2014 North American PRRS Symposium

Final Program

Intercontinental Hotel
Chicago, Illinois

December 5-6, 2014

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David A. Benfield Student Travel Fellowships

David A. Benfield received his BS and MS degrees from Purdue University and a PhD from the University of Missouri-Columbia. He has a distinguished 24 years in research related to virus diseases of food animals. In 1990, he was the co-discoverer of the cause of "mystery swine disease" or porcine reproductive and respiratory syndrome virus (PRRSV). He has remained a role model and mentor to many of those who are currently in the PRRS field. He is a member of the American Society for Virology, American Association of Veterinary Laboratory Diagnosticians, and Honorary Diplomat of the College of Veterinary Microbiologists, American Association of Swine Veterinarians and the American Association for the Advancement of Science. Currently, he is the Associate Director of the Ohio Agricultural Research and Development Center, The Ohio State University and a Professor in the Food Animal Health Research Program in the College of Veterinary Medicine. It is his generous donation that initiated this fellowship program. It is his hope that these fellowships provide students with the experience of attending the International PRRS Symposium to present their work on PRRS.

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

2014 Student Travel Fellowship Recipients

Andreia Arruda	University of Guelph
Basavaraj Binjawadagi	The Ohio State University
Junru Cui	University of Connecticut
Jenelle Dunkelberger	Iowa State University
Jiming Gao	Northwest A&F University
Rui Guo	Kansas State University
Mingyuan Han	University of Illinois
Young Nam Kim	Kyungpook National University
Yanhua Li	Kansas State University
Qinfang Liu	Kansas State University
Graham Lough	Roslin Institute
Zexu Ma	University of Maryland
Mahesh KC	Tribhuvan University
Predrag Novakovic	University of Saskatchewan
Kang Ouyang	The Ohio State University
Nick Serao	Iowa State University
Ana Maria Stoian	University of Bucharest
Pablo Valdes-Donoso	University of Minnesota
Leyi Wang	Ohio Dept. of Agriculture
Huajian Zhao	Nanjing Agricultural University

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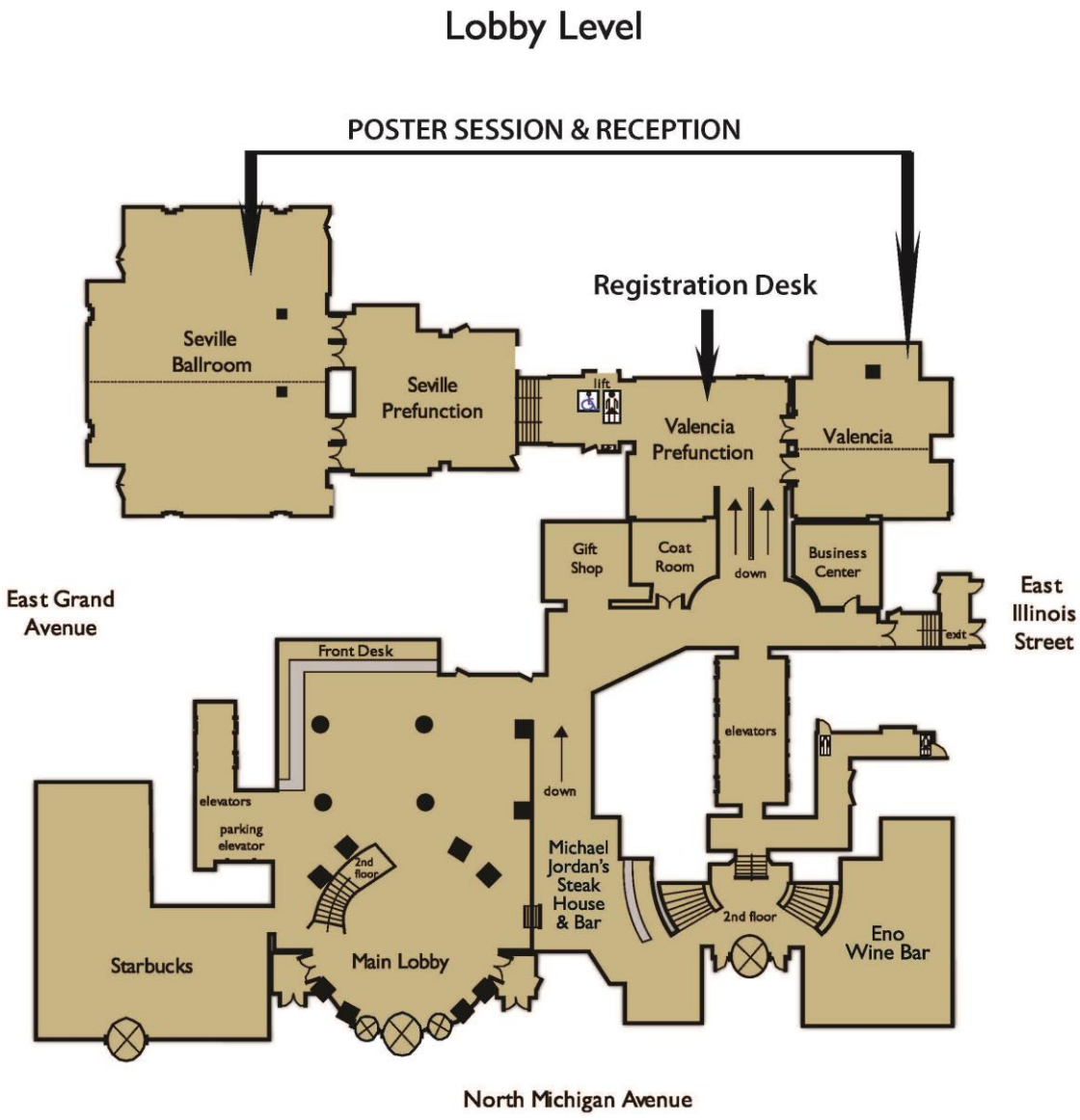
The 2014 North American PRRS Symposium Program has been approved for 12 hours of continuing education (CE) credit as approved by the Kansas Board of Veterinary Examiners. Kansas Board of Veterinary Examiners approves continuing education hours based on the guidelines set forth by the AAVSB RACE approval program for veterinarians and veterinary technicians. Signed CE confirmation forms will be available at the Registration Desk.

Intercontinental Hotel Maps



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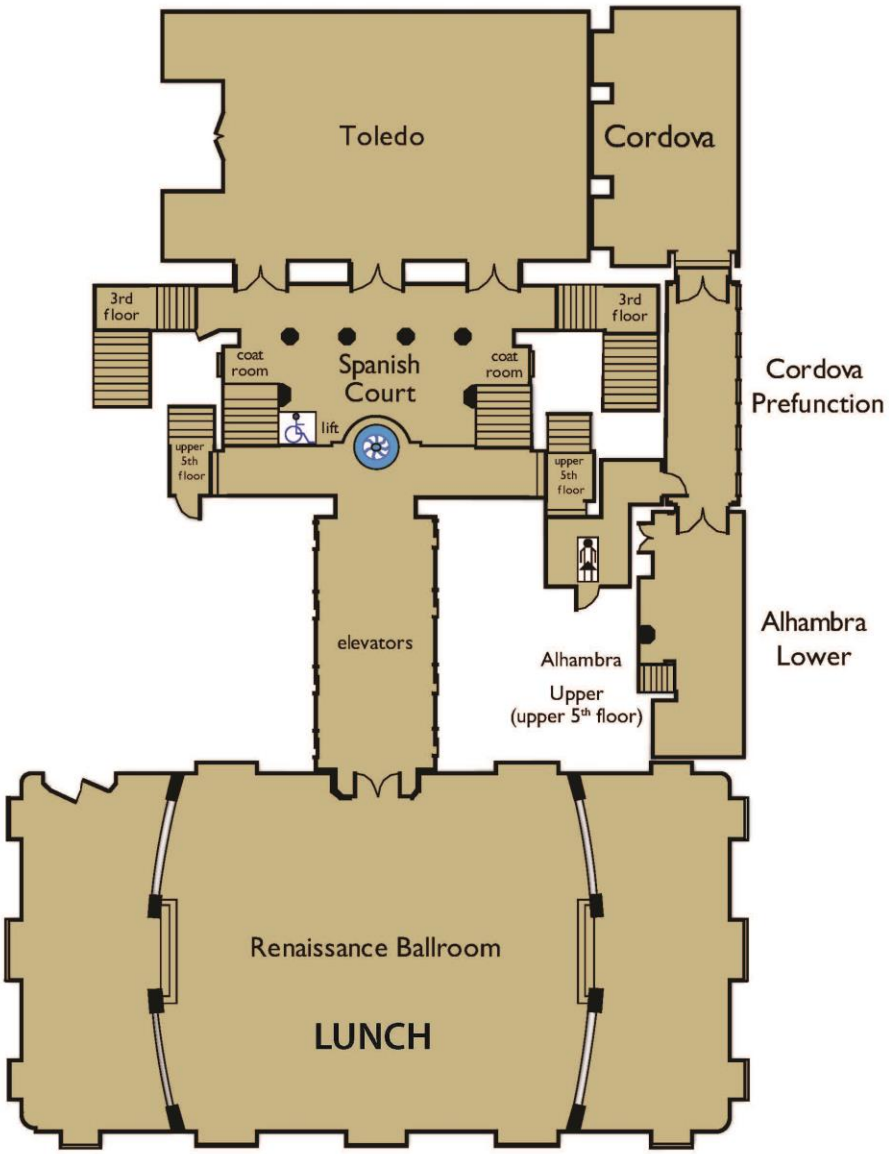
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Cordova
3rd floor
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coat room
Spanish Court
coat room
Cordova Prefunction
lift
upper 5th floor
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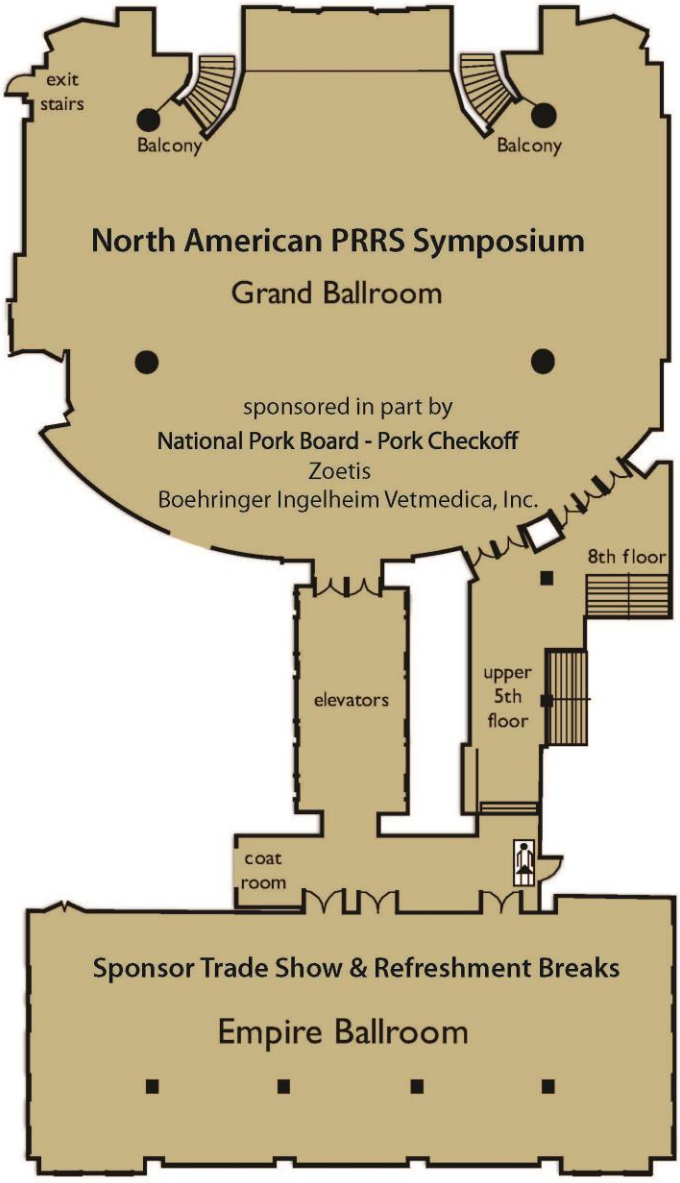
elevators
Alhambra Upper (upper 5th floor)
Alhambra Lower
Renaissance Ballroom
LUNCH

7TH Floor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2014 North American PRRS Symposium Program

Friday, December 5th

11:00am-3:00pm	Registration Open: Valencia Prefunction (Lobby Level)
11:00am-5:00pm	Poster Session Set-up: Seville and Valencia Rooms (Lobby Level)
Boehringer Ingelheim Sponsored PRRS Session: Efforts in Area Coordinated Disease Control **Grand Ballroom-Seventh Floor**	
1:00pm	Welcome and Introduction
1:05pm	Coordinating our Efforts for Area Disease Control *Dr. Jim Lowe-Session Moderator*
	Historical Perspective of Area Regional Control-PRV: What Lessons Apply to PRRSv Control and Elimination *Dr. Joe Connor, Carthage Vet Service*
	PRRS Epidemiology: closing some of our knowledge gaps *Dr. Andres Perez, University of Minnesota*
	Application of a System-Based PRRS Control Methodology – "a systematic approach for PRRS control within a region that is cost effective and brings documented, repeatable value" *Dr. John Kolb, BIVI*
	Q&A w/ speaker panel - moderated by Dr. Jim Lowe
3:00pm	Break (Grand Ballroom-Seventh Floor)
3:30pm	Tools and Their Application to Coordinated Disease Control
	How can we better evaluate sequences? The phylogenetic chart *Dr. Enrique Mondaca, BIVI*
	How can we view and communicate information easily? The Dashboard and Report Builder *Dr. Enrique Mondaca and Dr. Erin Johnson, BIVI*
	How can we collect important information on-farm? I-FormBuilder *Dr. Erin Johnson, BIVI*
	How can we work together to succeed? ARC and Vaccine *Dr. Poul Rathkjen, BIVI, Denmark*
4:50pm	Dr. Jim Lowe wrap up with summary take-homes
5:00pm	Adjourn session
5:30pm-7:30pm	**Poster Session-Seville and Valencia Rooms (Lobby Level)** *Special poster talks will be given from 6:00pm-7:00pm*

Saturday, December 6th

Special Session on PED and Coronaviruses **Grand Ballroom-Seventh Floor**	
8:00am	Opening Keynote Address- PEDV Immunology: Applications to Diagnostics and Vaccines *Michael Murtaugh, University of Minnesota*
8:45am	Nidoviruses: Making the PRRSV-Coronavirus Connection *Ying Fang, Kansas State University*
9:15am	PEDV in the US: Diagnostic Tools and Research Application in Diagnostics *Jinqiang Zhang, Iowa State University*
9:45am	Porcine Deltacoronavirus: Koch's Postulates Fulfilled *Dick Hesse, Kansas State University*
10:15am	Break (Empire Ballroom-Seventh Floor)
10:45am	Characterization of PDCoV and PEDV Infection in China *Yaowei Huang, Zhejiang University*
11:15am	Closing Keynote Address- Lactogenic Immunity and Vaccines for PEDV: Lessons from TGEV Trials and Tribulations *Linda Saif, The Ohio State University*
12:00pm-1:00pm	Lunch (Renaissance Ballroom-Fifth Floor)
General PRRS Session **Grand Ballroom-Seventh Floor**	
1:00pm	Surveillance in Today's Swine Industry *Jeff Zimmerman, Iowa State University*
1:40pm	The Use of Transgenic Pigs in PRRSV Research *Randy Prather, University of Missouri*
2:10pm	Reverse Genetics: 101 Uses for PRRSV Infectious cDNA Clones *Jay Calvert, Zoetis*
2:40pm	Break (Empire Ballroom-Seventh Floor)
3:00pm	Genetics of Host Response to PRRS *Jack Dekkers, Iowa State University*
3:30pm	Translational Genomics of PRRS *Joan Lunney, APDL, BARC, ARS, USDA*
4:00pm	Innate Immunity to PRRSV and Application to New Vaccines *Dongwan Yoo, University of Illinois*
4:30pm	Analysis of PRRSV Immunity at the Herd Level *Bob Rowland, Kansas State University*
5:00pm	Adjourn

Selected Abstracts for Short Presentations during Poster Session

Friday, December 5th

6:00-6:10pm: Martine Schroyen: Blood transcriptomics in response to porcine reproductive and respiratory syndrome (PRRS). Abstract #58.

6:10-6:20pm: Hiep Vu: Development of a synthetic porcine reproductive and respiratory syndrome virus strain that confers broader cross-protection. Abstract #84.

6:20pm-6:30pm: Pablo Valdes-Donoso: Spatial and temporal dynamics of porcine reproductive and respiratory syndrome (PRRS) in a voluntary regional project (N212). Abstract #106.

6:30pm-6:40pm: Qingzhan Zhang: Suppression of type I interferon response by nonstructural protein 1 of PEDV through degradation of CREB-binding protein. Abstract #61.

6:40pm-6:50pm: Daniel Fredrickson: Safety and antibody response of pigs to an experimental Porcine Epidemic Diarrhea Virus (PEDV) Vaccine, Killed Virus. Abstract #69.

6:50pm-7:00pm: Sakthivel Subramaniam: In vivo targeting of porcine reproductive and respiratory syndrome virus antigen through porcine DC-SIGN to dendritic cells elicits antigen-specific CD4 T cell immunity in pigs. Abstract #82.

ABSTRACTS

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44	GP5 expression in Marc-145 cells inhibits PRRSV infection by inducing beta interferon activity. *J. Gao[1, 2*], P. Ji[1, 2], M. Zhang[1, 2], X. Wang[1, 2], N. Li[1, 2], C. Wang[1, 2], S. Xiao[1, 2], Y. Mu[1, 2], Q. Zhao[1, 2], T. Du[1, 2], Y. Sun[1, 2], J.A. Hiscox[3], G. Zhang[1, 4], E.M. Zhou[1, 2]. [1]Department of Preventive Veterinary Medicine, College of Veterinary Medicine, Northwest A&F University, Yangling 712100, China. [2]Experimental Station of Veterinary Pharmacology and Veterinary Biotechnology, Ministry of Agriculture of the People's Republic of China, No.22 Xinong Road, Yangling, Shaanxi 712100, China. [3]Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, Liverpool L3 5RF, UK. [4]College of Animal Science and Veterinary Medicine, Henan Agriculture University, Zhengzhou 450002, China.*	p.66
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46	A comparison of genetic parameters and effects for a major QTL between piglets infected with one of two isolates of porcine reproductive and respiratory syndrome virus. *A.S. Hess[1*], N.J. Boddicker[2], R.R.R. Rowland[3], J.K. Lunney[4], G.S. Plastow[5], J.C.M. Dekkers[1]. [1]Department of Animal Science, Iowa State University, Ames, Iowa; [2]Genesus, Inc., Canada; [3]Department of Medicine & Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas; [4]USDA, ARS, BARC, APDL, Beltsville, Maryland; [5]Department of Agricultural, Food & Nutritional Science, University of Alberta, Alberta, Canada.*	p.68

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49	Essential role of extracellular signal-regulated kinase activation in porcine epidemic diarrhea virus replication. *Y. Kim*, C. Lee. Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, Republic of Korea.*	p.71
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	J.C.M. Dekkers[2], R.R.R. Rowland[1]. [1]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, 66506, USA, [2]Department of Animal Science, Iowa State University, Ames, IA, 50011, USA.	
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58	Blood transcriptomics in response to porcine reproductive and respiratory syndrome (PRRS). *M. Schroyen[1]*, J.P. Steibel[2,3], I. Choi[4], J.E. Koltes[1], C. Eisley[5], E. Fritz-Waters[1], J.M. Reecy[1], J.C.M. Dekkers[1], R.R.R. Rowland[6], J.K. Lunney[4], C.W. Ernst[2], C.K. Tuggle[1]. [1]Department of Animal Science, Iowa State University, Ames, IA, [2]Department of Animal Science, Michigan State University, East Lansing, MI, [3]Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, [4]APDL, BARC, ARS, USDA, Beltsville, MD, [5]Department of Statistics, Iowa State University, Ames, IA, [6]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.*	p.80
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60	Blood biomarkers for growth performance in gilts infected with Porcine Reproductive and Respiratory Syndrome (PRRS) virus. *N.V.L. Serão[1]*, T.E. Burkey[2] G. Gourley[3], T.E. Weber[1], M. Fitzsimmons[3], K. Schwartz[1], C. Sparks[4], J. Odle[5], J.C.M. Dekkers[1], N.K. Gabler[1]. [1]Department of Animal Science, Iowa State University, Ames, IA, [2]Department of Animal Science, University of Nebraska, Lincoln, NE, [3]Swine Graphics Enterprises L.P., Webster City, IA, [4]Choice Genetics USA LLC, West Des Moines, IA, [5]Department of Animal Science, North Carolina State University, Raleigh, NC.*	p.82
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62	Microscopic evaluation of lungs from pigs experimentally infected with type 1 PRRSV strains of different virulence. *T. Hući[1*], R. Sapierzyński[1], M. Czopowicz[1], C.K. Hjulsager[2], J. Nielsen[3], T. Stadejek[1]. [1]Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 161, 02-776 Warsaw, Poland, [2] DTU-VET National Veterinary Institute, Bülowsvej 27,*	p.84

	1870 Frederiksberg, Denmark, [3]Statens Serum Institut, Artillerivej 5, 2300 Copenhagen, Denmark.	
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69	Safety and antibody response of pigs to an experimental Porcine Epidemic Diarrhea Virus (PEDV) vaccine, killed virus. *D. Fredrickson*, M. Bandrick, L.P. Taylor, D.W. Coleman, T. Ricker, A. Pfeiffer, C.R. Locke, M.J. Huether, J. Zhang, R. Verhelle, T.K. Hildebrand, J.M. Hardham, V.J. Rapp-Gabrielson. Zoetis Veterinary Medicine Research & Development and Global Manufacturing & Supply, Kalamazoo, MI and Lincoln, NE.*	p.91
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Reverse genetics: 101 uses for PRRSV infectious cDNA clones

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Reverse genetics is the opposite of forward or classical genetics, and attempts to characterize the phenotypic effect of a known genotypic change. For DNA viruses and cellular organisms, a reverse genetics system may consist of direct manipulation of the DNA genome. Equivalent methods for manipulating RNA genomes are either difficult or non-existent. Thus, reverse genetics systems for viruses with RNA genomes require use of the enzyme reverse transcriptase and the production of a DNA copy of the genome. The resulting cDNA clone is readily modified, and must then be converted back to RNA through the action of an RNA polymerase and introduced into a cell in order to generate a mutant virus. Nature has shown us how to do this with retroviruses, and we have been using this tool for studying PRRS viruses for nearly 20 years. In traditional "RNA-launched" infectious cDNA clones, RNA transcripts are generated by *in vitro* transcription from a phage promoter and transfected into cells. Once in the cytoplasm of the cell the transcripts behave like viral genomes and initiate the replication cycle, culminating in the release of infectious progeny virus. In contrast, "DNA-launched" infectious cDNA clones eliminate the need for in vitro transcription by replacing the phage promoter in the plasmid with a eukaryotic promoter such as the immediate early promoter from cytomegalovirus (CMV promoter). In this case the infectious cDNA clone plasmid is directly transfected into cells. More than 100 peer-reviewed publications describe studies utilizing PRRS infectious clones. These include (1) knockouts or down regulation of PRRS ORFs to determined gene function, to enhance vaccine immunogenicity, or to make DIVA markers, (2) construction of chimeric viruses to study cell tropism, pathogenesis, or to generate vaccines with enhanced cross-protection, and (3) using PRRSV as a vector to express marker proteins (such as GFP), cytokines, or genes from other porcine pathogens to create multivalent vaccines.

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Historical perspective of area regional control – PRV: What lessons apply to PRRSv control and elimination?

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Can the lessons we learned from the successful eradication of Aujeszky's (PRV) in the US apply to Porcine Respiratory and Reproductive Syndrome (PRRS)? PRRS continues to be costly with annual estimates of $664,000,000 and cumulative losses of $16,494,193,192. The cost of PRRS continues to accumulate, and is likely underestimated if reproductive and respiratory losses, vaccine expenses, and costs of human stress are aggregated and also considered.

PRV is transmitted primarily via respiratory contact and generally over short distances with a limited period of viremia for transmission from one pig to another. PRV is sequestered in ganglion for a long period of time, but veterinarians quickly understood stressors that contributed to recrudescence. Environmental survival was limited, resulting in a narrow risk window. Vaccine played a pivotal role in PRV eradication, and the development of a DIVA (differentiating infected from vaccinated animals) vaccine accelerated eradication. Movement restrictions and quarantining were effective, and other species were dead end hosts.

In contrast, PRRS is shed in body fluids, easily travels with pigs and in contaminated transport, and transmits via aerosol and fomites. PRRS exhibits a long period of viremia resulting in prolonged shedding; pigs may shed virus for 25-30% of their growing cycle. The prolonged shedding increases the probability of PRRS transmission with contact between shedding and susceptible pig populations.

The industry embarked on a 13 year plan to eradicate PRV following a series of highly controversial meetings. Industry consensus was that US producers could not live with the cost of the virus and remain competitive to other available protein sources. The eradication stimulus was also driven by transmission among multiple species and not just pigs thereby generating support for eradication from non-farmers. The key drivers of the eradication were a strong cooperation of federal, state, and industry programs led by a producer/veterinary advisory committee, a project coordinator, user friendly herd cleanup plans, effective and differential vaccines, and funding from national and state sources for the effort.

The success seen in PRRS ARC (Area Regional Control) projects in the last years indicates management of PRRS in an area can lead to successful control and potential future elimination of the virus. Even without a differential and sterilizing vaccine, the industry and profession are better equipped for successful elimination compared to the early period of PRV eradication. Our industry has primarily segregated flows; our veterinarians are experienced in a multitude of pathogen eliminations; biosecurity is at the highest industry level; there is an increased utilization of premise identification numbers on diagnostic submissions particularly following the recent outbreak of PED and recent experience with PRRS ARC provide optimism for the future.

This presentation will compare and contrast steps for a successful elimination program.

3
Genetics of host response to PRRS

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Initial work under the PRRS Host Genomics Consortium (PHGC) identified a region on chromosome 4 of the pig with a large effect on blood PRRS viremia and growth rate of commercial crossbred nursery pigs following experimental infection with one specific strain of the PRRS virus (NVSL-97-7895). These findings offer exciting opportunities for marker-assisted selection but are based on challenge in clean facilities using naïve pigs and a specific virus strain. Thus, an important problem with translation to application is that disease in industry is uncontrolled and often involves prior or concurrent exposure to other pathogens. The purpose of this presentation is to describe ongoing efforts to investigate opportunities to use the genetics of the pig as a tool in the fight against PRRS, including experimental infections with a second strain of the PRRS virus, experimental co-infections of PRRS and PCV-2, preceded by PRRS vaccination, experimental infections of pregnant gilts, and field studies using nursery pigs and replacement gilts. Using deep phenotyping and genotyping approaches that combine state of the art genomics with state of the art virology, the ultimate goal is to find genomic markers and other biomarkers that can be employed in the development of breeding programs to lessen the impact of PRRSV on the commercial pig industry. Although genetic selection will not offer a single 'magic bullet' solution, especially given the complexity and variability of the PRRS virus, host genetics can be an additional and complementary approach to fight the impact of PRRSV on pork production.

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Nidoviruses: Making the PRRSV-Coronavirus connection

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The order *Nidovirales* includes the distantly related coronaviruses, arteriviruses, toroviruses, and roniviruses, in which porcine reproductive and respiratory syndrome virus (PRRSV; arterivirus) and porcine epidemic diarrhea virus (PEDV; coronavirus) are economically important swine pathogens. In spite of striking differences in genome size and virion structure, the genome organization and expression of arteri- and coronaviruses are strikingly similar, and key replicase (proteases) domains were postulated to share a common ancestry. Nidoviruses contain positive-stranded RNA genome, and a prominent feature of the genome expression is the nested set of subgenomic (sg) mRNAs that was the basis for the order name *Nidovirales* (Latin nidus=nest). The viral genome encodes large replicase polyproteins, which are posttranslational processed by viral proteases to generate the nonstructural proteins (nsps) for directing viral replication and transcription. Replicase gene expression is under the principle control of ribosomal frameshifting signals and a complex proteolytic cascade that is directed by ORF1a-encoded proteinase domains. The viral papain-like proteases are critical in the proteolytic processing of the replicase. Our recent analysis of the papain-like proteinase domain (PLP2) of PRRSV demonstrated that the arterivirus PLP2 is a dual-specificity proteinase that not only cleaves the nsp2/3 junction in the replicase polyproteins, but is also able to disrupt innate immune signaling by removing ubiquitin (Ub) and Ub-like modifiers from host cell substrates. The biological significance of this activity was supported by the fact that PLP2 deubiquitinating (DUB) activity could inhibit type I IFN production by interfering with NF-kB activation, which is regulated by polyubiquitination. In comparison of these studies to that of coronaviruses, it is revealed that the viral protease/DUB activity is also conserved in many family members of coronaviruses. In PEDV, PLP2 was identified possessing DUB activity, which deubiquitinates RIG-I and STING, the key components of the signaling pathway for IFN expression. Using PRRSV reverse genetics, targeted mutagenesis was used to inhibit DUB activity without affecting nsp2/3 cleavage. The resulting virus mutants displayed reduced DUB activity that translated into enhanced innate immune signalling in infected cells, suggesting that this approach could be applied to next-generation vaccine development. Further understanding of the nidovirus common features and multifunctionality of the replicases may provide a rational basis for future disease prevention and control.

5
Porcine Deltacoronavirus—Koch's Postulates fulfilled

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Porcine Deltacoronavirus (PDCoV) is an emerging virus which has recently been detected in US and Canadian swine herds associated with outbreaks of diarrhea in the absence of other etiologic agents. Most reports associating PDCoV with enteric disease are from field cases, and thus controlled experimental evidence of PDCoV as an etiologic agent is lacking. The objective of this study was to develop a challenge model for controlled experimental PDCoV infection in neonatal piglets and to characterize the clinical course of disease, virus distribution in tissues, and histopathology subsequent to PDCoV infection.

A PDCoV challenge inoculum was derived from pooled intestinal content obtained from a field case that was found to be free of other common etiologic agents of neonatal porcine diarrhea. This inoculum was amplified in individual nursing age pigs to confirm infectivity and purity, and subjected to quantitative Real Time PCR to estimate viral concentration. Following successful preliminary infection, nine sows were obtained from a high health commercial source. The sows were farrowed and housed in five BSL2 biosecure isolation rooms at the University of Nebraska for the 42 day study period. The litters and dams were divided into four experimental groups: PDCoV oronasal inoculated, contact control, aerosol transmission control, and sham inoculated control. The inoculated animals were inoculated at 2-3 days of age via intranasal and oral routes. After inoculation, all animals were observed daily for any signs of disease and antemortem samples were collected for testing. Sequential post mortem examination was conducted, including collection of a complete set of fresh and formalin-fixed tissues on days 1, 2, 3, 4, 5, 6, 7, 10, 14, 21, 28, 35, 42 post-challenge.

