- α .

The DON mycotoxin had a significant effect on the survival of PRRSV infected cells and on virus replication, in a dose dependant manner. For the moment, more experiment will be needed to determine the significance of these results, on PRRSV infection, *in vivo*. In order to answer this question, the effect of DON is currently evaluated on *ex vivo* PRRSV infected pulmonary alveolar macrophages.

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Enhancement of innate immunity with granulocyte colony-stimulating factor did not prevent disease in pigs infected with a highly pathogenic Chinese PRRSV strain

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Chinese highly pathogenic PRRSV (HP-PRRSV) strain JXwn06 has been shown to produce high fevers, loss of body condition, respiratory distress and death in pigs. Necropsy reveals extensive interstitial pneumonia, multi-systemic pathology and a high occurrence of secondary bacterial infections. The fulminant bacterial disease suggests dysregulation of the immune system that allows subclinical bacterial infections to become a major cause of morbidity and mortality in HP-PRRSV infected swine. We hypothesized that by enhancing innate immunity through administration of granulocyte colony-stimulating factor (G-CSF) we might attenuate secondary bacterial disease and the clinical effects of HP-PRRSV infection. G-CSF is a cytokine that stimulates production and release of functional granulocytes from the bone marrow and is used in humans to reduce the chance of infection in selected cancer patients receiving chemotherapy medications that induce neutropenia. Four-week-old pigs obtained from a PRRSV free herd were randomly assigned to treatment groups in a 2 X 2 design. Pigs received either an intramuscular injection of 10^{10} TCID₅₀/ml adenovirus vectored G-CSF (Ad5-GCSF, n=30) or adenovirus vector with no insert (Ad5-empty, n=30) on day -3, and on day 0 received an intranasal challenge with 2 ml HP-PRRSV 10^4 TCID₅₀/ml. A combination of Ad5-GCSF treated (n=5) and Ad5-empty treated (n=10) pigs served as sham inoculated controls. At the time of HP-PRRSV challenge, pigs treated with Ad5-GCSF exhibited a significant neutrophilia in response to the vectored G-CSF (>6 times increase in neutrophils by day 0). Despite G-CSF treatment, all pigs challenged with HP-PRRSV began exhibiting signs of disease by day 2 and had a rapid paradoxical decline in neutrophils to baseline levels. Disease progressed to 100% morbidity and mortality in both HP-PRRSV challenged groups. In contrast, non-virus challenged, Ad5-GCSF treated control pigs remained healthy and had a neutrophilia for the duration of the study. This study confirms the highly virulent nature of HP-PRRSV and its ability to easily overcome host defenses leading to disease and secondary bacterial infection.

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Fulminant sepsis is a cardinal sign of HP-PRRSV in pigs

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In 2006, a unique syndrome with high morbidity and mortality was recognized in growing pigs in China that became known as porcine high fever disease (PHFD). One consistent finding in affected pigs was the detection of porcine reproductive and respiratory syndrome virus (PRRSV) that had unique nsp2 gene mutations. Experimental infection of pigs with these novel isolates reproduced the clinical disease providing strong evidence for the role of PRRSV as the causal agent of PHFD. However, there was still a question if there was some unknown agent in the PRRSV preparations that increased the severity of the clinical disease over what was expected for a “routine” PRRSV infection. This question was resolved when PHFD was reproduced with virus derived from an infectious clone of the JX143 PRRSV isolate. These studies demonstrated that PRRSV isolates with a common genetic motif had a causal role in PHFD leading to this lineage of virus being called highly pathogenic PRRSV (HP-PRRSV). We imported a plasmid containing a full-length clone of the JXwn06 HP-PRRSV isolate. Infectious virus (rJXwn06) was rescued from the clone and used to inoculate young pigs in a series of studies. All non-vaccinated pigs given the rJXwn06 virus developed a pronounced fever about 2 days post inoculation (dpi) followed by anorexia and listlessness in most pigs and vomiting in some. From about 5 dpi until the end of each experiment pigs became very sick developing respiratory distress, ataxia, cachexia, diarrhea, red-blue discoloration of extremities and many became moribund at which time the pigs were euthanized for humane purposes. In contrast, pigs given VR-2332 challenge virus were much less affected with a mild to moderate fever and no mortality. Vaccination with a commercially available vaccine attenuated the clinical effect of the rJXwn06 challenge when compared to non-vaccinated challenge controls. However, many of the vaccinated pigs became very sick following challenge but were recovering by the conclusion of the experiment. Control pigs appeared clinically normal in all experiments with no microscopic or macroscopic lung lesions. VR-2332 infected pigs developed lung lesions consistent with a PRRSV infection. Severe pneumonia was observed in most of the HP-PRRSV infected pigs. Bacteria were isolated from lung lavage of 9/40, 0/29, and 86/100 control, VR-2332, and HP-PRRSV infected pigs, respectively. *Bordetella bronchiseptica* and *Haemophilus parasuis* were the only bacteria isolated from the controls (6/9 from one experiment), whereas a wide variety of typical respiratory bacterial pathogens as well as less frequently isolated opportunists were isolated from the HP-PRRSV infected group.

In summary, our studies with rJXwn06 virus are consistent with other HP-PRRSV reports. Based on our experience with experimental PRRSV infections, the HP-PRRSV isolate has a potent immunomodulating capacity that negatively affects the pig’s homeostasis, this was most striking in some experiments that had 100% mortality in the HP-PRRSV group.

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Non-structural proteins 1 α and 1 β down modulate tumor necrosis factor-alpha expression after porcine reproductive and respiratory syndrome virus infection

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes late-term abortion in sows and pneumonia in piglets. PRRSV evades the host immune response by several mechanisms, including the modulation of cytokine secretions in infected pigs. Particularly, PRRSV infection reduces the secretion of the pro-inflammatory cytokine, tumor necrosis factor- α (TNF- α). In this study, we characterized the viral proteins and amino acid sequences that are important for modulation of TNF- α expression after PRRSV infection. The pathogenic strain FL12, derived from a PRRSV infectious clone, consistently suppresses TNF- α in infected swine macrophages. Using a TNF- α promoter-reporter gene construct, we demonstrated that the viral non-structural proteins, Nsp1 α and Nsp1 β reduce the TNF- α promoter activity in transiently transfected cells, mainly by inhibiting the cellular transcription factors Nuclear Factor- κ B (NF- κ B) and Specificity Protein 1 (Sp1) respectively. Furthermore, screening of Nsp1 α mutant constructs revealed that five amino acid residues (Gly90, Asn91, Arg97, Arg100, and Arg124) are important for inhibiting the TNF- α promoter activity. Nsp1 α also reduced TNF- α protein levels in an *in vitro* translation assay, and Gly90 was important for this activity. Screening of Nsp1 β mutant constructs showed that multiple amino acid stretches in all domains are important for inhibiting the TNF- α promoter activity. We obtained two mutant vFL12 strains with substitutions at Nsp1 α Gly90 and Nsp1 β ⁷⁰SMVRE⁷⁴ positions. Both mutant viruses increased TNF- α mRNA levels at early times after infection when compared to parental vFL12 strain in infected macrophages. However, only Nsp1 α mutant virus induced higher TNF- α protein levels when compared to vFL12 in infected macrophage cultures. Moreover, Nsp1 β mutant virus was attenuated in infected pigs. In summary, we have identified viral protein sequences that are necessary for modulating the expression of the pro-inflammatory cytokine TNF- α after PRRSV infection.

