NORTH AMERICAN PRRS SYMPOSIUM

2021

Program and Proceedings

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December 4, 2021

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Welcome to 2021 NA PRRS/NC229 Symposium Emerging and Foreign Animal Diseases of Swine

NORTH AMERICAN PRRS SYMPOSIUM

Dear friends,

It is our great pleasure to warmly welcome you to the 2021 North American PRRS/NC229 Symposium!

The North American PRRS Symposium was initiated in November 2003 as the “International Workshop on PRRS”. The conference continues to focus on the latest knowledge and novel tools for the control of PRRS, the costliest viral disease to ever face a global swine industry. In recent years, the emergence and spread of new swine viruses, such as PEDV and ASFV, have expanded the focus to include emerging and foreign diseases. Integration with the USDA NC229 multi-state project on swine diseases provides the means to deliver a much larger and more comprehensive meeting experience.

The 2021 NA PRRS/NC229 symposium includes a variety of plenary and poster sessions. The program covers different aspects of basic and applied research on swine diseases and creates invaluable opportunities for scientists from academia and industry to share their research findings, exchange ideas, and develop collaborations. We believe this event will enhance our capabilities to develop effective control and prevention measures for both endemic and emerging swine diseases.

The executive directorship, scientific committee, and conference staff hope that this symposium will provide all attendees with an informative and scientifically rewarding experience. We also encourage you to enjoy the beautiful city of Chicago!

— Ying Fang, Sheela Ramamoorthy, & NA PRRS/NC229 Organizing Committee

2021 North American PRRS Symposium Organizing Committees

NA PRRSS/NC229 Advisor:

Dr. Raymond (Bob) Rowland
University of Illinois

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Co-Chairs, Joint Scientific Committee:

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Dr. Ying Fang
University of Illinois

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Dr. Zhengguo Xiao
University of Maryland

A tribute to the career of Fernando A. Osorio

— Prepared by Drs. Raymond (Bob) Rowland, Hiep Vu, and Ying Fang

Dr. Fernando Osorio completed his MV (DVM equivalent) degree at the Buenos Aires National University in Argentina in 1973. Between 1973 and 1979, he was a Researcher in Animal Virology (Foot- and-Mouth Disease Virus) at the Institute of Virology, INTA- Castelar, Argentina. He received an M.S. degree in veterinary virology at Iowa State, followed by a Ph.D. degree in 1984 from the same institution. In 1986, he became a Diplomate in Veterinary Microbiology. In 1984, he joined the faculty at the University of Nebraska Lincoln (UNL) where he remained until his retirement in 2021.

While at UNL, Dr. Osorio served in several leadership positions, including the head of the Reference Laboratory for Vesicular Disease and Vaccine Control for the Americas (1992-1993), head of the Diagnostic Virology, Veterinary Diagnostic Center, Department of Veterinary and Biomedical Sciences (1984-2005). In 2005, he joined the faculty in the Nebraska Center for Virology & School of Veterinary and Biomedical Sciences. He served in several advisory capacities including, NCRA/ NIMSS Administrative Advisor for NC229 Multi-State Project. He was one of the original founders of NC229.

Dr. Osorio is globally recognized as an outstanding scientist and a passionate educator who has devoted his entire career to the study of swine viruses and the training of veterinary scientists. His research centers on elucidating pathogenic mechanisms exhibited by several important swine viruses, including pseudorabies virus and porcine reproductive and respiratory syndrome virus (PRRSV). He was the first to create a pig model for the study herpesvirus latency and pioneered the application of PCR to the study of experimental pathogenesis and diagnostic detection of latent pseudorabies virus. His research on PRRSV produced new insights into immuno-pathogenesis, virulence, and mechanisms of protective immunity. He was the first to demonstrate that PRRSV can establish a long-term persistence in pigs, hallmarked by virus replication in the testes. The testicular tropism of PRRSV explained the ability of the virus to circulate rapidly worldwide through the exchange of “liquid genetics” (artificial insemination). He was the first to establish unequivocal evidence that virus-neutralizing antibodies provide full protection against PRRSV infection. His research resulted in the publication of 110+ papers in refereed journals in the fields of virology, pathology, and immunology. Dr. Osorio has four patents, one of which is being developed by a veterinary biologics company. Perhaps Dr. Osorio’s greatest legacy is his life-long advocacy and support in the career development of scientists from all over the world. During his career, he trained 29 graduate students, 11 post-doctoral research associates, and mentored 5 junior faculty. In recognition of his leadership and outstanding service to international students, he was honored by Dermot Coyne Award by International Affairs at University of Nebraska-Lincoln.

2021 Travel Fellowship Recipients

Each year a special set of donors provide funding to assist the participation of students to present their research at the Symposium.

This year the Symposium welcomes a generous donation from:

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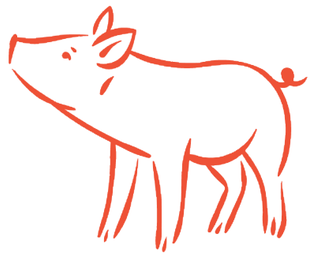

Symposium Invited Presenters

Jeff Zimmerman
Iowa State University

Understanding the Right Surveillance Tools for Emerging and Foreign Diseases

Mariana Kikuti
University of Minnesota

Latest Epidemiology Research on PRRSV Lineage 1C 144 in the US

Tyler Holck
Swine Health Information Center

Preparedness Plan for Prevention of ASF Introduction in the US

Diego Diel
Cornell University

Experimental Inoculation of Swine with SARS-CoV-2

Qiuong Wang
The Ohio State University

Study Swine Coronaviruses to Understand Coronavirus Tissue Tropism and Interspecies Transmission

Raymond (Bob) Rowland
University of Illinois

PRRS Virus Glycoprotein Interactions With CD163 and Targets of Virus Neutralization

Scott Dee
Pipestone

PRRSV L1C: A Rapid Response to a National Crisis

Symposium Program

Friday, December 3

6:30 – 8:30 pm: NA PRRS/NC 229 Organizing Committee Meeting

Saturday, December 4

7:00 – 8:30 am – Zoetis Breakfast Special Session: Field Outreach – Emerging and Foreign Disease Threats

Chicago Ballroom D/E

Moderator: Jose Angulo

- 7:05 – 7:25 am (S01): Understanding the Right Surveillance Tools for Emerging and Foreign Diseases
  Jeff Zimmerman, Iowa State University
- 7:25 – 7:45 am (S02): Latest Epidemiology Research on PRRSV Lineage 1C 144 in the US
  Mariana Kikuti, University of Minnesota
- 7:45 – 8:05 am (S03): Preparedness Plan for Prevention of ASF Introduction in the US
  Tyler Holck, Swine Health Information Center
- 8:05 – 8:25 am: Q & A Session

8:30 – 8:40 am – Opening Remarks

Chicago Ballroom D/E

Ying Fang and Sheela Ramamoorthy

8:40 – 9:40 am – Plenary Session 1: Viral Infection and Gene Expression: New Features in Nidoviruses