Inoculated piglets developed soft to diarrheic feces with vomiting and were fecal PCR positive for PDCoV by day 2 post-challenge. Negative control animals remained PCR negative throughout the study period. Virus detection via PCR demonstrated that: There was no significant difference between detection rates of inoculated animals versus contact control animals. The sham inoculated group remained free of virus throughout the duration of the study. Viremia was detected at one day post inoculation in 60% of the pigs tested, remained present in all of the pigs tested until four days post-inoculation, at which time the percent positive fell to 25% at day five and all animals were negative for viremia thereafter. Virus detection rates in fecal and nasal samples were generally similar, with fecal samples tending to have a higher concentration of virus than the nasal samples. In both sample types, virus was detected at high to moderate levels from 2 to 10 days post-inoculation. Approximately 70% of the samples were positive at day 14, 25% of the samples were positive at day 21, and no samples were positive at 28 days post-inoculation and thereafter. Oral fluid samples were positive at the first collection point at 14 and 21 days post-inoculation, day 28 samples contained low levels of virus in 4 of the 6 infected litters and were negative for virus at 5 weeks post-inoculation. Viral antigen detection via immunohistochemistry (IHC) demonstrated positive staining of PDCoV in enterocytes in the small intestine 24 hours post inoculation. The complete testing of tissues collected during this sequential sacrifice study is currently underway. Antibody response via the indirect fluorescent antibody (IFA) and serum neutralization (SN) along with PCR results demonstrated sham inoculated animals (sows and their pigs) remained serologically and PCR negative throughout the entire study. Inoculated animals (sows and their nursing pigs), contact controls and room aerosol controls all sero-converted and were shown to be IFA and SN positive by 14 days post-inoculation and remained positive through the conclusion of the study at 42 days post inoculation. These data indicate PDCoV is capable of producing significant enteric lesions and clinical diarrhea in neonatal pigs in the absence of other etiologic agents.

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Characterization of PDCoV and PEDV infection in China

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Although the prototype of porcine deltacoronavirus (PDCoV), HKU15, was first identified in 2011 in Hong Kong Special Administrative Region (HKSAR), China, the existence of PDCoV in Mainland China and if any, the prevalence rate and the phylogenetic relationships with the US PDCoVs have not been investigated. We report here, for the first time, the identification of Chinese PDCoVs in three separately geographic locations in China in 2014. A total of 1344 fecal or small intestinal samples from diarrheal piglets collected from 11 provinces of China during 2014 were screened for PDCoV RNA positive by RT-qPCR or RT-PCR. Twelve PDCoV-positive samples, including seven from 1 farm in Jiansu province (East China), four from 1 farm in Hunan province (South China), and one from 1 farm in Sichuan province (West China), were identified. Eight of these 12 samples were positive for porcine epidemic diarrhea virus (PEDV). The sequences of structural protein genes (S-E-M-N) of three representative strains (JS1, HN1 and SC1) were subsequently determined. Phylogenetic analysis showed that these Chinese strains were clustered more closely related to the US strains than HKU15. The low prevalence rate of PDCoV infection in Mainland China implies that PDCoV may not have a great transmissibility compared to PEDV and may not be responsible for large-scale outbreaks of porcine diarrheal diseases in China recently.

We also report here the retrospective investigation of the potentially evolutionary process by which US PEDV strains hypothetically descended from precursors in China. The US PEDV strains identified in 2013 are most closely related to the strain AH2012, which was isolated in east China. We conducted a molecular epidemiologic analysis using 96 PEDV-positive samples collected on 26 farms in 4 provinces from east China during January 2012-July 2013. Phylogenetic analysis of the structural protein genes (S-ORF3-E-M-N) revealed a new sublineage, represented by strains CH/HuBWHYQ/2012, CH/JXZS-1223L/2012, and CH/JXZS-3L/2012, together with the published strain CH/ZMDZY/11, is most closely related to the 2013 US strains. The AH2012 strain was clustered closely with the 2013 US strains in the ORF1ab and the partial N gene region. Recombination in these two strain sublineages was likely associated with the emergence of PEDV in the US in 2013.

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

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Introduction: Three collaborative large-scale, long term PRRS control projects applying a systems-based methodology were conducted. These projects occurred in three different geographical locations. The objective of these projects was to improve PRRS stability and overall growing pig performance through the strategic use of modified-live vaccine (MLV) compared to 15 to 24 months of prior production data.

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

Results: All three projects demonstrated significant improvements in ADG and mortality, following the interventions for PRRS control in the growing pig phase of production. Results are summarized in Table 1.

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

	System			
	A	A-FP	B	C
ADG, % change				
Nursery	+23%	-	+7%	0.0%
Finisher	+6%	+5%	+7%	+2%
WTF	-	-	-	+6%
Mort, % change				
Nursery	-63%	-	-23%	-38%
Finisher	-33%	-32%	-35%	-30%
WTF	-	-	-	-45%

FP= Feeder Pig Flow

Discussion and Conclusions: Improved health and performance as measured by reductions in mortality in the nursery period (23-63%), finisher period (30-35%), and WTF period (45%) were realized. Improvements in ADG in the nursery phase (7-23%), finisher phase (2-7%), and WTF (6%) were consistent and repeatable across the three systems. These three systems-based PRRS control projects demonstrate that the consistent implementation of a methodology utilizing herd closure and modified-live vaccine for the control of wt-PRRSv in BH's and weaned pigs can mitigate the consequences of infection; improving health and performance.

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Translational genomics of porcine reproductive and respiratory syndrome (PRRS)

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Our goal is to understand transcriptional control of viral disease responses focusing on the economically most important disease of pigs, porcine reproductive and respiratory syndrome (PRRS) (annual U.S. losses of $664M). The PRRS Host Genetics Consortium (PHGC) was established to combine efforts of scientists from university, government and commercial pig genetics and animal health companies to assess the role of genetics in determining pig resistance/ susceptibility to PRRS virus (PRRSV) infection, pathology and growth effects. We utilized a nursery pig PRRSV infection model with deep sampling for phenotypic analyses, extensive genotyping (60K SNPchip) and a shared database http://www.animalgenome.org/lunney/. We have completed 15 trials using ~200 PRRSV-infected pigs each and identified a genomic region on SSC4 which has a significant impact on variation in viral load and growth responses following challenge with each of 2 different PRRSV isolates. To address disease resistance mechanisms we probed the blood transcriptome of PHGC pigs using RNAseq. We have verified genes that are differentially expressed in PRRS resistant versus susceptible pigs. We are now expanding our efforts to probe for alternate control and regulatory networks by comparing pigs with differential PRRSV responses, i.e., those with slow or fast post viral peak control responses. This data will help us identify new resistance pathways that may be used for vaccine design and novel biotherapeutics. Support: US National Pork Board, USDA ARS and NIFA, Genome Canada, Genome Alberta, pig breeding companies.

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Porcine Epidemic Diarrhea Virus immunity

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Porcine Epidemic Diarrhea Virus (PEDV) is the etiologic agent of an enteric viral infection of intestinal epithelial cells that is spread by fecal-oral transmission. Intestinal immunity is typically dependent on secretory IgA in the lumen that neutralizes infectivity, and that is derived from B cells that are induced and differentiated in local gut-associated lymphoid tissues. Model enteric antigens such as cholera toxin induce strong secretory IgA responses that can be detected also in distant mucosal sites such as oral-nasal tissues and the reproductive tract. By contrast, systemic antibody responses characterized by IgG-secreting B cells in spleen and peripheral blood are limited.

Pigs infected with PEDV rapidly develop systemic IgG and intestinal IgA antibody responses. Using a practical feedback challenge model of viral inoculation, it was demonstrated that systemic responses equally strong in the presence and absence of clinical signs of disease.
Working with commercial sow herds in North Carolina, Iowa, and Minnesota, we found that, after one good feedback exposure, sows with or without clinical signs routinely became infected and shed PEDV, but amount of shedding was less in non-clinical sows. Sows seroconverted but, surprisingly, serum antibodies to nucleocapsid, the ELISA antigen, declined rapidly and were frequently negative at nine weeks after feedback. The findings indicate that clinical signs are not necessary for immunity, and may put more PEDV into the environment unnecessarily.

Immunity is durable even though serological tests turn negative relatively quickly. We looked at the feedback response of newly introduced, naïve gilts and previously exposed sows in a herd that had a PEDV outbreak and whole-herd feedback 4 months (134 days) earlier. Two days after the feedback challenge test, all gilts showed clinical signs and shed virus in feces, with 90% showing Ct values less than 30. By contrast, no previously exposed sows showed clinical signs, 50% never shed virus, and the Ct values of shedders were >30. Thus, previously exposed sows were resistant to disease after re-exposure, and the duration of solid immunity was at least 4 months.

On the day of re-challenge by feedback, 40% of previously exposed sows were ELISA-positive to nucleocapsid. Five weeks later, all naïve gilts had seroconverted, and 75% of sows were positive. ELISA-negative sows had anti-PEDV antibodies, though, since all sows and all gilts, were IFA-positive. Hence, one good exposure to PEDV induces immunity in sows, previously exposed sows are resistant to diarrheal disease months later, but get a boost in immunity, duration of immunity is prolonged, with the full duration not yet known, and measuring nucleocapsid antibodies in serum does not give a full picture of immune status.

A key question concerns mechanisms by which intestinal immunity leads to secretory IgA in sow milk that provides passive protection to suckling piglets. Answers to these and related questions will help guide development of vaccines effective to enteric viral diseases of swine, including PEDV.

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Epidemiology of PRRS: closing some of our knowledge gaps

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Despite much time and money have been invested over the last 25 years on porcine reproductive and respiratory syndrome virus (PRRSV) research in North America, critical aspects of PRRSV epidemiology are yet-to-be elucidated. The paper here summarizes results of and future plans for research conducted at the University of Minnesota on PRRSV incidence, molecular epidemiology, and modeling.

PRRSV incidence: Weekly PRRSV status data from a convenience sample of 371 farms, in 14 unique production companies, representing 1.2 million sows in 15 states, have been collected since 2009. The project, which has been referred to as National Incidence Project, revealed that every year from 2009 to 2012, the number of PRRSV outbreaks exceeded the baseline during the middle week of October. However, in the fall of 2013 and for the first time in four years, the epidemic started three weeks later and the annual cumulative incidence was lower compared to the previous four years. This difference in 2013, compared to previous years, may be explained by a variety of porcine epidemic diarrhea (PED)-related or –unrelated factors.

PRRSV molecular epidemiology: Accuracy of two alternative methods for measuring PRRSV diversity was assessed, namely, 1. the pairwise identity, and 2. the genetic distance determined by phylogenetic trees constructed by Bayesian methods, referred to as cophenetic distance. In contrast to the pairwise identity, which is the most frequently used method for computing PRRSV diversity, Bayesian analysis allows to incorporate different parameters such as time of virus collection. There was a moderate correlation (R=0.7) between the results obtained by both methods. Results suggest that the pairwise identity may overestimate the extent of the relation between pairs of sequences with a similar genetic composition, but for which the evolutionary distance was relatively high. In lay terms, if a PRRSV with a given genetic composition ("virus A") mutates into another genetic composition ("virus B") twice in, say, 10 years, then the pairwise identity method would suggest that both viruses B are related to each other. However, by considering the effect of time, the cophenetic distance would relate each virus to its ancestor, revealing the true phyhlogenetic relation. This distinction is important for highly mutant viruses, such as PRRSV. Additionally, this year a number of type 1-3-4 PRRSV sequences, genetically similar but phylogenetically distant to other 1-3-4 PRRSV strains identified in previous years, have been detected.

PRRSV modeling: A project intended to model PRRSV space-time clustering and transmission in the N212 regional project of Minnesota will start this fall. The project goal will be to elucidate the nature and extent of the association between PRRSV spread and socio-economic factors hypothesized to influence disease spread.

In summary, this presentation will provide an update of research activities at the University of Minnesota intended to close some of the knowledge gaps on PRRSV epidemiology, and with the ultimate goal of mitigating the impact and supporting control of the disease in the U.S.

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Genetic engineering the pig to better understand PRRSv infection

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Characterization of the molecular interactions between the porcine reproductive and respiratory syndrome virus (PRRSv) and the host may lead to treatments and therapies to prevent or alleviate the effects of infection. One model to describe viral entry begins with PRRSv interacting with heparin sulfate on alveolar macrophages, followed by attachment to SIGLEC1. The bound virus is endocytosed. The pH within the endosome then drops and the virus interacts with CD163. The viral genome is subsequently uncoated and released. However, in a whole animal model, deletion of *SIGLEC1* had no effect on development and progression of PRRSv infection demonstrating that sialoadhesin is not absolutely required for infection *in vivo*. The second host susceptibility gene, CD163, is a member of the scavenger receptor cysteine-rich (SRCR) superfamily. The protein has 9 extracellular SRCR domains, a transmembrane domain and a cytoplasmic tail. There are 17 exons. SRCR domain 5 (exon 7) of the protein appears to be the domain responsible for unpackaging of the viral genome as deletion of other domains in an *in vitro* system did not inhibit infection. Additionally replacement of domain 5 with domain 8 of human CD163L also abolished infectivity (Note that CD163L has 12 domains and the numbering of the domains begins at the N-terminal end; and thus domain 5 of CD163 is analogous to domain 8 of CD163L). We have created founder animals that have a number of different genotypes. These include: **A**. knockout of *CD163*, **B**. deletion of both domains 5 and 6 of CD163, **C**. deletion of domain 5 of CD163, **D**. deletion of 35 amino acids in the core of domain 5 of CD163, **E**. a partial domain swap of domain 8 of CD163L with domain 5 of CD163, and **F**. a knockout of both *CD163* and *SIGLEC1*. PRRSv challenge of offspring of these pigs should determine if CD163 is the entry mediator, or if SIGLEC1 and CD163 are co-entry mediators. If CD163 is the entry mediator, then we should be able to determine which portion(s) of CD163 mediates infectivity. A definitive description of the molecules involved in PRRSv infection should facilitate the development of preventatives, treatments and therapies for swine.

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Area elimination of PRRSVgenotype II by using Ingelvac® PRRS MLV applying load, close and homogenize concepts in combination with coordinated and strategic piglet vaccination: A pilot project at Horne peninsula, Denmark

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Objective: The objective of this study is to evaluate a PRRSV elimination project in Horne peninsula, Denmark. The aim of the elimination project was to achieve SPF status for all herds in the area, and avoid lateral spread of PRRS virus between herds. Thirteen farms are located in this small pig dense area (10 x 7 km); 4 sow farms (400 sows in avg.), 2 wean to finish (W-F) farms (producing about 14000 finishers a year) and 6 finisher farms (together producing 36000 finishers a year). No pigs from outside are introduced in this area, except purchased PRRS negative gilts.
Materials and Methods: In the peninsula area, there are two pig flows. Pig-flow 1, consisting of two sow sites with nurseries and three finisher sites. In this pig flow PRRSV genotype II circulation was identified during late nursery and finisher sites before the start of the project. The other pig flow, Pig-flow 2, is structured with two sow sites with traditional nurseries, two W-F sites, one finisher site and a gilt acclimatization + dry sow barn; PRRSV genotype II infection was active in all Pig-flow 2 sites.
In Pig-flow 2, the acclimatization site was loaded with gilts down to 10 woa. The sites with sows and gilts were closed for 29 weeks. The first batch of gilts introduced after herd closure were vaccinated and placed in quarantine for 3 month, before introduction to the sow site. The first PRRS negative gilts were introduced in May 2014. All sows, gilts, boars, and piglets in the farrowing room and the nursery, were homogenized by vaccination with Ingelvac® PRRS MLV during the first week of July 2013. Each of the following 3 weeks, 7 day old piglets were vaccinated until the mass vaccination of sows, gilts and boars was repeated four weeks after the initial mass vaccination. Following the second mass vaccination, piglet vaccination was moved to immediately post-weaning, in the nursery for the next 10 weeks.
In pig-flow 1, the oldest nursery rooms were emptied and simultaneously with piglet vaccination in pig-flow 2, the growing pigs in pig-flow 1 were vaccinated before arrival to the finisher sites. In pig-flow 2, the nursery in the sow unit was depoped after vaccination stop. The nursery on the W-F site was buble depoped. During this time only PRRSV vaccinated pigs were housed in finisher sites in the area, with the intention of reducing viral shedding to surrounding sites. Monthly monitoring by PRRS PCR and IDEXX 3X Elisa was implemented in both pig-flows. In Pig-flow 1, suckling piglets (n:10) and end of nursery pigs (n:10) were monitored and in Pig-flow 2, cross sectional profiles in nursery and finisher sites (avg 29 pigs) were implemented during the project.
Results and Conclusion: Results from monthly monitoring by PCR and ELISA showed negative batches at nursery, W-F and F sites 2 weeks after piglet vaccination was stopped. The breeding herd was negative 13 weeks post LCH, and all the nurseries (W-F) were negative 22 weeks after piglet vaccination stopped. One year post LCH only 1 site (W-F) remains positive. The implementation of LCH model in combination with strategic piglet vaccination at weaning or end of nursery with depopulation of problematic sites has successfully eliminated PRRSV from 12 out of 13 herds on Horne peninsula 64 weeks after LCH was initiated. The challenges in this project, although not the number of animals in total, but rather the number of farms/sites and the coordination and communication of multiple parties, were overcome and elimination was achieved.

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Analysis of PRRSV immunity at the herd level

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The PRRS Host Genetics Consortium (PHGC) was established as a means to incorporate genomic and genetic tools to lessen the impact of PRRS. The PHGC model incorporates the experimental infection of 200 young pigs with well-characterized PRRS and PCV2 viruses. One spinoff of the PHGC approach is the opportunity to investigate PRRSV infection at the population level, including the identification of pigs that possess unique responses. As part of one experimental trial, pigs were evaluated for virus neutralizing (VN) activity at 42 days after infection. One half of the pigs were vaccinated with a PRRS MLV prior to challenge with a PRRSV isolate, KS62, and PCV2. Results for VN assays showed that pigs could be placed into distinct categories or groups based on the number of virus isolates neutralized. Approximately 13% of pigs, regardless of vaccine status, possessed no detectable VN activity. A single pig, 16-45, was able to neutralize a panel of 7 viruses, including a Type 1 virus. Therefore, 16-45 was described as possessing broadly neutralizing activity. A neutralization resistant KS62 variant was obtained by growing KS62 in the presence of 16-45 serum. After six rounds of antibody selection, the resulting virus, KS62-R4, was resistant to neutralization by16-45 and a second broadly neutralizing serum, 15-4. However, KS62-R4 remained sensitive to neutralization with 16-21, a serum with homologous VN activity against the KS62 isolate. DNA sequencing identified a single mutation in the structural genes, ORFs 2-6; the deletion of a tyrosine codon, TAC, in ORF6 at amino acid position 10 (Y10) in the M protein. Since M is a highly conserved protein, the presence of a deletion was unexpected. To confirm the neutralization properties of Y-10 mutation, an infectious clone was prepared that possessed the Y-10 deletion. The recombinant virus, P129-EGFP-KS62-R4, possessed almost identical neutralization properties as the KS62-R4 parent strain, including the escape from neutralization by broadly neutralizing sera 16-45 and 15-4, and sensitivity to homologous serum, 16-21. The results confirmed the role of the Y10 deletion in neutralization by 16-45. The deletion of a tyrosine at position 10 in M suggests that the loss of neutralization by 16-45 is the result of the deletion of a critical antibody contact residue. The analysis of 573 Type 2 PRRSV M protein peptide sequences from GenBank showed several amino acid substitutions at position 10, including the presence of asparagine (87.4%), histidine (9.2%), tyrosine (2.6%), and arginine (0.7%). No deletions were identified within the GenBank sequences. In this study, the viruses incorporated in the neutralization assays possessed histidine, tyrosine and asparagine substitutions at position 10. Therefore, the presence of different amino acids at position 10 suggests that peptide sequence variation is not sufficient to escape from broadly neutralizing serum, 16-45. In this study, the screening of populations of infected pigs was used to identify unique naturally occurring antibody responses, which can be incorporated as tools to probe the nature of the immune response to PRRSV. The results demonstrate that epitopes recognized by sera with different neutralization properties are distinct, which can explain the large number of putative neutralizing epitopes previously reported in the literature. The results also point to the importance of thoroughly characterizing the neutralization properties of polyclonal and monoclonal antibodies utilized for mapping neutralization epitopes.

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Lactogenic immunity and vaccines for Porcine Epidemic Diarrhea Virus (PEDV): Lessons from Transmissible Gastroenteritis Virus (TGEV) trials and tribulations

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The alphacoronavirus PEDV emerged in the US in April 2013 as a new, devastating diarrheal disease in swine. It spread rapidly throughout the US, infecting swine of all ages. Genetically, US PEDV strains resemble recent highly virulent strains from China (China AH2012). For unknown reasons, vaccines based on European and historic PEDV strains failed to control the recent virulent PEDV outbreaks in Asia. Like TGEV, PEDV infects intestinal villus enterocytes causing severe villous atrophy and dehydration, resulting in fatal (80-100% mortality) diarrheal disease in seronegative suckling piglets. Thus vaccination strategies for both diseases must focus on induction of mucosal immunity to protect the target intestinal enterocytes. This necessitates protective levels of mucosal immunity in neonates at birth and throughout the nursing period. Maternal immunity induced in the sow and passively transferred to piglets via suckling of colostrum and milk (lactogenic immunity) is crucial for immediate protection of neonates against enteric infections. Maternal vaccination strategies to induce lactogenic immunity against TGEV will be reviewed. These vaccination principles are also applicable against emerging enteric infections such as PEDV. TGEV research has provided both a basic understanding of the concept of lactogenic immunity, as well as vaccination strategies for its induction in seronegative pregnant swine. Our discovery of the gut-mammary SIgA axis (trafficking of IgA immunocytes from the gut to the mammary gland) in swine was a predecessor for the concept of a common mucosal immune system. It provided an explanation for why sows that recovered from TGEV infection, or ones orally inoculated with live TGEV, had high persisting levels of IgA antibodies in milk that protected their piglets from TGEV. However sows systemically immunized with inactivated TGEV vaccines had mainly IgG antibodies that declined rapidly in milk and provided little passive immunity to piglets. Subsequent studies of enteric virus vaccines (TGEV, rotavirus in swine; polio in children) suggested that after effective priming of the gut by natural infection or oral live vaccines, parenteral booster vaccines, including subunit or inactivated vaccines, could enhance and maintain mucosal or lactogenic immunity. The oral prime/parenteral booster approach may explain why such parenteral vaccines are effective in some scenarios in sows recovered from TGEV or rotavirus infections or after oral live vaccines. The need for oral live vaccines to prime gut immunity in sows requires development of attenuated strains of PEDV. This can be accomplished by classical methods of serial passage of PEDV in cell culture as done for prior Korean PEDV vaccines. Alternatively the use of infectious clones of PEDV and reverse genetics will allow targeted mutations in virulence genes and engineering of genomic changes that limit PEDV replication in vivo to rationally attenuate PEDV. In spite of such advances, many unanswered questions remain about development of TGEV or PEDV vaccines to induce lactogenic immunity. They include: 1) Level of attenuation of the vaccine required to infect the gut of the sow, but not induce disease and still evoke active (sow) and passive (piglets) immunity; 2) Role of parity, sexual maturation (gilts), stage of pregnancy and virus dose on stimulation of the gut-mammary SIgA axis and induction of SIgA antibodies in milk. The devastating effects of PEDV may provide the impetus to address these fundamental questions.

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Innate immunity to PRRSV and application to new vaccines

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PRRSV undergoes high rates of genetic variations and rapid evolution, resulting in antigenic diversity and heterogeneity. The paradigm of PRRSV infection is initiation of weak and delayed immune response which may explain the viral persistence for a prolonged period. Current obstacles to developing an effective vaccine for PRRSV include antigenic heterogeneity and viral persistence. An immunological hallmark during PRRSV infection is poor induction of type I interferons (IFNs). The type I IFN system is an important antiviral component of the host innate immunity. Additionally, type I IFNs participate in a wide range of immunological functions including cellular resistance to viral infection, expression of innate cytokines, dendritic cell maturations, and regulation of cellular immunity and adaptive responses. Evidence is accumulating that removal of IFN antagonism from virus may induce heterologous protection and effective cellular responses, suggesting a novel way to developing a future vaccine candidate for PRRSV. At least six viral proteins have been identified as IFN antagonists, and among them, nsp1-alpha, nsp1-beta, nsp11, and nucleocapsid (N) protein have been characterized in my laboratory. Nsp1-alpha inhibits production of IFNs by degrading CREB-binding proteins in the nucleus whereas nsp1-beta interferes IFN production and ISRE expression. Nsp11 participates in suppression of RIG-I signaling and degrades IPS-1 mRNA in the cytoplasm while N protein blocks nuclear translocation of STAT2 and activates NF-kB and interleukin-10. A SAP (for SAF-A/B, Acinus, and PIAS) domain has been identified in the nsp1-beta subunit, and this domain is highly conserved in PRRSV, equine arteritis virus, lactate dehydrogenase-elevating virus, and simian hemorrhagic fever virus. When critical residues in the SAP domain are mutated to alanine, IFN suppression by nsp1-beta is reverted and IRF-3-dependent transcription is recovered from suppression. These SAP mutants also exhibit altered subcellular localization of nsp1-beta. The mutations contributing to impaired IFN induction have been individually introduced into the PRRSV infectious clone, and SAP mutant viruses have been generated. In comparison with wild-type PRRSV, SAP mutant viruses show effective induction of IFN in infected-cells, suggesting that the SAP domain contributes to PRRSV replication and plays a role for viral immune evasion. A clinical study in pigs using the SAP mutant is in progress.

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PEDV in the U.S.: diagnostic tools and research applications in diagnostics

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In North America, porcine epidemic diarrhea virus (PEDV) was detected for the first time in the U.S. in April 2013 and then in Canada and Mexico. The objectives of this update are to overview current laboratory diagnostics, challenges related to detection and characterization of PEDV, and research relevant to the interpretation of diagnostic results and the circulation of PEDV in swine.

Several singleplex PEDV-specific real-time RT-PCRs targeting the nucleocapsid, membrane, spike, or ORF1b genes and some multiplex PEDV/TGEV or PEDV/PDCoV PCRs have been developed. These assays can test large numbers of samples in a timely manner for PEDV diagnosis and surveillance. However, the diagnostic situation is complicated by the fact that different PCRs are used across laboratories. Since there are no data comparing the various PEDV RT-PCRs, it is not known if the cycle threshold (C_T) values obtained from different RT-PCRs performed in different laboratories are equivalent. It should also be recognized that establishing the ideal C_T cut-off and the definitive interpretation of PEDV PCR results in different sample matrices is not a simple process. For that reason, the clinical relevance of PEDV RT-PCR results in different specimen types should be interpreted cautiously. Whole genome and/or spike gene sequencing, together with comparative and phylogenetic analyses, have been used to study virus evolution and determine genetic relatedness of U.S. PEDVs. Currently there are at least two strains of PEDV (U.S. prototype and U.S. variant) circulating in U.S. swine.

Gross and histological evaluation of tissues, together with immunohistochemistry and/or *in situ* hybridization, are useful procedures for establishing a diagnosis of enteritis caused by PEDV. Although several laboratories have successfully isolated PEDVs in cell culture, the overall success rate of PEDV isolation in cell culture has been low (<10%). Thus, pig bioassay still remains the most reliable means to determine if infectious PEDV is present in a clinical specimen or if "X" treatment will inactivate the virus. Experimental infection studies showed that naïve neonatal piglets are more sensitive than weaned pigs as a PEDV bioassay model.

Several serological assays have been developed to detect PEDV-specific antibody. These include immunofluorescence antibody (IFA) assay, virus neutralization, and various ELISAs based on recombinant spike protein, recombinant nucleocapsid protein, or purified whole virus. The performance (sensitivity, specificity, and accuracy) of these PEDV ELISA assays have not yet been compared. Several laboratories are currently developing fluorescent microsphere immunoassays (FMIA) targeting selected antigens of PEDV and/or other porcine coronaviruses to detect virus-specific antibodies. In particular, studies are needed to identify the antibody isotypes and levels associated with protective immunity, as well as the specimen most reflective of lactogenic immunity.

Although much progress has been made in the development of PEDV diagnostics, a great deal of work remains to be done. In particular, comparison and standardization of PEDV PCRs and PEDV antibody assays across the laboratories is needed.

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Preparing for the next incursion: efficient, cost-effective on-farm surveillance

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We discuss solutions to the challenge of implementing an efficient, cost-effective on-farm surveillance system in a very complex industry. In the purest sense, "monitoring" is not the same as "surveillance", but for convenience we will use the two terms interchangeably to mean, "*An ongoing effort at assessing the health and disease status of a given population*" (Salman, 2003).

The basics of surveillance are as follows:

- Surveillance must have a clear purpose, e.g., to prove freedom from a target, to detect the presence of the target, or to support production process control (De Vries & Reneau, 2010).
- Effective disease surveillance produces data. With the possible exceptions of smallpox and measles, surveillance based on syndromics does not work. If it did, we should not have spent the last 150 years training pathologists and developing diagnostic technology.
- Actions/responses designed to achieve the purpose of surveillance should be based on the analysis of surveillance data. "*It is not enough to do your best; you must know what to do, and then do your best.*" (W.E. Deming).

The requirements for a viable on-farm swine surveillance system are simple:

1. It must provide a return on investment (ROI) to producers. This implies a clear focus on things that matter. "*Just because you can measure everything doesn't mean that you should.*" (W.E. Deming).
2. The people using the system must believe that it has value.
3. It must be easy to implement. This means from sample collection to data analysis.
4. It must provide timely and accurate results. In particular, assays used in surveillance must be specific and reproducible because false positives destroy confidence in the system. Because there are no perfect tests, a seamless confirmatory testing procedure must be in place to evaluate unexpected/questionable positive results.
5. It must be flexible. That is, it must be useful for more than one purpose and able to provide data on a variety of targets.

On-farm collection of statistically appropriate numbers of blood or nasal swab specimens from individual pigs does not meet any of the requirements listed above. Therefore, on-farm surveillance either is not done or it is done incorrectly. Although surveillance data has historically been too expensive and too difficult to collect routinely, this need not be the case.

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Detection of PRRSV and specific seroconversion in oral fluid and serum of pigs in a longitudinal study in three Polish farms

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Oral fluid testing using PCR and ELISA has recently become a common method in routine PRRSV diagnosis, surveillance and monitoring. It is widely used in North America but much less so in Europe. The objective of our study was to monitor PRRSV status of growing pigs from three Polish farms over time, using PCR and ELISA on oral fluid and serum.

The study was performed in three Polish farms, reportedly PRRSV positive. Farm A was a multisite system with 800 sows. Replacement gilts were vaccinated with Porcilis® PRRS at the beginning of quarantine. Farm B was a multisite system with 800 sows. Vaccination against PRRSV was implemented with Porcilis® PRRS in piglets at 3 weeks of age. Farm C was a farrow to finish farm with 1000 sows and no vaccination against PRRSV. In farms A and B, three pens of pigs, and in farm C four pens of pigs were randomly selected, and oral fluid samples were collected at 5, 7, 9, 11, 13, 15 and 17 or 18 weeks of age. Additionally, serum samples from four pigs from each pen were collected at 5, 9, 13 and 17 or 18 weeks of age. Oral fluid was collected using the Oral Fluid Collection Kit (IDEXX), according to the manufacturer's instructions. The RNA extraction was performed after completion of all samplings using a QIAmp Viral RNA Mini Kit (Qiagen). Real-time PCR for the detection of PRRSV was performed with EZ-PRRSV™ MPX 4.0 (Tetracore) using the Rotor Gene-Q 6000 (Qiagen). Detection of PRRSV antibodies in oral fluid and serum was performed using IDEXX PRRS X3 and IDEXX PRRS Oral Fluid antibody tests, respectively.

PRRSV was not detected by PCR in any sample from farm A. In farm B, Type 1 PRRSV was detected in oral fluid in pigs from nearly all pens from 5 to 15 weeks of age, but was negative in pigs at 17 weeks of age. In farm C, (the unvaccinated group), Type 1 PRRSV was detected in samples from pigs at 5 to 17 weeks of age. The results of serum and oral fluid testing were in agreement. Seroconversion in farm A was detected in serum of 5 week old pigs in all 3 pens sampled. In farms B and C seroconversion was detected in all pens of pigs from 5 to 17 weeks of age.