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Molecular markers important for immunological responses during Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infection

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Host immune responses to PRRSV infection may be correlated with genetic control. The PRRS Host Genetics Consortium (PHGC) studies are aimed at identifying genes and pathways that are associated with pigs that clear PRRS virus while continuing to gain weight. Analyses of data from each PHGC trial [viral load from 0-21 days post infection (dpi) and weight gain from 0-42 dpi] were used to statistically identify four groups of pigs: those with the best phenotype, low virus and high growth (LvHg), high virus and high growth (HvHg), high virus and low growth (HvLg), and, the worst, low virus and low growth (LvLg). All RNA samples were converted to cDNA and subjected to real time PCR using primers corresponding to markers important for immune system activations involved in T helper 1 (Th1), Th2, and immunological tolerance pathways. Markers included the following: transcription factors TBX21 (T-bet), GATA3 (presumed Th1 and Th2 regulators, respectively), FOXP3, cytokines IL10, interferon gamma (IFNG), CD163, the PRRSV receptor, and CD69, the early marker for T lymphocytes (T-cells) activation and proliferation as well as primers for PRRSV detection.

LvHg animals at 4-10 days post infection (dpi) exhibited increased in ratio of TBX21/GATA3. LvHg animals demonstrated a robust increase in expression of IL10 and IFNG within 4-14 dpi, with high basal expression of CD69 relative to other groups at 0 dpi. However one LvHg animal was responsible for these changes. Although in HvHg the ratio of TBX21/GATA3 was the highest during PRRSV infection relative to other groups, the up regulation and expression of TBX21 and GATA3 was minimal, as well as that for other markers. HvLg animals had high base line expression (0 dpi) of GATA3 and FOXP3 transcription factors and delayed increase (10 dpi) of TBX21/GATA3 ratio. Similarly in LvLg animals a high basal expression of transcription factors GATA3 and FOXP3 was detected with delayed increased of TBX21/GATA3 ratio. In LvLg animals however higher baseline expression for CD163 and cytokine IL10 markers was detected as well. Taken together the HvLg and LvLg animals exhibited up regulation of the markers responsible for Th2 type immunity during PRRSV infection.

In summary, our data correlated basal expression of CD69 as a factor leading to up regulation and activation of markers responsible for Th1 type immune response; however, high basal expression of transcription factors GATA3 and FOXP3 demonstrated activation of the markers responsible for Th2 type pathway during PRRSV infection. These studies were supported by USDA NIFA Functional Genomic grant #2010-65205-20433 and National Pork Board grants.

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The role of PRRSV in the experimental reproduction of porcine circovirus associated disease

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Porcine circovirus associated disease (PCVAD) represents a group of diverse multi-factorial syndromes, characterized by clinical signs such as wasting, diarrhea, respiratory distress, dermatitis, progressive weight loss and reproductive failure. Overall, the onset of disease requires infection with porcine circovirus type 2 (PCV2), however, the lack of experimental models that can faithfully reproduce PCVAD has hindered the investigation of pathogenesis and vaccine development. In this study, a dual infection disease model was used to investigate the interaction between PCV2 and PRRSV. The experimental model involved 49 conventional pigs divided into 7 groups balanced according to weight and sex. Treatments for each group are as follows: Group 1-mock challenge only, Group 2-PCV2 vaccine only, Group 3-PRRSV challenge only, Group 4-PCV2 challenge only, Group 5-PCV2 vaccine-PCV2 challenge, Group 6-PCV2 challenge-PRRSV challenge, and Group 7-PCV2 vaccine-PCV2 challenge-PRRSV challenge. Vaccination with a commercial baculovirus expressed PCV2 capsid protein product was carried out on Day 0 and Day 21 of the study. On Day 35, pigs were challenged with 10^5 tissue culture infectious doses of PRRSV (Group3), PCV2 (Groups 4 and 5) or both PCV2 and PRRSV (Groups 6 and 7). Mortality during the 77 day trial reached 43% in the dual-challenged (Group 6) pigs. The principal effect of PRRSV infection was to increase the mean serum titer of PCV2 by almost 2 logs, along with massive accumulation of PCV2 antigen and lymphocyte depletion in lymphoid organs compared to pigs challenged with PCV2 alone (Group 4). PCV2 failed to show a reciprocal effect on PRRSV replication. Vaccination with baculovirus-expressed PCV2 CP prior to dual challenge prevented disease, blocked PCV2 infection, and delivered, according to the measurements of this study, sterilizing immunity. Overall, the results of this study highlight the significance of secondary factors, such as PRRSV, for the experimental reproduction of PCVAD, while providing a mechanistic role for PRRSV in the onset PCVAD. Moreover, the efficacy of a two-dose subunit PCV2 CP vaccine was demonstrated.

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Evaluation of immune responses to porcine reproductive and respiratory syndrome virus in pigs at day two post-infection

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes a persistent, economically devastating disease in pigs of all ages. Irrespective of regular vaccinations, control of PRRS has become a difficult task to swine farmers, and remained as a challenge. To comprehensively elucidate both cellular and innate immune cytokine responses at very early stage of PRRSV infection, seven weeks old pigs in a commercial research setting were infected and analyzed. One pig in a pen of 25 was PRRSV infected and responses were assessed two days later. All the 25 infected and a few of the 25 contact neighbor pigs were viremic. A majority of viremic pigs had more than 50% reduction in NK cell mediated cytotoxicity. At day 2 post-infection, in plasma nearly one fold increase in innate IFN- α production was detected. An enhanced secretion of IL-4, IL-10, and IL-12 (but not IFN- γ) in a majority of infected pigs was also observed. In addition, a reduced frequency of myeloid cells, CD4⁻CD8⁺ and CD4⁺CD8⁺ T cells and upregulated frequency of Foxp3⁺ T-regulatory cells were detected in viremic pigs. Interestingly, all the viremic contact pigs also had comparable immune cell modulations. The replicating PRRSV present in both infected and incontact pigs was found to be responsible for rapid modulation in NK cell function and modulation in secretion of innate immune cytokines, resulting in rapid subversion of host innate immunity. As the study was performed in pigs maintained in natural commercial environmental settings, results of this study have practical implications in developing protective PRRSV vaccines. This project is supported by USDA-NIFA PRRS CAP2 award to MPM and RJG.

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Ontogeny of PRRSV nucleocapsid-specific antibody isotypes in pigs after experimental infection

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Effective use of serology to determine the stage of PRRSV infection is one of challenges for prevention and control of PRRS. The objective of this study was to characterize kinetics of virus-specific IgG, IgM and IgA in pigs over time after experimental infection with VR2332 strain. Sera were collected from 27 pigs in 14-day intervals from 0 to 202 dpi and tested for various isotypes specific for viral nucleocapsid. The pigs comprised 4 groups based on the presence and absence of initial inoculation at 0 dpi and re-inoculation at 193 dpi: A) inoculated/re-inoculated; B) inoculated/not re-inoculated; C) not inoculated/inoculated; and D) negative control. All samples were randomized first and, after 1:5 dilution, tested on IDEXX PRRS ELISA X3 kits using swine IgG-, IgM- or IgA-specific goat antibody labeled with HRP and TMB substrate. Optical density was measured at 450nm. Three sera with known isotype status and 2 kit controls were included in each plate to assess plate-to-plate variation. All samples and controls were run in duplicate. Group D stayed seronegative until 202 dpi. After inoculation, pigs developed IgG by 7-14 dpi. The IgG lasted at high level until 202 dpi and was not boosted up by re-inoculation, suggesting that IgG is a good indicator of exposure. IgM appeared by 7 dpi and then rapidly declined. By 28 dpi, no IgM was detected. Re-inoculation did not elicit IgM response whereas group C developed IgM antibody sharply, indicating that IgM response can be indicative of first and recent exposure in naïve pigs. IgA appeared by 14 dpi, started to decline after 70 dpi to negative level by 182 dpi, and was boosted up by re-inoculation, suggesting that IgA response may align better with host immune status to PRRSV. In conclusion, isotype-specific serologic assessment can be useful to determine infection and immune status of pigs.