Chicago Ballroom D/E

Moderators: Diego Diel and Hiep Vu

- 8:40 – 9:00 am (S04): Novel PRRSV Proteins and Expression Mechanism: Evolutionarily Conserved Signatures in Arteriviruses
  Ying Fang, University of Illinois
- 9:00 – 9:20 am (S05): Experimental Inoculation of Swine with SARS-CoV-2
  Diego Diel, Cornell University
- 9:20 – 9:40 am (S06): Study Swine Coronaviruses to Understand Coronavirus Tissue Tropism and Interspecies Transmission
  Qiuhong Wang, The Ohio State University

9:40 – 10:10 am – Break

10:10 – 11:30 am – Plenary Session 2: Virus-Host interaction and Host Responses

Chicago Ballroom D/E

Moderators: Scott Kenney and Zhengguo Xiao

- 10:10 – 10:30 am (S07): PRRS Virus Glycoprotein Interactions With CD163 and Targets of Virus Neutralization
  Raymond (Bob) Rowland, University of Illinois
- 10:30 – 10:45 am (S08): Expression Levels of CD169, CD163 and CD151 in PRRSV-Infected Peccaries
  Christine Harness, Iowa State University
- 10:45 – 11:00 am (S09): A Virulent and Pathogenic Infectious Clone of Senecavirus A
  Diego Diel, Cornell University
- 11:00 – 11:15 am (S10): Mediation of Type 1 IFN Signaling by PRRSV nsp5-related Protein
  Chia-Ming Su, University of Illinois
- 11:15 – 11:30 am (S11): Phenotypic Effect of a Genetic Variant Linked to DIO2 on Fetal Outcomes in PRRSV-2 Infected Gilts
  Haesu Ko, University of Alberta

11:30 am – 1:00 pm – Lunch Break

1:00 – 2:30 pm – Plenary Session 3: Vaccines and Therapeutics

Chicago Ballroom D/E

Moderators: Andreia Arruda and Federico Zuckermann

- 1:00 – 1:20 pm (S12): Development of an Effective Vaccine Against the Pandemic Strain of African Swine Fever Virus That Grows in Cell Culture
  Douglas Gladue, PIADC-USDA-ARS
- 1:20 – 1:40 pm (S13): Targeting Suicidal Replication to Enhance the Safety of Attenuated Viral Vaccines
  Sheela Ramamoorthy, North Dakota State University
- 1:40 – 2:00 pm (S14): Effective Protection Induced by an Experimental Subunit DIVA Vaccine Against PRRS Virus
  Federico Zuckermann, University of Illinois
- 2:00 – 2:15 pm (S15): Intranasal Delivery of Inactivated Influenza Virus and Poly(I:C) Adsorbed Corn-Based Nanoparticle Vaccine Elicited Robust Antigen-Specific Cell-Mediated Immune Responses in Maternal Antibody Positive Nursery Pigs
  Veerupaxagouda Patil, The Ohio State University
- 2:15 – 2:30 pm (S16): Amplification of IgG Genes from Classical Swine Fever Virus C-strain E2 Glycoprotein Specific Single Porcine B Cells
  Lihua Wang, Kansas State University

2:30 – 3:00 pm – Break

3:00 – 4:05 pm – Plenary Session 4: Diagnostics and Disease Control

Chicago Ballroom D/E

Moderators: Roman Pogranichniy and Mariana Kikuti

- 3:00 – 3:20 pm (S17): PRRSV L1C: A Rapid Response to a National Crisis
  Scott Dee, Pipestone
- 3:20 – 3:35 pm (S18): Investigation of Vesicular Lesions in Pigs With Unknown Causative Agents
  Ethan Aljets, Iowa State University
- 3:35 – 3:50 pm (S19): Development of a Blocking Enzyme-Linked Immunosorbent Assay for Detection of Antibodies against African Swine Fever Virus
  Fangfeng Yuan, University of Illinois
- 3:50 – 4:05 pm (S20): Differentiation of Animals Vaccinated with Flag T4G Against Classical Swine Fever Virus from Infected Pigs Using a Dendrimeric Peptide-Based Approach
  Jose A. Bohorquez, IRTA-CReSA

4:05 – 5:00 pm – NC 229 Business Meeting

Chicago Ballroom D/E

Moderators: Roman Pogranichniy and Sheela Ramamoorthy

6:00 – 8:00 pm – Benfield Poster Session and Reception

Chicago Ballroom A/B/C

- 6:00 – 7:45 pm: Poster Viewing
- 7:45 – 8:00 pm: David Benfield Award Ceremony

NA PRRS Symposium
Oral Presentations

Session Number	Name	Company/Business	Country	Title
S01	Jeff Zimmerman	Iowa State University	United States	Understanding the Right Surveillance Tools for Emerging and Foreign Diseases
S02	Mariana Kikuti	University of Minnesota	United States	Latest Epidemiology Research on PRRSV Lineage 1C 144 in the US
S03	Tyler Holck	Swine Health Information Center	United States	Preparedness Plan for Prevention of ASF Introduction in the US
S04	Ying Fang	University of Illinois	United States	Novel PRRSV Proteins and Expression Mechanism: Evolutionarily Conserved Signatures in Arteriviruses
S05	Diego Diel	Cornell University	United States	Experimental Inoculation of Swine with SARS-CoV-2
S06	Qiuhong Wang	The Ohio State University	United States	Study Swine Coronaviruses to Understand Coronavirus Tissue Tropism and Interspecies Transmission
S07	Raymond (Bob) Rowland	University of Illinois	United States	PRRS Virus Glycoprotein Interactions With CD163 and Targets of Virus Neutralization
S08	Christine Harness	Iowa State University	United States	Expression Levels of CD169, CD163 and CD151 in PRRSV-Infected Peccaries
S09	Diego Diel	Cornell University	United States	A Virulent and Pathogenic Infectious Clone of Senecavirus A
S10	Chia-Ming Su	University of Illinois	United States	Mediation of Type 1 IFN Signaling by PRRSV nsp5-related Protein
S11	Haesu Ko	University of Alberta	Canada	Phenotypic Effect of a Genetic Variant Linked to DIO2 on Fetal Outcomes in PRRSV-2 Infected Gilts
S12	Douglas Gladue	PIADC-USDA-ARS	United States	Development of an Effective Vaccine Against the Pandemic Strain of African Swine Fever Virus That Grows in Cell Culture
S13	Sheela Ramamoorthy	North Dakota State University	United States	Targeting Suicidal Replication to Enhance the Safety of Attenuated Viral Vaccines
S14	Federico Zuckermann	University of Illinois	United States	Effective Protection Induced by an Experimental Subunit DIVA Vaccine Against PRRS Virus
S15	Veerupaxagouda Patil	The Ohio State University	United States	Intranasal Delivery of Inactivated Influenza Virus and Poly(I:C) Adsorbed Corn-Based Nanoparticle Vaccine Elicited Robust Antigen-Specific Cell-Mediated Immune Responses in Maternal Antibody Positive Nursery Pigs
S16	Lihua Wang	Kansas State University	United States	Amplification of IgG Genes from Classical Swine Fever Virus C-strain E2 Glycoprotein Specific Single Porcine B Cells
S17	Scott Dee	Pipestone	United States	PRRSV L1C: A Rapid Response to a National Crisis
S18	Ethan Aljets	Iowa State University	United States	Investigation of Vesicular Lesions in Pigs With Unknown Causative Agents
S19	Fangfeng Yuan	University of Illinois	United States	Development of a Blocking Enzyme-Linked Immunosorbent Assay for Detection of Antibodies against African Swine Fever Virus
S20	Jose Alejandro Bohorquez Garzon	IRTA-CReSA	Spain	Differentiation of animals vaccinated with Flag T4G against classical swine fever virus from infected pigs using a dendrimeric peptide-based approach