The results of oral fluid and serum testing by ELISA and PCR provided comparable results. In farm B PRRSV circulation was extended from 5 to 15 weeks of age despite piglet vaccination with Porcilis® PRRS, which might indicate lack of efficacy of the control protocol. On the other hand, the gilt vaccination in farm A performed with Porcilis® PRRS was successful in controlling PRRSV. RNA sequencing is ongoing to better understand the strain circulating in farm B in relationship to the vaccine used.

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Evaluation of an immunoflourescence antibody assay for detection of Type 1 and Type 2 PRRSv infection

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Study objective: The study objective was to evaluate the suitability of an immunofluorescence antibody assay (IFA) for detection of antibodies directed against Type 1 and Type 2 PRRS virus.
Materials and methods: Sixty (60) seven-week old SPF piglets were included in this study. They were divided into 6 groups of 10 piglets each, and each group was infected with one of 6 different wild-type PRRSv strains of Type 1: I2, BS, Lit, SP, BE or Type 2: VR2332.Virus was applied intranasally at doses of $10^{3.5}$ to $10^{5.8}$ $TCID_{50}$ per 2 ml, 1 ml for each nostril. Blood samples were collected for each individual piglet at day 0 and day 21 after infection, and antibody titers were determined by an IFA performed at the R&D Service Lab, MSD AH, Netherlands. Type 1 PRRSv or Type 2 PRRSv infected MA-104 monolayers in a 96-well tissue culture plate were incubated with two-fold dilutions of the test serum sample. Following incubation at 37 °C, the presence of PRRSv-specific antibody in the serum was detected using a FITC-conjugated anti-swine antibody. The titer of a sample was defined as the reciprocal of the highest sample dilution showing specific PRRSv fluorescence and was expressed as $\log_2$. An IFA titer <4 $\log_2$ was considered negative, and a titer ≥4 $\log_2$ to ≤6 $\log_2$ was considered suspect. Both cut-offs were therefore included in the calculations.
Results: On day 0, all titers of animals later infected with PRRSv Type 1 were negative in IFA PRRSv Type 1 and 2 (IFA-1 and IFA-2), as were 9 of 10 animals later infected with PRRSv Type 2. The positive animal had an IFA-2 titer of 7 $\log_2$ on day 0. This IFA-2 titer rose to 11 $\log_2$ by day 21. Tables 1 and 2 show the results from the samples on day 21 after infection.

Table 1: Sensitivity and specificity of the IFA for both PRRSv types.

	n		Cut-off < 4 $\log_2$	Cut-off ≤ 6 $\log_2$
PRRSv Type 1	50	Sensitivity IFA-1	0.98	0.77
		Specificity IFA-1	0.98	1.00
PRRSv Type 2	10	Sensitivity IFA-2	1.00	0.90
		Specificity IFA-2	0.90	0.90

Table 2: Average and median IFA values.

	IFA-1 titer		IFA-2 titer		Wilcoxon
	Average	Median	Average	Median	paired test
PRRSv Type 1	8.1	9.0	1.7	0.0	$p < 0.001$
PRRSv Type 2	1.8	0.0	10.3	11.0	$p = 0.006$

In conclusion, this IFA is suitable for detecting antibodies against PRRSV as well as differentiating between antibodies directed against PRRSv Type 1 and 2.

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Sensitivity improvement of pan-viral DNA array and high-throughput sequencing with propidium monoazide (PMA) for the identification of viruses from tissue samples using PRRS virus as a model

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Pan-viral DNA array (PVDA) and high-throughput sequencing (HTS) are useful tools to identify novel virus of emerging diseases. Although PVDA and HTS work well with isolated virus, they are less sensitive to detect viruses in tissue samples. This is because of host genomic DNA and RNA (HGD/R) contaminating nucleic extract from tissue samples. Both propidium monoazide (PMA) and ethidium bromide monoazide (EMA) have the capacity to bind free RNA and DNA but are cell membrane-impermeable and thus are unable to bind protected RNA/DNA such as virion protected viral genomic material. DNA permanently linked to PMA or EMA following photolysis is not amplifiable by RNA/DNA polymerase. Thus a PMA or EMA treatment before nucleic extraction could lower HGD/R contamination. To validate this hypothesis, lung tissue homogenates were spiked with porcine reproductive and respiratory virus (PRRSV) and were processed with different combination of treatment: with/without ultracentrifugation and incubation with/without different concentration of EMA or PMA. Following each treatment, total DNA/RNA was extracted. Quantitative PCR (qPCR) was used to evaluate HGD/R contamination (beta-actin) and PRRSV presence in each DNA/RNA sample. Finally, PVDA and HTS were used to detect PRRSV in each DNA/RNA samples. Both EMA and PMA treatment increased beta-actin quantification at least by 11.40±0.52 Ct ($p<0.001$), indicating an important loss of HGD/R contamination in these samples. While EMA caused a dose-dependent decrease of PRRSV qPCR detection, no significant differences were seen in PRRSV qPCR quantification following PMA treatment. Ultracentrifugation pre-treatment (with/without PMA or EMA treatment) has no effect on HGD/R and PRRSV quantification. PRRSV was not detected by PVDA and by HTS in untreated or ultracentrifugated only samples. However, PRRSV was detected by PVDA in EMA and PMA treated samples and the best results were obtained following PMA treatment. HTS sensitivity was also improved by EMA or PMA treatment and the number of reads was significantly higher in PMA treated samples. The PMA treatment was also used on PRRSV positive clinical samples in order to confirm its efficiency to increase sensitivity of both PVDA and HTS. An increase of sensitivity was obtained in PMA treated clinical samples when HGD/R contamination was significantly lower compared to PMA treated clinical samples with higher HDG/R contamination. Overall, these results support the use of PMA as a treatment to increase sensitivity of PVDA and HTS.

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Real-Time PCR reagents for the detection of Porcine Epidemic Diarrhea Virus and Porcine Deltacoronavirus

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Porcine epidemic diarrhea virus (PEDV) and the porcine deltacoronavirus (PDCoV) represent new threats to the swine industry. Emerging in the United States in 2013 and continuing in 2014, these swine enteric coronaviruses have had a significant impact on producers. To aid in early detection of virus, monitor viral shedding, or differentiate viral species, PCR has been shown to be a useful diagnostic tool. To this end, IDEXX has developed three sets of real-time PCR reagents to detect the presence of viral RNA from i) PEDV, ii) PDCoV, and iii) TGEV, with a fourth set of reagents for the detection and differentiation of PEDV and PDCoV in a multiplexed format. Each test utilizes a multiplexed internal sample control (ISC) for the detection of host swine RNA to ensure proper sample collection, sample addition, nucleic acid extraction, and cDNA production and amplification. All four tests have efficiencies between 95-100% and maintain analytical sensitivities of ≤ 10 copies per reaction even in the presence of an artificially high concentration of the internal control (host swine RNA). All PEDV and PDCoV reagents have been evaluated with oral fluid and fecal swab clinical samples and each demonstrates 100% sensitivity and specificity for the samples tested to date. As part of the IDEXX RealPCR modular system, these tests use a shared RNA master mix and a pooled positive control. Compatibility with the IDEXX RealPCR modular system allows these tests to be run side-by-side with other RealPCR reagents maintaining fast run protocols and increasing laboratory workflow efficiency.

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Evaluation on the detection of viral pathogens in porcine lung biopsy with next-generation sequencing approach

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Objective: The current study is to evaluate the efficiency of next-generation sequencing (NGS) on the detection of viral pathogens, viral genome assemble, genotyping and identification of co-infection events in porcine lung biopsy specimens.
Methodology: Total RNA was extracted from lung biopsy isolated from two individual pigs (#15 and #17) followed by double-stranded cDNA (dscDNA) synthesis. The respective shotgun DNA libraries were constructed and a pyrosequencing run comprising two libraries was performed on 454 GS Junior Platform (Roche) at 200 cycles run module. Full processing for shotgun sequences was performed in the post-run processing algorithm. Sequences obtained were trimmed and mapped to NCBI complete RefSeq release of viral and viroid sequences with 454 GS Reference Mapper.
Result: The sequencing run recovered a total of 201,820 reads with an average read length of 399.5 bp, yielding a total of 80,627,524 bases. A total of 70,588 and 130,879 reads were retrieved from two biopsy specimens respectively after the quality trimming process. Reference mapping against viral and viroid database reveals that a very high proportion of reads (72.2%) matched to porcine circovirus 1 and 2 genomes in pig #15 which showed obvious symptoms of porcine reproductive and respiratory syndrome (PRRS), while 2.2% of unique reads matched to porcine reproductive and respiratory syndrome virus (PRRSV) genome. Viral genome assemblies reveals complete genomes of PCV1 and PCV2, whereas an incomplete genome of PRRSV was also obtained comprising 15 contigs with a total of 10,615 bases sequence (69.4% of the reference PRRSV genome). Besides, the finding indicates that, apart from the co-infection of PCVs and PRRSV, a number of non-pathogenic viruses also exist in this animal and these viruses may also contribute to the development of the observed disease symptoms. On the other hand, though pig #17 also showed similar PRRS symptoms, neither PCV nor PRRSV sequences were identified in the dataset. Majority of the sequence reads were found to be host-specific, and this vast amount of host sequence is thought to be masking the viral sequence signals and hence only limited potential viral sequences can be retrieved from the data.
Conclusion: NGS provides a rapid and efficient mean to identify viral pathogens and to construct respective viral genomes simultaneously. However, the efficiency may be hammered by the presence of host sequences and also affected by the viral titer itself. In order to elevate the sensitivity, host RNA subtraction, viral component enrichment and utilization of a higher throughput platform should be considered when employing NGS for pathogen detection.

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Application of a broad-spectrum microbial detection array for the analysis of pig pathogens

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Many of the disease syndromes challenging the commercial swine industry involve the analysis of complex problems caused by polymicrobial, emerging/re-emerging and transboundary pathogens. Currently circulating transboundary diseases, such as African swine fever and classical swine fever, are constant threats and raise concern about the possibility of both intentional as well as unintentional introduction. The best assurance of timely identification for known and unknown threats is to employ techniques that can track known disease threats, as well as rapidly identify the introduction of new pathogens before they become established.

In this study, we employed a novel and comprehensive microbial detection technology, the Lawrence Livermore Microbial Detection Array (LLMDA) that was designed to detect >8000 species of microbes. We evaluated the utility of LLMDA to analyze the microbial composition in serum, oral fluid and tonsil samples from pigs co-infected with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). The assay was compared to standard PCR methods for the detection of PRRSV and PCV2.

Under conditions of experimental infection, the array identified PRRSV and PCV2, but at a lower sensitivity compared to standard polymerase chain reaction (PCR) detection methods. The pen-based oral fluid sample was the most informative, possessing signatures from several porcine-associated viruses and bacteria, which may contribute to the severity of PRRSV and PCV2 diseases.

Since PRRSV and PCV2 are immunosuppressive, a second goal was to evaluate the presence of other agents that may contribute to disease. In addition to PCV2 and PRRSV, the LLMDA also detected other viral co-infections including porcine parainfluenza, astrovirus, and porcine stool-associated virus from oral fluid samples. Common bacterial co-infections detected by LLMDA are Streptococcus suis, clostridium sp., Staphylococcus sp., and Enterococcus sp.

This study demonstrates the utility of Lawrence Livermore Microbial Detection Array in routine clinical diagnostics and surveillance. Even though microarrays are not as sensitive as standard PCR assays, they create the opportunity to query hundreds of thousands to several million sequence-specific DNA signatures, all in parallel. As the cost of microarrays decrease, the application for use in routine diagnostics and disease surveillance in veterinary livestock is expected to increase, especially in the analysis of syndromes that result from polymicrobial interactions.

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Sero-prevalence of porcine reproductive and respiratory syndrome (PRRS) in pigs of different developmental regions of Nepal

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Porcine Reproductive and Respiratory Syndrome (PRRS) is a highly infectious viral disease of swine caused by PRRS Virus of Arteriviridae family. This disease is characterized by pneumonia and respiratory distress in neonatal pigs and reproductive failure like abortion, mummification, still birth and infertility in adults. As the prevalence of this disease in the country has not been evaluated so far, a cross sectional study was performed in four developmental regions of Nepal. A total of 200 blood samples were collected from major pig production pockets of four developmental regions of Nepal and serum is tested for antibody against PRRS virus by immunochromatographic test (rapid test kit) using colloidal gold immunoassay method. Out of 200 serum samples 37 (18.5%) were found to be positive. The prevalence rate in Eastern development region, Central development region, Western development region and Mid-Western development region was found to be 6%, 22%, 32% and 14% respectively. Statistically significant difference ($p<0.05$) was observed in prevalence of disease in different development regions. District wise 12%, 24%, 20%, 16%. 48%, 20% and 8% of the samples were found to be positive in Sunsari, Kathmandu valley, Chitwan, Kaski, Rupandehi, Banke and Surkhet districts respectively. All the samples were found to be negative from Dhankuta district. The presence of antibodies in the serum and the fact that there had been no vaccination against this disease clearly indicates that the pigs have already been exposed to the PRRS virus. Though this test is not confirmatory test it gives some indication of prevalence of the disease in Nepal. The vaccination against this disease has not been introduced in Nepal. As two strains of the virus are prevalent in other country so the strain prevalent in Nepal has to be identified before introducing the vaccination programme. The vaccine has to be as per the strain and type of virus prevalent in Nepal. It is necessary to carry out detail studies on molecular characteristics of the virus for proper implementation of prevention and control strategies.

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The optimized herd concept (OHC) – A comprehensive solution strategy to control PRRSV

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PRRSV outbreaks are feared in the swine industry because of the devastating economical impact. Even though various diagnostic tests for PRRSV including ELISA and PCR systems exist, correct result interpretation with respect to the actual PRRSV status pose a major challenge. Noteworthy are the genetic diversity of PRRSV isolates, the prolonged PRRSV persistence and the complex immunological behavior of the virus. Vaccination against PRRS has shown to be an effective tool to reduce clinical disease, however PRRSV infection is not prevented. MLV vaccine virus may be shed and transmitted to non-vaccinated contact pigs and vaccine virus can persist in boars and be disseminated through semen. The complex nature of the PRRSV disease indicates that single diagnostic tests may not be enough to successfully subdue PRRS virus but rather comprehensive solution strategies are needed for the effective control of the disease. In particular to discriminate type I from type II on herd level with an ELISA approach, to detect single or mixed infections of different PRRSV types, assess the chronological order of different infections, estimate the time of infection and the virus load and finally support decisions to choose the optimal time for PRRSV vaccination and the respective schedule.
Here we demonstrate the reliability and robustness of PRRSV detection using the PrioCHECK® PRRSV Ab porcine and the newly developed PrioCHECK® PRRSV VIA ELISA to discriminate PRRSV type I from type II. By combination of herd as well as site information and results of the PrioCHECK® PRRSV Ab porcine and the PrioCHECK® PRRSV VIA, optimized measures for a comprehensive PRRSV control can be implemented.
Using Type I and Type II specific antigens an indirect ELISA was developed being able to discriminate Type I from Type II PRRS called PrioCHECK® PRRSV VIA.
In combination with results of PrioCHECK® PRRSV Ab porcine, PRRSV PCR as well as herd / site information the PrioCHECK® PRRSV VIA is able to

- Discriminate type I from type II on herd level
- Detect single or mixed infections of different PRRSV types on herd level
- Assess the chronological order of different infections
- Estimate the time of infection and the virus load based on the serological titre level
- Help to decide over the optimal time for PRRSV vaccination and the respective schedule

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Development of an indirect ELISA, a fluorescent microsphere immunoassay (FMIA) and a blocking ELISA for detection of antibodies against porcine epidemic diarrhea virus (PEDV)

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PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the *Coronaviridae* family. PEDV was first identified in the U.S. in May 2013 and has since been confirmed in at least 30 states and additional countries. PCR assays provide an important tool in control of the virus; however, well-validated, high-throughput serological assays would provide a valuable additional diagnostic tool for the swine industry.

In response to these needs, we developed three separate serological assays for the detection of antibodies against PEDV: (1) an indirect ELISA (iELISA), a fluorescent microsphere immunoassay (FMIA) and a blocking ELISA (bELISA). All three tests use *E. coli* expressed, full length PEDV nucleoprotein (NP) as detection antigen. For the FMIA, a two-step carbodiimide coupling procedure was used to couple PEDV-NP protein to Luminex™ microspheres. For the bELISA, monoclonal antibodies (SD6-29 & SD17-103) against the PEDV-NP were biotinylated and included to serve as the competitive, detection moiety. All three tests were optimized in a checkerboard fashion to maximize signal-to-noise ratios using samples of known serostatus. Known PEDV negative sample sets included samples from selected high biosecurity herds with no history of PEDV and archived serum samples collected prior to the emergence of PEDV in the U.S. Next, multiple comparison analysis was performed to assess sensitivity, specificity and testing agreement against a previously developed immunofluorescence assay (IFA). Receiver operating characteristic analysis (ROC) was performed using swine serum samples, (FMIA n=1420, bELISA n=1186 and iELISA n=1486). Results of ROC analysis for FMIA showed estimated sensitivity and specificity of 98.2% and 99.2%, respectively. The bELISA and iELISA showed a sensitivity and specificity of 98.2% and 98.9%; and 97.9% and 97.6% respectively. Inter-rater (kappa) agreement, a statistical measure of test agreement, was calculated to be 0.932 between FMIA and IFA, 0.945 between bELISA and IFA and 0.941 between FMIA and bELISA. Similar comparative kappa values were observed between the FMIA, bELISA and the iELISA which demonstrated a significant level of testing agreement between the three assays. Time-course experiments demonstrated seroconversion between 7-10 DPI. Antibody kinetics of individual isotypes were also assessed via a PEDV-NP FMIA using time-course collected serum samples. Results showed an early and robust IgM response (7 DPI) followed by increasing levels of IgG. Development of neutralizing antibody responses was monitored using a fluorescent focus neutralization (FFN) assay. Also, increasing levels of spike-specific IgM, IgA, & IgG signals were detected in serum with the latter two showing similar concentrations. Additionally, none of the known positive TGEV or PRCV samples tested (n=>50) were shown to cross-react on either test. These high throughput assays should be of value in controlling the spread of PEDV and in seroprevalence studies. Ongoing studies include adapting the assays to oral fluid and milk testing and multiplexing the FMIA for the simultaneous detection of antibodies to other swine pathogens including porcine deltacoronavirus.

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Swine Enteric Coronavirus evolution and phylogenetic diversity in the United States

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The three known swine enteric disease coronaviruses (SEDCs), Transmissible Gastroenteritis Virus (TGEV), Porcine Epidemic Diarrhea Virus (PEDV), and Porcine Deltacoronavirus (PDCoV) have caused severe problems for pork producers. PDCoV was identified in January 2014 within the United States (US), which was globally discovered during surveillance for new Deltacoronaviruses in Hong Kong. Due to control measure, TGEV prevalence in the US has greatly reduced. However, TGEV continues to circulate in the US swine population. The genetic diversity of SEDCs has yet to be understood in the US. The object of this study was to identify the genetic diversity of PEDV, PDCoV, and TGEV in the US. The US SEDC strains were selected for complete genome sequencing using the Illumina Miseq. In North America, PEDV strains cluster within 2 distinct phylogenetic clades while the INDEL strains, which contain a spike gene deletion similar to the prototype strain CV777, form a distant cluster within North American clade II. In addition, another PEDV strain has been identified with spike gene nucleotide deletion at position 164–169 (TTGGTG), which has yet to be described. Additionally, PDCoV was identified in January 2014 within the US. Thus far, 23 US PDCoV strains have been sequenced and share a 99.5-100% nucleic identity, clustering separately from the Hong Kong strains. The current TGEV strains from the US generate new branch in the phylogenetic tree and share a nucleotide percent identify of 98-99.9% while only sharing a 96-97% to the historic TGEV strains. Lack of global sequence data impedes our understanding of the evolution and diversity of SEDCs. This study highlights the genetic diversity of SEDCs in the US and interrupts the results within a global setting. The evolution of TGEV in the US may shed light on the evolution of PEDV and PDCoV.

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Performance assessment of a real-time polymerase chain reaction assay for porcine epidemic diarrhea virus to assess PEDV transmission in growing pigs

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PEDV was first diagnosed in the U.S. in April 2013 as sporadic cases of diarrhea in young piglets with high mortality. Real-time RT-PCR is a high throughput test system that has potential to detect PEDV during the acute phase of the infection or pre-seroconversion.

A study in nursery pigs was conducted to assess the transmission potential of young pigs experimentally-infected or experimentally-exposed to PEDV. On day 0 (D0), a 4-week-old pig was challenged with PEDV and 14 naïve contacts were comingled. On D7, 9 contact pigs were moved to a new room to serve as the principal virus reservoir group (PG), and comingled with 1 naïve age-matched sentinel (S1). Three days later, the S1 pig was moved to a separate room until necropsy. This process was repeated on D14, 21 and 28 with pigs S2, S3 and S4. On day 49, 5 naïve age-matched pigs (N) and the PG were challenged (N/C, PG/C) with homologous virus and euthanized on D78. A daily rectal swab was collected from each pig and tested for PEDV using real-time RT-PCR to detect the N gene (gN) and the S gene (gS) using commercial chemistry.

Detection limits and threshold cycle (Ct) values of real-time RT-PCR were assayed for PEDV samples and positive controls for both gN and gS. The coefficient of variation determined based on the replicates (intra-assay variation) ranged from 0.00% to 2.65% and inter-assay variation had an average of 2.75%. Real-time RT-PCR PEDV assay results are visualized in a heat map where positive is $Ct \leq 34.99$. All PG pigs were real-time RT-PCR-positive from D3-11, with some intermittently positive to D42. Following challenge at D49, all PG pigs were negative post-challenge (PC) and all N/C pigs were positive by D52 days. 3/5 pigs until 13 days PC. PEDV RNA was detected in S1 and S2 within 1 day of contact, but not detected in S3 or S4. Real-time RT-PCR positive rectal swabs collected after D21 were tested for infectious virus using bioassay techniques.

A critical need for the current PEDV surveillance program is the rapid detection of PEDV. The present study evaluated the real-time RT-PCR assay to detect PEDV infection in the transmission potential of young pigs experimentally-exposed to PEDV.

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Development of serological assays for detection of antibodies against porcine deltacoronavirus (PDCoV)

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A novel porcine deltacoronavirus (PDCoV) was reported in China in 2012. In February 2014, the Ohio Department of Agriculture announced the identification of PDCoV in the U.S. Since then, PDCoV has been identified in a number of U.S. states and linked with apparent clinical disease including acute diarrhea and vomiting in the absence of other identifiable pathogens. PDCoV is currently diagnosed by real-time PCR and clinical symptoms, along with elimination of other viral pathogens known to cause similar disease. Since PDCoV was just recently linked with clinical disease, limited serological capabilities are currently available to detect an antibody response to this virus. Therefore, the objective of this study was to develop and optimize several serological assays including indirect ELISA, fluorescent microsphere immunoassay (FMIA), indirect fluorescent antibody (IFA) and virus neutralization assays.

An indirect fluorescent antibody (IFA) test was first developed using cell culture adapted PDCoV provided by the National Veterinary Services Laboratories. Serum samples from swine herds with recent documentation of PDCoV infection and samples from expected naïve herds were used for initial assay optimization. For development of ELISA and FMIA test formats, the full-length nucleoprotein (NP) of PDCoV was cloned and expressed in *E. coli* as a 41 kDa polyhistidine fusion protein. This protein was purified by Nickel-NTA affinity column chromatography and was recognized in Western blotting by convalescent serum and two separate monoclonal antibodies developed in our laboratory. A refolded version of the protein was used as an antigen in both assays as it was shown to impart significantly higher antibody capture efficacy, 27 times compared with non-refolded antigen. ELISA microtiter plates were coated with 250 ng of antigen while Luminex microspheres were coupled at a concentration of 12.5 ug nucleoprotein per 6 $x10^6$ microspheres. The tests were optimized in a checkerboard fashion to reduce signal to noise ratios using expected seronegative and seropositive serum samples (n=375). The expected positive samples used were submitted field serum samples (n=110) from herds previously testing PDCoV positive by PCR. Expected negative samples included archived experimental serum collected prior to 2009 (n=108) and field samples from high-health herds with no known history of PDCoV exposure (n=157). Preliminary ROC analysis was performed and diagnostic sensitivities and specificities were approximately 95% and 94%, respectively as compared to a validated IFA test. Once validated, the assays were used to assess the kinetics of a time course antibody response. Both FMIA and ELISA showed seroconversion between 8-14 DPI. In addition, neutralizing antibody titers in serum are being quantified via the development and validation of a fluorescent focus neutralization assay (FFN). These results confirm the utility of these new diagnostic tests to aid in the control and surveillance of porcine deltacoronavirus outbreaks.

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Objective assessment of the extent of lung lesions in pigs infected with Porcine Reproductive and Respiratory Syndrome Virus, a novel approach

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Given the many strains of PRRSV with differing virulence and the enormous financial strains of PRRS on the pork industry, much effort has been directed at assessing disease severity in pigs infected with PRRSV, especially when evaluating viral virulence and vaccine efficacy. However, many of the parameters used in disease assessment are either subjective and/or require considerable pathological skill and expertise. The goal of this study is to validate a simple and objective method, based on measuring the lung's volumetric mass density (ρ), for assessing the extent of lung lesions in pigs infected with PRRSV and its application to measure vaccine efficacy. Seventy-four pigs were divided into 3 groups. Group 1 served as the untreated strict control, group 2 was vaccinated and challenged with PRRSV, and group 3 was challenged with PRRSV and not vaccinated. At 14 days post-infection, all of the pigs were euthanized and their lungs evaluated for the extent of gross and microscopic pathology using the method described by Halbur et al. (1995). The density of the lung was determined by dissecting the lung in 4 sections. The weight of each quarter was divided by its corresponding volume, which was measured by completely submerging each lung section into a 2L size graduated cylinder partially filled with water and measuring the volume of water displaced. The average of the four calculated density measurements was used as the lung density for each pig. The gross and microscopic lesions observed were consistent with the presence of virus-induced interstitial pneumonia. The histopathology scores positively correlated with the density of the lungs, resulting in a Pearson's correlation value of 0.75. The mean density of lungs of the pigs in group 3 (0.72±0.06) was significantly higher ($p<0.05$) than those in groups 1 (0.66±0.04) and 2 (0.67±0.06). These results validate the objective measurement of lung density as a method for assessing the extent of lung lesions in pigs infected with PRRSV and its use to assess vaccine efficacy.

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Development of monoclonal antibodies and other reagents for detection of porcine deltacoronavirus (PDCoV)

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Coronaviruses are enveloped, positive sense RNA viruses divided among four genera, including the recently described genus *Deltacoronavirus*. A novel porcine deltacoronavirus (PDCoV) was reported in China in 2012. In February 2014, the Ohio Department of Agriculture announced the identification of PDCoV in the U.S. Since then, PDCoV has been identified in a number of U.S. states and linked with apparent clinical disease including acute diarrhea and vomiting in the absence of other identifiable pathogens. PDCoV is currently diagnosed by real time PCR and clinical symptoms along with elimination of other viral pathogens known to cause similar disease. Since minimal specific antibody-based reagents are available to assist in diagnosis of PDCoV, the purpose of this study was to develop readily available reagents for detection of PDCoV antigen in diagnostic tests, such as virus isolation, immunohistochemistry and fluorescent antibody techniques.

The full-length nucleoprotein (NP) of PDCoV was cloned and expressed in *E. coli* as a 41 kDa polyhistidine fusion protein. This protein was purified by Nickel-NTA affinity column chromatography and is recognized in Western blotting and ELISA by convalescent serum from infected pigs. Both denatured and refolded versions of this protein were used to immunize rabbits for hyperimmune serum and mice for monoclonal antibody production. Rabbit hyperimmune sera specifically recognize the NP and can be used in indirect fluorescent antibody staining at dilutions of 1:1000 to 1:5000. In addition, the polyclonal antisera was used successfully in immunohistochemical staining procedures for the detection PDCoV antigen in intestinal tissues. To date, a total of 12 hybridoma clones producing monoclonal antibodies against the PDCoV-NP have been isolated and are currently being fully characterized. The resulting monoclonal antibodies all recognized native viral protein in infected Vero-76 cells, demonstrated by bright cytoplasmic immunofluorescent staining. All monoclonal antibodies of the IgG_1 isotype recognize full length recombinant PDCoV-NP in addition to native, whole virus via Western blotting.

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

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Development of the Inmunofluorescence Assay (IFA) for the detection of Porcine Reproductive and Respiratory Virus (PRRSv) endemic strains in Mexico

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The PRRSv is considered the most important disease causing huge economic losses worldwide (Karniychuk 2011, Nieuwenhuis 2012, Prather 2013, Franzo 2014). The high genetic variability (15% and 12.5% between genotypes) of the virus along with the nucleotide substitution rate (between 4.7×10^{-2} and 1.55×10^{-3}) and the animal trading favoring recombination affects the control and accurate diagnosis of the disease. (Darwich 2010, Franzo 2014, Drigo 2014). Implementation of accurate diagnostic tests plays an important role in the surveillance and control programs for the disease. The objective of this study was to implement the Inmunofluorescence Assay (IFA) at the laboratory to be available for further investigation and public diagnosis. MARC 145 cells were grown in 96 well plates at 37°C with 5% CO^2 for 24 hours or 80-90% confluence. Cells were infected with different concentrations, of six wild virus obtained from different counties of Mexico, in quadruplicates for 96 hours. Plates were washed three times with 100 µl of sterile PBS and fixed with 50 µl of 80% acetone. Blocking was made with 200 µl of PBS-BSA for 30 minutes at room temperature. Decanted and 30 µl of the fluorescent antibody (Rural Technologies SDOW-F 1009282 for the Nucleocapsid protein, ORF 7) were added at a 1:10 dilution till 1:100 in PBS 5% BFS and allowed to incubate at 37 ° C. Finally decanted and washed three times with 100 µl PBS at 4° C leaving 50 µl to observe the plate under a Life Technologies EVOS FL (11212-1626-043) microscope. Green fluorescence was observed in all viral dilutions while negative control cells did not show any fluorescence at all. All six strains show positive results with fluorescence observed in MARC 145 cells. Assay was standardized for dilution 1:100 to optimize the reagent. The IFA was standardized at a concentration of 1:100 of the antibody. Diagnosis was achieved with the IFA for the six wild virus strains that are a representative sample of endemic strains circulating in México, which cytopathic effect has found to be varying and in some cases difficult to observe, providing one more tool for the accurate diagnosis.

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Detection of Porcine Reproductive and Respiratory Syndrome virus in swine farms from rural Romania using one-step Real-Time PCR for the reference gene ORF7

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The main objective of the current study was to evaluate the sensitivity and accuracy of the Real-Time RT-PCR method and to genetically analyze diverse PRRSV isolates from Romanian farms, in tissue homogenate samples.

The secondary purpose of this study was to identify the molecular variation of the PRRSV based on the sequence of a low pathogenic PRRS strain characteristic for the Romanian territory.

The biological material consisted of 75 samples from pig farms from different regions of Romania. The samples were represented by organs homogenates (liver, lungs, and kidney) collected from specimens that present signs of infection.

The estimation of the pathogen load in samples with clinical signs of PRRS virus was obtained by Real Time PCR method, followed up by Sanger sequencing and data collection and interpretation.

The PRRSV is one of the most variable and rapidly evolving RNA viruses, the probe hybridization being the key point in this assay.

The primers used for the detection of PRRS virus by Real Time PCR were represented by primers with a specific sequence for the ORF7 gene of the PRRS virus. More important, the primers attachment process was influenced by punctual mutations of the viral strand belonging to the reference ORF7 gene.

We identified 35 of the tested samples that were positive to the PRRSV, EU genotype. According to these results, only the samples which presented these specific punctual mutations at the genome level of ORF7 tested as positive due to primer sequence specificity and complementarity.