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Antiviral effect of various mutagens against PRRS Virus

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Porcine reproductive and respiratory syndrome (PRRS) caused by PRRS virus (PRRSV) is a disease of swine characterized by severe reproductive failure in adults and respiratory distress in all ages of pigs. Modified live vaccines (MLVs) are widely used to protect PRRS virus infection because inactivated vaccines do not provide sufficient protection against PRRSV. Because PRRSV mutates at a high rate in nature and MLV can revert to virulent wild-type virus, the safety of MLV has become a great concern. In the current study, the replication of VR2332, a prototype of North American PRRS virus, in MARC-145 cells was assessed in the presence of four different types of antiviral mutagens (Ribavirin, 5-Fluorouracil, 5-Azacytidine and Amiloride hydrochloride hydrate) which are known to increase mutation frequency of RNA viruses above the tolerable error threshold during viral replication and drive the viruses into extinction. To determine the effect of mutagens on PRRSV replication in MARC-145 cells, the cells were treated with each mutagen before or after virus inoculation (B or A method, respectively). For B method, MARC-145 prepared in 48 hrs was incubated with growth media containing each of four antiviral mutagens at the concentration of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mM for 4 hrs before virus inoculation. On the other hand, the cells were replenished with growth media containing each of the antiviral mutagens at the same 6 concentrations described above after virus inoculation for A method. Then supernatants were collected every 24 hrs up to 4 days and tested for virus titration. In addition, cytotoxicity of the mutagens in MARC-145 was measured by a cytotoxicity assay. Among the four different mutagens, Ribavirin and 5-Fluorouracil effectively suppressed the replication of PRRSV without causing high cytotoxicity, whereas 5-Azacytidine and Amiloride hydrochloride hydrate showed antiviral effect against PRRSV replication but were highly cytotoxic. As Ribavirin and Fluorouracil have significant antiviral effect against PRRSV without causing high cytotoxicity, these might be good candidates for therapeutic agents against PRRSV infection though their efficacy and safety need to be evaluated based on *in vivo* assessment.

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Interaction of PRRSV genotype I and II with plasmacytoid dendritic cells

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PRRSV has been considered as a virus evading innate immune recognition in its primary target cells through interaction of particular viral proteins with the IFN signalling cascade. This is of importance for local replication, but the overall IFN type I load at the level of the organ or the whole organisms is probably linked to the interaction of PRRSV with plasmacytoid dendritic cells (pDC). These cells represent the most potent source of IFN type I and are considered the primary source of systemic IFN type I during virus infections. This IFN type I is known to influence innate and adaptive immune responses. Although during PRRS IFN responses are generally low when compared to influenza virus, transmissible gastroenteritis virus and classical swine fever virus, they have been detected both in the serum of infected animals and in the lung. Consequently, we are investigating the ability of PRRSV to activate or to suppress pDC including the biological elements controlling these responses. Our results demonstrate that both genotype I and II strains of PRRSV can activate pDC to produce IFN- α responses in the same range as classical swine fever virus. Addition of certain cytokines such as IFN- α , IFN- β , Flt3-Ligand or GM-CSF can enhance these responses up to 5-fold. On the other hand in only certain experiments we observed a suppression of CpG-induced pDC activation reaching levels of a maximum 30-50% inhibition. Current investigations focus on factors of importance in pDC activation by PRRSV including the involvement of TLR7, the requirement of live virus and replication, the interaction of infected macrophages and macrophage products with pDC, and the impact of PRRSV-specific antibodies on pDC responses. The knowledge obtained represents a critical part of the puzzle to understand how PRRSV interacts with the porcine immune system.

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Substantial isolate-dependent variability in the type I interferon response of a porcine alveolar macrophage cell line to porcine reproductive and respiratory syndrome virus

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The molecular mechanism(s) used by porcine reproductive and respiratory syndrome virus (PRRSV) to modulate the type I interferon (IFN) responses of a targeted cell in the pig, namely alveolar macrophages, has not been detailed. Such efforts in part have been hindered by the susceptibility of only a fraction of the population to PRRSV infection. In contrast, when utilizing the unorthodox but permissive MARC-145 cell as the host, the activities of its transcriptional factors CREB/ p300, NF κ B, IRF3 and SP1 that are responsible for IFN α/β gene expression were found to be inhibited in the presence of PRRSV nsp1. However, these studies relied on the protein from a single virus isolate and thus did not consider the possibility of strain variation in regards to the extent of the virus's ability to suppress an otherwise expected production of type I IFN by the infected cells. Consequently, here several PRRSV isolates were evaluated in regards to their effect on the secretion of IFN- α by ZMAC cells, a line that was established from fetal alveolar macrophages and is readily infected by this virus. Interestingly, the impact on IFN- α production was strain-dependent in that while the cells appeared non-responsive in the presence of either of two field isolates, copious amounts of this cytokine was released by ZMAC cells exposed to a third individual. This "unconventional" isolate was also unique in that it induced type I gene transcription and stimulated the phosphorylation of IRF3 to a greater extent than was observed in those Z-MAC cells infected with either "conventional" virus. Since the phosphorylation of a second transcriptional factor, NF κ B, also involved in the initiation of IFN- α/β gene transcription was comparatively unaffected in any of the virus-infected cells, the "conventional" viruses may be blocking IFN- α production by interfering with a step(s) in the pathway leading to the activation of IRF3.

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Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on U.S. pork producers

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An economic analysis published in 2005 estimated that productivity losses from clinical porcine reproductive and respiratory syndrome (PRRS) virus infections cost U.S. pork producers \$560 million dollars annually. Since the 2005 study, pig production and health strategies have evolved, PRRS virus control/elimination strategies have improved, and structural adjustments have occurred in the industry. Because of these developments, it was reasonable to question whether the incidence, severity, and/or impact of PRRS outbreaks on pig health and productivity in the U.S. herd may have changed since the 2005 study was conducted. The primary objective of the 2011 study was to estimate the current economic impact of PRRS virus in the U.S., taking into account the noted changes in the industry. Data for the economic analysis was compiled from several sources: (1) swine health surveillance data collected by the USDA National Animal Health Monitoring System (NAHMS) from commercial U.S. pork producers; (2) a survey of swine veterinary experts on the incidence and impact of clinical PRRS on pig production efficiency; and (3) a survey of production records recorded during the period 2005 to 2010 from commercial farms with known PRRS virus status.

Breeding herds were categorized both by their current PRRS status and whether they had experienced a PRRS outbreak in the previous 12 months. Groups of growing pigs were categorized according to their PRRS status at placement and at the time of marketing. Similar to the 2005 analysis, a budgeting model was applied to each of the breeding herd categories using estimates for productivity measures obtained from the survey of production records. An estimate of the percent of breeding herds in each category was obtained from the expert opinion survey. A “CURRENT” scenario was defined as the average outcome for all PRRS categories, weighted according to the percentage of swine breeding herds and groups of growing pigs in each PRRS category. The “WO PRRS” scenario assumed 100 percent of breeding herds in the U.S. were in PRRS virus-free herds and 100 percent of groups of growing pigs were negative at placement and at closeout.