Novel PRRSV Proteins and Expression Mechanism: Evolutionarily Conserved Signatures in Arteriviruses

Ying Fang[1] [2]*, Rui Guo[2], Xingyu Yan[1] [2], Yanhua Li[2], Jin Cui[1], Saurav Misra[3], Andrew E. Firth[4], Eric J. Snijder[5]

[1]Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, Urbana, Illinois, USA, [2]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA, [3]Department of Biochemistry and Molecular Biophysics, College of Arts and Sciences, Kansas State University, Manhattan, Kansas, USA, [4]Department of Pathology, University of Cambridge, Cambridge, UK, [5]Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands.

Arteriviruses are enveloped positive-strand RNA viruses that assemble and egress using the host cell's exocytic pathway. In previous studies, we demonstrated that PRRSV and most of other arteriviruses use a unique -2/-1 programmed ribosomal frameshifting (PRF) mechanism to translate two additional viral proteins, nonstructural protein 2TF (nsp2TF) and nsp2N. This -2/-1 PRF mechanism is transactivated by a viral protein, nsp1β, and cellular poly(rC) binding proteins (PCBPs). Like full-length nsp2, the N-terminal domain of frameshift products contains a papain-like protease (PLP2) that has deubiquitinating (DUB) activity, in addition to its role in proteolytic processing of replicase polyproteins. In cells infected with porcine reproductive and respiratory syndrome virus (PRRSV), nsp2TF localizes to compartments of the exocytic pathway, specifically endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and Golgi complex. Remarkably, nsp2TF interacts with the two major viral envelope proteins, the GP5 glycoprotein and membrane (M) protein, which drive the key process of arterivirus assembly and budding. The PRRSV GP5 and M proteins were found to be poly-ubiquitinated, both in an expression system and in cells infected with a nsp2TF-deficient mutant virus. In contrast, ubiquitinated GP5 and M proteins did not accumulate in cells infected with the wild-type, nsp2TF-expressing virus. Further analysis implicated the DUB activity of the nsp2TF PLP2 domain in deconjugation of ubiquitin from GP5/M proteins, thus antagonizing proteasomal degradation of these key viral structural proteins. Our findings suggest that nsp2TF is targeted to the exocytic pathway to reduce proteasome-driven turnover of GP5/M proteins, thus promoting the formation of GP5-M dimers that are critical for arterivirus assembly.

Session 05

Experimental Inoculation of Swine with SARS-CoV-2

Alexandra Buckley[1]*, Shollie Falkenberg[2], Mathias Martins[3], Melissa Laverack[3], Mitchell V. Palmer[4], Kelly Lager[1], **Diego G. Diel[2]***

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Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the susceptibility of animals and their potential to act as reservoirs or intermediate hosts for the virus has been of significant interest. Pigs are susceptible to multiple coronaviruses and have been used as an animal model for other human infectious diseases. Research groups have experimentally challenged swine with human SARS-CoV-2 isolates with results suggesting limited to no viral replication. For this study, a SARS-CoV-2 isolate obtained from a tiger which is identical to human SARS-CoV-2 isolates detected in New York City and contains the D614G S mutation was utilized for inoculation. Pigs were challenged via intravenous, intratracheal, or intranasal routes of inoculation (n=4/route). No pigs developed clinical signs, but at least one pig in each group had one or more PCR positive nasal/oral swabs or rectal swabs after inoculation. All pigs in the intravenous group developed a transient neutralizing antibody titer, but only three other challenged pigs developed titers greater than 1:8. No gross or histologic changes were observed in tissue samples collected at necropsy. In addition, no PCR positive samples were positive by virus isolation. Inoculated animals were unable to transmit virus to naïve contact animals. The data from this experiment as well as from other laboratories supports that swine are not likely to play a role in the epidemiology and spread of SARS-CoV-2.

Session 06

Study Swine Coronaviruses to Understand Coronavirus Tissue Tropism and Interspecies Transmission

Qiuhong Wang[1] [2]

[1] Center for Food Animal Health, Department of Animal Sciences, CFAES, The Ohio State University
[2] Department of Veterinary Preventive Medicine, CVM, The Ohio State University

Six coronaviruses (CoVs) infect pigs: transmissible gastroenteritis virus (TGEV) and its respiratory variant porcine respiratory coronavirus (PRCV), porcine epidemic diarrhea virus (PEDV), swine acute diarrhea syndrome coronavirus (SADS-CoV), porcine hemagglutinating encephalomyelitis virus (PHEV), and porcine deltacoronavirus (PDCoV). Among them, TGEV, PEDV, SADS-CoV and PDCoV are enteric pathogens, PRCV causes sub-clinical or mild respiratory infections, and PHEV may cause diverse clinical signs, including respiratory disease, encephalomyelitis, and vomiting and wasting disease. Except for SADS-CoV, the other porcine CoVs infect both respiratory and intestinal epithelial cells. In addition, PHEV infects neurons in the peripheral and central nervous systems.

Among these porcine CoVs, PEDV and SADS-CoV originated indirectly or directly from bat CoVs. TGEV likely evolved from canine CoVs, and it could be one of the parental strains for the emergence of several recombinant canine CoVs. PRCV is the mutant of TGEV. PHEV is evolutionally closely related to bovine CoVs and human common cold CoV OC43. Finally, PDCoV probably evolved from sparrow and other avian delta-CoVs. To date, TGEV, PRCV, PEDV, SADS-CoV and PHEV exclusively infect pigs, although SADS-CoV can infect different human primary cells in vitro, suggesting its zoonotic potential. PDCoV has the broadest host range, including sparrows, pigs, Asian leopard cats, Chinese ferret badgers, and humans.

Studies of these swine CoVs have revealed their tissue tropisms for both the respiratory and intestinal tracts, and they occasionally show neurotropism, like human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that has tissue tropism for respiratory and intestinal tracts and neurons (e.g., olfactory neurons). Interspecies transmission and recombination occur frequently, resulting in the emergence of new CoVs. Continually monitoring CoVs in animals and humans is important for spillover risk assessment, and prevention and control of CoV diseases in both animals and humans.