One of the purposes of this study was the evaluation and verification of the Real-Time RT-PCR method. This method is fast, sensitive and – unlike other methods that use probes (TaqMan) – is not able to tolerate mismatches in the probe region.

Secondly, the genomic sequences variation of the tested samples in this current study revealed a high identity to the low pathogenic PRRS strain that is currently evolving in Romania.

In conclusion, for the future studies it is strongly suggested to share and determine sequences of new emerging strains from all over the world, in order to provide a source of continuous information for the molecular diagnostic methods.

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Characterization of two porcine reproductive and respiratory syndrome virus isolates with deletions in the GP2 gene

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In this study two novel PRRSV isolates, Henan-A10 and Henan-A11 were obtained from sera of aborting sows from an all-in, all-out swine farm practicing routine HP-PRRSV vaccination, in Henan Province in 2013. Both isolates could replicate in primary porcine alveolar macrophage cells but not in MARC-145 cells confirmed by RT-PCR and indirect immunofluorescent assays. The complete genomic sequences of Henan-A10 and Henan-A11 were determined, and submitted to Genbank (accession no. KJ609516 and KJ609517). A discontinued 90 nucleotide deletion in the non-structural protein (NSP) 2 gene was found in their genome, which is an unique feature of the HP-PRRSV. A phylogenetic tree based on the complete genomic sequences of 62 PRRSV strains was constructed to investigate the genetic relationships between the two isolates and other PRRSV strains, and the results revealed that both viruses were most closely related to HP-PRRSV.

However, genomic sequence analysis showed that Henan-A10 and A11 shared only 96.8–97.8% nucleotide identity with the representative HP-PRRSV strain JXA1, meanwhile they shared 88.4-88.9% nucleotide identity with PRRSV strain VR2332. Notably, the amino acid sequence analysis showed that both viruses have a 10 aa deletion in the C-terminal of the GP2 protein. A nucleotide mutation (TTA–TAA) results in a stop codon in this region, which makes GP2 shorter other PRRSV strains. In order to investigate the influence of the deletion, a chimeric clone with the corresponding deletion in GP2 was constructed based on a full-length, infectious cDNA clone of HuN4-F112. We found that the deletion did not affect viral growth in MARC-145 cells, indicating that the endodomain of PRRSV GP2 may be variable.

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Industry and academic collaborations for maintenance of the *virotype*® PRRSV RT-PCR reagents for reliable pathogen detection: Continual employment of scientific tools are essential

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Introduction: Accurate pathogen detection is essential in pathogen mitigation strategies and Molecular detection assay sensitivity during development and over time is commonly complicatedly in the context of rapidly evolving RNA viruses. Additionally, the evolution of emergent clades coupled with the ever-present threat of novel emerging viruses with clinical similarity require constant consideration. Recent and relevant examples include Influenza A virus evolution and matrix gene recombination in multiple hosts including human, avian, and swine and Porcine Enteric Corona virus spread and evolution in swine populations globally. Porcine Respiratory and Reproductive Syndrome virus (PRRSV) is no exception for this pathogen has persisted and continues to evolve globally, continually causing significant economic consequences and dismay with regard to accurate molecular based detection. Continual and vigorous commitment of industry and diagnosticians is paramount for ongoing accurate diagnostic reagents of this high consequence pathogen.
Methods: Virotype PRRSV NA/EU real-time PCR reagents are designed to detect North American and European PRRSV strains in a multiplex assay which includes an internal positive control. Reagents are derived from the Friedrich-Loeffler-Institut validated virotype PRRSV Kit and are being translated for detection of PRRSv in the United States.
Reagent evaluation with academic partners which includes diligent bioinformatics including pathogen genomic characterization from clinical samples identified critical sequence changes that might affect accuracy of the virotype PRRSV NA/EU detection. In silico PCR identified design modifications which if implemented would maintain accurate detection of the regional PRRSV strains, and maintain detection accuracy of known strains. Moreover, ongoing studies with academic/diagnostic partners facilitate assay maintenance to help insure persistent sensitivity. Analytical sensitivity was evaluated for improved sensitivity of detection utilizing purified RNA, from relevant PRRSV strains, and serum samples from infected swine, and clinical samples.
Results: Assay redesign demonstrate improved sensitivity (1-3 logs) over currently utilized diagnostic methods PRRSv detection of atypical or emergent field strains without affecting detection of known strains.
Conclusion: Academic collaboration and attainment of sequence information for atypical virus isolates coupled with diligent deployment of bioinformatics aided in assessment and successful redesign of oligonucleotides utilized in the QIAGEN virotype PRRSV EU/NA Reagents. Moreover, work amplifies the necessity of solid working relationships between the pork and the biotechnology industry and academia in further advancement and maintenance of veterinary diagnostic assays for the sustained detection of high consequence pathogens of swine including PRRSv.

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Genomic sequencing and analysis of a new porcine reproductive and respiratory syndrome virus isolate from Ohio: virus continues change

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Porcine reproductive and respiratory syndrome (PRRS) virus is a single stranded, positive sense, enveloped RNA virus, belonging to the family *Arteriviridae*. PRRS was first recognized in the United States in 1987 and PRRS virus (PRRSV) was first identified in the Netherlands in 1991. Genetic characterization demonstrated that there are two genotypes, European (type I) and North American (type II), currently circulating in the world. PRRSV contains a 15kb-size genome encoding structural proteins in the 3' end. In 2013, six blood samples from pigs experiencing severe respiratory diseases in an Ohio pig farm were submitted to the Animal Disease Diagnostic Laboratory of Ohio Department of Agriculture. All samples were tested positive for PRRSV using a real-time RT-PCR assay. The CT values of these samples ranged from 15.85 to 28.38. Virus isolation was carried out using MARC-145 cell line. Cytopathic effect was observed after 4 days post inoculation. Whole genome sequencing was subsequently completed for the samples. The whole genome analysis showed that this PRRSV strain (OH28372) has 92% or even lower nucleotide similarity to the published sequences in GenBank including XW001 and NADC30. The ORF5 gene of OH28372 has 96% nucleotide similarity to the other sequences in GenBank. In addition, OH28372 has a deletion of 13 nucleotides in the 3' untranslated region. In consistent with genomic analysis, phylogenetic study of the complete genome showed that OH28372 is more closely related to the two recent US strains (XW001 and NADC30) under the North American lineage. Our study highlights the importance of continued monitoring of PRRSV evolution using genomic sequencing.

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PCV-2 and PRRSV is Common in Henan, China from November 2013 to October 2014

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Objective:
To investigate the viruses that cause porcine reproductive and/or respiratory failure in Henan, China from November 2013 to October 2014.
Methods:
A total of 67 porcine lungs were collected from pigs that showed reproductive and/or respiratory failure in different places of Henan, China from November 2013 to October 2014.The viral detection was based on polymerase chain reaction (PCR) with seven pairs of primers specific to porcine circovirus type 2 (PCV-2), porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever virus (CSFV), porcine parvovirus (PPV), porcine pseudorabies virus (PRV), swine influenza virus (SIV), and Japanese encephalitis virus (JEV) respectively.
Result:
The positive rate of PCV-2, PRRSV, PPV, SIV, PRV and CSFV was 76.12%, 38.81%, 10.45%, 5.97%, 2.99% and 2.99% respectively, while no JEV was detected in this study. The result reveals that PCV-2 and PRRSV are the predominant viruses in the specimens; moreover, PCV-2 and PRRSV can be detected in all seasons. Infection with PRRSV alone accounted for only 1.49% (1/67) of cases, although PRRSV is one of the major viruses detected in the specimens. The co-infection rate of PCV-2 and PRRSV is up to 31.34%, suggesting that co-infection of PCV-2 and PRRSV is common. Even though co-infection with three or even four viruses was detected, the rates are very low compared with the co-infection rate of PCV-2 and PRRSV; for example, the co-infection rate of PCV-2, PRRSV, PPV and CSFV is only 1.49%.
Conclusion:
PCV-2 and PRRSV were commonly found in pig lungs that it is thought to be responsible for porcine reproductive and/ or respiratory failure and the co-infection event of these two viruses were not rare in Henan, China from November 2013 to October 2014.

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Virulence of Porcine Epidemic Diarrhea Virus (PEDV) for weaning-age pigs

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A study was conducted to evaluate the virulence of 2 isolates of PEDV when administered to weaning-age pigs (Data on File, Study Report No. B82W-US-14-279, Zoetis Inc). Pigs, sourced from a farm free of PEDV, were randomly allotted to seven treatment groups; each group consisted of 10 piglets. One treatment group was not challenged (T01) and the others were challenged via esophageal gavage with 10^3 (T02), 10^4 (T03), or 10^5 (T04) $TCID_{50}$ per 10 mL of Isolate #1 or 10^3 (T05), 10^4 (T06), or 10^5 (T07) $TCID_{50}$ per 10 mL of Isolate #3. Clinical observations and fecal swabs were collected daily post-challenge and pigs were euthanized and necropsied 7 days post-challenge. Fecal swabs were analyzed for PEDV nucleic acid via RT-qPCR. Intestinal tissues were submitted to the Iowa State University Veterinary Diagnostic Laboratory, Ames, IA, and examined for villous atrophy and for PEDV staining via IHC. The study was valid in that the T01 control pigs remained negative for PEDV-associated clinical signs, intestinal lesions and fecal shedding. Pigs across all PEDV challenge groups showed clinical signs and had lesions consistent with PEDV infection (Table 1). Clinical signs were observed beginning 3 days post-challenge and continued until necropsy. Fecal shedding of PEDV was detected as early as Day 2 post-challenge in pigs inoculated with the higher doses (T03, T04, T06 and T07) and on Day 5 or 6 in pigs challenged with the lower doses (T02 and T05). Fecal shedding in high copy numbers was still apparent in all groups at necropsy on Day 7. Microscopic and macroscopic lesions associated with PEDV were present in pigs in all challenged groups. Under the conditions of this study, neither field isolate produced mortality in weaned pigs; however, both isolates, at all doses tested, induced clinical signs, intestinal lesions and persistent fecal shedding of the virus. In vivo procedures occurred according to state, national, or international regulations and after ethical review by Zoetis's IACUC.

Table 1. Abnormal fecal scores and fecal shedding following challenge with PEDV.

Trt	PEDV Isolate	Dose	Fecal scores*	Fecal shedding*
T01	na	na	0.78	0
T02	#1	10^3	18.40	15.54
T03	#1	10^4	46.67	42.60
T04	#1	10^5	51.02	71.50
T05	#3	10^3	19.96	19.35
T06	#3	10^4	54.13	59.75
T07	#3	10^5	42.44	76.53

* back transformed mean % days

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Analysis of B-cell repertoire related to PRRSV neutralization

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Neutralizing antibodies (nAbs) play an important role in protective immunity against PRRSV infection; however, PRRSV infection and vaccination usually induce weak and delayed nAbs. Lack of knowledge about PRRSV-specific nAbs has become a big obstacle in developing effective PRRSV vaccines. Within the PRRS Host Genetics Consortium (PHGC) project, pigs producing a wide range of humoral immune responses from no detectable nAbs to broad nAbs have been identified. In this study, we analyzed the B cell repertoires from pigs that produced no nAbs, homologous nAbs, and broad nAbs. Swine VDJ gene segments were amplified using a single primer pair, cloned into TOPO pCR2.1 vector and submitted for high-throughput sequencing. A total of 385 VDJ sequences were obtained from mock-infected and PRRSV-infected pigs. Sequence alignment showed that the diversification of the VDJ gene was mainly due to the variation in CDRs, especially CDR3, which is the main determinant of antibody-antigen binding. Seven major V_H genes accounted for >70% of the antibody repertoires from mock-infected pigs, whereas, the percentages were <50% in PRRSV-infected pigs. In addition, one, six and two lineages were considered as the candidates associated with non-nAbs, homologous nAbs, and broad nAbs, respectively. This study provided a simple straightforward method to analyze swine immunoglobulin VDJ repertoires and identified potential lineages associated with different antibody responses.

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PRRS virus shedding and IgA antibody response in feces of vaccinated pigs

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Porcine reproductive and respiratory virus (PRRSV) is small 15 kb, enveloped, single-stranded and positive sense RNA virus belonging to the family *Arteriviridae* and order *Nidovirales*. PRSSV causes economically important disease in pigs of different age, especially in sows and young pigs. The virus affects respiratory and reproductive systems and causes abortions, stillbirth, cyanosis of the ear and vulva and respiratory distress. PRRSV is spread worldwide. Based on the genetic and antigenic differences PRRSV strains can be divided into European and North American genotypes. They diverge approximately by 40 % at nucleotide sequence level.

The main aim of our study was to quantify the amount of virus shed in feces of pigs vaccinated with attenuated or inactivated vaccines and in unvaccinated pigs following challenge with Lelystad PRRS virus strain. Another aim was to detect IgA antibodies in fecal samples and to prove possible relationship between the amount of shed virus and the titer of IgA in feces.

Seventeen piglets divided into five groups were used in our study (three animals in each vaccinated group, five animals in control group). Commercially available inactivated (In-A, In-B) and attenuated (MLV-A, MLV-B) vaccines were used. Control group was left unvaccinated. Subsequently all groups were exposed to 10^6 $TCID_{50}$ of PRRSV (Lelystad strain). Fecal samples were collected in one week intervals. Virus RNA was detected by qRT – PCR test, ORF7 specific IgA antibodies by ELISA test.

The virus was present in feces in almost all (but one) animals vaccinated with attenuated vaccine or following experimental infection. The titer of shed virus was not influenced by the type of used vaccine and reached 10^5 virus genome copies/g in all vaccinated and control groups of piglets. Nonetheless in some animals intermittent shedding was observed.

All vaccines were able to induce IgA antibody response in the gut of piglets. The titer of IgA antibodies didn´t correlate with the amount of shed virus.

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Porcine reproductive and respiratory syndrome virus infection in pigs with severe combined immunodeficiency

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Although there is increased knowledge in the pathogenesis of PRRS, further understanding of the role of the adaptive immune responses during PRRSV infection is needed. Severe combined immunodeficiency (SCID) is a genetic syndrome observed in humans, mice, horses, and dogs and is characterized by lack of cellular components of the adaptive immune system. Affected animals are incapable of mounting a specific immune response and typically succumb to secondary infections as a result of the severe immunodeficiency. We recently discovered a line of pigs with naturally occurring SCID syndrome and they have proven very valuable in studying the pathogenesis of PRRSV infection. In this study, seven SCIDs and two normal littermates were inoculated with PRRSV at 21-days of age to determine the differences in responses of the adaptive immunity to the virus infection and to assess the presence of lesions during acute viremia. SCID in pigs was determined by flow cytometry immunophenotyping performed at 2-days of age and was confirmed by histopathology at the end of the study. The most significant microscopic lesions were observed in lungs of normal pigs, characterized by lymphoplasmacytic interstitial pneumonia and perivascular edema. No PRRSV-related pneumonia was observed in SCID pigs. Serum samples were taken 0, 4, 7, and 10 days post-infection (dpi). Viremia levels were measured by real time RT-PCR at 0, 4, 7, and 10 dpi and revealed no significant differences between SCID and normal littermates, showing that both normal and SCID pigs were permissive to PRRSV infection. Multiplex analysis of serum antibodies revealed an increase of PRRSV- IgM and IgG in normal pigs but not in SCID pigs, demonstrating that only normal pigs were capable of mounting a PRRSV-specific immune response. Overall, these findings indicate that the microscopic lesions observed in the lungs of normal pigs are the result of the response of the adaptive immune system to PRRSV infection in immune competent pigs and not by the sole presence of the virus, highlighting the need of deeper understanding of the role of cellular and humoral responses to PRRSV infection. Current laboratory efforts are targeted at developing a line of T-cell reconstituted SCID pigs that will serve as a model to further understand the role of T-cell mediated immunity during PRRSV infection.

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Dendritic cell innate immunity to PRRSV VR-2385 and VR-2332

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Dendritic cells are the professional antigen presenting cells of the immune system. They are responsible for transporting antigen to peripheral lymphoid organs, directly stimulating naïve T cells to mount a specific response. It is not well understood which subpopulation of DCs is susceptible to infection with PRRSV, but infection is highly likely and could provide the missing information that will allow us to further understand PRRSV immunopathogenesis. Significant gaps exist in the knowledge surrounding pathogenesis and immune regulation by PRRSV, and viral persistence in peripheral lymphoid organs and in the spleen may be indicators of an expanding viral tropism. We believe that specific strains of PRRSV possess unique immune regulatory mechanisms only observable in DCs. The aim of the current study was to determine the susceptibility of DCs and to characterize the innate response to PRRSV infection in a strain specific manner, according to variable pathogenicity. By shedding light upon PRRSV immunopathogenesis, we hope to provide information that could aid in the development of an efficacious vaccine.

Flt3L bone marrow derived DCs (BMDCs) were differentiated from hematopoietic stem cells and infected with two strains of PRRSV (ATCC VR-2385 and VR-2332). Specific DC populations were isolated based on the expression of CD163. A comparative analysis was launched between the populations. Flow cytometry, fluorescent microscopy, confocal microscopy, and real-time quantitative PCR were performed to analyze infection and to assess the subsequent immune response.

Specific subsets of DCs (those expressing CD163) are targeted by both strains of PRRSV. The more virulent strain (VR-2385) showed elevated levels of TNF-alpha and IFN-alpha mRNA in comparison to VR-2332, which stimulated IL-10 expression. Both strains appeared to induce similar levels of IFN-beta and CCR7, but VR-2385 infection resulted in suppressed MHCI and MHCII expression. The CD163 negative DCs seemed to be incapable of responding to PRRSV in the absence of a susceptible cell population.

Our results indicate that although infection is restricted to CD163 expressing DCs, the innate response of DCs to infection appears to be strain specific. The more virulent strain (VR-2385) appears to possess the ability to potentially compromise DC antigen presentation, exhibited by down-regulation of MHCI and MHCII. Furthermore, viral persistence may be attributable to lack of MHCI expression. Increased virulence may be attributable to higher levels of pro-inflammatory cytokines, in comparison to VR-2332, which primarily showed increased levels of IL-10. Future studies will help to identify new virulence factors, which are only observable in DCs and during their interaction with T-lymphocytes.

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Effect of WUR genotype and PRRS vaccination on pigs co-infected with PRRS and PCV2b

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Porcine reproductive and respiratory syndrome (PRRS) is the most costly disease to the US pork industry. PRRS virus (PRRSV) weakens the immune system, thereby increasing the likelihood of co-infection with other diseases, such as porcine circovirus type 2b (PCV2b). The objectives of this research were twofold: to determine the effect of genotype for the WUR PRRS resistance Single Nucleotide Polymorphism (SNP) on chromosome 4 and PRRS vaccination on growth rate and viral load (VL) in nursery pigs co-infected with PRRSV and PCV2b. Since the AB WUR genotype has been associated with increased weight gain and lower PRRS VL in previous studies, we hypothesized that AB pigs would grow faster and have less PRRS and PCV2b VL after co-infection. We also hypothesized that vaccinated pigs would grow slower following vaccination but faster when co-infected, and have lower PRRS and PCV2b VL following co-infection. Commercial nursery pigs (n=392) from two PRRS Host Genetics Consortium (PHGC) trials were used for the analysis of average daily gain (ADG) and PRRS and PCV2b VL. Pigs originated from the same breeding company and were pre-selected for WUR genotype (50% AA and 50% AB per trial). Weaned pigs were shipped to Kansas State University and randomly sorted into pens in one of two rooms. Pigs in one room were vaccinated using a modified live virus PRRS vaccine. After 28 days, all pigs in both rooms were co-infected with field strains of PRRSV and PCV2b. Pigs were weighed weekly and serum samples were collected at 0, 4, 7, 11, 14, 21, 28, 35, and 42 days post-infection (dpi). PRRS and PCV2 VL were defined as area under the curve of the log of PCR-based serum viremia from 0-21 dpi for PRRSV and 0-42 dpi for PCV2b. Due to availability of data, PCV2b VL was analyzed for one trial only (n=203). A mixed model was used to analyze VL and ADG by fitting trial, WUR genotype, vaccination (vx), WUR genotype*vx, age at vx, weight at vx, and PRRS viremia at 0 dpi as fixed effects. Interactions with trial were also fitted and removed if not significant (P>0.1). The random effects dam, sire, and pen (trial) were also fitted. ADG was analyzed for the pre co-infection period (-28 to 0 dpi) and for 0 to 21, 21 to 42, and 0 to 42 dpi of the co-infection period. Prior to co-infection, AB pigs had greater ADG (P=0.02) than AA pigs, regardless of vaccination status, and vaccinated pigs had lower ADG than non-vaccinated pigs (P=0.003). Vaccinated pigs also tended (P=0.08) to have lower ADG than non-vaccinated pigs for the entire co-infection period (0-42 dpi) and had significantly lower ADG (P=0.02) for the later part of the co-infection period (21-42 dpi). However, vaccinated pigs had lower PRRS VL post co-infection (P<0.0001), as did AB pigs (P<0.0001). WUR genotype had no significant effect on PCV2 VL post co-infection in the non-vaccinated group, but in the vaccinated group, AB pigs had lower PCV2b VL than AA pigs (P=0.004). In conclusion, vaccination for PRRS resulted in slower growth prior to co-infection and during the later phase of co-infection with PRRSV and PCV2b. The AB WUR genotype was associated with faster growth prior to co-infection, overall reduced PRRS VL, and reduced PCV2b VL in vaccinated pigs. This work was supported by the USDA ARS NIFA award 2012-38420-19286 and by PIC/Genus and Choice Genetics.

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GP5 expression in Marc-145 cells inhibits PRRSV infection by inducing beta interferon activity

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Objective: The major neutralizing epitope of PRRSV is mainly located on glycoprotein GP5. Immunization with exogenous GP5 or exposure to native GP5 by means of DNA immunization can provide some degree of immune protection to PRRSV infection in pigs. However, during PRRSV infection in pigs, the production of neutralization antibodies induced by GP5 is delayed or suppressed. This suggests that the synthesis of GP5 is late than some PRRSV proteins or other PRRSV proteins interfering with the function of GP5 in inducing host responses during virus infection. The objective of this study was to exclude the impacts of the other PRRSV proteins and determine the role of GP5 in the replication of PRRSV in Marc-145 cells.

Methods: A Marc-145 cell line stably expressing GP5 (Marc-145-GP5^{Flag}) was constructed by lentviral transduction. Cell viability was tested by cell proliferation and cell apoptosis measurement. PRRSV infective to Marc-145, Marc-145^{puro} and Marc-145-GP5^{Flag} cell lines was analyzed by real time RT-PCR, $TCID_{50}$ and flow cytometry assay. Type I interferon mRNA and protein level, promoter activity were detected by real time RT-PCR, indirect ELISA and luciferase activity assay. To confirm beta interferon induced by GP5 expression reduced PRRSV infection siRNA was introduced into Marc-145-GP5^{Flag} cell following the PRRSV infective to this cell was analyzed by real time RT-PCR and Western bolting.

Results: Cell proliferation and cell apoptosis measurements indicated that the expression of GP5 in Marc-145 cells did not disturb the cells' viability. Following infection with different PRRSV strains PRRSV replication in Marc-145-GP5^{Flag} cells was inhibited significantly. Type I interferon assay results showed that beta interferon in the Marc-145-GP5^{Flag} cells were increased in both mRNA and protein level. When introduced siRNA into cells to knock down beta interferon mRNA, PRRSV infection to the cells was recovered.

Conclusion: These data suggest that early GP5 expression is not favorable for further infection by PRRSV, because it not only stimulates production of neutralization antibodies in pigs, but also induces beta interferon production in host cells. Therefore, GP5 is an important protein in the induction of self-protection responses from the host.

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IFN phenotype mutant PRRS viruses with a modified regulatory role of SAP-like motif in nsp1-beta

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Nonstructural protein (nsp) 1-beta of porcine reproductive and respiratory syndrome virus (PRRSV) is a viral antagonist modulating the host innate immune response by blocking the expression of type I interferons and inflammatory cytokines including tumor necrosis factor-α. Bioinformatics analysis identified a SAP (for SAF-A/B, Acinus, and PIAS) domain of 126-LxxxLxxxGL-135 in the nsp1-beta subunit, a protein structure associated with DNA-binding and nuclear retention of molecules involved in transcriptional control. Sequence alignments of nsp1 of PRRSV, equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV) indicated that the SAP domain was highly conserved in arteriviruses. The residues at L126, L130, G134, and L135 in the SAP domain were mutated to alanine to determine their role for IFN modulation. Luciferase reporter assays indicated that the IFN suppression by nsp1-beta was reverted when SAP mutants of L126A, L130A, and L135A were expressed in cells, and either IFN regulatory factor (IRF) 3-dependent or NF-kappaB-dependent transcriptions were recovered from suppression. G134A mutation did not alter the IFN suppression of nsp1-beta. These SAP mutants also exhibited altered subcellular localization from the nucleus to the cytoplasm in gene-transfected cells with the exception of G134A. The mutations contributing to impaired IFN regulatory activities were individually introduced into the PRRSV infectious clone, and infectious mutant viruses were generated for SAP mutants L126A and L135A. The nsp1-beta protein of L126 and L135 mutant viruses was not detected in the nucleus, which was consistent with the results in gene-transfected cells. In comparison with the wild-type virus, the SAP mutant viruses showed an impaired growth in cells, suggesting that the SAP domain contributes to PRRSV replication and plays an important role for viral immune evasion.

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A comparison of genetic parameters and effects for a major QTL between piglets infected with one of two isolates of porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome (PRRS) is the most costly disease to the North American pork industry; previous methods for containment have had limited success. The aim of the PRRS Host Genetics Consortium (PHGC) is to identify genomic markers and pathways associated with host response to PRRS virus (PRRSV) infections, which can be used for genetic selection of pigs for increased resistance or reduced susceptibility to the virus. Previously, the Single Nucleotide Polymorphism (SNP) WUR10000125 (WUR) on chromosome 4 was found to be associated with viral load (VL), defined as the area under the curve of Log viremia from 0-21 days post-infection (dpi), and weight gain (WG), defined as weight gained from 0 to 42 dpi, during infection with the NVSL-97-7895 PRRSV isolate (NVSL).

This study used data from nine PRRSV infection trials with NVSL and four trials with the KS-2006-72109 isolate (KS06). Each trial had ~200 commercial crossbred piglets experimentally infected at 28-35 days of age. Blood samples and weights were collected periodically for up to 42 dpi. The objectives of this study were to 1) estimate genetic parameters and the effects of SNP WUR on VL and WG of piglets experimentally infected with these two PRRSV isolates, and 2) estimate the genetic correlations of pig responses (VL and WG) between KS06 and NVSL.

Heritabilities and the genetic correlation between VL and WG were estimated using pedigree information in ASREML 3.0, using the following model: parity nested within trial as a fixed effect, age and weight at infection as covariates, and animal, litter, and pen nested within trial as random effects. Heritability of VL was 0.31±0.12 for NVSL and 0.40±0.09 for KS06; heritability of WG was 0.30±0.09 for NVSL and 0.24±0.11 for KS06. VL and WG had negative phenotypic (NVSL=-0.25±0.03; KS06=-0.17±0.04) and genetic (NVSL=-0.32±0.23; KS06=-0.47±0.20) correlations. Including WUR genotype as a fixed effect showed that heterozygous individuals had lower VL than AA pigs for both isolates (NVSL=-4.6±0.4 units, $p<0.0001$; KS06=-3.6±0.7 units, $p<0.0001$), but significantly higher WG was only observed for pigs infected with NVSL (NVSL=2.0±0.2 kg, $p<0.0001$; KS06=-0.6±0.4 units, $p=0.11$).

Genetic correlations for VL and WG between isolates were estimated with the same model but using marker-based relationships since no pedigree was available to connect pigs in the NVSL and KS06 trials. The genetic correlation of response to NVSL with response to KS06 was high for both VL (0.95±0.20) and WG (0.78±0.28). Consistent with the WUR effect estimates, adding WUR as a fixed effect reduced the estimate of the genetic correlation for VL (0.8±0.25) but increased the estimate of the genetic correlation for WG (0.8±0.30).

These results suggest that selecting for increased resistance or reduced susceptibility to one PRRSV isolate will increase resistance or reduced susceptibility to another PRRSV isolate. Selecting for the AB genotype for WUR is expected to reduce VL across PRRSV isolates but its effect on WG during infection may differ between virus isolates. This work was supported by Genome Canada, USDA ARS, and breeding companies of the PHGC and PigGen Canada.

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Antiviral activity of *Sasa quelpaertensis* Nakai extract against porcine reproductive and respiratory syndrome virus by modulating virus-induced cytokine production

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Sasa quelpaertensis Nakai, known as Jeju-Joritdae, is a type of bamboo grass that widely distributed on Mt. Halla on Jeju Island in South Korea. Its leaf extract possess a number of health promoting activities, including anti-ulcerogenic, anti-obesity, anti-inflammatory, and anticancer properties. To date, there is still no report regarding an effect of *Sasa quelpaertensis* Nakai extract (SQE) against viral infections in human or veterinary subject. Porcine reproductive and respiratory syndrome virus (PRRSV) represent one of devastating viral pathogens of swine causing substantial economic impacts on the global pork industry. In the present study, therefore, we tried to investigate the antiviral activity of SQE and its mechanism of action upon PRRSV infection in cultured porcine alveolar macrophage (PAM) cells. Our results demonstrated that SQE treatment efficiently suppresses the replication of PRRSV in a dose-dependent manner. Treatment of cells with SQE at -1, 0, and 1 hpi resulted in more than 80% decrease in PRRSV production, while its addition between 2 and 12 hpi led to 72% to 33% inhibition of PRRSV infectivity. In contrast, no significant impairment of PRRSV propagation was observed when SQE was added at 24 hpi. These data indicated that the inhibitory effect of SQE is exerted mainly during the initial period of infection, suggesting that its action occurs at early time points after PRRSV infection. Further experiments revealed that SQE blocks post-entry steps of the replication cycle of PRRSV, including viral genomic and subgenomic RNA synthesis, viral protein translation, and virus progeny release. The addition of SQE decreased a titer of PRRSV to $10^{2.6}$ $TCID_{50}$/ml (>4 log reduction compared with the control). The growth kinetics study demonstrated that the overall process of PRRSV replication was dramatically delayed in the presence of SQE. Since SQE has been reported to possess the anti-inflammatory property, we examined whether SQE regulates the transcriptional activation of immune-response genes upon PRRSV infection to establish potential mechanism(s) of action for its antiviral activity. Quantitative real-time RT-PCR was conducted to determine the RNA levels of immune-related genes in PRRSV-infected PAM cells in the presence and absence of SQE. It was found that numerous cytokine genes regulated by PRRSV infection, including IL-1α, IL-6, IL-8, IL-15, TNF-α, and AMCF-1, were significantly altered by treatment of the cells with SQE. This finding indicates that the antiviral activity of SQE is involved in the modulation of inflammatory responses during PRRSV infection. Altogether, our results indicate that SQE has the potential for an excellent therapeutic agent against PRRSV.