The present study estimated the total cost of PRRS in the U.S. national breeding and growing pig herd at \$664 million annually (\$1.8 million per day); an increase of approximately \$104 million from the \$560 million annual cost estimated in 2005. The 2011 study differed most significantly from the 2005 study in the allocation of losses between the breeding and the growing pig herd. Specifically, losses in the growing pig herd accounted for 88% of the total cost of PRRS in the 2005 study compared to 55% in the current analysis. Differences between the 2005 and the 2011 studies may be attributed to changes in the prevalence of PRRS virus, incidence of outbreaks, pathogenicity of virus strains, production and animal health management practices, inflation and other pathogens that have emerged since 2005 such as porcine circovirus type 2 (PCV2). In addition, information on veterinary costs, biosecurity costs, and other costs from the survey of expert opinion were used to estimate an annual costs attributed to PRRS virus. The total additional costs attributed to PRRS for veterinary, biosecurity and other outbreak related costs were \$477.79 million putting the cumulative cost of the disease at more than \$1 billion per year when added to production-related losses.

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Adjusting disease freedom confidence for imperfect diagnostic accuracy: A review of the evidence for non-traditional diagnostic specimens tested for PRRSV

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PURPOSE: Regional Area Control and Elimination projects require cost effective options to monitor for PRRSV including diagnostic specimens and collection tools other than venipuncture. Provided the test performance is known and reproducible when run on alternative samples, the interpretation of disease freedom confidence can account for imperfect performance. This process must be transparent because the results can affect the entire region. This review was undertaken to determine if sufficient evidence was available in published literature to make such adjustments.

METHODS: Structured searches were applied to citation databases. Relevant publications between 2000 and 2010 were reviewed to obtain test sensitivity, specificity, validity, reliability and reproducibility for PRRSV diagnostic tests on the following venipuncture-alternatives: PCR: pooled blood swabs, blood on filter discs (FD), oral fluids (OF), OF on FD, milk, meat transudate and tonsil swabs; ELISA: pooled serum, blood swabs, blood on FD (individual and pooled), OF, milk, and meat transudate.

RESULTS: No option had comparable yet independent peer-reviewed publications from which diagnostic sensitivity, specificity, or reliability distributions could be estimated without substantial assumptions. Tests for OF with PCR and pooled serum with ELISA had sufficient validation detail reported to recommend research focus on reproducibility. Remaining options require continued research on reliability and reproducibility.

DISCUSSION: Despite promising developments for monitoring PRRSV, there was insufficient evidence to make recommendations on how the confidence in results from venipuncture-alternatives should be adjusted for test performance. Future publications describing test validation should adhere to recommended standards to ensure that results are maximally useful. Laboratories are encouraged to report test validation results to enable further statistical evaluation of test accuracy.

Diagnostic performance of a commercial PRRS serum antibody ELISA adapted to oral fluid specimens: longitudinal response in experimentally-inoculated populations

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Previous work (AAVLD abstract “Adaptation of a commercial PRRS serum antibody ELISA to oral fluid specimens”) showed that a commercial PRRS ELISA (HerdChek® PRRS X3 ELISA) could be adapted to detect anti-PRRSV IgM, IgA, and IgG in oral fluid specimens. Further, the protocol for the IgG ELISA for oral fluid samples was readily amenable to the routine performance of the assay in high throughput diagnostic laboratories. This suggests the possibility of a cost-effective method to routinely monitor commercial swine populations for maternal antibody, vaccination compliance, and herd immune parameters using oral fluid sampling.

The purpose of the present study was to evaluate the ability of the PRRS oral fluid IgG ELISA to detect anti-PRRSV IgG antibody in pen-based oral fluid samples from experimentally inoculated pigs over time. In nine trials, ~200 pigs per trials were intramuscularly (IM) inoculated with PRRSV isolate NVSL 97-7895. Oral fluid samples were collected on 0, 5, 7, 9, 11, 14, 17, and 21 days post inoculation (DPI). All oral fluid samples were randomized and tested for anti-PRRSV antibodies using the PRRS ELISA protocol for oral fluids: 1:2 oral fluid sample dilution, 16 hour incubation at 4°C, reaction detected using anti-swine IgG_{FC}). Anti-PRRSV IgG antibodies were detected as early as 7 DPI and all samples were positive by DPI 9. These results indicated that the ontogeny of anti-PRRSV antibodies in oral fluid is amenable to rapid detection of infection. Testing based on oral fluids could provide an efficient, cost-effective approach to PRRSV monitoring in commercial herds and surveillance in elimination programs.

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Adaptation of a commercial PRRS serum antibody ELISA to oral fluid specimens

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Oral fluid samples are increasingly used for the surveillance of PRRSV infection in commercial swine operations using PCR-based assays (Chittick et al., 2011; Kittawornrat et al., 2010). While PCR-based assays are useful for detecting the circulation of PRRSV, antibody-based assays are informative regarding herd immunity and history of prior infection. The feasibility of detecting antibody in oral fluids has already been addressed: antibody-based assays using oral fluid specimens are already widely available in human diagnostic medicine for a variety of pathogens (Prickett et al., 2010). The purpose of the present study was to optimize a commercial PRRS ELISA (HerdChek® PRRS X3 ELISA) to the oral fluid matrix. ELISA parameters assessed in the optimization process included: sample volume, sample dilution, incubation time, secondary antibody isotype (IgM, IgA, IgG_{H&L}, IgG_{Fc}), and secondary antibody dilution. To reduce oral fluid sample-to-sample response variation during this process, 11 oral fluids (“Reference Standards”) were used in the optimization process to measure the effects of changes in parameters. Reference standards were collected from one commercial wean-to-finish barn (1,100 pigs) prior to the day of PRRS vaccination (Ingelvac® PRRS MLV) and on DPV 0, 10, 15, 20, 28, 35, 41, 49, 56, 75, and 91. (Reference standards available upon request.) Results showed that the ELISA was readily adapted to detect IgM, IgA, and IgG in oral fluid specimens. The protocol that we have developed for detection of IgG is readily amenable to the routine performance of the assay in a diagnostic laboratory.

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Cross-reaction between PRRSV variants isolated in Korea

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a significant economic damage on the swine industry in Asia. High rate of genetic change and diverse genetic variant infection were most serious obstacles to the effective vaccination and prevention of PRRS. Especially china highly pathogenic PRRSV also be found one of the genetic variants. Thus, the following study was conducted to corss reactivity between genetic heterologous strain of PRRS

Materials and methods

Komi-NA263 and Komi-NA316, PRRSV field strains were isolated from tissue samples and total RNA was extracted from each sample. The ORF5 gene was amplified and sequenced. Multiple sequence alignment was carried out using sequence analysis software, Lasergene1 to determine sequence homology and phylogenetic relationship among the viruses. A total of 15 PRRSV seronegative 3 weeks old pig purchased and randomly allotted to three groups. Pigs in each group were inoculated intranasally with 2 ml of PRRSV komi-NA263 isolate ($10^{5.0}$ TCID₅₀/ml), komi-NA316 isolate ($10^{5.0}$ TCID₅₀/ml) or DMEM media as placebo. At 7, 14, 21 and 28 days post-injection, pig serum were collected to perform ELISA and serum neutralizing assay. Antibody titers (IgG) were determined using the commercially available PRRSV antibody detection kit (HerdCheck PRRS; IDEXX, USA) according to the manufacturer's instructions and SN titre was assessed by visual determination of cytopathic effects and taken as the highest dilution of serum.