Session 07

PRRS Virus Glycoprotein Interactions With CD163 and Targets of Virus Neutralization

Raymond (Bob) Rowland

Department of Pathobiology, University of Illinois, Urbana-Champaign

Potential vaccine antigens are found in the five proteins located on the surface of the PRRS virion, which include GP2, GP3, GP4, GP5, and M. These proteins form covalent and noncovalent interactions and possess hypervariable peptide sequences. One consequence of this complex surface structure antigenic variation and subsequent escape from immunity, including escape from vaccination. The net result presents challenges in the development of new vaccines capable of generating broadly protective immunity. One potential vaccine target is the induction of antibody that disrupts the interactions between the macrophage CD163 receptor and the corresponding GP2, GP3, GP4 heterotrimer. The importance of CD163 is demonstrated by the ability of pigs lacking CD163 to completely resist virus infection. Further studies identified specific domains in CD163, such as SRCR5, as important for conferring resistance to PRRSV infection in transfected cells and CD163-modified pigs. This current model predicts that escape from neutralizing antibody should incorporate mutations within the GP2-GP3-GP4 heterotrimer. However, experiments designed to understand the PRRSV-cell interaction by mapping mutations that appear following the escape of virus from neutralizing antibody identify the ectodomain regions of GP5 and M as sites of mutation. The short GP5 ectodomain peptide sequence possesses several features characteristic of a broadly neutralizing epitope including a short, conserved oligopeptide flanked by N-glycosylation sites and hypervariable regions, a pattern shared by neutralizing epitopes of other viruses. Together, these data create opportunities to explore effective vaccine strategies and to better understand how PRRSV interacts with the macrophage.

Session 08

Expression Levels of CD169, CD163 and CD151 in PRRSV-Infected Peccaries

C. Harness[1]*, J. Zimerman1, R. Molina-Barrios[2], J. Hernández[3], L. Giménez-Lirola[1], P. Piñeyro[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA. [2]Departamento de Ciencias Agronómicas y Veterinarias, Instituto Tecnológico de Sonora (ITSON), Cd. Obregón, Sonora, México. [3]Laboratorio de Inmunología, Centro de Investigación en Alimentación y Desarrollo A.C (CIAD), Hermosillo, Sonora, México

PRRSV is an enveloped, positive-sense, single-strand RNA virus that causes extensive losses to the swine industry. PRRSV infection has been widely studied in swine, but peccaries (Pecari tajacu) are also susceptible to PRRSV infection, developing a prolonged and sustained viremia and antibody response. In swine, the virus targets pulmonary and intravascular macrophages and differentiated monocytes, interacting with cells through a complex of cell surface receptors including CD151, sialoadhesin (CD169), CD 209, and CD163. The objective of this study was to evaluate the gene expression levels of CD169, CD163 and CD151 receptors in pulmonary and lymphoid tissues collected from PRRSV experimentally infected peccaries.

The modulatory effect of CD169, CD163, and CD151 genes was evaluated on lung, lymph node, tonsil, and spleen. Tissues from peccaries experimentally infected with PRRSV (n=3), PRRSV negative conventional pigs (n=3), and PRRSV positive conventional pigs (n=3) were collected and stored at -80°C. In brief, mRNA expression levels were quantified as the difference between each target gene and a reference housekeeping gene (Beta-actin), i.e., the ΔCt method, and then compared between groups. Real-time RT-PCR was performed by elution of total RNA and primers, mixed with the commercial Power SYBR Green RNA-to-CT™ 1Step Kit. All samples were tested in triplicate. In situ detection of CD163 mRNA was evaluated by RNAscope and cellular antigen expression by CD163 immunohistochemistry.

Gene expression analysis showed that CD163 and CD151 mRNA levels are significantly lower on peccaries than PRRSV positive and negative pigs in all tissues evaluated. No significant differences in mRNA levels on these two receptors were observed between positive and negative PRRSV pigs. No difference was detected in mRNA levels for CD169 receptor between either group of pigs or peccaries. RNAscope in situ detection of CD163 mRNA showed an intense intracytoplasmic signal on both positive and negative PRRSV pigs but was not detected in PRRSV positive peccaries. The cell surface CD163 antigen was highly expressed on positive and negative pigs but not on PRRSV positive peccaries.

In conclusion, CD163 and CD151 mRNA levels differ significantly during PRRSV infection in peccaries compared with conventional pigs. Further studies are necessary to evaluate if this is a specie specific difference or perhaps a more exacerbated regulatory response during PRRSV infection on peccaries. These receptors play a role in mediating pro-inflammatory cytokines; therefore, the lack of inflammatory response observed during PRRSV infection in peccaries could be due to the low expression levels during PRRSV infection on peccaries. Experimental inoculation of peccaries produced a sustained PRRSV viremia and antibody response; therefore, viral replication occurs but is perhaps not entirely dependent on CD163 and CD151 receptors. Further studies to evaluate structural and functional differences on PRRSV entry receptors on peccaries are necessary to understand the mechanism of viral replication in this species.

Session 09

A Virulent and Pathogenic Infectious Clone of Senecavirus A

Maureen H.V. Fernandes[1] [2]*, Marcelo de Lima[2] [3], Lok R. Joshi[1] [2], **Diego G. Diel[1] [2]**

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Senecavirus A (SVA) is a picornavirus that circulates in swine populations worldwide causing outbreaks of vesicular disease (VD). Vesicular diseases are among the most economically important diseases of livestock and could have catastrophic economic consequences due to international trade restrictions. Thus, a better understanding of SVA infection biology and the viral mechanisms is critical to establish control strategies for SVA and for other picornaviruses. To begin to study the molecular interactions of SVA, we generated a reverse genetics system for SVA based on the wild type SVA strain SD15-26. The full-length cDNA genome of SVA was cloned into a plasmid under a T7 RNA polymerase promoter. Following in vitro transcription, the genomic viral RNA was transfected into BHK-21 cells and rescue of infectious virus (rSVA SD15-26) was shown by inoculation of highly susceptible H1299 cells. In vitro characterization of the rSVA SD15-26 showed similar replication properties and protein expression levels as the wt SVA SD15-26. A pathogenesis study was conducted in finishing pigs to evaluate the pathogenicity and infection dynamics of the rSVA SD15-26 virus in comparison to the wt SVA SD15-26. Animals from both rSVA- and wt SVA SD15-26-inoculated groups presented characteristic SVA clinical signs followed by the development of vesicular lesions on the snout and/or feet. The clinical outcome of infection, including disease onset, severity and duration was similar in rSVA- and the wt SVA SD15-26-inoculated animals. All animals inoculated with rSVA or with wt SVA SD15-26 presented a short-term viremia, and animals from both groups shed similar levels of virus in oral and nasal secretion, and feces. Our data demonstrates that the rSVA SD5-26 clone is fully virulent and pathogenic in pigs, presenting comparable pathogenesis and infection dynamics to the wt SVA SD15-26 strain. The SVA infection clone generated here is a useful platform to study virulence determinants of SVA, and to dissect other aspects of SVA infection biology, pathogenesis and persistence.