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Evaluation of GBP1 and CD163 gene polymorphisms as genetic markers for PRRS susceptibility

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One of the major problems in modern pig industry is an infectious disease. Susceptibility to infectious diseases is influenced by both constitutional (e.g. genotype, age, gender and reproductive status) and environmental factors (e.g. nutrition, management, infections, and other forms of stress). Genetic variation within individual animals or herds can cause differences in the execution of immune functions against infectious agents in the domestic pigs. The objective of present study was to identify genetic factor(s) responsible for susceptibility of pigs to PRRSV under commercial field condition. Polymorphisms of guanylate-binding proteins (GBPs) genes have been evaluated for associations with viral load and weight gain after the PRRSV infection. Experimental PRRSV infections to commercial herds under controlled nutrition and environmental conditions were also performed to accurately collect disease–related impacts like virus load and growth measurements with *GBPs* and CD163polymorphisms. The results demonstrated that polymorphisms within GBP1 and CD163 genes are significantly associated with susceptibility to PRRS infection and will be useful markers to improve disease resistance or tolerance in pig breeding programs

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Essential role of extracellular signal-regulated kinase activation in porcine epidemic diarrhea virus replication

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Since viruses are obligate intracellular parasites, they have developed several strategies to manipulate a variety of host cell signal transduction pathways for successful virus survival. However, to date, little is known about the intracellular signaling mechanisms involved in porcine epidemic diarrhea virus (PEDV) replication. The extracellular signal-regulated kinase (ERK) pathway that transduces signals to modulate a wide range of cellular functions has been shown to regulate a number of viral infections. The present study therefore aimed to determine the role of the ERK pathway during PEDV infection. We found that PEDV infection induces the activation of ERK1/2 by 12 h postinfection and thereafter the progressive decease of its phosphorylation by western blot analysis and Fast Activated Cell-based ELISA (FACE) assay. Notably, UV-irradiated inactivated PEDV, which is capable of allowing viral attachment and internalization but incapable of pursuing viral gene expression, was sufficient to trigger ERK1/2 phosphorylation, suggesting that PEDV-cell interaction is responsible for its activation. To confirm that ERK1/2 following PEDV infection is truly activated, its downstream substrate Elk-1 was measured in the infected cells by western blot analysis. The PEDV-induced ERK1/2 activation was found to lead to phosphorylation of Elk-1 with kinetics that paralleled those observed for ERK1/2. Direct inhibition of ERK activation by chemical inhibitors, U0126 and PD98059, significantly suppressed PEDV replication by affecting viral RNA synthesis, viral protein expression, and progeny release. Furthermore, knockdown of ERK1/2 by siRNA effectively blocked PEDV replication and resulted in approximately 3-fold decrease in infectious progeny titers. We previously demonstrated that PEDV induces caspase-independent apoptosis through the activation of mitochondrial apoptosis-inducing factor (AIF) to facilitate viral replication and pathogenesis. Next, it was investigated whether ERK1/2 activation is associated with the PEDV-induced mitochondrial AIF-medicated apoptosis pathway. Independent treatment with ERK-specific inhibitors did not inhibit PEDV-induced apoptotic cell death. Furthermore, the mitochondrial-to-nuclear translocation of AIF, a specific hallmark of PEDV-triggered apoptosis, was verified in the presence of each ERK inhibitor by confocal microscopy analysis and fractionation assay. These results indicate that ERK1/2 signaling is not involved in the apoptosis pathway during PEDV infection. Taken together, our data suggest that the ERK signaling pathway play an important role in post-entry steps of the PRRSV life cycle and beneficially contributes to virus replication.

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Nsp2 recruits BAG6 to target itself, mediate ER-stressed apoptosis and promote PRRSV replication

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Non-structural protein 2 (Nsp2) of porcine reproductive and respiratory syndrome virus (PRRSV) is a multi-domain and multi-functional replicative protein, which can play important roles in the replication and pathogenesis of PRRSV. The interaction of Nsp2 with host protein BCL2-associated athanogene 6 (BAG6) was validated recently. However the role of this interaction contributing to viral replication and its molecular mechanism underlying is still not fully elucidated. Here, by using confocal microscopy analysis, we demonstrated that Nsp2 interacted with BAG6 and caused BAG6 translocation from nucleus to the cytosol. And the N-terminus but not the CP active sites of Nsp2 was found to interact with the N-terminal domain of BAG6. Further experiment also indicated that the cytosolic re-localization of BAG6 provided advantages for itself to assist Nsp2 targeting to the Endoplasmic reticulum (ER)-originated double-membrane vesicles (DMVs). Additionally, the ER-stress mediated apoptosis was discovered in PRRSV infected cells in this research, and Nsp2 was confirm to be able to induce the ER-stress mediated apoptosis by deubiquiting BAG6-bound proteins and preventing them from degrading during PRRSV infection. At last, by using over expressing and RNAi to modify the expressing level of BAG6 in PRRSV infected cells, we found that BAG6 played a crucial role in promoting PRRSV replication, but not release. In conclusion, the Nsp2 can recruit host protein BAG6 to target itself, mediate ER-stressed apoptosis and promote PRRSV replication. These findings will shed new light on the molecular mechanism involved in PRRSV infection and may yield a novel target for further antivirus research.

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Attenuation of porcine reproductive and respiratory syndrome virus (PRRSV) by inactivating the expression of ribosomal frameshifting products nsp2TF and nsp2N

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The ribosomal frameshift products of PRRSV, nsp2TF and nsp2N, are novel proteins recently identified. In this study, they were determined having ability to interfere with cellular protein ubiquitination (Ub) and ISGylation activities that associated with innate immune suppression function of the virus. Two recombinant viruses (KO1 and KO2), generated by partially or completely inactivation of nsp2TF/nsp2N expression, displayed impaired de-Ub and de-ISGylation ability and induced higher levels of IFN-α and ISG15 expression in infected cells. When tested in a nursery pig model, these mutants showed reduced level of viremia compared to wild-type (WT) virus. After challenge, mutant virus-immunized pigs demonstrated improved protection with reduced lung lesion and viral load in lung and tonsil tissues, in comparison to pigs immunized with WT virus. Our data strongly implicate PRRSV nsp2TF/nsp2N in viral immune evasion and demonstrate that nsp2TF/nsp2N-deficient viruses are attenuated *in vivo*. Thus, manipulation of nsp2TF/nsp2N expression may be used in the rational design of improved PRRSV vaccines.

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Mutations in the highly conserved GKYLQRRLQ motif of nsp1β protein impairs the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV)

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The PRRSV nonstructural protein 1β (nsp1β) has been identified to be a strong innate immune antagonist. Recently, this protein was determined to function as a transactivator for expression of -2/-1 programmed ribosomal frameshifting (PRF) products, nsp2TF and nsp2N. Both nsp2TF and nsp2N contain a papain-like protease domain (PLP2) that has been implicated in disrupting innate immune signaling by acting as a deubiquitinating enzyme, which is associated with innate immune suppression function of the virus. Embedded in nsp1β's papain-like autoproteinase domain (PLP1β), we identified a highly conserved GKYLQRRLQ motif that is critical for PRF transactivation and innate immune suppression function of the virus. In this study, we further investigated the function of three basic residues (K124, R128, R129) in GKYLQRRLQ motif that are exposed on the surface of the protein. Site-directed mutagenesis analysis showed that R128A or R129A mutation impaired PRF transactivation function of nsp1β, as well as reduced the ability of nsp1β to suppress IFN-β and reporter gene expression. However, only R128A mutation affects the ability of nsp1β to suppress "self-expression" *in vitro*. Subsequently, three viable recombinant viruses, vSD95-21-R128A, vSD95-21-R129A and vSD95-21-R128R129AA, carrying single or double mutations in the GKYLQRRLQ motif, were created using reverse genetics. In comparison to the wild-type virus, vSD95-21-R128A and vSD95-21-R129A show similar growth ability, while vSD95-21-R128R129AA mutant had slightly reduced growth ability with about 0.5 log10 decrease in viral titer in MARC-145 cell. When tested in porcine alveolar macrophages, a 5-10 fold higher level of IFN-α and ISG15 expression were detected in mutant-infected macrophages comparing to wild type virus-infected macrophages. The result demonstrates that R128 and R129 residues are critical for nsp1β function, and modifying these key residues in the GKYLQRRLQ motif could attenuate virus growth and improve the cellular innate immune responses.

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Novel phenotypes for capturing genetic variation in resistance and tolerance of pigs to PRRSv

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There are two alternative host response strategies to PRRSv: resistance, which is the host's ability to limit or inhibit pathogen replication, thus reducing infection severity; and tolerance, which is the host's ability to limit the impact of infection on performance, without necessarily having an impact on pathogen burden. Tolerance is a difficult trait to measure accurately using current statistical methodology. Whilst resistance can be measured at an individual level, tolerance can only be measured at a group level, as multiple measurements are required from closely-related individuals to produce one family specific estimate. Further complications arise from the fact that resistance and tolerance are dynamic traits, which interact over the time-course of infection. This study aimed to identify new phenotypes to allow joint analysis of changes in viral load (resistance) and its impact on performance to PRRSv at an individual level, and the effect of a previously identified resistance QTL on these novel phenotypes. Data were from eight separate PRRS Host Genetics Consortium trials, where groups of 200 crossbred nursery pigs (total of 1524) were infected with PRRSv NVSL on day 0. Weekly individual measures of average daily gain (ADG) and blood viremia were obtained weekly up to 6 weeks post infection. We adopted a novel methodology which permits analysis of the interplay between resistance and tolerance over time in a 2D-space, generated by plotting pair-wise (longitudinal) individual measurements of log-transformed viral load against ADG, producing a trajectory of 2D vectors for every pig. These trajectory vectors give rise to two novel phenotypes: 1) the rate of simultaneous changes in ADG and virus load between two consecutive weeks, represented by the magnitude of each vector, and 2) the change in ADG with respect to change in viral load obtained by the vector angles, where positive/negative angles correspond to increase/ decrease in growth rate with respect to reduction in viral load, respectively. Weekly measures of each novel phenotype were analysed using animal models in ASReml. Furthermore, linear mixed models, containing genotype as a fixed effect, were used to identify if these new phenotypes were also conferred by the previously identified WU10000125 SNP locus conferring lower viral load (resistance) to PRRSv. We found evidence of genetic variation in vector magnitude for all weekly intervals, and in vector angles from week 3 onwards, with statistically significant heritabilities ranging from 0.07-0.30. We found evidence of variation based on the resistance QTL in magnitude of vectors between 1 and 3 weeks, and in vector angle at week 3 between genotypes AA and AB ($p<0.05$), where the B allele conferred a higher recovery in performance per unit reduction in viral load, and faster rate of change. This indicates that, in early stages of infection, viral load and its impact on performance may be under partial control of the resistance QTL. Subsequent infection processes, whilst still genetically determined, are not explained by the QTL. Resistance-tolerance trajectories, and their derived phenotypes, provide deeper insight into how resistance and tolerance together regulate the impact of infection on performance over the time-course of infection, and may provide new comprehensive disease traits for genetic selection.

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Interferon induction of a PRRSV strain sustained after serial passaging

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Type I interferons (IFNs), such as IFN-alpha and IFN-beta, are critical to innate immunity against viruses and play an important role in the modulation of adaptive immunity. An exceptional PRRSV strain, A2MC2, is able to induce type I IFNs in the cultured cells. Moderate virulence was observed in piglets with experimental infection of this strain. To attenuate it, we conducted serial passaging in MARC-145 cells. The rationale was that the serial passaging of A2MC2 would reduce or minimize previously observed mild virulence in pigs while retaining its competency of IFN induction. Here, we report that the interferon induction feature is sustained after the virus was propagated for 90 serial passages. The presence of interferons in the virus samples was monitored by a bioassay in Vero cells using NDV-GFP as an indicator virus as it is sensitive to interferons. Pretreatment of the Vero cells with the A2MC2 culture supernatant made the cells become resistant to the NDV infection. The bioassay results showed that the virus kept its capacity in inducing IFNs. The culture supernatant of A2MC2 passage 90 (P90) still induced antiviral response at dilution up to 1:8. The stability of this IFN induction during the virus passaging suggests that the viral genomic feature activating IFN synthesis was well preserved. The virus yields and multistep growth curve were monitored. RNA isolation from A2MC2 P25 virus was conducted. Reverse transcription and PCR were performed to amplify the cDNA for DNA sequencing. The sequencing results show that there are 15 nucleotide mutations in the P25 RNA genome in comparison to its parent virus. The mutations result in 10 amino acid changes. These mutations appear to have no effect on the feature of IFN induction. As P90 also induces IFN induction, any further nucleotide mutations are not expected to correlate with the feature. These results indicate that A2MC2 can be further explored for development of an improved vaccine against PRRS.

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Effects of porcine reproductive and respiratory syndrome (PRRS) modified live virus vaccine on the host response of nursery pigs to co-infection with PRRS virus and porcine circovirus type 2b

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Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) contribute to a number of disease complexes. Even though vaccination has resulted in the effective control of PCV2, several countries remain affected by the virus resulting in significant economic impacts to the swine industry. The level of PCV2 viremia is increased during co-infection with PRRSV, which can result in higher morbidity and mortality. To evaluate the host response to PRRSV vaccination followed by co-infection, 226 seven-week old pigs were challenged with PCV2b and PRRSV and monitored for 42 days. The pigs were not vaccinated for PCV2. Half of the pigs were vaccinated with PRRS modified live virus vaccine 28 days prior to challenge and housed separately. Two clinical syndromes became apparent post-infection. Between 8 and 17 days post-infection, clinical signs of aural cyanosis consistent with PRRSV infection occurred primarily in the unvaccinated group. Overall, 38% of unvaccinated pigs and 19% of vaccinated pigs were documented with aural cyanosis. An unvaccinated pig was 3.02 times (95% CI [1.7, 5.9]) more likely to present with aural cyanosis than a vaccinated pig ($p = 0.0011$). These results demonstrated the effectiveness of vaccination. Between days 22 and 35 post-infection, clinical signs consistent with porcine circovirus associated disease (PCVAD) including tachypnea, dyspnea, pyrexia, muscle wasting, rhinorrhea, lethargy, and pale discoloration occurred primarily in the vaccinated group. Histopathologic lesions included interstitial pneumonia, lymphohistiocytic infiltration in the alveolar septa and peribronchiolar areas, and lymphoid depletion. Overall, 12% of unvaccinated pigs and 26% of vaccinated pigs received systemic veterinary treatment during peak PCVAD presentation. A vaccinated pig was 2.67 times (95% CI [1.23, 5.80]) more likely to receive systemic veterinary treatment during this time compared to an unvaccinated pig ($p = 0.0136$). Vaccinated pigs had increased mortality, increased PCV2 viremia, decreased average daily weight gain, and required more systemic veterinary treatment compared to unvaccinated pigs. In this disease model, the protective effects of PRRSV vaccination were outweighed by the vaccine-associated amplification of PCVAD.

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Relationship of CD163 and CD169 positive macrophages in type 2 PRRSV infected maternal-fetal interface

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Fetal death in reproductive Porcine Reproductive and Respiratory syndrome (PRRS) is hypothesized to be a consequence of transplacental PRRS virus infection during the late gestation. However it still remains unclear how PRRSV crosses the pig epitheliochorial placenta and infects the fetus. Entry-mediator CD163 and CD169 (sialoadhesin) receptors are considered essential factors in PRRSV replication in porcine macrophages and initial spread of the infection. In this study, our objectives were to identify, quantify susceptible CD163 and CD169 macrophages within the PRRSV infected uterus and fully attached fetal placenta, and to evaluate their potential use in predicting fetal outcome. A total of 113 PRRS virus-naïve high-health pregnant gilts were intramuscularly and intranasally inoculated with PRRSV (10^5 $TCID_{50}$ total dose) and 19 negative control gilts were sham inoculated on gestation day 85±1. At 21days post inoculation, dams and their litters were humanely euthanized for necropsy examination. Samples of uterus with fully attached placenta, as well as fetal thymus were collected and analysed by an in-house qPCR to quantify PRRS viral load. Based on the PRRSV RNA concentration in the uterine/placental tissue adjacent the umbilical stump of each fetus, 3 groups of samples: negative (not detected), low (quantifiable, below mean), and high (quantifiable, above mean) were formed (n=40/group; 120 total). The corresponding paraffin-embedded uterine section was subjected to immunohistochemical analysis of CD163 and CD169 markers. Immunohistochemical staining was performed by using human monoclonal antibodies directed against CD163 and CD169 on automated immunostainer (DAKO), followed by antibody detection using the DAKO EnVision+System and AEC (3-amino-9-ethylcarbazole) as a chromogen. Numbers of CD163 and CD169 positive macrophages per $1mm^2$ area of the endometrium and fetal placenta were determined by Image ProPlus software. Results of immunohistochemistry revealed that the numbers of CD163 positive macrophages in endometrium differed among negative, low and high viral load groups and in the fetal placenta between negative and low viral load groups ($P<0.001$; $P<0.05$, respectively; Linear Mixed Model). Also, we found that numbers of CD169 positive macrophages in the endometrium and fetal placenta were positively associated with PCR viral load groups ($P<0.001$). Our findings confirm significant increase in the numbers of CD163 and CD169 positive macrophages in the endometrium and fetal placenta during PRRSV infection in pregnant gilts. Funding for this project is provided by Genome Canada, Genome Prairie, and BIVI PRRS Research Award. The authors acknowledge Jan Shivers from University of Minnesota, LaRhonda Sobchishin from Department of Veterinary Pathology at WCVM, Melissa Koehnlein, and Dr. Dale Godson from Prairie Diagnostic Services for their technical assistance in immunohistochemistry.

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Interaction of interferons and mTOR signaling underlying PRRSV infection

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Specific objective: Animal immune and metabolic systems interact to elicit effective immune responses during viral infections. The signaling pathway mediated by the mammalian target of rapamycin complex (mTOR), is key to cellular metabolism and implicated in various disease responses. Recent studies highlight the critical role of mTOR signaling in regulating heterosubtypic protection against viral infection. Little is known about how interferons coordinate immunometabolic involvement in antiviral responses. We have examined the involvement of mTOR signaling in infection by porcine reproductive and respiratory syndrome virus (PRRSV) and determined the components in mTOR signaling that were regulated by interferons and PRRSV infection. Our data establish a model to evaluate IFN regulation of immunometabolism, and elicit a novel means to manage effective anti-PRRSV responses via regulating IFN-mTOR interaction.
Methods: The effects of type I IFNs and the mediators of mTOR pathway (including mTOR1 inhibitor Rapamycin, and non-selective mTOR inhibitor PP242 and activator MHY1485) on PRRSV infection and mTOR signaling were tested in Marc-145 cells, porcine monocyte-derived dendritic cells (mDCs) and alveolar macrophages (PAMs). PRRSV infection was assayed by florescence-facilitated spectrometer or microscopy. Gene expression of mTOR pathways was examined using real-time RT-PCR, RNA-Seq and immunoblotting for protein analyses. The production of type I IFNs was examined with both bioassays and ELISA. RNA interference was applied for knockdown transcripts per different mTOR complexes, mTOR1 or mTOR2.
Results and conclusions: Suppression of mTOR signaling by PP242, a non-selective inhibitor for both mTOR1 and mTOR2, significantly inhibited PRRSV infection in Marc-145 cells (70-80%), alveolar macrophages (30-50%), and monocyte-derived dendritic cells (30-50%) cells. The PRRSV-suppressive effect of PP242 at 2.0 μM was comparable to porcine IFN-α1 and IFN-β at 0.1 and 100 ng/ml, respectively. The mTOR activator, MHY1485, reversed the antiviral effect of PP242; however, rapamycine, a selective inhibitor for mTOR1, had less effect on PRRSV suppression in the Marc cells. Further studies showed that expression and activation of several critical components of mTOR signaling, including protein kinase B (Akt), Raptor, Rictor, and P70S6K were co-regulated by both IFN treatment and PRRSV infection. For example, phosphorylation of Akt was stimulated by type I IFNs, but suppressed by PRRSV infection and, IFN-α1 and IFN-β exerted subtype-specific regulation of Akt phosphorylation. In summary, these findings support that mTOR signaling has a bi-directional loop with the type I IFN system and suggest that some components of mTOR signaling may serve as targets for studying immunometabolic regulation of IFNs and implicate for antiviral regulation. (This work was supported in part by USDA AFRI NIFA/DHS 2010-39559-21860 and USDA NIFA 2013-67015-21236.)

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Blood transcriptomics in response to porcine reproductive and respiratory syndrome (PRRS)

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The existing variability in pig response to Porcine Reproductive and Respiratory Syndrome virus (PRRSv) infection, and recent demonstration of significant genetic control of such responses, leads us to believe that selection towards more resistant pigs could be a valid strategy to reduce the economic impact of PRRS on the swine industry. We are interested in finding biomarkers that could predict more resistant pigs. As blood is a well-established source of biomarkers, we collected whole blood in three PRRS host genetics consortium (PHGC) PRRSv challenge trials of ±200 animals at 0, 4 and 7 days post infection (dpi). From three trials, 100 animals representing the extremes for growth rates and viremia levels were selected, whole blood RNA was prepared, and microarrays run on 4 or 7dpi versus 0dpi RNAs. Phenotypes examined were weight gain and viral load. We also examined the effect of the SSC4 WUR10000125 (WUR) genotype, which is associated with 15% of the genetic variability in responses to PRRSv. To further examine the genotype effect, an RNAseq experiment was performed on globin reduced RNA from whole blood collected on 0, 4, 7, 10 and 14dpi of eight littermate pairs of AB (favorable) and AA (unfavorable) WUR genotype. Little or no information could be obtained through differential expression (DE) analyses that contrasted the extreme phenotypes examined in the microarray experiment, or comparing the two genotypes examined by microarray as well as RNAseq. However, in both studies, on several dpi, interesting clusters of genes associated with phenotypes or WUR genotype were found when applying Weighted Gene Co-expression Network Analysis (WGCNA). Specific modules of similarly expressed genes were correlated with weight gain, viral load and/or WUR genotype and were annotated with GO terms such as innate immune response, adaptive immune response, cell cycle regulation, or DNA damage response. In addition, Partial Correlation and Information Theory (PCIT) analysis of these datasets found differentially wired hub genes that could explain the clustering of some genes in a module. We conclude that there are molecular differences between pigs with extreme phenotypes or different WUR genotype in response to PRRSv infection. However, these differences can be quite subtle and more difficult to discover with conventional DE expression analyses; co-expression methods can be used to reveal those network differences. Support: US National Pork Board, USDA ARS and NIFA grant #2010-65205-20433, and pig breeding companies.

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Antibody response during a Porcine Reproductive and Respiratory Syndrome (PRRS) outbreak can be predicted using high-density SNP genotypes

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Previous genetic analyses of PRRS antibody (Ab) response, measured as PRRS ELISA Sample-to-Positive (S/P) ratio, showed this trait to be positively genetically correlated with favorable sow reproductive performance during a PRRS outbreak, being mainly controlled by two Quantitative Trait Loci (QTL) on *Sus scrofa* chromosome (SSC) 7. One of these QTL encompassed the Major Histocompatibility Complex (MHC) region and the other was at ~130 Mb on SSC7. The objective of this work was to evaluate the use of high-density Single Nucleotide Polymorphism (SNP) genotypes to predict PRRS Ab response of sows during an outbreak. Two datasets were used. The training dataset included data on 1,648 F1 (Landrace x Large White) replacement gilts that were sourced from 17 multiplier herds with high-health status from 6 genetic sources and introduced into 22 commercial farms with historical cases of natural disease challenges. Blood samples were collected 40.1±14 days after entry, while animals followed standard acclimation procedures. The validation dataset included 580 sows from a commercial multiplier herd that had undergone a PRRS outbreak. Blood samples were collected 46 days after the estimated day of the outbreak and used for semi-quantification of PRRSV Ab (S/P ratio) by commercial ELISA. All pigs were genotyped, using different versions of the Porcine SNP Chip (60K v1, 60K v2, and 80K). After quality control, 38,678 SNPs that were common to all versions were used for analyses. The training dataset was used to estimate the effects of SNPs on S/P ratio using the Bayes-B method in five scenarios: all SNPs across the genome (ALL_SNP), only SNPs in the two QTL (SSC7_SNP), only SNPs in the MHC QTL (MHC_SNP), only SNPs in the 130 Mb QTL (130Mb_SNP), and all SNPs outside the two QTL (Not7_SNP). SNP estimates were used to predict S/P in the validation dataset. Accuracy of genomic prediction was calculated as the correlation between predicted and S/P ratio pre-adjusted for fixed effects, divided by square root of heritability. Moderate to high accuracies were observed for all scenarios except Not7_SNP (0.15), indicating that the rest of the genome has little predictive ability for S/P ratio. The highest accuracy was for SSC7_SNP (0.63). Slightly greater accuracy was obtained with MHC_SNP (0.55) compared to using all SNPs (0.49). Lastly, 130Mb_SNP had a moderate accuracy of 0.30. These results demonstrate that antibody response during a PRRS outbreak can be predicted using genetic markers in the two QTL on SSC7. In addition, the high accuracy of MHC_SNP suggests that the SNP effect estimates are consistent across different Landrace x Large White populations. Financial support from Genome Canada, the Canadian Swine Health Board, and PigGen Canada is appreciated.

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Blood biomarkers for growth performance in gilts infected with Porcine Reproductive and Respiratory Syndrome (PRRS) virus

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The objective of this work was to identify blood biomarkers that can be used to discriminate growing gilts with divergent growth performance during infection with PRRS virus (PRRSV). A total of 76 gilts (16.1±0.43 kg and 47-60 days old) were inoculated intramuscularly with a high-virulence field strain of PRRSV in a commercial finishing unit (19 pigs/pen). Blood samples were collected at 0, 7, 14, 28, and 56 days post infection (dpi) and used for determination of PRRSV viremia, PRRSV antibody titer, and complete blood cell counts. Body weights (BW) were collected at 0, 7, 14, 21, 28, 42, and 56 dpi to calculate individual pig ADG as the slope across BW measurements. Two growth rate groups (GRG) of 12 gilts each were created after adjusting each pig's ADG for the effects of pen, initial BW, and age. One group included animals with high (High-GRG) and one with low (Low-GRG) adjusted ADG, and their blood samples were longitudinally analyzed for 89 biomarkers (blood cytokine, acute phase proteins, cell counts, lipids, and metabolites). Statistical analysis was performed by a mixed model that included the fixed effects of GRG, dpi, GRG*dpi, pen, and initial age (covariate), and the random effect of animal, using a first-order autoregressive (co)variance for residuals. Initial analyses indicated that biomarker levels at 56 dpi were not different (P>0.05) from those at 0 dpi and thus, data on 56 dpi were excluded. Canonical Discriminant Analysis (CDA) of the remaining biomarker data was used to discriminate animals from the two GRG groups: Low-GRG and High-GRG. Biomarkers were pre-selected based on significance (P<0.10) of the effect of GRG at 0 dpi (GRG-Pre) and across 7, 14, and 28 dpi (GRG-Post). Biomarkers selected using Stepwise-CDA (P<0.05) were then included in the final CDAs. The number of pre-selected blood biomarkers was 14 for GRG-Pre and 24 for GRG-Post. For CDA-GRG-Pre, serum alkaline phosphatase (ALP) was the only selected biomarker and had an R^2 of 0.37, with higher levels of ALP prior to PRRSV resulting in classification of gilts into the High-GRG. Six animals (29% error) were misclassified. In addition to ALP, three biomarkers were selected for CDA-GRG-Post: acetate, alanine, and haptoglobin. Gilts with high levels of ALP and alanine were more likely classified into High-GRG, whereas those with high levels of acetate and haptoglobin were more likely classified into Low-GRG. All gilts were correctly classified into their original GRG (0% error). These results suggest that plasma ALP, acetate, alanine, and haptoglobin have the potential to discriminate animals with low and high growth performance during PRRSV infection. Financial support from Iowa Pork Producers Association (IPPA#12-113), Genome Canada, and PigGen Canada is appreciated.

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Suppression of type I interferon response by nonstructural protein 1 of PEDV through degradation of CREB-binding protein

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Type I interferons (IFN-α/β) play a key role for the host antiviral state. Some viruses of the family *Arteriviridae* and family *Coronaviridae* in the order *Nidovirales* have been shown to down-regulate the production of type I IFNs during infection. Porcine epidemic diarrhea virus (PEDV) has emerged in the United States in 2013 and spread rapidly to most producing States posing significant economic concerns. To study the ability of PEDV for immune modulation and to identify viral components, all 16 nonstructural proteins (nsp) of PEDV have been cloned and screen for their modulation of IFN-β responses. Nsp1 to nsp16 genes were individually amplified from viral genome and inserted into pXJ41 eukaryotic expression vector using FLAG as a tag for detection. Their gene expression was confirmed by western blot analysis and immunofluorescence (IFA) and the modulation of IFN production was determined by luciferase reporter assay, vesicular stomatitis virus (VSV)-GFP bioassay and real-time RT-PCR. The results showed that the expression of IFN-β mRNA was obviously suppressed after infection with PEDV. Of 16 nsps, nsp1, nsp7, nsp14, and nsp15 were found to inhibit IFN, interferon regulatory factor 3 (IRF3), and NF-κB promoter activities. The inhibition was not due to the inhibition of IRF3 nuclear translocation, suggesting a nuclear event. Of note, nsp1 did significantly impede the activation of IFN-β promoter when stimulated by IPS-1, TRAF3, and IRF3. Furthermore, nsp1 degraded the CREB-binding protein (CBP) in the nucleus, inhibiting the formation of enhanceosome thus resulting in the suppression of IFN production. In conclusion, our data show that PEDV inhibits IFN induction. Nsp1, nsp7, nsp14, and nsp15 are the IFN antagonists of PEDV, and at least one mechanism for IFN inhibition is CBP degradation by nsp1.

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Microscopic evaluation of lungs from pigs experimentally infected with type 1 PRRSV strains of different virulence

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Porcine reproductive and respiratory syndrome virus (PRRSV) type 1 strains belong to several genetic subtypes, namely 1 through 4 (Stadejek et al. 2013). Individual strains from Eastern European subtypes 2 and 3 studied to date proved to be significantly more virulent than the strains from the most prevalent subtype 1 (Karniychuk et al. 2010; Morgan et al., 2013; Hjulsager et al. 2013). The aim of the present study was to assess microscopic changes in lungs of pigs infected with type 1 PRRSV strains of different virulence.

Lung sections were collected from 28 pigs free from more than 30 common pig pathogens, at 24 days post inoculation with subtype 1 strain 18794 isolated in Denmark (7 pigs), subtype 2 strain Ili6 isolated in Russia (7 pigs), subtype 2 strain Bor59 isolated in Belarus (7 pigs) or cell culture medium (7 control pigs). Clinical assessment showed that Bor59 strain was significantly more virulent for pigs than the two remaining strains (Hjulsager et al. 2013). Lung sections stained with hematoxillin and eosin were evaluated microscopically. The evaluation included the severity of interstitial pneumonia, cellular infiltrations, proliferation of type 2 pneumocytes, BALT hyperplasia and proliferation of connective tissue. Additionally, the measurement of thickness of epithelium of bronchiolar mucosa and thickness of myocytes in bronchiolar wall were performed. Statistical evaluation was performed with Statistica 10 program (StatSoft Inc.), using ANOVA, Tukey's HSD or Kruskal-Wallis tests.

At 24 days post infection different level of interstitial pneumonia was observed in pigs of the PRRSV inoculated groups, but surprisingly, also in some of the control pigs. The inflammatory infiltration, proliferation of connective tissue, and also the intensity of eosinophil infiltration, were higher in the groups of PRRSV infected pigs than in the control groups. The most noticeable lesions were observed in Bor59 infected pigs, where three out of seven pigs exhibited severe lesions. Also BALT activity and infiltrations around bronchioli, were most prominent in Bor59 infected pigs. Intense myocyte proliferation was observed in the pigs with the most severe interstitial pneumonia lesions. Due to the fact the groups were small, the differences in most of the analyzed parameters were statistically insignificant. Only the intensity of eosinophil infiltration, was significantly higher in Bor59 pigs than in the remaining groups.

Despite the very good sanitary status of pigs used in the experiment, most control animals exhibited some degree of pulmonary lesions. Generally, the more prominent lesions in Bor59 infected pigs were consistent with the clinical status of those pigs. The lungs of Bor59 infected pigs revealed strong fibrosis of stromal connective tissue, total disappearance of organ structure and most importantly, the strongest infiltration of eosinophils. Thus, the subtype 2 Bor59 strain can be considered as pathogenic and pneumotropic and as such must be considered as a valuable challenge strain in vaccine studies.