Results

To confirm the genetic differency of the 2-isolated virus, the ORF5 gene sequence of PRRSV was amplified by RT-PCR using the North American type PRRSV-specific primers, and a 703-bp product was specifically amplified. These were compared to each other Korean isolates in GenBank belong to the NA-type, Komi-NA 263 and Komi NA 316 were equivalent korea segment 1 and 4 (Fig. 1). Two field isolated PRRSV Komi-NA263 and Komi-NA316 shared 94% and 87% nucleotide sequence homology with VR-2332 for ORF5 (Data not shown). In komi-NA263 and komi-NA316-infected pigs, specific antibody was detectable by 7 dpi, increased quickly by 14 dpi and thereafter remained high for both groups(Fig. 2). Serum samples were further evaluated for the ability to neutralize against PRRSV strain Komi NA263 and Komi-NA316 *in vitro* using serum neutralization assays. As shown in Table 1, pigs infected with Komi-NA263 developed significantly increased up PRRSV-specific neutralizing antibody titer (1: 8 or 16) against Komi-NA263 but against PRRSV strain Komi-NA316 was a significantly lower cross-neutralizing activity (1: 2 or not).

Table 1. Neutralizing antibody assay against isolated PRRSV strain Komi-NA 263(A) or Komi-NA 316(B)

Group	Neutralizing antibody titer (2 ⁿ)									
	1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B
infected with Komi-NA263	2 ⁴	2 ¹	2 ⁴	ND	2 ³	2 ¹	2 ⁴	2 ¹	2 ⁴	ND
infected with Komi-NA316	2 ¹	2 ⁴	ND	2 ³	ND	2 ³	2 ²	2 ⁴	2 ¹	2 ¹
Control (as placebo)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

a* Infected with Komi-NA 263 pig serum

b* Infected with Komi-NA 316 pig serum

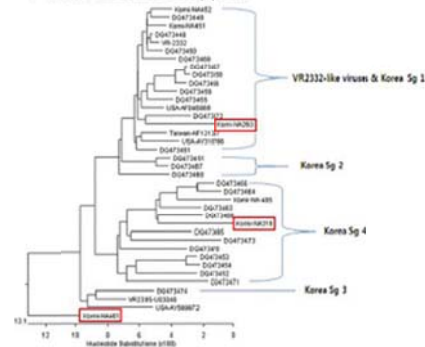


Fig. 1. Phylogenetic tree of isolated PRRSV viruses and selected sequences of other Korea segment and North American genotypes based on GP5 sequence.

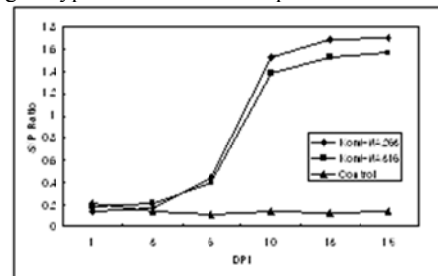


Fig. 2. Serum antibody response for PRRSV in pigs infected with the Komi-NA263 or Komi-NA316. The antibody response was investigated using the Herd chek PRRS antibody test kit. S/P ratios greater than 0.4 are considered positive.

Discussion

This study provides clear relationship between different PRRS genotypes and the cross reactivity of them. Moreover through this study, we can get that how we can develop effective vaccines against genetically diverse PRRSV infection

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Evaluation study of interventions for reducing the risk of PRRSV introduction into filtered farms via retrograde air movement (back-drafting) through idle fans

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Purpose

The objectives of this study were to demonstrate that the entry of PRRSV-contaminated aerosols into a filtered facility via retrograde air through unfiltered points (i.e. idle fans) is a true risk; to titrate the minimum air velocity necessary to introduce PRRSV-contaminated aerosols via retrograde air; and to validate commercially available interventions that have been designed to prevent this risk.

Methods

The study, conducted at the UMN SDEC production regional model site, used an empty facility under negative pressure ventilation. In order to create retrograde air movement through an idle fan, one of the 2 fans was intentionally stopped while the other continued to operate. The retrograde air moved through a common plastic shutter intervention at several fan stages. Each stage was challenged with 10 replicates of different PRRSV concentrations (1 through 7 logs each) which were generated using a cold-fog mister located on the exterior of the facility. A cyclonic collector was used to collect a sample from each replicate. To titrate the air velocity needed to transfer PRRSV, an anemometer was utilized. The measurements of retrograde air velocities and static pressures were collected for each fan stage.

Interventions evaluated included the standard plastic shutter, plastic shutter plus canvas cover, nylon air-chute, aluminum shutter plus air-chute and, double shutter system (aluminum and plastic shutters). All 5 interventions were challenged as described in order to determine whether aerosolized PRRSV could penetrate the different treatments.

Results

The results of this study suggest that a real risk of PRRSV entry may exist when there is a minimum retrograde air speed of 0.76 m/s. Performance of treatments according to challenge dose are shown in Table 1.

Table 1.

PRRSV concentration	A	B	C	D	E
10 ¹ TCID ₅₀ /L	10/10	4/10*	0/10*	0/10*	0/10*
10 ³ TCID ₅₀ /L	10/10	3/10*	0/10*	0/10*	0/10*
10 ⁵ TCID ₅₀ /L	9/10	3/10*	0/10*	0/10*	0/10*
10 ⁷ TCID ₅₀ /L	10/10	6/10	0/10*	0/10*	0/10*

A-E: Treatments

*: significantly different when compared to plastic shutter alone (p<0.05)

Conclusion

Results from this study indicate that unfiltered retrograde air movement is a risk for PRRSV introduction in filtered farms and that it requires a minimum velocity of air flow. Finally, this study confirms that not all interventions designed to reduce this risk are effective.

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Molecular epidemiology of highly pathogenic Porcine Respiratory and Reproductive Syndrome (HP-PRRS) variants in Southeast Asian countries (Vietnam, Laos and Cambodia)

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Porcine respiratory and reproductive syndrome (PRRS) is an economically important infectious disease in the swine industry all over the world. The causative virus is a member of the *Arteriviridae* family in the order of *Nidovirales*. In 2006, a highly pathogenic PRRS (HP-PRRSV) outbreak occurred in China killing approximately 2 million heads of pig. The progeny variants of this outbreak not only continue to affect Chinese swine industry from 2006 till now, but the virus has spread across border to several Southeast Asian (SEA) countries including Vietnam, Laos, Cambodia, Thailand and Myanmar. In Vietnam, the highly pathogenic PRRS was first recognized in the northern part of the country in 2007 and later led to a countrywide epidemic affecting at least 65,000 pigs. The subsequent epidemic of HP-PRRS also started in the northern Vietnam in April 2010, and the virus rapidly spread southward in Vietnam (July 2010). HP-PRRS outbreaks were also confirmed in Laos PDR (June 2010) and in Cambodia (July 2010). To characterize the epidemiology of these PRRSV outbreaks in Southeast Asia, we sequenced 211 PRRSVs from Southeast Asia and incorporated them into current Chinese HP-PRRSV genome diversity database. Majority of sampling (n=187) were originated from a single introduction, which subsequent spread throughout the three SEA countries including Vietnam, Cambodia and Laos. Other SEA PRRSV sequences formed small clusters in the tree that are probably originated from several independent introductions. However, epidemiological impact remained unknown. In addition, our analysis indicated that the spreads of HP-PRRS among SEA countries are relatively frequent, probably due to connections of the pig industries in these countries. In summary, since the HP-PRRSV outbreak in China, the virus has been introduced more than three times into southeast Asia, followed by inter-country transmission of virus that amplified the intensity of their impact.