Session 10

Mediation of Type 1 IFN Signaling by PRRSV nsp5-related Protein

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Type 1 interferons (IFNs-alpha/beta) play a critical role in the innate immune response to viral infection. Porcine reproductive and respiratory syndrome virus (PRRSV) has been shown to inhibit the expression of type 1 IFNs and interfere the IFN signaling pathway. Since signal transducer and activator of transcriptions (STATs) are crucial mediators in the IFN signaling, the STATs are hypothesized to be the target of the virus to hamper the antiviral response induced by the IFN signaling. PRRSV has been shown to interfere with the STAT signaling, but the relationship between the IFN suppression and PRRSV-mediated STAT signaling still needs to be investigated. PRRSV viral proteins have been investigated for inhibiting IFN-mediated STAT signaling, and nonstructural protein (nsp) 5 has been reported to induce the STAT3 degradation. However, nsp5-mediated type 1 interferon signaling remains unclear. Besides, the previous report showed that nsp4-8 region of PRRSV has an alternative cleavage pathway depended on the presence of nsp2. In the major processing pathway, nsp4-5 junction is cleaved, and nsp5-7beta is not cleaved. In the minor processing pathway, nsp4-5 is not cleaved, but nsp5/6, nsp6/7alpha, nsp7alpha/7beta, nsp7beta/8 is cleaved. In the present study, the basis for IFN inhibition by PRRSV nsp5 was investigated. Since nsp4-8 of pp1a is cleaved via either major processing or minor processing pathways, four different nsp constructs were first made and expressed to represent nsp5, nsp4-5, nsp5-7, nsp5-8. Then, suppression of IFN responses by these nsp5-fusion constructs was examined by luciferase assay. While the control showed an increase of the interferon-sensitive responsive element (ISRE) activity by IFN stimulation, only nsp5 exhibited strong suppression of ISRE activity, and other nsp5-fusion constructs did not suppress the ISRE activity. The immunofluorescent staining results showed that nsp5 alone blocked the STAT1 nuclear translocation, but neither nsp4-5, nsp5-7, nor nsp5-8 showed the inhibition of STAT1 nuclear translocation. Our results demonstrate that PRRSV nsp5 interferes the IFN signaling by blocking the STAT nuclear translocation, but nsp4-5, nsp5-7, and nsp5-8 did not participate in the STAT signaling pathway.

Session 11

Phenotypic Effect of a Genetic Variant Linked to DIO2 on Fetal Outcomes in PRRSV-2 Infected Gilts

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Our initial genome-wide association study (GWAS) was conducted to understand the genetic basis of fetal response following maternal PRRSV infection. This GWAS identified an intergenic single nucleotide polymorphism (SNP) (DRGA0008048) that was significantly associated with fetal viability in response to maternal PRRSV infection. The iodothyronine deiodinase 2 (DIO2) gene is located about 14 kilobases distal to this DRGA SNP on SSC7. Thus, the main objective of this study was to confirm the association between this SNP and fetal viability in PRRSV infected gilts using an independent fetal population. We hypothesized that genomic variants in the SSC7 region would be related to variation in fetal thyroid function and metabolism, and therefore might affect fetal growth and developmental responses in PRRSV infected gilts. Yorkshire gilts (n=27) and Landrace sires (n=6) were selected to be either AA (13 gilts, 3 sires) or BB (14 gilts, 3 sires) for the DRGA SNP and bred to produce homozygous AA or BB F1 crossbred fetal groups. At 86±0.9 days of gestation, gilts were inoculated either with 1 × 105 TCID50 NVSL 97–7895 total dose (n=22) or minimum essential media for control (n=5). At 21 days post inoculation, necropsy was conducted and a total of 84 control fetuses (30 AA, 54 BB) and 303 fetuses from inoculated litters (150 AA, 153 BB) were collected. Fetal phenotyping included fetal viral load in placenta, serum and thymus, thyroid hormone levels (T4, T3), biometric measurements (body weight, girth, crown rump length, nose rump length, 9 organ weights) and skeletal measurements (length and area of humerus, radius, ulna, and area of secondary ossification center in humerus and radius). Fetuses of AA and BB genotype were compared for fetal viability and survival using logistic mixed-effects regression models and estimated marginal means from linear mixed-effects regression models for the fetal measurements. Contrary to our expectation, the BB genotype was not associated with more viable or live fetuses. Viral loads and serum T3 level were also not related to the genotype, however, the BB genotype was significantly related to reduced serum T4 level (β (SE)=-10.31 (4.0), P= 0.01). Most of the fetal growth and bone measurements were not associated with the DRGA genotype, but BB fetuses had a significantly increased area of the proximal humeral secondary ossification center (β (SE)=321.5 (109.3), P=0.003). These results may imply a differential effect of the DIO2 gene between genotypes on fetal thyroid hormone metabolism, which may be at least partially involved in fetal bone ossification during maternal PRRSV infection.

Session 12

Development of an Effective Vaccine Against the Pandemic Strain of African Swine Fever Virus That Grows in Cell Culture

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African swine fever is currently causing a pandemic resulting in devastating losses to the swine industry worldwide. The only effective vaccines against this current highly virulent pandemic strain have been live-attenuated vaccines which contain one or more genetic deletions. However, a major limitation of all live-attenuated vaccines is that they rely on the production of the vaccine in primary swine cells, which are difficult to use for the production of a of commercial vaccine. To overcome the need for primary cells, a large-scale systematic approach to test for a cell line that could be used for vaccine production was performed. As observed in previous attempts of cell culture adaptation, large genomic deletions in the viral genome occurred in many cell lines, an occurrence that has been linked to a decreased growth in macrophages, and decreased ability to replicate in swine. However, one cell line PIPEC (Plum Island Porcine epithelial cells) was able to replicate live attenuated vaccines. ASFV experimental vaccines adapted to PIPEC cells resulted in a deletion in the left variable region(LVR), this deletion allows for growth in PIPEC cells while maintaining the ability to replicate in primary swine macrophages. In challenge studies, ASFV-G-ΔI177L/ΔLVR maintained the same level of attenuation, immunogenic characteristics, and protective efficacy as parental ASFV-G-ΔI177L. ASFV-G-ΔI177L/ ΔLVR is the first rationally designed ASF vaccine candidate that can be used for large-scale commercial vaccine manufacture

Session 13

Targeting Suicidal Replication to Enhance the Safety of Attenuated Viral Vaccines

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While inactivated vaccines are extremely safe, in general, attenuated vaccines can potentially revert to virulence but generally provide effective protection and a longer duration of immunity. Although porcine circovirus type 2 (PCV2), the causative agent of post-weaning multi-systemic wasting disease syndrome in piglets, is a DNA virus its mutation rates resemble RNA viruses. While inactivated and subunit vaccines against PCV2 are effective in preventing clinical signs they do not prevent viral shedding and may potentially influence viral evolution in the field. The hypothesis that the rapid mutation rates of PCV2 can be harnessed to target suicidal replication was tested with the goal of developing a safe and effective attenuated vaccine against PCV2. Selected serine and leucine codons in the PCV2 capsid protein were recoded such that single base pair changes during viral replication could result in stop codons. The recoded vaccine construct was rescued successfully by transfection and was stable in vitro. When subjected serial passaging under immune selection pressure with sub neutralizing antibodies in-vitro, 5 of the 22 recoded serine and leucine codons were converted to stop codons, as detected by deep sequencing. As hypothesized, the suicidal PCV2 vaccine was cleared from the serum of vaccinated pigs at 2 weeks post-vaccination. Vaccine efficacy was better than or comparable to a commercial vaccine based on challenge viral replication and tissue lesions and neutralizing antibody responses. Future efforts will focus on the development of directed suicidal vaccines for PCV3 and PRRSV and testing of the vaccines in a PCV3 and PRRS coinfection model.