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The phosphorylation of IRF-3 induced by HP-PRRSV infection upregulates SAMHD1 expression in PAMs

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Most recently, the studies of PRRSV are focus on the mechanism that how the virus to evade the recognition and elimination of host innate immune response. As an important innate immunity restriction factor, the sterile alpha motif and HD domain 1 (SAMHD1) protein blocks HIV-1 infection in myeloid-lineage cells. However, SAMHD1 protein has not been experimentally demonstrated during PRRSV infection. To explore the antiviral effect of SAMHD1 on PRRSV infection, we first explored the regulatory mechanism of SAMHD1 expression. The results showed that TLR-3 and RIG-I/MDA5 pathways are involved in SAMHD1 expression. Overexpression of TBK1 enhances the SAMHD1 promoter luciferase activity. The mutation in the phosphorylation site S172A of TBK1 impaired the inducible expression of SAMHD1. Overexpression of IRF-3 enhances the SAMHD1 promoter luciferase activity, but only active form of IRF-3 can induce SAMHD1 expression. Degradation of IRF-3 expression by RNA interference and inhibition of its phosphorylation, and nuclear translocation by BX 795 reveal that inducible expression of SAMHD1 by the interferon-alpha and Newcastle disease virus infection were significantly impaired. Complementary IRF-3 expression could restore the inducible expression of SAMHD1 to level as the control. Thus, the phosphorylation and nucleus translocation of IRF-3 plays major role in SAMHD1 expression. Next, SAMHD1 expression was detected in porcine alveolar macrophages (PAMs) infected with HuN4, a highly pathogenic PRRSV (HP-PRRSV) strain. The results showed that SAMHD1 is upregulated in PAMs by HuN4 infection. We further detected the regulatory pathways in PAMs. The phosphorylation of IRF-3 was significantly upregulated and the RIG-I/MDA5/TBK1 pathway participates in the activation of IRF-3 by HuN4 infection. Inhibition of the phosphorylation and nuclear translocation of IRF-3, the expression of SAMHD1 was significantly reduced. These findings indicate that SAMHD1 may play important roles in HuN4 virus proliferation in PAMs.

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Broad and homologous neutralizing antibodies recognize distinct epitopes in the PRRSV proteome

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The neutralizing antibody (nAb) response following infection with PRRSV is primarily directed against the isolate used for infection; i.e. homologous nAb (hnAb). The absence of nAb against genetically diverse isolates is a major impediment for the development of the next generation of vaccines. Following evaluation of over 1,200 sera samples from experimentally infected pigs, we identified a small number of pigs that neutralized a broad range of genetically diverse PRRSV isolates, including both Type 1 and Type 2 isolates. We categorize these samples as possessing broadly neutralizing antibody (bnAb). Furthermore, we identified pigs possessing high titers of only hnAb. We hypothesize that the different nAb's recognize different PRRSV epitopes. To test this hypothesis, we used a checkerboard virus neutralizing assay to select for viruses that escaped neutralization with sera possessing homologous or broadly neutralizing activity. Viruses made resistant to hnAb *in vitro* retained sensitivity to bnAb, and conversely, viruses made resistant to bnAb remained sensitive to hnAb. Sequencing identified mutations in a short hypervariable domain of GP5 associated with escape from homologous neutralization. For viruses resistant to broadly neutralizing serum, non-conserved amino acid changes were identified in GP3 and GP5, as well as an amino acid deletion in the ectodomain region of M. The deletion in M that resulted in the bnAb resistant phenotype was confirmed using reverse genetics with a PRRSV infectious clone. The results demonstrate that homologous and broadly neutralizing responses are distinct and recognize different epitopes. Furthermore, the mutations in GP3, GP5, and M suggest that these proteins may interact to form a conformational neutralizing epitope. The results also show that the properties of neutralizing antibodies must be taken into consideration when evaluating neutralization epitopes. Furthermore, the results demonstrate the utility of screening populations of infected pigs to identify unique naturally occurring responses as tools to probe the properties of PRRSV immunity.

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DDX19A senses viral RNA and mediates NLRP3-dependent inflammasome activation in Porcine Reproductive and Respiratory Syndrome Virus-infected porcine alveolar macrophages

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The NLRP3 inflammasome plays a major role in innate immune responses by activating caspase-1, which leads to the mature and secretion of IL-1-beta and IL-18, and ultimately caused inflammatory responses. High pathogenic *porcine reproductive and respiratory syndrome virus* (HP-PRRSV) infection induces severe inflammatory responses. However, the underlying mechanism is still poorly understood. Here we found that PRRSV infection and transfected its genomic RNA alone could sufficiently trigger NLRP3-dependent inflammasome activation in primary porcine pulmonary alveolar macrophages (PAMs). Using unbiased proteomic and targeted biochemical approaches, we identified DDX19A, a DExD/H-box RNA helicase family member, senses and binds PRRSV genomic RNA and polyI:C. In addition, we found that DDX19A interacts with NLRP3 NACHT and LRR domains via its helicase ATP binding domain and forms the DDX19A-NLRP3 inflammasome together with apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1 following PRRSV infection. Furthermore, knockdown of DDX19A efficiently inhibits the activation of caspase-1 and secretion of IL-1-beta in PRRSV-infected PAMs. Finally, we identified that DDX19A involved in PRRSV replication complex through interacting with PRRSV Nsp2, Nsp9, Nsp10 and Nsp12 and knockdown of DDX19A significantly inhibits the viral replication in MARC-145 cells and PAMs. Taken together, we identified DDX19A as a novel RNA sensor that binds PRRSV genomic RNA, activates the NLRP3-dependent inflammasome and regulates viral replication during PRRSV infection.

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Production and evaluation of porcine reproductive and respiratory syndrome virus like particles in pigs

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Porcine Reproductive and Respiratory syndrome (PRRS) is an economically devastating problem plaguing the global swine industry. Since early 1990s both live attenuated and inactivated vaccines are in use, but still control of PRRS is a major problem. Most of the inactivated virus and subunit vaccines are poorly immunogenic due to their soluble nature and rapid degradation *in vivo*. Alternatively, virus like particles (VLPs) generated using viral surface proteins mimic the morphology of the native virus, and are non-infectious as they lack the genetic material. Until now only two reports on PRRS-VLPs are available, GP5 and M containing VLPs and influenza nucleocapsid protein and PRRSV GP5 chimeric (NA/GP5) VLPs, but their immunogenicity was not evaluated in pigs. Since putative PRRSV neutralizing epitopes are not limited to GP5 and M, we hypothesized that PRRS-VLPs comprising all the six viral membrane proteins (GP2a, E, GP3, GP4, GP5 and M) will be a potent candidate vaccine. We cloned full-length genes of the surface proteins of a type 2 PRRSV (strain SD09200) in to a baculovirus transfer vector, transformed in to competent *E. coli,* and transfected into Sf9 cells to generate recombinant baculoviruses (rBVs). High titered rBVs stocks were used to co-infect Sf9 cells in different combinations to generate PRRS-VLPs. Our *in vitro* results revealed that VLPs were formed from co-infection of GP5-M and GP2a-GP3-GP4-GP5-M with 40-80 nm sized particles, measured by transmission electron microscopy. Further, we also observed VLPs when rBVs containing genes of PRRSV E with three minor glycoproteins GP2a-GP3-GP4 were coinfected in Sf9 cells. In a pilot vaccine trial in nursery pigs, BEI inactivated PRRS-VLPs entrapped in the biodegradable nanoparticles or unentrapped were coadministered intranasally twice with a potent mucosal adjuvant, *Mycobacterium tuberculosis* whole cell lysate, and subsequently challenged with a virulent heterologous PRRSV strain 1-4-4. Analysis of immune correlates of protection and viral load are in progress. In conclusion, we generated PRRS-VLPs containing all the six membrane glycoproteins, and that could be a potential candidate vaccine when delivered in nanoparticles with a potent adjuvant. This project was supported by NPB and OARDC, OSU to RJG.

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Responses of swine to mosaic DNA vaccines for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

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Objectives: Design and construct T cell epitope mosaic DNA vaccines to improve the breadth and depth of protection towards genetically and antigenically diverse PRRSV strains. Evaluate immunogenicity of the vaccine candidates in pigs.

Methods: Two DNA vaccine candidates that encode mosaic T cell epitopes derived from 748 genotype II ORF5 sequences encoding for GP5 protein were constructed. The mosaic DNA vaccines were tested in pigs in two pilot vaccination/challenge trials. Gene gun and electroporation were utilized as delivery systems in trials 1 and 2, respectively. Lymphocyte proliferative responses were detected using the MTT based method after 48h of incubation at 37°C. Viral loads in blood and tissues were quantified using a standard curve generated by SYBR Green based quantitative real-time PCR method. The expression of interferon-gamma and interleukin-10 mRNA in virus-stimulated PBMCs was measured in real-time PCR using the delta-delta method. Antibody responses were monitored in vaccinated animals by indirect-ELISA.

Results: Protein expression of the mosaic DNA vaccines was detected i*n vitro* by western blot and was confirmed by induction of specific antibody responses in vaccinated animals. The levels of virus-specific antibodies detected in vaccinated animals were higher than those of control animals in trial 2 ($p<0.05$). Lymphocyte proliferative responses detected in virus-stimulated PBMCs of mosaic-vaccinated pigs were higher than the baseline level in both studies ($p<0.05$). Viral RNA was detected in serum, tissues, porcine alveolar macrophages (PAMs) and bronchoalveolar lavage (BAL) fluids of vaccinated and control animals. There were no differences between groups in viral copy numbers in serum, BAL fluids, lungs or tracheobronchial lymph nodes (TBLNs) in trial 1. In trial 2, the initial viral copy numbers in mosaic-vaccinated animals decreased 269 fold in 48h while viral copy numbers increased by 1.2-5 fold in control animals in the same period. There were no significant differences in viral loads in lungs or TBLNs between groups. Viral loads in inguinal lymph nodes (ILNs) and spleens of mosaic-vaccinated animals were lower compared to those of control animals ($p<0.05$). There were no significant differences between groups in viral copy numbers in PAMs or BAL fluids. There were no significant differences between vaccinated and control animals in expression of interferon-gamma or interleukin-10 mRNA by virus-stimulated PBMCs. Lesions in lungs are being evaluated.

Conclusions: The data suggest that in general electroporation-induced immune responses were better than those induced by gene gun administration. The rapid decrease in viral loads in serum was consistent among all tested animals in the mosaic-vaccinated group and deserves further attention. The immunogenicity of these mosaic vaccines needs to be further improved.

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The role of TLR7 in PRRSV infection of porcine alveolar macrophages

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Objective: PRRSV can down-regulate host innate and adaptive immune responses. Due to the limited control measure for PRRSV infection, the new strategies for controlling PRRSV infection are needed. Toll-like receptors (TLRs) are pathogen recognition receptors that play pivotal roles in the innate immune system, which trigger antimicrobial immune responses by recognizing the Pathogen Associated Molecular Pattern (PAMP). It has been documented that ssRNA viruses can be recognized by TLR7. Our hypothesis is that activation of TLR7 could control PRRSV infection. Our previous study has shown that TLR7 agonist (T7) induced the high level of interferon-gamma and interleukin 4 in human peripheral blood lymphocytes. The objective of this study was to determine the effect of TLR7 agonist in PRRSV infection of porcine alveolar macrophages (PAMs).

Methods: PAMs were obtained by postmortem lung lavage of 4-week-old PRRSV negative pigs. PAMs were co-cultured with highly pathogenic PRRSV SD16 strain at the 0.01 MOI and different concentrations of T7 (0, 5, 10, 20, 40, 80mg/L) in DMEM. One hour later, the supernatant of PAMs culture was discarded and the cells were cultured in the medium containing T7 at the above concentration for 12, 24, and 36h, respectively. The PRRSV N protein expression were detected by indirect immunofluorescence assay (IFA).

Results: As shown in Table 1, the suppression ratios of PRRSV infectivity were between 50% to 80% with the presence of T7 at 20mg/L or higher.

Table 1. T7 suppressed PRRSV infection of PAMs

Culture Time (h)	Suppression of PRRSV infection at various concentration of T7 (mg/L) (%)					
	0	5	10	20	40	80
12	0	0	0	0	0	10
24	0	0	0	30	**50**	**60**
36	0	0	10	**50**	**70**	**80**

Conclusion: T7, the TLR7 agonist, can suppress PRRSV infection of PAM at dose dependent manner. Further study is needed to elucidate the mechanism of T7 in suppression of PRRSV infection.

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Safety and antibody response of pigs to an experimental Porcine Epidemic Diarrhea Virus (PEDV) vaccine, killed virus

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A study was conducted to evaluate the safety and antibody response of an experimental killed PEDV vaccine in non-pregnant sows. PEDV negative sows were vaccinated twice, 3 weeks apart with the experimental vaccine (n=23) or an adjuvant placebo (n=3). Sera, collected on Days -1, 21, 30 and 35, were tested for PEDV antibodies by 4 methods. An indirect ELISA to detect antibodies to purified recombinant S1 protein, a Beta serum neutralizing (SN) assay and an indirect fluorescent antibody (IFA) assay were conducted at Zoetis. Sera were also sent to the Animal Disease Research and Diagnostic Laboratory at South Dakota State University, Brookings, SD to test for fluorescent focal neutralization (FFN) titers. Pearson correlations between the assays were calculated. *In vivo* procedures occurred according to state, national, or international regulations after ethical review by Zoetis's IACUC. No untoward local or systemic reactions attributable to the vaccine were observed in the vaccinated sows. Antibody titers of the placebo controls remained at pre-vaccination levels at all post-vaccination time points and vaccinated sows had detectable antibodies to PEDV at all post-vaccination time points as measured by the 4 assays. There were correlations between the assays (Table 1). Under the conditions of this study, the experimental vaccine was both safe and immunogenic, inducing neutralizing antibodies (SN and FFN), antibodies to the whole virus (IFA) and antibodies to the S1 protein.

Table 1. Correlation of PEDV antibody assays

	Pearson Correlation Coefficients Prob > |r| under H0:Rho=0			
	Beta SN	FFN	IFA	S1 ELISA
Beta SN	1.00000	0.89143 <0.0001	0.87981 <0.0001	0.82158 <0.0001
FFN		1.00000	0.85397 <0.0001	0.70330 0.0024
IFA			1.00000	0.87191 <0.0001
S1 ELISA				1.00000

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Inhibition of PRRSV live attenuated vaccine by deoxynivalenol (DON) naturally contaminated feed

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Cereal commodities are frequently contaminated with mycotoxins produced by the secondary metabolism of fungal infection. Among these contaminants, deoxynivalenol (DON), also known as vomitoxin, is the most prevalent type B trichothecene mycotoxin worldwide. Pigs are very sensitive to toxic effect of DON and are frequently exposed to naturally contaminated feed. Recently, DON naturally contaminated feed has been shown to decreased porcine reproductive and respiratory syndrome virus (PRRSV) specific antibody responses following experimental infection. The objective of this study was to determine the impact of DON naturally contaminated feed on the immune response generated following immunisation with (PRRSV) live attenuated vaccine. 18 Pigs were randomly divided into three experimental groups of 6 animals based on DON dietary content of served diets (0, 2.5 and 3.5 mg DON/Kg). They were fed these rations 1 weeks prior to the immunisation and for all duration of the immune response evaluation. All pigs were immunized intra-muscularly with one dose of Ingelvac® PRRSV modified live vaccine (MLV). Blood samples were collected at day -1, 6, 13, 20, 27 and 35 post immunization (pi) and tested for PRRSV RNA by qPCR and for virus specific antibodies by ELISA. Results showed that ingestion of DON contaminated diets significantly decreased PRRSV vireamia. All pig fed control diet were viremic while only 1 (17%) and 3 (50%) out 6 pigs were viremic in groups receiving 3.5 and 2.5 mg of DON/Kg, respectively. As consequence, all pigs fed control diet developped PRRSV specific antibodies while only the viremic pigs, i.e 1 (17%) and 3 (50%) pigs that were fed with 3.5 and 2.5 mg of DON/Kg, respectively, have developped PRRSV specific antibodies. These results suggest that fedding pigs with DON contaminated diet could inhibit the vaccination efficiency of PRRSV MLV by severely impairing viral replication. Further studies will be needed to discover the exact mecanism of this inhibition.

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The Epitope Content Comparison (EpiCC) Tool: application to PRRSv

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PRRSv (Porcine Reproductive and Respiratory virus) is an enormous economic burden to pork producers. Like many RNA viruses, PRRSv has considerable genetic and antigenic variability that has made the disease difficult to prevent with standard vaccines and an efficacious, broadly cross-protective formulation has yet to be developed. While methods for comparing existing vaccines to PRRSv strains have been informative, the 'whole gene' approach fails to estimate cross-reactivity because it does not consider the T cell epitopes that are presented to the immune system, and whether they are conserved between the vaccine and the challenge strain. We developed an Epitope Content Comparison (EpiCC) informatic tool to better define the degree of conservation between PRRSv vaccines and circulating strains.

We previously developed a set of Swine Leukocyte Antigen (SLA)–restricted epitope prediction tools (PigMatrix). We further modified this tool to define relatedness based on T cell epitope content. Using EpiCC, we screened GP5 sequences from 8 PRRSv and 2 modified live virus (MLV) vaccines. Epitopes predicted to bind to common class I SLA alleles were identified and compared pairwise between strains for calculation of an epitope-based relatedness score (EpiCC score). A distance score matrix was constructed and used to built an 'EpiCC-based tree' that depicts the relatedness between GP5 proteins.

EpiCC provides an objective approach to aid pork producers in vaccine selection when a PRRSv strain is introduced into a herd, and to select viral epitopes for incorporation into a MLV vaccine. Finally, EpiCC may also be used for analyses of evolutionary drift via epitope deletion and for bio-surveillance.

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Oral immunotherapy of chicken egg yolk antibody against recombinant S1 domain of the porcine epidemic diarrhea virus spike protein in piglets

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Porcine epidemic diarrhea virus (PEDV) is a highly contagious enteric pathogen of swine causing high mortality in neonatal piglets. PEDV outbreaks have continuously occurred in most swine-producing Asian countries and recently emerged in the United States, leading to tremendous economic losses to the Asian and U.S. pig industry. The spike (S) protein of PEDV is a type 1 transmembrane envelope glycoprotein and consists of S1 and S2 domains responsible for virus binding and fusion, respectively. Since the S1 domain is involved in a specific high-affinity interaction with the cellular receptor and induction of neutralizing antibody in the natural host, it is a primary target for the development of effective vaccines and therapeutics against PEDV. Passive immunization by oral administration of specific antibodies has been an attractive approach against gastrointestinal (GI) pathogens in both humans and animals. Egg York antibodies (IgY) from immunized chickens has been demonstrated to be a convenient source for specific antibodies on a large scale and shown to be safe and effective against PEDV in newborn piglets. In this study, we aimed to produce IgY against the PEDV S1 protein and investigate its immunoprophylaxis in neonatal piglets. A codon-optimized PEDV S1 gene containing residues 25-749 was synthesized used to establish a stable porcine cell line constitutively expressing the PEDV S1 protein with a chicken immunoglobulin Fc fragment at the C terminus of the S1. The expression of a recombinant fusion protein (designated S1-cFc) was confirmed from cell culture supernatants by western blot analysis. Two groups of 10-week-old hens (n=16) were immunized intramuscularly with a BEI-inactivated PEDV vaccine strain or the purified recombinant S1 protein emulsified in ASA70 adjuvant followed by booster inoculations at 3-week intervals. IgY was extracted from the egg yolk using highly acidic ionized water (pH 2.0) and concentrated by the general procedures. Virus neutralization assay found that the titers of IgY against the whole PEDV and S1 protein are 32 and 16, respectively. To evaluate prophylactic efficacy of IgY, a total of 15 newborn piglets were obtained from seronegative pregnant sows in a farm without PEDV vaccination and outbreak and divided into three groups [group 1 (n=6); IgY against PEDV, group 2 (n=6); IgY against S1, group 3 (n=3); control)]. Piglets were initially administered orally with 2 ml of IgY at birth and challenged orally with 1 ml of small intestine homogenate containing 104.5 TCID50/ml PEDV field virus at 5 days following birth. After challenge exposures, all piglets except the control were provided with oral administration of IgY at day 1 and 2 post-challenge. Clinical signs and the mortality of the piglets monitored daily throughout the study. Stool samples were collected every day with 16-inch cotton-tipped swabs and subjected to real-time RT-PCR. All piglets were euthanized at 7 days after challenge for post-mortem examination. Results of in vivo assessment for immunoprophylatic effect of IgY against the S1 protein will be discussed.

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Packaging of Porcine Reproductive and Respiratory Syndrome Virus replicon RNA by a stable cell line expressing its nucleocapsid protein

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Porcine reproductive and respiratory syndrome virus (PRRSV), a member of the *Arteriviridae* family, is one of the most common and economically important swine pathogens. Although both live-attenuated and killed-inactivated vaccines against the virus have been available for a decade, PRRSV is still a major problem in the swine industry worldwide. To explore the possibility of producing single-round infectious PRRSV replicon particles as a potential vaccine strategy, we have now generated two necessary components: 1) a stable cell line (BHK/Sinrep19/PRRSV-N) that constitutively expresses the viral nucleocapsid (N) protein localized to the cytoplasm and the nucleolus and 2) a PRRSV replicon vector (pBAC/PRRSV/Replicon-ΔN) with a 177-nucleotide deletion, removing the 3'-half portion of ORF7 in the viral genome, from which the self-replicating propagation-defective replicon RNAs were synthesized *in vitro* by SP6 polymerase run-off transcription. Transfection of this replicon RNA into N protein-expressing BHK-21 cells led to the secretion of infectious particles that packaged the replicon RNA, albeit with a low production efficiency of 0.4×10^2 to 1.1×10^2 infectious units per ml; the produced particles had only single-round infectivity with no cell-to-cell spread. This *trans*-complementation system for PRRSV provides a useful platform for studies to define the packaging signals and motifs present within the viral genome and N protein, respectively, and to develop viral replicon-based antiviral vaccines that will stop the infection and spread of this pathogen.

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Expression of PED coronavirus spike glycoprotein in *Pichia pastoris*, baculovirus and in a mammalian cell line

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The porcine epidemic diarrhea virus (PEDV) is an infectious and highly contagious virus of pigs. Recently, outbreaks of the severe PED disease have been reported in the USA and Canada affecting pigs of all ages. Characterized by high mortality rates among suckling piglets, the economic impact of the disease is very high. To establish a method for production of recombinant PEDV antigens, the gene encoding spike (S) protein of PEDV was expressed in different expression systems. To this end, the S1 fragment (amino acid residues 1-736) was cloned into plasmid vectors to express the gene in *Pichia pastoris*, baculovirus and in human embryo kidney (HEK) 293T cells. We also added C-terminal histidines for affinity chromatography purification of the recombinant protein. The protein expressed in *P. pastoris* was soluble and glycosylated, but it was proteolytically cleaved; the yield of the purified recombinant protein was 180 microgram/l. Soluble S1 protein yield from the baculovirus infected Sf9 cell culture was 3.87 mg/l, whereas the secreted S1 protein yield of 29.6 mg/l was achieved in HEK 293T culture supernatants. The purified S1 protein from baculovirus and mammalian cells migrated in denaturing gel as 80.9 and 82.7 kDa bands, respectively. In addition, these proteins were recognized by anti-S1 peptide serum in Western blot assay. Taken together, these data indicate that HEK 293T expression platform is the best for expression of PEDV S protein.

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Vaccination of PEDV-naive dams with replicon RNA particle vaccine protects suckling piglets from challenge

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An alphavirus-derived replicon RNA Particle vaccine has recently received conditional regulatory approval for vaccination against PED. Vaccination of dams is likely to play an important role in the control and eradication efforts. This study evaluated the potential of dam vaccination with a PED RNA Particle vaccine to protect suckling piglets from challenge.

Ten PEDV-naive pregnant sows were vaccinated intramuscularly with two doses of PED RNA Particle vaccine (n=6) or with placebo (n=4). Booster vaccination was approximately two weeks prior to farrowing, and four weeks after initial vaccination. At approximately five days of age, all piglets were challenged orally with cell culture grown PEDV strain Colorado/2013. Piglets were observed for mortality for two weeks post-challenge.

RNA Particle-vaccinated dams had statistically significant reductions in litter mortality when compared to placebo-treated dams. All vaccinated dams developed PEDV-neutralizing antibodies prior to challenge, which were passively acquired by their suckling piglets. Additionally, PEDV-neutralizing antibodies were detectable in the milk of vaccinated sows for two weeks post-farrowing.

This study, consistent with earlier experiments conducted by our group, supports the conclusion that suckling piglets can be protected from PEDV challenge by vaccination of naive sows with nonliving, recombinant vaccine.

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Impact of Fostera® PRRS on ADG of piglets after heterologous PRRSv challenge

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Introduction: Fostera PRRS is licensed for the vaccination of healthy, susceptible swine as young as one day of age or older as an aid in prevention of respiratory disease associated with PRRSv with a 26 week duration of immunity. This paper will summarize the results of three studies in which Fostera PRRS had a positive impact on the average daily gain (ADG) of piglets after heterologous PRRS challenge.
Materials and Methods: Study 1: 24 PRRS seronegative pigs were vaccinated at 3 weeks of age with Fostera PRRS and 24 controls received placebo vaccine IM. The pigs were housed separately in BSL-2 rooms and were comingled 4 days prior to challenge. Pigs were individually challenged IM and IN with virulent, heterologous PRRSv (NADC20) 7 weeks after vaccination. Pigs were necropsied 10 days post-challenge and lung scores were assessed. All pigs were weighed one day prior to re-housing and 10 days post-challenge. Study 2: One group of 24 pigs received a single 2 mL IM injection of Fostera PRRS vaccine and the other group of 24 pigs received 2 mL of placebo vaccine IM. Pigs were housed separately and commingled immediately prior to challenge in a BSL-2 facility. Each pig was challenged 24 weeks post-vaccination IM and IN with medium containing PRRSv NADC20. Pigs were necropsied 10 days post-challenge and lung scores were measured. Pigs were weighed 1 day prior to challenge and again immediately prior to necropsy. All pigs were observed daily and clinical observations were recorded by personnel blinded to treatment group assignments. Study 3: 62 PRRS seronegative barrows were randomly assigned to three treatment groups (vaccinated, positive and negative controls) at 4 weeks of age. One group of 20 pigs was vaccinated with a single 2 IM dose of Fostera PRRS while the positive and negative control groups (21 pigs each) were administered 2 IM of sterile saline. Treatment groups were housed in separate BSL2 rooms throughout the study. 4 weeks post vaccination, pigs in the vaccinated and positive control groups were challenged IM and IN with a virulent, heterologous contemporary lineage 9 PRRSv isolated in 2012.. All pigs were necropsied 12 days post-challenge and lung lesions were measured. Pigs were weighed 3 days prior to challenge and 15 days later.
Results: Study 1: Control pigs had 43.9% mean lung lesions while the vaccinated pigs had 0.7% ($P \leq 0.0001$). ADG for vaccinates post-challenge was 2.5 times higher in vaccinates versus controls (1.0 vs. 0.4 lb./day, ($P \leq 0.0001$).) Study 2: Vaccinated pigs demonstrated a 92% reduction in lung lesions versus the controls (12.1% vs. 1.0%, $P \leq 0.0001$). Only mild, low incidence of clinical signs was noted in control animals post-challenge with no significant differences between treatment groups. Despite this, there was a 1 kg/day difference between the treatment groups during the post-challenge period. Non-vaccinated animals lost weight post-challenge while vaccinated pigs continued to gain weight. Study 3: Mean percent lung lesion scores were lower in the vaccinated group (mean 10.2%) when compared to a mean 16.9% lung lesion in the positive (non-vaccinated, challenged) control group. Post-challenge ADG was numerically higher in the vaccinated animals versus the positive control group (0.71 kg/day vs 0.58 kg/day). The ADG of both challenged groups was lower than the negative (unchallenged) control group.
Discussion: Fostera PRRS helps defend growing pigs against a heterologous PRRSv challenge as evidenced by these results. Vaccination with Fostera PRRS helped prevent, lung lesions, which also resulted in post-challenge weight gain. It is notable that even when clinical signs of PRRS are minimal, as in study 2, that the impact on growth can be substantial (24.2 lbs. improvement). Pork producers can protect their investment by helping protect their pigs from dramatic production losses incurred with PRRSv challenges by vaccinating their pigs with Fostera PRRS.

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Safety, efficacy, and duration of immunity of a PRRSv MLV vaccine in 1 day-of-age pigs

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In studies with 1 day-of-age pigs, an experimental Porcine Reproductive & Respiratory Syndrome modified-live virus (MLV) vaccine demonstrated safety, efficacy, and duration of immunity.

An initial 1 day-of-age safety and efficacy study was performed. Pigs were vaccinated at 1 day-of-age (n=11) and controls (n=7) received a placebo. No biologically relevant differences in clinical observations were observed among vaccinates over controls during the vaccination phase. Pigs were challenged at 7 weeks of age with a heterologous PRRSv isolate NADC20 and were necropsied 10 days post-challenge. Vaccination demonstrated a 90% reduction in lung lesions (controls 33.0% vs. vaccinates 3.3%, $P \leq 0.0001$).

An efficacy study with a larger number of pigs was subsequently performed. Pigs were vaccinated at 1 day-of-age (n=24) and controls (n=22) received a placebo. Pigs were challenged at 7 weeks of age with a heterologous PRRSv isolate NADC20 and were necropsied 10 days post-challenge. Vaccination demonstrated a 98% reduction in lung lesions (controls 43.9% vs. vaccinates 0.7%, $P \leq 0.0001$).

Safety of the PRRSv MLV vaccine was assessed in 1 day-of-age pigs. Pigs were vaccinated at 1 day-of-age (n=91) and controls (n=100) received a placebo. Pigs were clinically observed for 10 days with general health observations collected to weaning. Clinical observations were minimal and present in both treatment groups. All pigs were serologically negative at day 1 (vaccination). All control pigs remained negative at weaning whereas all vaccinated pigs had seroconverted.

A reversion-to-virulence study was performed to assess vaccine stability of the PRRSv MLV vaccine in 1 day-of-age pigs. The vaccine was passaged five times through pigs (n=10 per passage). No observations (level and duration of viremia, rectal temperatures, clinical observations, lung lesions, and serology) indicated an increase in, or reversion to, virulence.

Duration of immunity (DOI) was assessed at both 18 weeks and 26 weeks after vaccination at 1 day-of-age. In the 18 week DOI study, pigs were vaccinated at 1 day-of-age (n=20) and controls (n=23) received a placebo. Pigs were challenged at 18 weeks of age with a heterologous PRRSv isolate NADC20 and were necropsied 10 days post-challenge. Vaccination demonstrated a 95% reduction in lung lesions (controls 21.1% vs. vaccinates 1.0%, $P \leq 0.0001$). In the 26 week DOI study, pigs were vaccinated at 1 day-of-age (n=24) and controls (n=24) received a placebo. Pigs were challenged at 26 weeks of age with a heterologous PRRSv isolate NADC20 and were necropsied 10 days post-challenge. Vaccination demonstrated a 93% reduction in lung lesions (controls 17.7% vs. vaccinates 1.2%, $P \leq 0.0001$).

Field safety of the PRRSv MLV vaccine was assessed in 1 day-of-age pigs. Pigs were vaccinated at 1 day-of-age (n=200) and controls (n=100) received a placebo; the control group represented the current herd health conditions. Pigs were clinically observed for 21 days following vaccination. The incidence of abnormal health events along with necropsy diagnosis and diagnostic laboratory results were not distinguishable between vaccinates and controls.

With the exception of the Field Safety study, all pigs were from PRRSv-naïve dams.