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Genetic diversity and evolution of PRRS virus strains circulated in pig herds during last 15 years in Slovakia

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded RNA virus belonging to the *Arteriviridae* family. It is the causative agent of significant respiratory and reproductive disease in swine worldwide. The virus is a recently emerged pathogen, being first identified as a cause of clinical disease in 1991 (Benfield, Wensvort et al., 1991). Virus PRRS was spread simultaneously in North America and Europe to evolve two distinct genotypes. Diversity within the two genotypes exists, and further division of PRRSV EU type into at least 3 subtypes has been suggested (Stadejek et al., 2006, 2008).

In Slovakia, surveillance program has been introduced in 1995 and immediately circulation of both genotypes PRRSV has been found. Sequence analyses of ORF7 from NA type of PRRSV shown close connection with strains circulated in Quebec (Psikal et al., 1999). During following years spreading of disease in the Slovakian pig population has been monitored with serological testing specific for particular genotypes. For detection of virus, virus cultivation, nested RT-PCR and later on real-time RT-PCR have been used.

To know more information about strains circulating during whole period, sequencing of ORF7 (Oleksiewicz et al., 1998) from selected strains has been introduced. Phylogenetic analyses have been performed together with sequences available in GenBank. Our results have confirmed spreading and long time perpetuation firstly introduced PRRSV strains together with new introductions of EU strains from different Europe countries. Together with field strains, spreading of vaccine strains have been confirm too. Sequences obtained in this study have been published in GenBank.

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Temporal evolution of PRRSV isolates in Sonora and analysis of recombination events

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The control and eradication of porcine respiratory and reproductive syndrome virus (PRRSV) is a future goal for the swine industry. In the present work we evaluated the temporal evolution of PRRSV from 2002 to 2009 in the state of Sonora, an important pig producing area in Mexico. The study was conducted with 148 ORF5 sequences that were obtained from farms of Hermosillo (HMO, n=14), Obregon (OBR, n=13) and Navojoa (NAV, n=5) from 2002 to 2009. Sequences retrieved from each farm were phylogenetically compared and classified in clusters. For a given cluster, an identity matrix provided the percentage of genetic variation within a given group of isolates within the farm. Two isolates were considered to be different when nucleotide similarity was $\leq 97\%$ and the isolates belonged to significantly different clusters (bootstrap values $>60\%$). The first sequence was obtained in Sonora in 2002 and corresponded with a strain that was only detected once in one farm. That isolate shared no more than 90% of similarity with any other PRRSV isolate in Mexico or abroad and was not detected again. In 2003 a new strain was introduced in several farms of the area of HMO belonging to the same company and disseminated to other companies and farms in HMO but was not detected in OBR or NAV. The closest isolates shared only 95% of similarity. This same strain was still detected in 2006 ($\geq 98\%$ similarity to the initial isolates). In 2005 a new isolate with no known related strains in Mexico or abroad ($<93\%$ similarity) disseminated in several farms of HMO. By 2009, an isolate sharing 97% similarity and belonging to the same cluster was still found in one farm indicating the persistence in the area of the 2005 isolate descendants. In 2009, most of the farms were infected by a new virus (1-18-2) which apparently has replaced the previous viruses circulating in the state.

Interestingly, in HMO, 6/14 farms had two or more different viruses co-existing in some of the years. In farms where vaccination was performed, the vaccine isolate could be detected along with one or more field isolates. Particularly, in two farms up to 6 different viruses were detected in a single year. These two farms shared some of the isolates with farms in OBR and NAV. In contrast, farms of OBR and NAV usually only have one virus at a time.

The use of a recombination detection program (RDP) suggested the presence of five potential viruses product of recombination; however, parental isolates were only established for two of them. This two recombinant isolates arose in one of the farms in HMO that was co-infected with multiple isolates simultaneously. Taking together the results of this study emphasize the importance of biosecurity as well as suggest that recombinant events can be frequent in multiple strains co-circulate within a farm.

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Effect of temperature and relative humidity on UV₂₅₄ inactivation of airborne PRRSV

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Objective In the last decade, researchers have verified the occurrence of airborne transmission of PRRSV over significant distances. The use of ultraviolet (UV₂₅₄) for the inactivation of airborne PRRSV is appealing due to the low cost of implementation relative to filtration, but data on the efficacy of the method is lacking. The objective of this experiment was to evaluate the UV₂₅₄ inactivation of airborne PRRS virus under a range of temperature (T) and relative humidity (RH) conditions.

Methods Airborne PRRSV was exposed to one of 4 levels of UV₂₅₄ under 9 defined conditions of T ($\leq 15^{\circ}\text{C}$, 16°C to 29°C , $\geq 30^{\circ}\text{C}$) and RH ($\leq 24\%$, 25% to 79% , $\geq 80\%$). Samples of air collected after UV₂₅₄ treatment were titrated for infectious PRRSV and the data used to calculate the UV₂₅₄ inactivation constants and UV₂₅₄ half-life ($T_{1/2}$) exposure doses for each combination of T and RH. Note: “inactivation constant” is the absolute value of the slope of the line describing the relationship between the survival fraction of the microbial population and UV₂₅₄ exposure dose.

Results The effects of UV₂₅₄ dose, T, and RH on PRRSV (TCID₅₀) recovered in air samples were all statistically significant ($p < 0.001$), as were the interactions between UV₂₅₄ dose*T ($p = 0.0475$), and UV₂₅₄ dose*RH ($p = 0.0204$). The derivation of inactivation constants and their associated UV₂₅₄ half-life doses for specific conditions of T and RH will make it possible to calculate the UV₂₅₄ dose required to inactivate airborne PRRSV under a range of laboratory and field conditions.

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Novel PRRSV ORF5a protein is not immunoprotective but drives GP5 glycosylation

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped RNA virus responsible for PRRS in swine; a disease with significant animal welfare and economic implications for which there is no specific treatment, and variable protection from vaccination due to viral genetic and antigenic diversity. Molecular mechanisms responsible for virulence, pathogenesis and protective immune response remain poorly understood. These factors limit progress towards development of effective measures for prevention and treatment of PRRS.

We have discovered a novel open reading frame (ORF) that is initiated upstream of and overlaps ORF5 encoding major envelope glycoprotein GP5. Presence of the ORF5a is evolutionarily conserved in all Arterivirus family members, and ORF5a protein is present in infected cells, incorporated into virions, and elicits antibody production in pigs infected with PRRSV. ORF5a protein has a highly conserved arginine-glutamine (RQ) rich motif arising from nucleotide sequence dually encoding the GP5 hypervariable glycosylation domain that is assumed to be driven by immunological selection. To investigate this paradox, 4900 PRRSV sequences were examined to determine codon usage and infer selective pressures on this region. We determined that purifying selection to maintain ORF5a protein drives GP5 reading frame variation through selective ORF5a RQ codon usage. This has implications for the variation in GP5 glycosylation pattern in this region where neutralizing epitopes have been described.