Session 14

Effective Protection Induced by an Experimental Subunit DIVA Vaccine Against PRRS Virus

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We demonstrate that the incorporation of PRRS virus envelope proteins from virions into soluble nanoscale membrane assemblies called nanodiscs (NDs) works as an effective vaccine. Notably, because these assemblies selectively incorporate transmembrane proteins and thus exclude the nucleocapside (N) protein, this biologic constitutes a DIVA vaccine against PRRS. The level of protective immunity obtained in cohorts of weaner pigs vaccinated with NDs displaying PRRS virus envelope proteins (P-ND) was compared to pigs vaccinated with either an in-house inactivated whole virus (IWV) vaccine, or NDs that were void of viral envelope proteins, i.e. empty NDs (E-ND), as a mock vaccine. The P-ND and IWV vaccines were prepared using the PRRS virus strain G16X, which belongs to the lineage 5 North American PRRS genotype. The P-NDs, E-NDs and IWV preprations were mixed with an oil-in-water adjuvant before being administered intramuscularly to groups of pigs (each n=8) twice at a 25-day interval. An additional pig cohort (n=5) was immunized once with a commercial modified live virus (MLV) vaccine (Prevascent, Elanco). A strict control group (n=3) consisted of pigs that were neither vaccinated nor challenged. Forty days after the first immunization, all the vaccinated pigs were challenged intranasally with virulent virus strain 16244B which, based on the amino acid sequence of GP5, is <97% homologous to G16X virus. The level of protection induced by the vaccines was assessed by measuring parameters indicative of protective immunity including: viremia, gross lung pathology, peripheral blood oxygen saturation (SpO2), and weight gain. As compared to the strict control group, animals immunized with E-NDs and challenged with 16244B exhibited: a three-fold decrease in their rate of weight gain; a significant level of lung dysfunction reflected by hypoxemia (oxygen saturation level of <90%); a sizable area of the lung with gross pathology (45±9); and a sustained level of viremia (>3 log10 TCID50/ml of serum) for 12 days. Pigs immunized with the P-NDs exhibited a significantly ($p<0.05$) improved rate of weight gain, no hypoxemia, and a major reduction in gross lung pathology. The level of protective immunity attained in the P-ND vaccinated pigs based on these parameters was not significantly different from the protection afforded to pigs immunized with either the IWV or the MLV vaccine. While the viremia in all the pigs in the MLV vaccinated cohort ended at 10 days after the virus challenge, the viremia in the P-ND vaccinated pigs was extinguished by 12 days after the challenge in 3 out of 8 pigs, with the other 5 pigs exhibiting a clear trend towards an impending viral termination. Pigs in the IWV vaccinated group exhibited a similar rate of viremia elimination as the P-ND vaccinated group. At day 12 post-challenge, the viremia in all three groups of vaccinated pigs was significantly lower ($p<0.05$) than the viremia in the E-ND vaccinated group. Before challenge, all of the pigs vaccinated with either the IWV or the MLV vaccines had antibodies against both the N protein and the envelope glycoprotein 5 (GP5) in their serum. In contrast, none of the pigs immunized with the P-ND had antibodies against the N protein but were positive for antibodies against GP5. These observations demonstrate that a vaccine based solely on viral envelope proteins incorporated into NDs are capable of providing a significant level of protective immunity against a virulent virus challenge and can serve as a DIVA vaccine.

Session 15

Intranasal Delivery of Inactivated Influenza Virus and Poly(I:C) Adsorbed Corn-Based Nanoparticle Vaccine Elicited Robust Antigen-Specific Cell-Mediated Immune Responses in Maternal Antibody Positive Nursery Pigs

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Swine influenza A virus (SwIAV) constitutes a significant economic and health risk in pigs. Current swine flu vaccines are not completely effective against evolving SwIAVs. There is a need to develop an effective killed vaccine against SwIAV infection. Furthermore, maternal derived antibody (MDA) interference to commercial injectable SwIAV vaccination in grower pigs is a problem. We developed intranasal deliverable sweet corn nanoparticle (Nano-11) adsorbed inactivated SwIAV and Poly (I:C) adjuvanted [Nano-11-KAg+Poly(I:C)] vaccine to augment mucosal immune response in MDA positive pigs. The candidate vaccine efficacy was assessed against a heterologous H1N1 SwIAV challenge infection and compared that to a commercial KAg vaccine performance. Our data indicated that Nano-11-KAg+Poly(I:C) vaccine delivered intranasal stimulated robust cross-reactive IgG and secretory IgA antibodies in the lungs and broadly protective polyfunctional cell-mediated immune responses to highly variant SwIAV, associated with reduction in viral load in the airways and amelioration of lung pathology comparable to a multivalent commercial vaccine. But the commercial vaccine appears to elicit a strong inflammatory response associated with accumulation of naïve T-helper cells in both the mucosal lymph nodes and systemic compartment suggesting possible inefficient antigen presentation in MDA positive pigs. Our data suggested the important role played by Nano-11 and Poly(I:C) based inactivated SwIAV vaccine in induction of polyfunctional, cross-protective cell-mediated immunity against swine flu in MDA positive pigs. This research was supported by USDA-AFRI 2019-67015-29814.

Session 16

Amplification of IgG Genes from Classical Swine Fever Virus C-strain E2 Glycoprotein Specific Single Porcine B Cells

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*Email: lihua@vet.k-state.edu; Key words: Classical swine fever (CSF), C-strain, E2 glycoprotein, single porcine B cell.

Introduction: Neutralizing antibodies (nAbs) can be used before or after infection to prevent or treat viral diseases. However, there are few efficacious nAbs against classical swine fever virus (CSFV) have been produced because most nAbs against CSFV are derived from mouse hybridoma cells and these murine nAbs can induce the production of anti-mouse antibodies in vivo, leading to a short half-life. Here, we report the amplification of porcine immunoglobulin (IgG) genes from CSFV C-strain E2 (CE2) glycoprotein specific single porcine B cells aiming to facilitate the development of CSF vaccines or antiviral drugs that offer the advantages of stability and low immunogenicity.

Methods: Pigs were immunized with CSFV subunit vaccine, KNB-E2. At 42 days post of vaccination (DPV), CE2 specific single B cells were isolated via fluorescent-activated cell sorting (FACS) baited by Alexa Fluor™ 647 labeled CE2 (positive), Goat Anti-Porcine IgG (H+L)-FITC antibody (positive), PE Mouse Anti-Pig CD3ε (negative) and PE Rat Anti-Mouse CD8a (negative). The full-length of IgG heavy (H) chains and light (L) chains were amplified by reverse transcription-polymerase chain reaction (RT-PCR) and nested RT-PCR.