These studies demonstrate that the use of an experimental PRRSv MLV vaccine can be adopted into pig populations as young as 1 day-of-age.

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Comparative analysis of routes of immunization of a live PRRS virus vaccine in a heterologous virus challenge study

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Purpose: Porcine reproductive and respiratory syndrome (PRRS) has been an economically important disease since 1991. Effective mucosal vaccination induces both mucosal and systemic immunity compared to parenteral vaccination. PRRSV causes disease primarily in the respiratory tract and thus intranasal (IN) delivery of a potent vaccine adjuvant formulation has a promise.
Methods: PRRS-MLV (Boehringer Ingelheim) (strain VR2332) was coadministered with an adjuvant *Mycobacterium vaccae* whole cell lysate (*M. vaccae* WCL) or CpG ODN through intramuscular (IM) or IN route (as an aerosol), followed by a heterologous PRRSV (strain 1-4-4) challenge at day post-vaccination (DPV) 42 by IN route (as an aerosol). The viral load and immune correlates were determined in the blood, lungs, and tonsils of pigs.
Results: At DPV 14, 26, and 42 the vaccine virus RNA load was greater in the plasma of pigs received the vaccine through IM than IN route, though the data was not statistically significant; and the replicating virus was detected only in IM vaccinated groups. At DPC 7, 10 and 14 of MLV vaccine +/- adjuvant through IM received pigs, reduced viral RNA load and the absence of detectable replicating virus was detected compared to similar vaccine adjuvant IN received pig groups. Similarly, the viral RNA load in the lungs of IM vaccine +/- adjuvant groups at DPC 14 was lower than IN trial groups, but in tonsils it was comparable. Immunologically, virus neutralizing antibody titers in the plasma and lungs of IM vaccine groups was higher than IN groups, though the data was not significant. Concomitantly, at DPV 26 PRRS-MLV IM (without adjuvant) received pigs had significantly increased frequency of $CD4^{-}CD8^{+}$ and $CD4^{+}CD8^{+}$ T cell subsets in PBMCs compared to IN administered similar trial groups. At DPC 14, in PRRS-MLV IM (without adjuvant) received pigs PBMCs restimulated with the challenge virus *in vitro* significantly increased frequency of IFN-$gamma^{+}$ $CD4^{-}CD8^{+}$ and $CD4^{+}CD8^{+}$ T cell subsets compared to mock-challenged group; and significantly increased IFN-$gamma^{+}$ gamma delta T cell frequency compared to all the IN vaccinated with adjuvant groups was observed. When total IFN-$gamma^{+}$ lymphocytes were analyzed, significantly increased frequency of IFN-$gamma^{+}CD3^{-}$ cells were observed in PRRS-MLV IM (without adjuvant) received pigs compared to mock and IN vaccinated virus-challenged pigs.
Conclusions: Our data suggested that PRRS-MLV administered by IM route induces the virus specific T-cell response and reduces the challenge virus load compared to IN vaccinated pigs. But, to induce robust cross-protective PRRSV immune response using PRRS-MLV, co-administering the vaccine IN with a potent adjuvant like *M. tuberculosis* WCL is required (Dwivedi et al., 2011, Vaccine, 29, 4058-4066). Otherwise, IM delivery of PRRS-MLV without adjuvant itself is beneficial. This project was supported by USDA PRRSCAP2 and OARDC, OSU to RJG.

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Construction and characterization of a self-propagating replicon of PRRSV

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Porcine reproductive and respiratory syndrome virus (PRRSV) replicates primarily in the alveolar macrophages in infected pigs. The immune response pigs infected with this macrophage-tropic wt PRRSV is weak and delayed for weeks. We hypothesized that if PRRSV can replicate in many other cells and tissues in addition to the macrophages, it may be possible to induce a robust and early immune response against the virus. Since viruses encoding and having the vesicular stomatitis virus (VSV) G protein on the envelope are known to infect many different cell types and have broad cell tropism, we further hypothesized that a PRRSV encoding VSV G protein could confer pantropic replication phenotype in cultured cells as well as in infected pigs. To test our hypothesis, we engineered the complete coding sequence of VSV G protein into the full-length infectious clone of PRRSV (FL12) by replacing the coding regions of the viral glycoproteins (GPs) 2 to GP4. Transfection of BHK-21 cells with *in vitro* synthesized RNA from the ensuing plasmid resulted in generation of infectious PRRSVs containing VSV G protein on the viral envelope. The recombinant PRRSV, called FL12-Gdelta2-4, was found to infect many different cell types in culture including those that are typically not susceptible to PRRSV infection. The FL12-Gdelta2-4 virus replicated well in cultured mammalian cells and was able to spread from cell to cell in the culture. This self-propagating virus or replicon could grow to titers of nearly one million plaque forming units per ml in culture supernatant of infected BHK-21 cells. Additionally, the virus was found to be stable for at least 10 successive passages in BHK-21 cells. Current studies are directed at infecting pigs with this virus and examining the ensuing immune responses in pigs.

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Field efficacy of an experimental Porcine Epidemic Diarrhea (PED) vaccine administered to pregnant sows

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A placebo-controlled field study was conducted at a commercial production sow farm that was experiencing an outbreak of PED to determine if an experimental PED killed-virus vaccine would help reduce pre-weaning piglet mortality in an actively infected herd. (Data on File, Study Report No. B826R-US-13-258, Zoetis Inc). Pregnant gilts and sows were randomly assigned by parity to placebo (T01) or vaccinated (T02) groups (N=120 each) in a split plot design. Vaccinations were administered 5 and 2-weeks pre-farrowing, with enrollment occurring over a period of 8 weeks. The farm used common industry protocols for exposure of all sows and gilts to the virus prior to initiation of the study and the first block of animals was vaccinated one month after the farm initially broke with PEDv. Serum samples, collected from a randomized subset of sows prior to each vaccination and at the time of piglet processing, were tested for PEDv serum neutralizing (SN) antibodies. Pooled fecal samples from piglets from the same sows were collected at processing and weaning to confirm the presence of PEDv by RT-qPCR. Personnel performing daily clinical observations and laboratory testing were masked to the treatment group allocations. This study was conducted according to state, national, or international regulations and after review by Zoetis's Ethical Review Board. Vaccinated litters had a 0.6% pre-weaning mortality rate due to PED compared to a 6.3% pre-weaning mortality rate due to PED in litters of placebo controls (Table 1). The reduction of pre-weaning mortality was apparent in all parity groups (P0, P1-3 and P ≥4). The favorable impact of vaccination was also demonstrated by the percentage of litters with no piglet deaths attributable to PED: 92.0% in the T02 vaccinated group versus 76.3% in the T01 control group. Sows and gilts had a strong neutralizing serologic response to vaccination. The mean beta SN titer in the T02 vaccinates increased ~ 2.5 fold compared to baseline levels after the first vaccination, at which time the mean titers were also ~ 2.8 times higher than the mean titers of the T01 controls. These data present the possibility of a vaccine effect under field conditions in a herd that has been previously infected with PEDv.

Treatment	Mean (SD) percent litter pre-wean mortality due to PED*	Percent litters with no piglet deaths due to PED	Mean (SD) percent litter pre-wean mortality due to non-PED causes*	Percent litters weaned**
T01 – Placebo	6.3% (24.89)	76.3%	15.9% (27.15)	78.1%
T02 - Vaccinates	0.6% (4.33)	92.0%	13.9% (19.86)	93.8%

*Back-transformed mean percent litter pre-wean mortality.

**Litters not weaned had 100% mortality due to PEDv or any other reason.

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Enhanced immune responses in pigs by DNA vaccine coexpressing GP3 and GP5 of European type porcine reproductive and respiratory syndrome virus

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Background: Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant viral diseases in the pork industry. The etiological agent is PRRS virus (PRRSV), which was first isolated in the United States in 1987 and in Europe in 1990. The disease subsequently became panzootic and spread worldwide. Infection with this virus can cause reproductive failure, respiratory disease and growth retardation in pigs, thereby causing significant economic losses to the pork industry in North America, Europe and Asia. Vaccination is a predominant strategy for the prevention and control of PRRS for the purpose of reduction of clinical losses, but not for prevention of virus infection. Two types of PRRS vaccines are commercially available, modified live-attenuated vaccines and killed virus vaccines. However, neither of them not able to protect completely against PRRSV infection, and they both have inherent drawbacks.

Results: In this study, three recombinant DNA vaccines, pVAX1-EU-ORF3-ORF5 (coexpressing EU type PRRSV GP3 and GP5), pVAX1-EU-ORF3 and pVAX1-EU-ORF5, were constructed and evaluated for their abilities to induce humoral and cellular responses as well as to protect piglets against homologous virus challenge. All piglets were given booster vaccinations at 21 days after the initial inoculation and then challenged 14 days later. Pigs inoculated with pVAX1-EU-ORF3-ORF5 developed significantly higher ($P < 0.05$) PRRSV-specific antibody responses, neutralizing antibodies and levels of IL-4 and IL-10 than those given pVAX1-EU-ORF3, pVAX1-EU-ORF5 or pVAX1. Moreover, pigs immunized with pVAX1-EU-ORF3-ORF5 had markedly increased levels of IFN-gamma and IL-2 in serum and T-lymphocytes ($CD3^+CD4^+$ and $CD3^+CD8^+$ T cells) in peripheral blood. Thus, EU-type PRRSV GP3 and GP5 proteins demonstrated good immunogenicity and reactogenicity and could induce cellular immunity in pigs. Following challenge with the Lelystad virus (LV) strain, piglets inoculated with pVAX1-EU-ORF3-ORF5 showed viremia and virus load distributed in organ tissues that were significantly lower ($P < 0.05$) than those in the pVAX1-EU-ORF3 group and control group, and slightly lower than those in the pVAX1-EU-ORF5 group ($P > 0.05$). As GP3 could effectively enhance humoral- and cell-mediated immune responses to GP5, the results of this study suggested that these two proteins delivered by a vaccine can synergistically induce immunity against PRRSV.

Conclusions: In summary, vaccination of pigs with DNA constructs co-expressing GP3 and GP5 linked by the 2A sequence of FMDV along with the Quil A adjuvant could induce significantly stronger PRRSV-specific cellular and antibody responses in pigs and conferred a more efficient reduction in virus load in challenged pigs, compared with delivery of either antigen alone. Thus, the DNA vaccine carrying ORF3 and ORF5 showed a significant synergistic effect and may be used as a strategy to develop of a candidate vaccine against EU-type PRRSV in the swine industry.

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In vivo targeting of porcine reproductive and respiratory syndrome virus antigen through porcine DC-SIGN to dendritic cells elicits antigen-specific CD4 T cell immunity in pigs

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Immunogenicity of protein subunit vaccines may be dramatically improved by targeting them through antibodies specific to c-type lectin receptors (CLRs) of dendritic cells in mice, cattle, and primates. This novel vaccine development approach has not yet been explored in pigs or other species largely due to the lack of key reagents. In this study, we demonstrate that porcine reproductive and respiratory syndrome virus (PRRSV) antigen was targeted efficiently through antibodies specific to a porcine CLR molecule DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) in pigs. A recombinant PRRSV antigen (shGP45M) was constructed by fusing secretory-competent subunits of GP4, GP5 and M proteins derived from genetically-shuffled strains of PRRSV. In vaccinated pigs, when the PRRSV shGP45M antigen was delivered through a recombinant mouse-porcine chimeric antibody specific to the porcine DC-SIGN (pDC-SIGN) neck domain, porcine dendritic cells rapidly internalized them *in vitro* and induced higher numbers of antigen-specific interferon-γ producing CD4 T cells compared to the pigs receiving non-targeted PRRSV shGP45M antigen. The pDC-SIGN targeting of recombinant antigen subunits may serve as an alternative or complementary strategy to existing vaccines to improve protective immunity against PRRSV by inducing efficient T cell responses.

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The deubiquitinase function of arterivirus papain-like protease 2 suppresses the innate immune response

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

To dissect this dual specificity, which relies on a single catalytic site, we determined the crystal structure of equine arteritis virus (EAV) PLP2 in complex with Ub (1.45 Å). PLP2 binds Ub using a zinc finger that is uniquely integrated into an exceptionally compact OTU-fold, which we believe represents a new subclass of zinc-dependent OTU DUBs. Notably, the Ub-binding surface is distant from the catalytic site, which allowed us to mutate this surface to significantly reduce DUB activity without affecting polyprotein cleavage. Viruses harboring such mutations exhibited wild-type replication kinetics in cell culture, confirming that PLP2-mediated polyprotein cleavage was intact. However, the loss of DUB activity strikingly enhanced innate immune signaling, resulting in nearly an order of magnitude increase in beta-interferon mRNA expression by primary equine lung cells infected with the mutant. Furthermore, *in vivo* studies revealed that horses vaccinated with the mutant virus showed lower viremia after challenge with the heterologous virulent EAV KY84 strain compared to animals vaccinated with wild-type EAV.

Our findings not only establish PLP2 DUB activity as a critical factor in arteriviral innate immune evasion, the selective inactivation of DUB activity also opens new possibilities for developing improved live attenuated vaccines against arteriviruses like PRRSV and other viruses encoding similar dual-specificity proteases.

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Development of a synthetic porcine reproductive and respiratory syndrome virus strain that confers broader cross-protection

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Substantial genetic variation among PRRSV strains represents a major obstacle for the development of a broadly protective vaccine. We describe here a novel approach to generate a PRRSV vaccine strain that could confer broad cross-protection against divergent PRRSV isolates. We had initially obtained a set of 60 non-redundant, full-genome sequences of type-II PRRSV. After that, we generated the consensus genome (designated as PRRSV-CON) by aligning the 60 PRRSV full-genome sequences, followed by selecting the most common nucleotide found at each position of the alignment. Our analysis demonstrates that the PRRSV-CON has the highest degree of sequence identity to the PRRSV field-isolates when compared to any current PRRS vaccine strains, both at the full-genome level and the individual gene level. Next, we chemically synthesized the PRRSV-CON genome and assembled it into a bacterial plasmid under the control of the T7 promoter. The resulting PRRSV-CON cDNA clone is fully infectious. Viable virus is consistently produced after MARC-145 cells are transfected with the RNA transcript produced from the PRRSV-CON cDNA clone. Moreover, the PRRSV-CON virus replicates as efficiently as our prototype PRRSV strain FL12, both *in vitro* and *in vivo*. Importantly, primary infection of pigs with PRRSV-CON virus confers significantly broader protection than the prototype PRRSV strain FL12 when tested upon subsequent challenge with a third unrelated heterologous PRRSV strain. Collectively, our data demonstrate that the PRRSV-CON virus can serve as a potential vaccine candidate for the development of a novel PRRS vaccine with broader cross-protection.

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Anti-GP5 idiotypic antibodies reduce efficacy of the attenuated vaccine against highly pathogenic PRRSV infection

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Objective: At present, both live attenuated and inactivated PRRSV vaccines cannot provide sustainable disease control. The efficacy of PRRSV vaccination against PRRSV infection may be related to the cellular immune responses induced by vaccination. In addition, the live attenuated vaccines have protective value against PRRS clinical disorders, but cannot prevent the disease or shorten persistent infection in individual pigs, resulting in long-term circulation of virus within swine herds and the potential to revert to virulence. The inability of current vaccines to provide effective protection against PRRSV infection is not fully understood. Our previous studies demonstrated the presence of auto-anti-idiotypic antibodies (aAb2s) specific for GP5 antibodies in pigs infected with PRRSV. The objective of this study was to evaluate the present of anti-GP5 idiotypic antibodies interfered with the efficacy of the attenuated PRRSV vaccine (CH-1R) to protect against highly pathogenic (HP) PRRSV infection.

Methods: PRRSV negative piglets were injected with a monoclonal Ab2 (Mab2-5G2) and aAb2s that are specific for anti-GP5 antibody, vaccinated with the attenuated PRRSV vaccine CH-1R and then challenged with the highly pathogenic PRRSV HuN4 strain. The animals were evaluated for clinical signs, pathological changes of the thymus and lungs, viremia, levels of serum antibodies and cytokines.

Results: The piglets injected with Mab2-5G2 or aAb2, and who received the attenuated PRRSV vaccine CH-1R before challenge, produced high levels of anti-N antibodies, IL-2 and IL-4, but low levels of neutralizing antibodies. After PRRSV HuN4 challenge, the animals showed obvious clinical signs, including lung lesions, severe thymus atrophy and decreased production of IL-4 and higher level of viremia.

Conclusion: When anti-GP5 Ab2s are present, the use of attenuated PRRSV vaccine CH-1R against HP-PRRSV infection is not recommended. It can result in poor health status with pneumonia and thymus atrophy.

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Assessment of cross-protective immunity elicited by a novel PRRS live virus vaccine against a highly virulent type 2 PRRS belonging to lineage 1

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This study assessed the efficacy of the type 2 PRRS virus vaccine strain G16X belonging to lineage 5 against a highly virulent isolate LTX1 belongs to lineage 1. The novel PRRS live virus vaccine G16X was derived from a naturally non-virulent virus and has the unique ability to stimulate an interferon (IFN)-alpha response *in vivo*. The LTX1 virus used as the challenge is closely related to the RFLP 1-22-2 family of type 2 PRRS virus that caused numerous outbreaks in South West Ontario in 2010/11. In the American Midwest, the LTX1 virus was responsible for a PRRS outbreak in a sow farm that was characterized by a group of swine practitioners as the worse they had experienced. A group of 7 week-old pigs (n=20) was immunized parenterally with the G16X PRRS vaccine and a second group (n=10) was mock-vaccinated. Four weeks later all of these animals were challenged by the administration of LTX1 virus via both intranasal and parenteral routes. A group non-vaccinated and non-challenged pigs (n=6) served as strict (environmental) controls. Following the virus challenge, mock-vaccinated animals exhibited a major drop in oxygen saturation (SpO_2) levels, which decreased from the normal 96±2% to 91.5% at 6 days post-challenge (pc) and remained at <91% in this group throughout the rest of the observation period. In contrast, the lowest average SpO_2 level in the vaccinated animals (92.9%) was observed at 9 days pc and returned to normal SpO_2 levels by 11 days pc. Animals immunized with the G16X vaccine exhibited a major reduction both the peak fever and the duration of pyrexia after challenge. As compared to the weight gain observed in the strict control group, pigs in the mock-vaccinated group exhibited a 75% reduction in weight gain from the day of challenge to 14 days pc, while the G16X vaccinated exhibited only a 20% reduction in growth. The significant reduction in the severity of all three objectively measured clinical parameters (fever, SpO_2 and weight gain) in the G16X vaccinated pigs correlated with a much-reduced severity and duration of clinical signs observed (depression and labored breathing) as compared to those observed in the mock-vaccinated and challenged pigs. Four days pc all of the mock-vaccinated animals exhibited a high level of viremia, which peaked at 7 days pc, with all but one of the animals in this group remaining viremic until the end of the observation period at 14 days pc. In contrast, the group of vaccinated animals exhibited a lesser viremia, which peaked at 4 days pc and began to subside thereafter, with only 25% of the animals exhibiting viremia at 10 days pc and becoming non-viremic 14 days pc. While the bronchoalveolar lavage (BAL) fluids of all the mock-vaccinated animals collected at the time of euthanasia exhibited detectable PRRS virus, 25% of the vaccinated animals had no detectable virus in their BAL fluid, and the average viral load in the positive samples was 30 fold-lower than the mock-vaccinated animals ($p<0.02$). The gross lung lesion score (i.e., typical of interstitial pneumonia induced by PRRS virus) in the mock-vaccinated pigs was 46±5.7%, while the same score in the vaccinated group was significantly ($p<0.001$) lower, i.e. 18±2.7%. Consistent with our previously postulated notion of the influential role of IFN-alpha during the developmental phase of a protective immune response against PRRS virus, the G16X strain, which elicited a significant IFN-alpha response soon after vaccination, promoted the development of a significant level of protective immunity against a genetically divergent and highly virulent contemporary PRRS virus isolate. The ability of the G16X vaccine virus to provide protective immunity to a field strain of a different lineage is consistent with the growing evidence that the degree of genetic homology between the challenge strain and the vaccine is not predictive of the degree of protective immunity elicited, but rather has more to do with the biological properties of the vaccine.

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Full genome sequence analysis of a wild, non-MLV-related type 2 Hungarian PRRSV variant isolated in Europe

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a widespread pathogen of pigs causing significant economic losses to the swine industry. The expanding diversity of PRRSV strains makes the diagnosis, control and eradication of the disease more and more difficult. In the present study, the authors report the full genome sequencing of a Type 2 PRRSV strain isolated from piglet carcasses in Hungary. Next generation sequencing was used to determine the complete genome sequence of the isolate (PRRSV-2/Hungary/102/2012). Recombination analysis performed with the available full-length genome sequences showed no evidence of such event with other known PRRSV. Unique deletions and an insertion were found in the nsp2 region of PRRSV-2/Hungary/102/2012 when it was compared to the highly virulent VR2332 and JXA-1 prototype strains. A majority of amino acid alterations in GP4 and GP5 of the virus were in the known antigenic regions suggesting an important role for immunological pressure in PRRSV-2/Hungary/102/2012 evolution. Phylogenetic analysis revealed that it belongs to lineage 1 or 2 of Type 2 PRRSV. Considering the lack of related PRRSV in Europe, except for a partial sequence from Slovakia, the ancestor of PRRSV-2/Hungary/102/2012 was most probably transported from North-America. It is the first documented type 2 PRRSV isolated in Europe that is not related to the Ingelvac MLV.

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The role of PRRSV non-structural protein 3 (nsp3) in modulation of apoptosis in infected cells

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PRRSV genetic determinants involved in the pathogenesis of disease and virus persistence in pigs are still not fully understood. It is thought that at the molecular level, specific interactions between proteins of the host primary target cell, the macrophage, and viral proteins drive the pathogenesis of PRRS. Using a GAL4-based yeast two-hybrid system to screen for PRRSV-host cell protein-protein interactions of a swine macrophage expression library, we have identified thirty host proteins that interact with PRRSV non-structural protein 3 (nsp3). Interestingly, twenty-one of these interacting host proteins are involved in apoptotic signaling pathway. Apoptosis is a hallmark characteristic of PRRSV infection and it is one of the host molecular pathways involved in pathogenesis. Apoptosis is observed in PRRSV infected and bystander cells, both *in vivo* and *in vitro*. However, responsible viral and host factors or mechanisms and consequences of apoptosis during infection are not completely understood. In order to understand the role of nsp3 in apoptosis, further studies were carried out with one of the nsp3 interacting proteins, FK506-binding protein 38 (FKBP38). FKBP38 is an immunophilin in the mTOR kinase pathway central to a wide array of cellular processes, such as cell survival, proliferation, and metabolism. Hepatitis C virus non structural protein NS5A interacts with FKBP38 and inhibits apoptosis in hepatoma cells. Activated FKBP38 regulates apoptosis signaling by targeting anti-apoptotic Bcl-2 to mitochondria. By introducing alanine substitutions to hydrophilic regions of nsp3, we mapped $_{181}$PYDIHHY$_{187}$ in nsp3 using yeast two hybrid system. This interaction is maintained in both type I (Lelystad) and type II (NVSL 97-7895) PRRSV isolates which are highly divergent, differing more than 50% at the amino acid level. This observation suggests a conserved function for nsp3-FKBP38 interaction. Modifications introduced into the interacting interphase of the nsp3 in the context of full length PRRSV infectious cDNA clone are deleterious for PRRSV suggesting that the function of nsp3 is essential to complete the virus life cycle.

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Nucleocapsid protein of Porcine Epidemic Diarrhea Virus enhances viral replication in vitro

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The recent outbreak of porcine epidemic diarrhea virus (PEDV) has caused significant challenge to US swine industry. The isolation and propagation of PEDV in cell culture is the first step toward development of vaccines and diagnostic tests. However, the natural characteristics of the virus pose certain difficulties on the adaptation and passage of PEDV in cell cultures. In this study, we demonstrated that nucleocapsid (N) protein of PEDV has the ability to enhance infectivity of PEDV. In transfected cells expressing N protein, the PEDV grows to 10-fold higher peak viral titer, in comparison to the virus titer in untransfected cells. Further analysis showed that N protein interacts with nonstructural protein 3 (nsp3) and localized to the viral replication-transcription complex (RTC). The nsp3-N interaction was mapped to the N-terminal 160 amino acids region containing ubiquitin-like domain of nsp3. These results support a previously established model of murine coronavirus, in which the N-nsp3 interaction serves to tether the viral genome to the newly translated RTC at early stage of infection, which could be a mechanism for N-protein dependent enhancement of PEDV infectivity.

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Economics effects of Porcine Epidemic Diarrhea (PED) in México

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Introduction

Porcine epidemic diarrhoea virus (PEDV) was first identified in the United Kingdom in 1971, and it caused mass epidemics in Europe in the 1970s and 1980s. It has since spread to Asia, where it has been considered endemic since 1982, causing substantial economic losses to pork producers. The first case of PED in the USA was confirmed on May 16, and by July 27, just borders to individual pig units. In México, according to information published by the OIE, the onset of the disease in Mexico was given on July 30, 2013 and pre-confirmation was held on August 8, 2013. Currently, 16 states of México have PED.

Materials and Methods

The study was based on information from 16120 sows in production of farms of México with continuous flow production. Production and economic records were for the years 2012, 2013 and 2014. Treatments for comparison, economical parameters of breeding área:

- Group I: Animals free of PED virus with subsequent infection
- Group II: Animals infected with PED virus.

Reproductive parameters were compared with T of Student to check statistically significant differences between means.

Results

We found significant differences in the number of weaned piglets and preweaning mortality(p=0.00). Table 1 and 2 show the reduction of weaned piglets and economic losses.

TABLE 1. Average of weaned piglets per sow per year and economic looses.

	Average of weaned Piglets/ sow/farrow				
	Without PED	With PED	Dif.	Farrows/ sow/year	Losses of weaned piglets /sow in production /year
Farm 1	10.39	8.69	1.70	2.41	4.11
Farm 2	9.52	7.39	2.14	2.28	4.87
Farm 3	9.66	7.88	1.78	2.13	3.79
Farm 4	9.67	8.69	0.98	2.41	2.37
AVERAGES	9.81	8.16	1.65	2.31	3.78

TABLE 2. Economic losses by PED virus in four farms with 16120 sows in production.

Piglets losses/sow/year	Sows in production	Total Piglets Losses	Piglet Price	Economic looses
3.78	16120	60934	US $37	US $2,256,800

Conclusions and Discussion

The Porcine Epidemic Diarrhea in México has a negative effect in the average of weaned piglets/sow/year generated and an important reduction in the economic income because it shows less fat pigs to sell.

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Economics effects of PRRS virus in pig herds in México

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Introduction

In Mexico, it has been estimated the economic impact of an outbreak of PRRS virus in farms of full cycle. At the International Symposium of PRRS virus: yesterday, today and tomorrow, CU, Mexico, 2012; Alberto Herrera believes that this disease in Mexico could be costing between US $80 and $120 million per year for the pork industry, provided that the economic impact of an acute outbreak of PRRS costs between US $208 and US $292 per sow per year, in the production line and when there are persistent infections it ranges from US $5 per pig for slaughter up to US $12.50 in acute cases. In the same forum Leonardo Perez, estimates that economic impact start at US $ 9.72 until US $ 21.08 per pig and US $ 185.4 to US $ 421.6 per female per year.

Materials and Methods

The study was based on information from 650 sows in production of farms of full cycle in the center of México with continuous flow production, production and economic records of the year 2009 (first period) and 2010 (second period), advice on feed production, genetics specialized in pig production, use of artificial insemination and weaning between 19 and 23 days and advice in pig production.

Treatments for comparison economical parameters of breedind área and the production line:

- Group I: Animals free of PRRS virus in the first period with subsequent infection
- Group II: Animals free of PRRS virus in the first period.
- Group III: Animals free of PRRS virus in the second period.
- Group IV: Animals with PRRS virus in the second evaluation period.

Reproductive, productive and economic parameters were compared with analysis of variance to check statistically significant differences between means. To find out which groups are statistically different, an additional Tukey-kramer test was performed.

Results

The values obtained in the cost per kilogram and costs per sow per year are shown in Table 1.

Figure 1. Values of cost per kilogram and cost per sow per year.

Variable	Group I	Group II	Group III	Group IV
Cost/ kilogram	US $1.62 ab	US $1.55 ab	US $1.36 a	US $1.85 b
Cost/ sow/ year	US $249.62 a	US $179.12 b	US $185.04 b	US $244.54 a

The averages of the groups sharing literal, show no significant statistical difference($p<0.05$).

Conclusions and Discussion

The results of the production costs presented in this study, only coincide with the upper range reported by Neumann, the analysis of this indicator increased between US $ 19 and US $42 per pig of 89 kilograms. The cost per sow in the group IV showed an increase of US $ 57 compared with group III, is the same range reported by Neumann et al (2005) and Nieuwenhuis et al. (2012) however smaller than the rest of the reports made in the nineties by various authors and Herrera and Pérez in 2012. The group of infected sows with PRRS virus showed increased production cost compared to the control groups and the group of sows without the PRRS virus. Finally we can say that we need to work hard in this field of work because there are very few publications Mexico and in the World.

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Utilization of data from a regional PRRS control project for practical field investigations into spread of PRRS

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Porcine reproductive and respiratory syndrome (PRRS) area regional control and elimination (ARC&E) programs became relatively frequent over the last years in the North American swine industry, with the first Ontario project commencing in 2010. In mid-2014, herd veterinarians from a specific region (Watford) expressed concerns with regard to spread of a specific PRRS virus (PRRSV) genotype within the region (so-called "RFLP 1-3-2"), therefore the primary goal of the present study was to make use of data available in the PRRS ARC&E database to investigate disease spread with this particular genotype. The objectives were to investigate spatial location and truck network membership as risk factors for being positive with the specific genotype, and to reconstruct the transmission chain of PRRSV within this region over time.

Site location, production system, trucking network and site demographics were collected at enrollment. Sampling was conducted by herd veterinarians, and all PCR samples from positive sites were attempted to be sequenced (ORF5 gene). A phylogenetic tree was constructed using R 2.15, and three kilometer buffers were constructed using ArcMap 10.1 for each of the sites. Network analysis of trucking data was conducted using UCINET, where truck companies and herds were nodes, and an edge was defined as an undirected connection between a site and a truck company. A network component was defined as a group of sites/ truck companies that were connected direct or indirectly. Multivariable exact logistic regression models were constructed in SAS 9.3 to investigate location and truck network membership as risk factors for being positive with "RFLP 1-3-2".

By the time of completion of this investigation, the Watford PRRS ARC&E had 136 swine sites enrolled. A total of 46 sequences were included in the phylogenetic tree. The so-called "RFLP 1-3-2" genotype was found to be located in two dissimilar clades. One of these clades had more than 96% similarity amongst its sequences; therefore this "genetic cluster" was used as the outcome for the risk factor analysis. The 3km buffers showed that approximately 20% of all sites did not have any neighbour within 3km, 45% had between one and three neighbours, and 35% had four or more. Thirty nine truck companies were involved in transporting pigs from all sites, one truck company transported animals for over 40% of the sites. Statistical analysis showed no association between being positive for this specific genotype and having a neighbour within the 3km radius positive for the same genotype, outside the production system ($P = 0.89$, OR = 0.88). There was a trend for a positive association between being positive for this specific genotype and being part of the same truck network component, outside the production system ($P = 0.08$, OR = 1.70). In conclusion, the Ontario PRRS database contains extensive data that can be utilized for regional disease investigations. According to this investigation, proximity to another herd that is positive with the same genotype (and is outside the same production system) was not identified as a risk factor, but being part of a truck network that has sites positive for a specific genotype might increase the odds of a herd being positive for that genotype. Complete assessment of likely transmission chains is pending, and is a promising approach for investigation of disease spread.