To determine if ORF5a was immunoprotective, pigs were immunized with ORF5a protein prior to virulent virus infection. Immunized pigs had consistent serologic responses which were not immunodominant. Antibodies did not neutralize virus, and robust antibody responses observed in some pigs did not translate into protection against viral challenge as evaluated by viremia. These findings indicate that ORF5a plays a role in viral biogenesis but does not elicit protective immunity.

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A high resolution characterization of spatial dissemination of Type 2 PRRSV in North America

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The evolution of PRRSV is highly dynamic in nature. An effective way to keep track of its dynamics is to link its epidemiological characterization with the underlying phylogenetic structure (i.e. evolutionary history). This approach has been proved to be successful in a previous study which characterizes the diversity and dynamics of Type 2 PRRSV based on the entire collection of GenBank ORF5 sequences. In this study, we increase the resolution of virus dynamics in North America with a more complete dataset (updated with more than 1000 Canadian samplings and 2008-2010 PRRSVdb sequences) and by using statistically robust Bayesian and maximum likelihood biogeographic approaches. After summarizing the viral transmission frequencies among pig producing regions and provinces, a more comprehensive picture of PRRSV traffic in North America is revealed. The estimated inter-regional PRRSV flows, as shown by the Mantel's R, has much higher correlation with the pig flow than with geographic distances or sampling size, which indicates transportation as a major driving force for long-distance PRRSV dissemination. In addition, we investigate the historical movement and predict the future expansion of PRRSV using an ancestral reconstruction approach. The results reveal an earlier separation of PRRSV population into Canada type (Lineage 1 and 2) and United States type (Lineage 6-9), followed by multiple unidirectional introductions (starting from late 1990s) of Canadian type PRRSV into the United States. The impact of these introductions is huge in Midwest US, where the Canadian type PRRSVs not only caused several major outbreaks (including the notorious MN184 outbreaks) but also are gradually replacing the indigenous diversity. Furthermore, independent invasions of these Canadian-type PRRSVs from Midwest to other regions are also observed, which poses potential risks of new outbreaks. In summary, this study reveals with high resolution the dynamics of Type 2 PRRSV in North America. The result provides direct implications for vaccine selection or design (for example, against Canadian type PRRSV) and disease control measures not only in global level but at individual farm as well. Our present investigation also calls for continuous surveillance through genetic sequences to keep in pace with future dynamics and evolution of this still uncontrollable virus.

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Analysis of circulation of porcine reproductive and respiratory syndrome virus in 22 Polish pig farms: implications for diagnosis and control

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Porcine reproductive and respiratory syndrome (PRRSV) is currently one the most important viruses of swine worldwide. Its control is difficult but even complete elimination is possible. Any control approach must involve careful evaluation of PRRSV circulation in the herd. PRRSV circulation depends on many factors of which herd management is likely the most important. In the present paper we communicate results of detailed analysis of PRRSV viremia and seroconversion performed in 22 Polish farrow to finish pig farms.

The analyzed herds were infected with PRRSV between 1996 and 2010. The farms represented enterprises with sow herds from 70 to 1400 heads. Different pig flow systems and PRRS control measures were applied. Serum was obtained from pigs of different age (between 2 and 23 weeks of age, depending on the farm, differing by 2-3 weeks). In most herds also sows were sampled. To assess homogeneity of the virological and serological status of a given age group pigs of the same age from several pens were sampled. In total, 3610 serum samples were obtained, (70 to 574 samples per farm). The sera were tested for the presence of PRRSV specific antibodies by in house indirect ELISA and for the presence of viral RNA by Real Time RT-PCR (PRRS NextGen, Tetracore). Serum samples were tested individually by ELISA and pooled (by 5) by PCR.

Results obtained in 9 farms (with sow herds of 70 to 850 heads) indicated no virus circulation in sows (PCR negative) nor in weaners (PCR negative and no, or waning colostral antibodies). In 5 of these farms PRRSV circulation was maintained in fattening units (PCR positive, increasing with age proportion of seropositive fatteners), while in remaining 4 farms no viremia nor seroconversion profile suggestive of infection was detected in any of the analyzed groups. In those, only seroconversion in sows and in young piglets was noted. In 6 farms of this group Porcilis PRRS (Merck Animal Health) and/or Progressis (Merial) vaccination was applied in gilts (3 herds) and/or sows (3 herds). In the remaining 13 farms viremia was detected by PCR in sows (4 herds) and piglets, before, or soon after weaning. Only 4 of these farms vaccinated sows and/or gilts against PRRS.

The PCR results showed differences in the PCR profiles between the farms. In some farms PRRSV was detectable from 4 up to 18 weeks of age. In others, positive PCR results were obtained only in few samplings. In one farm PCR result was negative despite serological evidence infection. On the other hand ELISA provided consistent results in all farms.

In summary, in farms where vaccination was used, in general better results of PRRSV control were obtained. However, herd management practices might contribute to the observed picture as well. Complete picture of PRRSV in a herd can be obtained only by testing large number of animals by serological and PCR methods. Oral fluid testing by ELISA and PCR can be a cost effective alternative to serum analysis.

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Development of indirect ELISA to detect antibodies to porcine reproductive and respiratory syndrome virus (PRRSV) in oral fluids of pigs

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Antibody detection in serum by ELISA is the most important method for porcine reproductive and respiratory syndrome (PRRS) diagnosis in pig farms. Accurate and useful results can be obtained only if large number of samples from pigs of different age are collected and analysed. Alternatively antibodies can be detected in oral fluids. However, no commercially available ELISA kit is designed for this. The aim of the present study is to present preliminary results on the development of indirect ELISA for the detection of PRRSV specific antibodies in oral fluids.

Serum and oral fluid samples were collected from 4 farms known to be free from PRRSV and 18 farms where PRRSV infection was previously identified. From 60 to 574 serum samples were obtained from each farm that represented pigs from 4 to 23 weeks of age. From each age group 10-25 serum samples collected from animals housed in 2-5 pens. From each pen one oral fluid sample was collected by hanging a piece of rope to which pigs had access for 30-60 minutes. From 12 to 36 oral fluid samples were collected from each farm. The antigen (capsid protein expressed in *E. coli*) for the oral fluid ELISA was produced and purified as described earlier, but the protocol was modified to address lower antibody concentration in oral fluid than in serum. The sensitivity of serum and oral fluid ELISAs to detect antibodies in individual pens (n=427) was compared using McNemar's test.

Specificity of oral fluids ELISA was high and only 1 of 55 samples from 4 PRRSV free herds reacted positive. Some discrepancies between the results of serum and oral fluid ELISAs regarding individual pens were observed in PRRSV positive farms but generally both methods provided similar serological profiles of these herds. Detailed statistical analysis will be presented during symposium.

The preliminary results indicate that the developed ELISA allows substitution of serum with oral fluid samples for surveillance and serological diagnosis of PRRS. This method can be a convenient, cost effective alternative to detection of PRRSV antibodies in serum. However, further studies in are needed to fully assess the test's specificity and sensitivity.

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**Proposal for ongoing targeted surveillance in the PRRS area
regional control and elimination (PRRS ARC&E) projects**

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Targeted surveillance has been recognized as one approach that can improve the efficiency of surveillance activities. The most straightforward application of the approach is when a veterinary authority is tasked with the goal of providing evidence of freedom from infection at a certain level. Surveillance activities are also important for endemic production diseases, but the framework within which the surveillance occurs is much different than for diseases exotic to this population. A particular challenge is the approach to surveillance during porcine reproductive and respiratory syndrome virus (PRRSv) area regional control and elimination projects (ARC&E). This is challenging because the surveillance system should be able to: (i) declare freedom from infection in a subset of herds that have successfully implemented elimination strategies, (ii) identify new infections in the area, particularly if they occur in herds that have a high risk of transmitting the infection, and (iii) monitor disease trends and other parameters over time. Fulfilling these objectives under conditions of scarce resources requires a strategic and structured approach regarding the inclusion of herds for ongoing surveillance, flexibility in accepting the results of different testing protocols, and the choice of appropriate testing procedures for specific tasks.