Results: Pigs immunized with KNB-E2 exhibited high levels of CSFV CE2 antibodies at DPV35. By pre-sort analysis, about 2% total CE2 specific B cells were obtained. The single B cells were deposited into single wells of four 96-well PCR plates. After reverse transcription and amplification, we obtained a total of 3 IgG H chains, 9 kappa L chains and 36 lambda L chains including three paired chains (two H+ κ and one H+ λ).

Discussion: This study is the first report to describe the amplification of whole-porcine IgG genes from single porcine B cells of KNB-E2 vaccinated pig. The method for direct isolation of full-length H and L chains from a single primary porcine B cell is versatile, sensitive, and reliable. The paired heavy and light chains can produce natural nAbs with lower risk for immunogenicity and longer period of stability.

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Session 17

PRRSV L1C: A Rapid Response to a National Crisis

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In partnership with Boehringer Ingelheim Animal Health USA Inc., Pipestone Research conducted a rapid response investigation to bring science-based answers to managing PRRSV 144 L1C Variant. Based on field observations from practitioners and producers, stating that, "This is the worst strain of PRRSV ever," "Vaccines don't work anymore", and "Biosecurity protocols are ineffective," the team completed 3 studies targeting these field concerns, including:

Study 1 (pathogenicity): Is PRRSV 144 L1C Variant more virulent than PRRSV 174?
Design: Naïve pigs were challenged with either PRRSV 144 or a highly pathogenic PRRSV 174. Outcomes included Average Daily Gain (ADG), percent mortality, viral load, clinical scores, pyrexia, and number of treatment events.
Results: Across all metrics, PRRSV 144 L1C Variant was not more pathogenic than the PRRSV 174 used in the study.

Study 2: Are vaccines effective against PRRSV 144 L1C Variant?
Design: Pigs were vaccinated either with Ingelvac PRRS® MLV (BI) or Prevacent® PRRS (Elanco) and compared to a non-vaccinated control group. Vaccination was applied according to label instructions and challenge occurred 28-day post-vaccination.
Results: Both vaccines were effective against PRRSV 144 L1C Variant. Growth performance was significantly better and clinical measurements were less severe in vaccinates vs. non-vaccinates.

Study 3: Can biosecurity protocols prevent PRRSV 144 L1C Variant introduction?
Design: Biosecurity protocols or products were tested on pigs challenged with PRRSV 144 L1C Variant.
Results:

- Disinfectants: Ag Forte Pro and Synergize™ neutralized PRRSV 144 after 60 minutes of contact.
- Contaminated feed: PRRSV 144 was transmitted through feed via natural feeding behavior. Both feed mitigants (Guardian™ or Sal CURB®) prevented infection.
- Feed transport: PRRSV 144 survived in the feed transport model and infected pigs.
- Survival in slurry: PRRSV survived in slurry for 14 days, but not 21 days.
- Contaminated fomites/Shower in protocol: Following 30 minutes of contact with infected pigs, PRRSV 144 was detected on hands, boots, and coveralls of personnel. In the absence of a shower and clothes/footwear change, virus was transmitted to contact controls. After a shower and clothes/footwear change, virus was not transmitted to contact controls.
- Filtration: PRRSV RNA was detected in 28%-43% of interior air samples during the challenge period. In contrast, there was no detectable PRRSV 144 in exterior air samples post-filtration.

Conclusions: Under the conditions of this study, PRRSV 144 L1C Variant, while highly pathogenic, can be managed using standard protocols and products previously validated against historical variants.

Session 18

Investigation of Vesicular Lesions in Pigs With Unknown Causative Agents

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Swine vesicular lesions may be caused by foot-and-mouth disease virus (FMDV), Senecavirus A (SVA), swine vesicular disease virus (SVDV), vesicular stomatitis virus (VSV), or vesicular exanthema of swine virus (VESV), some being foreign animal disease (FAD) pathogens to the USA. These cases submitted to the ISU VDL are always tested for FMDV and sometimes SVA via PCR, along with tests conducted at FADDL for FMDV, SVA, SVDV, VSV, and VESV. From 2015-2021, the ISU VDL had numerous swine vesicular cases that tested negative for all known vesicular viral pathogens. The objective of this study was to further investigate such cases to explore potential unrecognized causative agents of swine vesicular lesions.

Among the 228 swine vesicular cases submitted to ISU VDL from 2015 to 2020, all were FMDV-negative, 86 (37.7%) SVA-negative, 102 (44.7%) SVA-positive, and 40 (17.6%) SVA-not tested via PCR. Samples were available for 84 of these cases, which included 32 SVA-negative, 37 SVA-positive, and 15 SVA-not tested. The 32 SVA-negative and 5 selected SVA-positive cases were subjected to further investigations. From January to March of 2021, ISU VDL received >100 swine vesicular lesion cases; all tested negative for FMDV and SVA via PCR and 16 of these cases underwent further investigations, including bacterial culture, virus isolation (VI) using ST and PK-15 cells, metagenomics analysis via next-generation sequencing (NGS), and additional PCRs.

Six randomly selected vesicle swabs had no significant bacterial growth on routine culture.
Twenty-three randomly selected clinical vesicle swabs or fluids from the SVA-negative cases (11 samples from 2015-2020 and 12 from 2021) were tested by NGS. Various viruses were detected with different number of reads; however, no significant sequences were assembled. In contrast, full-length genome sequences of SVA were obtained from 4 SVA-positive vesicle swabs sharing 98.2-99.3% nucleotide identity.

Among the 48 SVA-negative cases, 18 were VI positive based on cytopathic effects, 21 were VI negative, and 9 had VI in progress. NGS was conducted on all 18 virus isolates. Nearly full-length genome sequences of porcine teschoviruses (PTV) were obtained from 16 cases, including different genotypes (PTV-2, 3, 4, 7, 9, 10 and 11). Porcine sapelovirus virus (PSV) sequences were obtained from 2 cases.

The 18 isolates and their clinical samples were tested for PTV, PSV, enterovirus G (EV-G), porcine parvovirus 1 (PPV1), and porcine parvovirus 2 (PPV2) via PCR. Although the virus isolates were strongly positive for PTV or PSV by PCR, their corresponding clinical samples either were negative or had high Ct values for PTV, PSV, EV-G, PPV1, and PPV2 suggesting these viruses may not be the causative agent. Experimental inoculation of pigs with these PTV and PSV isolates is needed to confirm their potential cause of the vesicular lesions. In addition, potential non-infectious factors should be investigated.

Session 19

Development of a Blocking Enzyme-Linked Immunosorbent Assay for Detection of Antibodies against African Swine Fever Virus

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The incursion of African Swine Fever virus (ASFV) into Eurasia presents a threat to the world's swine industry. Highly sensitive and specific diagnostic assays are urgently needed for the rapid detection during an outbreak, post-outbreak investigation, and for disease surveillance. In this study, a highly specific and repeatable blocking ELISA (bELISA) was developed using a recombinant p30 protein as the antigen combined with biotinylated mAb against p30 as the detection antibody. Initial test validation included sera from 810 uninfected animals and 106 animals experimentally inoculated with ASFV or recombinant Alphavirus/Adenovirus expressing p30. Receiver operating characteristic (ROC) analysis of the data calculated an optimal percentage of inhibition (PI) cut-off value of 45.92%, giving a diagnostic sensitivity of 98.11% and diagnostic specificity of 99.42%. The coefficient of variation of an internal quality control serum was 6.81% for between-runs, 6.71% for within-run, and 6.14% for within-plate. A time course study of infected pigs showed that the bELISA was able to detect seroconversion as early as 7 days post-inoculation. Taken together, these results demonstrated that the bELISA can be used as an alternative serological test for detecting of ASFV infection.