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Genetic diversity, frequency, and concentration of PRRS virus collected from air samples in high and low farm-density areas

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Porcine Reproductive and Respiratory Syndrome virus (PRRSV) can be transmitted between farms through airborne spread, especially, between those farms located within short distances from each other. Control and elimination of PRRSV in the US is challenging due to the high concentration of swine farms. Although airborne transmission has been documented, the amount and diversity of viruses present in areas where swine farms exist, has not been described.

The objective of this study was to describe and compare the amount and diversity of PRRSV collected in 4 different locations from air samples taken regularly during a high season of PRRSV occurrence (October to December 2012). Air sampling was located outside 4 large sow farms. The number of farms and animals present within a 10 km radius was variable for the 4 air sampling locations. The frequency of positive air samples, as well as the amount of virus isolated was recorded. Positive samples were also sequenced and used to reconstruct the phylogeny of the virus using Bayesian methods.

A wide variety of field and vaccine strain virus was detected in samples from air, in each of the sampling locations. The farm and pig density within 10 km of each sampling location was highly associated with the concentration of virus in air (mean=3.8 and 5.3 TCID50/ml in the location with lower and higher farm concentration, respectively) and genetic diversity.

The results here highlight the importance of airborne route of PRRSV transmission, and a higher risk in high farm density areas. This study contributes to understanding the mechanisms of PRRS transmission, which is essential to implement appropriate control measures.

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An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naïve pigs following consumption via natural feeding behavior: Proof of concept

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Since its initial detection in May 2013, porcine epidemic diarrhea virus (PEDV) has spread rapidly throughout the US swine industry. Initially, contaminated feed was proposed as a risk factor for PEDV; however, data were not available to support this theory. Here we provide proof of concept of this risk by describing a novel means for recovering PEDV-contaminated complete feed material from commercial swine sites and conducting an *in vivo* experiment to prove its infectivity. For on-farm detection of PEDV RNA in feed, paint rollers were used to collect material from at-risk feed bins from 3 clinically affected breeding herds. This material was tested by PCR and determined to be positive for PEDV-RNA (Ct = 19.50-22.20 range). To test infectivity, this material was pooled (Ct = 20.65) and a Treatment group of 3-week old PEDV-naïve piglets were allowed to consume it via natural feeding behavior. For the purpose of a Positive control, piglets were allowed to ingest feed spiked with stock PEDV (Ct = 18.23) while the negative control group received PEDV-free feed. Clinical signs of PEDV infection (vomiting and diarrhea) and viral shedding were observed in both the Positive control and Treatment group' post-consumption with virus and microscopic lesions detected in intestinal samples No evidence of infection was observed in the Negative controls. These data provide proof of concept that contaminated complete feed can serve as a vehicle for PEDV infection of naïve pigs using natural feeding behavior.

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An evaluation of a liquid antimicrobial (Sal CURB®) for reducing the risk of porcine epidemic diarrhea virus infection of naïve pigs during consumption of contaminated feed

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Since its initial detection in May 2013, porcine epidemic diarrhea virus (PEDV) has spread rapidly throughout the US swine industry. Recently, contaminated feed was confirmed as a vehicle for PEDV infection of naïve piglets. This research provides *in vivo* data supporting the ability of a liquid antimicrobial product to reduce this risk. Sal CURB® (Kemin Industries, Des Moines, IA, USA) is a FDA-approved liquid antimicrobial used to control *Salmonella* contamination in poultry and swine diets. To test its effect against PEDV, Sal CURB®-treated feed was spiked with a stock isolate of PEDV (Ct = 25.22), which PEDV-naïve piglets were allowed to ingest via natural feeding behavior *(ad libitum)* for a 14-day period. For the purpose of a positive control, a separate group of piglets was allowed to ingest non-treated (Sal CURB®-free) feed also spiked with stock PEDV (Ct = 25.22). A negative control group received PEDV-free feed. Clinical signs of PEDV infection (vomiting and diarrhea) and viral shedding in feces were observed in the positive control group 2-3 days post-consumption of non-treated feed. In contrast, no evidence of infection was observed in pigs fed Sal CURB®-treated feed or in the negative controls throughout the 14-day study period. In addition, the Sal CURB® -treated feed samples had higher ($p < 0.0001$) mean PEDV Ct values than samples from the positive control group.These data provide proof of concept that feed treated with Sal CURB® can serve as a means to reduce the risk of PEDV infection through contaminated feed. Furthermore, the results from the positive control group provide additional proof of concept regarding the ability of contaminated feed to serve as a risk factor for PEDV infection of naïve piglets.

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An evaluation of a liquid antimicrobial (Sal CURB®) for reducing the risk of viral proxies for foreign animal diseases in contaminated feed

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Porcine epidemic diarrhea virus (PEDV) is a trans-boundary pathogen confirmed to be spread through contaminated feed. Recent research has also provided *in vivo* data supporting the ability of a liquid antimicrobial product (SalCURB®, Kemin Industries, Des Moines, IA, USA) to reduce this risk. Other risks to the US swine industry include foot-and-mouth disease virus (FMDV) and classical swine fever virus (CSFV); however, the effect of SalCURB® on these agents is unknown. To gain an estimate of its effect, viral proxies for FMDV and CSFV were selected according to morphological similarities (classification, size, shape, structure, etc). Specifically, as a proxy for FMDV and CSFV, cultures of encephalomyocarditis virus (EMCV) and bovine viral diarrhea virus (BVDV), respectively, were established under laboratory conditions. An *in vitro* model was developed to test the ability of SalCURB® to reduce viral load and virus viability in contaminated feed. SalCURB®-treated feed (10 replicates: 15g feed + 0.05mL SalCURB®) was spiked with 2 mL of a stock isolate of EMCV (5 logs/mL) and BVDV (4 logs/mL) per 15 g of feed. The titrated level of SalCURB® was based on label claim of 6.5 lbs product/ton of feed. Positive controls (10 replicates, feed/virus/no SalCURB®) and negative controls (feed/saline placebo) were included in the design. Over a 10 day sampling period (day 0, 2, 4, 8 and 10), feed samples were collected and tested by PCR and VI. Results indicated that significant reductions ($p < 0.0001$) were observed in EMCV and BVDV quantities in treated feed versus positive control feed. In addition, virus isolation indicated recovery of infectious EMCV and BVDV in non-treated feed at 10 and 4 days respectively; however, viable virus of either variant was not detected at 2 days post-inoculation in treated feed. These data indicate that the treatment of feed with Sal CURB® negatively influenced the survival of viral proxies for foreign animal diseases in feed. While further studies are required, involving the actual viral pathogens, these data suggest that the liquid antimicrobial SalCURB® may be an important component of a feed biosecurity program.

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Assessing the viability of porcine epidemic diarrhea virus in dry feed at 4 degrees C

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Since its initial detection in May 2013, porcine epidemic diarrhea virus (PEDV) has spread rapidly throughout the US swine industry. Initially, contaminated feed was proposed as a risk factor for PEDV; however, data were not available to support this theory. Recently, proof of concept data have been published indicating the ability of contaminated feed to serve as a vehicle for PEDV transmission to naïve pigs. Based on this information, it now becomes critical to determine the duration of PEDV survival in feed. While current data indicate indefinite survival at -20 degrees C and out to 7 days (dry feed) and 28 days (wet feed) at 20 degrees C, no information is available regarding the survival of PEDV in dry feed at 4 degrees C. To answer this question, 22.7 kg of PEDV-negative dry feed was divided into 5 equal batches (4.5 kg/batch). Each batch was spiked with 100 mL of PEDV (Ct = 23) and placed at 4 degrees C storage for pre-determined periods of time (0 days, 7 days, 14 days, 21 days, 28 days and 35 days). Upon completion of each storage period, the designated batch of PEDV positive feed was fed to PEDV naïve piglets (4 piglets/group, 6 weeks of age). Over a 2-week period post-ingestion, daily rectal swabs were collected from each pig and tested by PCR for the presence of PEDV RNA and each group was observed for clinical signs of PED (vomiting & diarrhea). At the end of each 14 day period, piglets were necropsied and the intestinal tracts tested for the presence of PED lesions by histopathological examination and PEDV RNA by PCR and IHC. In addition, samples of feed were collected from spiked batches prior to feeding along with positive control samples of feed that were also stored at 4 degrees C. Results indicated that pigs ingesting day 0, day 7, day 14 and day 21 stored feed became infected, demonstrating shedding of PEDV in feces, clinical signs and lesions of PED. In contrast, pigs ingesting spiked feed stored out to day 28 or day 35 did not become infected. In both the batched feed and the positive control feed, Ct values remained stable (Ct = 23-24) between days 0 to 14, increased (Ct = 29-30) from day 14 to 21 and further increased (Ct = 34-35) from days 28 to 35 post-inoculation. These data indicate that PEDV can remain viable in feed at cold temperatures out to 21 days. This information limits the feasibility of using extended storage periods to reduce the risk of PEDV contaminated feed during cold weather on farms. It also raises questions whether PEDV could survive in feed and/or feed products during shipping periods that extend out to 21 days during cold temperatures.

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North East Illinois PRRS ARC&E Project

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Northeast Illinois PRRS ARC Overview

- Project Timeline:
  - Completed Phase 1-3 prior to 2010
  - Phase 4 & 5: Herd Plan Design & Execution – Ongoing
- Site Information
  - 86 sites housing pigs
  - 40 area producers
    - 90% producer participation (representing 95% of the pigs)

Success Stories of PRRSv Elimination

One new isolate has been introduced into our area each of the last two years (1-18-2 in 2013 and 1-22-2 in 2014); however neither have spread to neighboring farms despite 6 farms being located <3 miles away from the initially infected site. In addition, we have not found our area's previously dominant strain (1-18-4) in our control area for more than 2 years. Also of note, the wild type isolates in the area have remained isolated in specific densely populated, geographic areas within the project, leaving a majority of the land area free of wild type PRRSv.

Observations in the ARC Project Area

Participation and buy-in have been important to our area. When the project began late in 2009, virtually no pigs were vaccinated for PRRS in the area. That number steadily climbed to >75% where it has remained for the last two years. We feel that this is important for PRRS isolate containment and the reduction of active isolates in our area.

We feel that producer participation and buy-in are also reasons for our area's success in keeping PEDv exposure limited. Our ARC area had a 33% infection rate during the last PEDv season (July 1, 2013-July 1, 2014).

We believe these disease control programs can benefit the entire industry as we work to combat traditional swine diseases such as mycoplasma and influenza, and emerging threats like PEDv and Deltacoronavirus.

Challenges and Opportunities

- Keeping PRRS on the forefront in the face of PEDv
- Continuing to show farms the value in PRRSv Elimination
- Ability to eliminate PRRSv as everyone steps up biosecurity in the face of PEDv

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Northwest Indiana PRRS ARC project: What is success?

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Introduction

The Northwest Indiana (NW IN) Porcine Reproductive and Respiratory Syndrome (PRRS) Area Regional Control (ARC) project began in 2009 with a goal to develop a network of producers that voluntarily collaborate to control PRRS virus in a specific region. Growing confidence of participating producers has led to continual growth toward achieving the original goal. Individual producers are realizing how cooperation can ultimately improve the productivity of their farms.

Materials and Methods

Current boundaries of the NW project are from US 421 west to the Indiana/Illinois line and from SR 24 north to Lake Michigan. Each year the project has grown in number of producers, number of pigs represented, and geographical area. In 2010, 38 sites were tested, representing 19,850 sows and 144,705 grow-finish pigs. In 2013, 55 sites participated, representing 35,485 sows and 188,375 finishing pigs. There are currently 8 sites not in the project, representing about 5% of finisher pigs and < 3% of sows and several are small exhibition operations.

Results

Early implementation of PRRS control at individual sites has minimized outbreaks of PRRS in this area. There has been only one break in a negative sow farm since the project began. Testing in the summer of 2013 revealed 21 of the 55 sites were ELISA positive and 11 of the 55 were PCR positive. Currently 21 of 55 sites utilize modified live virus vaccine to help control PRRS on their farms. With ORF5 sequencing, 6 of 11 positive PCRs were determined to be vaccine virus at that time.

Results are reported monthly to one representative from each of the farms/companies that have signed participation agreements. If severe breaks occur between updates, neighbors in close proximity to the new positive farm are notified by phone.

Conclusion and Discussion

Joint meetings have occurred with the West Central Indiana PRRS ARC project in an attempt to find synergies that would strengthen both projects. Also, there are farms in the NW IN PRRS ARC project receiving pigs from out-of-state sow farms that participate in other PRRS ARC projects. The efforts of other projects can only strengthen the efforts in the NW IN PRRS ARC project. Perhaps more important than eradicating PRRS, the NW PRRS ARC project is building trust among neighbors, allowing the sharing of information not only for PRRS, but for other diseases as well. Several times in the past year, health status information has been shared between the participants when PEDV and TEGV outbreaks occurred. This helped neighboring farms develop updated trucking routes and biosecurity plans to keep disease from spreading. It is this crucial communication that will ultimately lead to area regional control not only in NW Indiana, but in area regional control projects across the country and world.

Acknowledgments: Indiana Board of Animal Health, Indianapolis, IN

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Area regional control program of Porcine Reproductive and Respiratory Syndrome virus in North Carolina

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) continues to be one of the most economically challenging diseases in the swine industry. Estimated costs of PRRSv as of 2012 were $664 million in production losses and $477.79 million in animal health, biosecurity and other outbreak related costs (D. Holtkamp 2012). Sampling of herds by using Polymerase Chain Reaction (PCR) provides continuous monitoring for the virus, and facilitates genetic sequencing of positive samples to track the virus within a region.

Materials and Methods

To determine PRRSv sequences infecting pig herds in Johnston County, NC, 61 sites including Murphy-Brown, Prestage, Goldsboro Milling, and independent producers at all stages of production are sampled quarterly. The sampling protocol for PRRSv testing of breeding herds focus on testing piglets only. PCR for PRRSv is performed on 30 pigs to obtain a 95% confidence with 10% prevalence in the weaned pig population at 7 days prior or 3 days after weaning. Sampling protocol for growing herds uses PCR for PRRSv and performed on pooled samples via one or more of the following methods: blood: up to 6 pools of 5 samples each (30 pigs); oral fluids: minimum of 6 ropes per barn or per site; and tissue. Positive PCR samples with a CT value <33 are submitted to South Dakota State University for PRRSv sequencing. All sequences are entered into a dendrogram to establish homology.

Results

In two years, 218 ORF5 sequences have been compiled into the project dendrogram. Eleven defined familial groupings have been assigned on the basis of on percentage of homology. Some farms appear as outliers that do not fit within a defined grouping. Most farms continue to produce sequences in the same grouping as previous submissions. Some farms have produced multiple, different sequences. 94.08% of virus changes are attributed to pig source changes, while 5.92% or virus changes are attributed to geographical distance.

Conclusions and Discussion

Many farms that have produced sequences in multiple familial groupings were associated with changes in pig flow/sow source. In fact, source change was the primary factor in farms turning PRRSv positive or a farm producing a different sequence. A few farms have produced a sequence that is unrelated to any other sequences found in Johnston County. The constant changing of sources can partially be attributed to swine producers exchanging sources of pigs in order to prevent area spread of PEDv. Furthermore, many nursery and finisher farms source from outside of this region; therefore, expanding of the project by sampling sow farm sources is necessary. Because this region is unique for the amount of pig source changes, additional analysis is needed to determine the effect of pig sources and distances seen on individual farms and within the region.

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PRRS case study: Is it the resident virus or is it a new introduction?

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In Porcine Reproductive and Respiratory Syndrome (PRRS) positive sow herds, veterinarians are challenged with the question, "Is this the resident virus, or a new introduction?" following the receipt of PRRS positive test results. Often after sequencing and comparison of the ORF5 region of the viruses, the answer is determined. Throughout the industry there are various rules of thumb (typically 2% or 3%) as to what percentage heterology among sequences is called the same or different. Although easy to apply, these thumb rules may not tell the complete story.

A 5000 sow, farrow to finish herd in a high density area, underwent PRRS field virus elimination by loading the farm with gilts, subsequent herd closure and mass vaccination with a modified live vaccine (MLV) in the spring of 2010. The sow herd maintained immunity following successful field virus elimination through quarterly mass vaccinations. The herd was subsequently re-infected with a virulent PRRS virus (RFLP= 1-4-4) in December of 2010. In early 2011, the herd again underwent elimination. In November of 2012, PRRS virus (also a 1-4-4) was detected in due to wean (DTW) piglet samples taken in response to clinical signs attributable to PRRS in the sow herd. Oral fluid samples from finishing pigs were also collected.

Three points support the diagnosis of resident virus. Comparisons of the 2010 to the 2012 viruses were 1.8% (DTW sample) and 2.2% (finishing sample) heterologous. This herd is farrow to finish and although piglets are vaccinated with MLV at weaning, field virus likely remained on-site as no changes in pig flow occurred. The sequence from finishing was 1% different than the virus detected from DTW piglets, so differences existed on-site. Finally, this system would typically close the herd until 9 consecutive negative DTW test results are received with bi-weekly or less frequent testing. Due to an already long closure (>1year) and an unsustainably low inventory, the herd was opened after just 5 negative DTW test results, sampled over 5 months.

There is also evidence to support a diagnosis of a new introduction including the moderate clinical signs noted in the previously exposed/immune sows. Further, as the farm was a participant in a PRRS Area Regional Control (ARC) project, the veterinarian had the following understanding of the area. Similar viruses (1-4-4) were introduced in other herds in this region in 2010 with evidence of continued area circulation, giving credence to lateral spread into the farm. A neighboring farm (1.58 mi) also detected a similar (0.7-1.3%) virus on the same day. Another similar (0.5-1.2%) virus had been found 7 months prior to this finding, just 3 miles to the SW of the case farm and 4.5 miles SW of the other newly infected farm.

Using a general rule of thumb, most would say that this 2012 isolate was the same as the previous resident virus. Although neither answer can be completely ruled out, given what is known about the farm and the context of the timing and location of similar viruses in the area, a successful elimination with new viral introduction seems just as likely. In this case, without the information available through the ARC, the producer would have likely concluded a failure of the elimination began in 2011. Access to information through ARC projects or other sharing networks gives producers and veterinarians additional evidence to inform their understanding of on-farm disease dynamics and the success of their farm, and area, PRRS control strategies. In this neighborhood, future attempts to eliminate virus on a single site are more likely to be successful if those attempts are made in concert with similar strategies on neighboring pig producing sites.

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South East IA PRRS ARC project: Washington County IA & surrounding areas

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SE IA Area Regional Control Project Coordinator

Background: Four neighboring producers and local veterinarians initiated the SE IA ARC project in an attempt to protect their own sow farms from PRRS virus in 2010. The group expanded after preliminary mapping by Boehringer Ingelheim Vetmedica, Inc. determined the primary and peripheral control areas of the region in 2011.

Objectives: Clear and measurable goals set by the working group

- Increase awareness of PRRSv and assoc. costs
- Reduce incidence & severity of PRRSv
- Fewer introductions of new highly pathogenic strains of PRRSv
- Decrease length of time of PRRSv shedding
- Improve bio-security
- Study PRRSv movement
- Create more consistent sow farm and grow finish production

Achievements:

- Cooperation from over 50 producers and veterinarians with PRRS status information collected over 3 years.
- Bi-weekly communication is conducted through updated bulletins and maps with veterinarian and producer meetings 3-4 times a year.
- Increased bio-security measures and overall enhanced awareness of PRRSv and other swine diseases
    - Informational session on bio-security practices, PRRSv, and PEDv with Allied Industry Partners to reduce transmission in the area
    - Farms have been filtered in the area
    - Shared educational materials on bio-security practices
- 89% of sites in the project have included their Premise ID with site information.
- Vaccination rate in reporting herds has increased over time with more sow herds choosing to maintain an immune population following stabilization rather than a naïve status, and more herds vaccinating growing pigs in the area
- 340 PRRS viral ORF5 sequences are shared and compared in a common project database with rapid outbreak reporting to veterinarians.
- Non-Participating producers are choosing to share PRRS ORF5 sequences with the project via their local herd veterinarian, which benefits them and benefits the project by better understanding the isolates in the area.
- Implemented PEDv outbreak reporting system and integrated PEDv into site mapping.

Challenges and Opportunities:

- Continuing the momentum of the project is a challenge, particularly in the face of new area sow herd breaks that lead to an increased number of downstream WTV positive sites and the perception that the project is not progressing.
- There is opportunity for further automation of reporting information (site status, diagnostic findings, sequence information, etc.) into and out of the project to participants.

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Cohort study investigating the risk of porcine epidemic diarrhea virus introduction through feed in Canada

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The first Canadian herd identified with porcine epidemic diarrhea virus (PEDV) was a closed herd in southwestern Ontario detected in late January 2014. The field investigation that followed in a series of subsequent PED cases identified feed from one feed company as a common source. The testing performed on the lots of imported spray-dried porcine plasma (SDPP) resulted in detection of PEDV nucleic acid. Soon thereafter (Feb 9th 2014), the feed company implemented voluntary recall of feed that contained SDPP, samples of which tested positive for PEDV nucleic acid. The challenge study that followed demonstrated that infection with PEDV could be reproduced when pigs were inoculated with PEDV-positive SDPP. Despite progress made in elucidating the conditions under which the PED incursion occurred, some questions still remain unanswered. This study had two objectives. First, to determine the probability of PEDV outbreak for producers that received the potentially contaminated feed with SDPP over the risk period; and second, to evaluate whether there was a difference in the likelihood of PED outbreak between producers that received potentially contaminated feed (exposed) and producers that did not receive such feed (non-exposed).

Three sources of data were used for this investigation. First, the feed company's database containing all sales of feed to customers for production categories that typically contain SDPP as ingredient, or of feed that contained SDPP alternative of non-animal origin. Second, the feed company's database containing data obtained as a result of feed recall. Thirdly, diagnostic data documenting all confirmed cases of PEDV from the source population was utilized. The analysis was performed at a customer level (i.e. production system). Production system was considered as exposed if it received at least one shipment of potentially contaminated feed (PCF) between January 9th 2014 and February 10th 2014. The date of first invoice related to PCF was considered as the start of exposure. Production system was considered as non-exposed if it did not receive any shipment of PCF. In the majority of cases these were production systems that received only a plant-based diet, or feed that was confirmed to have SDPP not affected by recall. The first shipment of any feed to non-exposed customers was assumed to be the start of the study for non-exposed customers. The date of clinical outbreak, confirmed with positive PCR assay, was considered as the date when outbreak occurred otherwise systems were considered censored on Feb 28th. Data were analysed using Kaplan-Meier survival curves, log-rank test, and Cox's proportional hazard model. Overall, 17.7% of systems receiving PCF experienced the PEDV outbreak over the study period defined in this study. Survival curves differed between exposed and non-exposed systems (log-rank $p<0.05$). The risk of PEDV introduction was associated with the exposure to PCF (Hazard Ratio=7.7, $p<0.05$). In addition, a dose-response relationship was detected (<0.01). Systems receiving greater than the median total quantity of SDPP in PCF had higher risk of PEDV outbreak than systems receiving no PCF (Hazard Ratio=12.2, $p<0.05$), or systems receiving below the median total quantity of SDPP in PCF (Hazard Ratio=3.5, $p<0.05$). At the same time, no association between number of feed deliveries and the risk of PEDV outbreak could be detected (log rank $p=0.13$). Additional analysis is being performed, but initial results indicate association between PCF with SDPP and likelihood of experiencing PEDV outbreak and are suggestive of dose-response relationship.

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Relative realtime RT-PCR quantification for UV-induced RNA damage of porcine respiratory and reproductive syndrome virus

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Introduction

Porcine respiratory and reproductive syndrome virus (PRRSV) as RNA virus causes devastating swine diseases with massive economic losses to the swine industry worldwide. In order to prevent economic losses from PRRS, many swine producers use UV light as a sterilizer for the workers, equipment, surface of farm units etc. However, it is very little known about its actual degree of UV-induced RNA damage and effectiveness. The aim of the experiment was to measure levels of UV-induced RNA damage by utilizing the property that damaged RNA of PRRSV can inhibit PCR.

Materials and Methods

PRRS virus strain, ATCC VR2332, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV lamp (Enputech Co., Ltd., Korea) with wavelength output at 254nm, 150uW•sec/Cm^2, for 0.5, 1, 2, 4, 8, 16, and 32 min, respectively. RT-PCR amplified a 100bp (1154-1253) region (Table. 1) and the amplicons were diluted 10^{-5}-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. Statistical evaluation was performed by Excel (Microsoft, USA), Using regression normalization (a linear trendline correction).

Table 1. The result of Ct value and inactivation ratio by exposure time using ReTi-PCR

Exposure Time (min)	Ct value	Inactivation ratio (%)
0.5	23.92	61.68
1	25,5	86.9
2	26.54	93.53
4	28.14	97.82
8	29.31	99.01
16	29.86	99.32
32	31.43	99.77

	Primer Seq.	Product Size
PRRSV-f	5'ACGGACCTATCGTCGTACAG3'	
PRRSV-r	5'AGGAGGTCCTCAAACCCAGA3'	100bp

Table 1. Oligonucleotide primer

Results

The inactivation ratio of PRRSV was relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control sample showed the linear correlation (R^2=0.999). The inactivation of PRRSV by UV light was dose dependent (Table 1). Table 2 showed inactivation ratio of PRRSV using a linear trendline correction.

Table 2. Calculated inactivation ratio of PRRSV

Virus	1D	2D	3D
PRRSV	10.72	72	638.2

*D=1log$_{10}$, unit is mW/cm^2 (Erwin *et al*., 2004)

Conclusions

In Table 2&3, PRRSV was sensitive to UV light irradiation. The UV light is very effective and environment friendly for disinfection in pig farm units. It is thought that these results can be a useful data for sterilizing of PRRSV.

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Making and measuring progress in a statewide disease control program

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Objectives

The Pennsylvania area regional control program has been in place for over a decade with the goal of controlling the spread of PRRS in the region. Over that time the program has grown to encompass upwards of 90% of the sows in the state and their downstream production. It has expanded to cover not only PRRS but PEDv as well. The majority of the producers in the state participate in and direct the program. The original challenge was to develop a structure for rapid information sharing amongst a wide variety of producers and allied industry representatives including those that do not use the internet. With this infrastructure developed, the current challenge has become using the information to make and measure progress toward to goal of preventing the regional spread of both PEDv and PRRS.

Methods

To try to prevent the regional spread of PEDv and PRRS the program has gone through several stages. During stage one, the group chose 3 regions in the state to designate for increased control and recommended that whenever possible PEDv and PRRS positive pigs would be kept out of barns within a 2 mile radius of sow units. During stage two, the group chose to move forward and implement these 2 mile buffer zones around all sow units and boar studs in the program with the goal of moving toward having no PRRS or PEDv positive pigs in those zones. The final element is measuring the progress. The program reporting strategy includes monthly and quarterly reporting on sites that have changed disease status as well as new metrics that track the number of positive sites in the buffer zones and the percent of positive sites over time.

Results

During stage one there was only one incident where PRRS positive pigs were placed in a 2 mile buffer zone that had been established in a control region. This site was owned by the same entity that owned the sow unit. For almost two years there were no other positive pigs placed in these buffer zones. During the past 12 months there was only one sow site that broke with PRRS, it was not in the control zones and it was determined to be a novel strain distinct from those in the area. Stage two has been implemented for less than six months. We have started charting the number of positive pigs within the two mile buffer zones. Currently there are 53 sites in the buffer zones. Of these sites, 7 are positive for PRRS and none are positive for PEDv.

Since we have started the program the level of PRRS is currently at its lowest level of 18.5% positive sites down from 21% positive sites. PEDv positive sites are down to 8 from a high of 35.

Conclusions

PA ARC continues to reap the benefits of almost a decade of producer cooperation as participation is highest and PRRS and PEDv prevalence at all-time lows. The decrease in disease mirrors a national trend and thus the exact role of the program in PA swine disease control remains to be elucidated. We will continue to monitor the disease trends over time, especially as seasonal rates of transmission increase, to see if the establishment of buffer zones around sow farms correlates with fewer sow sources becoming infected and a continued decrease in the number of positive sites in Pennsylvania.

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Spatial and temporal dynamics of porcine reproductive and respiratory syndrome (PRRS) in a voluntary regional project (N212)

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Regional strategies have been suggested as sound approaches to control and eventually eradicate porcine reproductive and respiratory syndrome (PRRS). However, the role of the industry structure on disease maintenance and spread is yet-to-be elucidated. Here, we propose a set of methodological approaches to evaluate the progress of regional control programs, to evaluate the role of the industry, and, ultimately, support its control.

By using information from the *Minnesota Voluntary Regional PRRS Elimination Project*, referred to as N212, between July 2012 and July 2014, we quantified the composition of herds, level of active participation in N212, and PRRS prevalence and cumulative incidence by yearly productive-cycle (2012-13 and 2013-14). An area (S) with high level of PRRS-report was selected for the study time (T) and a space-time inhomogeneous par correlation Kernel function (STIK) was used to assess whether there was spatio-temporal correlation in the data. Finally, an inhomogeneous Poisson model was fit to simulate the PRRS dynamics in N212.

From 47 counties included in N212, participation of producers increased steadily from 45.6% (n=332) in July 2012 up to 69.5% (n=536) in July 2014. Despite this variation, herd-type proportions remained constants with an average of 0.03, 0.07, 0.13 and 0.71 for genetic nuclei (gn), exclusive breeding (br), breeding with growing pigs (brgr) and exclusive growing (gr) farms respectively. For both productive-cycles, a slightly declining prevalence in br- and brgr-herds from 0.042 (95% CI: 0.024, 0.064) at the beginning of 2012-13 to 0.024 (95% CI: 0.01, 0.03) at the end of 2013-14 was reported. However, for gr-herds PRRS prevalence increased markedly in 2012-2013 reaching 0.192 (95%CI: 0.155, 0.229 and decreased strikingly by 2013-14 up to 0.063 (95%CI: 0.043, 0.084). Increasing cumulative incidence tendency for all type of herds was shown, however, the second productive-cycle (2013-14) was significantly lower than first one. In turn, the proportion of stable farms steadily increased from 1.2% in July 2012 to 20.4% in July 2014. An area selected with11 counties reported spatio-temporal density patterns indicating clustering of the disease at temporal distance of <10 days and spatial distance of <10 kilometers.

These results deliver insights of industry structure and PRRS occurrence in different herd-types and provide evidence of temporal and spatial aggregation of PRRS. In addition, parameters obtained are useful to construct a model that simulates PRRS' behavior in areas in which PRRS report was low.

Meetings of Interest

The **2014 Conference of Research Workers in Animal Diseases (CRWAD)** Meeting will be held December 7-9, 2014, at the Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois. http://www.cvmbs.colostate.edu/mip/crwad/

The 46th annual meeting of the **American Association of Swine Veterinarians** (AASV) is scheduled for February 28-March 3, 2015 in Orlando, Florida. http://www.aasv.org/annmtg/

World Pork Expo will be held June 3-5, 2015 at the Iowa State Fairgrounds in Des Moines, Iowa. https://www.worldpork.org/

International PRRS Congress will be held June 3-5, 2015 in Ghent, Belgium. http://www.prrs.ugent.be/

The **7th International Symposium on Emerging and Re-emerging Pig Diseases** is scheduled for June 21-24, 2015 at the Kyoto International Conference Center in Kyoto, Japan. http://emerging2015.com

European Federation of Animal Science 66th Annual Meeting will be held August 31-September 4, 2015 in Warsaw, Poland. www.eaap2015.org

Allen D. Leman Swine Conference will be held September 19-22, 2015. http://www.cvm.umn.edu/vetmedce/events/adl/home.html

The **24th International Pig Veterinary Society Congress** is scheduled for June 7-10, 2016 in Dublin, Ireland. http://www.ipvs2016.com

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