For the ongoing surveillance in the Niagara PRRS ARC&E we have proposed the implementation of a “high risk – high consequence” approach. The design and implementation of this approach will be presented. The approach requires the implementation of a short (25-question) survey that can be applied to all herd types without any modifications. The questionnaire assesses basic external biosecurity practices, willingness to eliminate the virus, and consequences of spread. A “surveillance algorithm” has been proposed regarding the incorporation of the most recent diagnostic information, external biosecurity level, potential infectiousness of herds, and phase of elimination into a targeted surveillance approach. Twenty eight “high risk – high consequence” herds within the Niagara PRRS ARC&E project have been selected for inclusion in the overall surveillance activity. Further refinements of the approach are expected.

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Baseline study in the Niagara region PRRS area regional control and elimination project (PRRS ARC&E)

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It is challenging to prevent the PRRSv infection of herds in pig dense areas, and disease elimination requires substantial resources. In addition, some of the recently-emerged PRRSv genotypes are more virulent. One strategy that is gaining popularity is a regional approach to PRRSv infection control. The first PRRS area regional control and elimination (PRRS ARC&E) program in Ontario has been initiated in the Niagara peninsula. The primary objective of the program is to implement a regional approach to infection control and to communicate the process both within the elimination area, and more broadly. The objectives of this study are to describe the baseline measurements and to report the findings of risk-factor analysis.

At the area-level, some inclusion criteria for this project were: moderate pig density, existing of administrative and natural borders, and the willingness of producers to participate. The project started in the summer of 2010. More than 95% of the producers in the Niagara area are participating, resulting in 76 herds in the program. Twenty two pigs on each site were blood sampled and tested with PRRS ELISA. Additional data obtained included demographic information and geographical coordinates.

Most of the herds (58%) included in the study were finisher barns. Twenty seven percent of the sites were sow barns. The distribution of other swine premises within a 3 mile radius around each of the sites varied bi-modally between a minimum of 0 (15%) and a maximum of 10 other sites (1.4%). The mode (19%) was 5 other sites. The herd level prevalence in the baseline study was 39%. In the positive sites, only one site was identified as having a single positive animal; all other positive herds had a minimum within-herd prevalence of 50%. Among other factors, the number of PRRSv-positive sites within a one mile radius was identified as a risk factor.

Distribution of within-herd prevalence suggests that a higher level of design prevalence could be justified for surveillance. Risk factor analysis should include membership in swine production networks, and data for such investigations should be included in routine data collection.

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Mathematical models of porcine reproductive and respiratory syndrome virus spread and control in sows and nursery pigs

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This research used observational data to estimate the basic reproductive number and other parameters of importance for dynamics of PRRS infection in a population. These parameters were then used to build mathematical models of PRRSv spread and control in sows and nursery pigs.

The observational data included results of PRRS Elisa test on blood samples from 795 newborn piglets sampled longitudinally from 7 swine herds in Ontario. Random effect linear model was used to estimate the duration of maternal immunity and random effect Poisson regression was used to estimate transmission parameter. Based on the parameters estimated from the data and additionally obtained from literature, the production stage-structured susceptible-infectious-resistant (S-I-R) deterministic mathematical model for sows and the age-structured maternally immune-susceptible-infectious-resistant (M-S-I-R) model for nursery pigs was built to include herd demographics and dynamics of PRRS virus infection in 1000-sows herd. The herd is assumed to be initially completely susceptible and seeded with one infectious sow. It is further assumed that transmission from piglets to sows does not occur, and that transmission between different batches of growing pigs does not occur. Control strategies included gilt acclimatization, herd closure, and mass immunization in different combinations.

Under the assumptions used in the mathematical model, herd-closure for at least 40 weeks can eliminate the virus if immunity against PRRSv in sows is assumed to be long-lasting. Mass immunization with 100% efficacy applied simultaneously to all sows could eliminate the infection if a herd was closed for at least 5 weeks after immunization. If the efficacy of mass immunization is below 100%, longer period of herd closure should be planned to control the disease.

Mathematical model is a useful tool to guide the control strategies. This study showed the possibility of PRRS elimination in a herd that is in general agreement with field observations. One of the critical parameters for PRRSv dynamics was a transmission parameter, which could be obtained from diagnostic data in a relatively simple way. Using diagnostic data, particularly from sow herds in early stages of infection, would allow building of stochastic models and further progress in this field. Duration of immunity was another parameter critical for the output of the examined control strategies.

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Association between PRRSV ORF5 genetic distance and differences in space, time, ownership, and animal sources among commercial pig herds

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Open reading frame 5 (ORF5) of the porcine reproductive and respiratory syndrome virus (PRRSV) genome is a variable region of the genome frequently used for diagnostic classification. The objective of this study is to test the association of genetic distance between pairs of PRRSV ORF5 sequences with the distance in space between the herds in which the viruses were collected; the separation in time between when the viruses were collected; and the distances in ownership, animal and semen suppliers between herds. Ownership and animal/semen supplier distances were binary measures of either the same or different between two herds. The partial Mantel test was used to test for correlation between genetic, spatial and temporal distances. Multivariable linear regression using simulation to generate *P*-values was used to test for associations of all variables with the outcome. Significant correlation was found between genetic distance and space ($r=0.10$) and time ($r=0.03$) after accounting for ownership similarity between herds. This correlation was limited to a threshold of < 30 km spatial separation. The slope of association of genetic distance with space and time was positive in herd pairs from different owners but for herds pairs for the same owner the relationship did not hold. Gilt and semen supplier distances were significantly associated with genetic distance in univariable models. After accounting for ownership the associations were overshadowed by the ownership effect. These findings are an indication that PRRSV is spreading among herds in Ontario, Canada. The spread between herds may be occurring by direct transmission between herds that are < 30 km from one another or via gilt and semen sources that are shared by many herds. The clustering of herds within ownerships is important in PRRSV spread and is important to consider in order to detect spatial patterns of PRRSV.

Meetings of Interest

The **2011 Conference of Research Workers in Animal Diseases (CRWAD)** Meeting will be held December 4-6, 2011, at the Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois.
For more information, go to: <http://www.cvmbs.colostate.edu/mip/crwad/>

Parco Tecnologico Padano will host a meeting on “Understanding and combating PRRS in Europe” on February 16-17, 2012 in Lodi, Italy. For more information, go to: www.tecnoparco.org/prrs

The 43rd annual meeting of the **American Association of Swine Veterinarians (AASV)** is scheduled for March 10-13, 2012 in Denver, Colorado, USA.
For more information, go to: <http://www.aasv.org/annmtg/>

European Federation of Animal Science 63rd Annual Meeting will be held August 27-31, 2012 in Bratislava, Slovakia. For more information, go to: www.eaap.org

The 4th annual **European Veterinary Immunology Workshop** will be held in Edinburgh, Scotland on September 2-4, 2012. For more information, go to: www.evig.org.uk

EUOPRRS 2012 will be October 10-12, 2012 in Budapest, Hungary. For more information, go to: www.euoprps.net

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