Session 20

Differentiation of Animals Vaccinated with Flag T4G Against Classical Swine Fever Virus from Infected Pigs Using a Dendrimeric Peptide-Based Approach

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Classical swine fever virus (CSFV) causes a viral disease of high epidemiological and economical significance that affects domestic and wild swine. Control of the disease in endemic countries is based on live-attenuated vaccines (LAV) that induce an early protective immune response against highly virulent CSFV strains. The main disadvantage of these currently available LAV is the lack of serological techniques to differentiate between vaccinated and infected animals (DIVA concept). A promising marker LAV prototype (named FlagT4G), based on the CSFV Brescia strain, has been developed. This LAV prototype has shown to be highly effective, inducing sterilizing immune response as early as 3 days after vaccination. However, it still lacks a reliable DIVA diagnostic test
Here, we describe the development of the FlagDIVA test, a serological diagnostic tool allowing to differentiate animals vaccinated with the FlagT4G candidate from those infected with CSFV field strains. The FlagDIVA test is a direct ELISA based on a dendrimeric peptide construct displaying two copies of a conserved epitope of CSFV structural protein E2 accompanied and one copy of an epitope found in the NS2-3 protein. In order to test the capacity of the FlagDIVA assay to detect antibody response against the FlagT4G, 15 pigs at six weeks of age were vaccinated with FlagT4G and sera samples were collected weekly until 28 days post vaccination (dpv). A boost immunization with the same vaccine dose was also carried out at 18 dpv. Sera samples from these pigs were evaluated for antibodies against CSFV by the FlagDIVA test and a commercial ELISA. Neutralizing antibody response was also assessed by neutralization peroxidase-linked assay (NPLA). The FlagDIVA was also used to evaluate the antibody response in 177 samples from a serum collection, including naïve pigs and animals infected with different field CSFV strains. All these serum samples had previously been tested for antibodies against CSFV by the commercial ELISA.

Specific anti-E2 antibody response was detected in all the FlagT4G-vaccinated pigs between 13 and 28 dpv by the commercial ELISA. Meanwhile, the FlagDIVA assay did not recognize the antibody response induced in the FlagT4G-vaccinated animals. In the samples from the serum collection, the FlagDIVA assay showed better performance than the commercial ELISA test in samples from pigs infected with low-virulence CSFV strains. These results show the efficacy of FlagDIVA for detecting anti-CSFV antibodies in infected animals, while it does not recognize the antibody response of FlagT4G-vaccinated animals. Therefore, the FlagDIVA test constitutes a valuable accessory DIVA tool in implementing vaccination with the FlagT4G candidate.

NA PRRS Symposium
Benfield Poster Session

Poster Number	Name	Company/Business	Country	Title
P01	Shamiq Aftab	South Dakota State University	United States	Antiviral Roles of Interferon Induced Transmembrane 3 (IFITM3) Protein on Seneca Virus A (SVA) Replication
P02	Shamiq Aftab	South Dakota State University	United States	Role of IFITM3 in PRRSV replication
P03	Ethan Aljets	Iowa State University	United States	Investigation of vesicular lesions in pigs with unknown causative agents
P04	Allison Blomme	Kansas State University	United States	Use of a viral isolation assay for detection of Porcine Reproductive and Respiratory Syndrome Virus in feedstuffs
P05	Jose Alejandro Bohorquez Garzon	IRTA-CReSA	Spain	Differentiation of animals vaccinated with Flag T4G against classical swine fever virus from infected pigs using a dendrimeric peptide-based approach
P06	Ting-Yu Cheng	The Ohio State University	United States	PRRSV detection in swine herds with different demographics and PRRSV management strategies
P07	Gerardo Ramon Diaz Ortiz	University of Minnesota	United States	Elaboration and evaluation of an autogenous inoculum as a PRRS control tool: An experience in Peru
P08	Diego Diel	Cornell University	United States	Experimental inoculation of swine with SARS-CoV-2
P09	Diego Diel	Cornell University	United States	A virulent and pathogenic infectious clone of Senecavirus A
P10	Ying Fang	University of Illinois	United States	A Novel Arterivirus Protein and Expression Mechanism
P11	Alba Frias-De-Diego	North Carolina State University	United States	Whole Genome or Single Genes? A Phylodynamic and Bibliometric Analysis of PRRSV
P12	Christine Harness	ISU-VDPAM	United States	Expression levels of CD169, CD163 and CD151 in PRRSV-infected peccaries
P13	Erin Ison	Purdue University	United States	Altered gene expression associated with thyroid hormone metabolism following in utero PRRSV infection
P14	Tobias Kaeser	North Carolina State University	United States	Modified-live virus vaccination induces heterologous immunity against different type-2 PRRSV strains.
P15	Mariana Kikuti	University of Minnesota	United States	Newly emerged Lineage 1C porcine reproductive and respiratory syndrome virus (PRRSV2) variant
P16	Mariana Kikuti	University of Minnesota	United States	Porcine reproductive and respiratory syndrome virus 2 (PRRSV-2) genetic diversity and occurrence of wild type and vaccine-like strains in the United States swine industry
P17	Haesu Ko	University of Alberta	Canada	Phenotypic effect of a genetic variant linked to DIO2 on fetal outcomes in PRRSV-2 infected gilts
P18	Haesu Ko	University of Alberta	Canada	A post-genome-wide association study testing the effect of a missense mutation in the DIO2 gene on fetal response following maternal PRRSV-2 infection
P19	Marie-Eve Koziol	Seppic	United States	Montanide™ Gel 01 PR, An Adjuvant For Safe and Efficacious Porcine Epidemic Diarrhea and Transmissible Gastroenteritis Bivalent Inactivated Vaccines
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P21	Abdullah Mahfuz	Feed Energy Company	United States	R2™ for mitigating the risk of PEDV, PRRSV and SVA -contaminated feed using an animal challenge model
P22	Margaret Mulligan	Purdue University	United States	Impact of fetal PRRSV2 infection on the organ-specific regulation of cell division
P23	Rahul Nelli	Iowa State University	United States	Primary porcine respiratory epithelial cells self-limit SARS-CoV-2 replication possibly by undergoing rapid apoptosis.

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P25	Angie Quinonez Munoz	BioSec	United States	Inactivation of two swine viruses on shoes by BioSec, a shoe-sanitizing station
P26	Sheela Ramamoorthy	North Dakota State University	United States	Oral delivery system for a rapid-response porcine epidemic diarrhea virus (PEDV) vaccine
P27	Sheela Ramamoorthy	North Dakota State University	United States	Targeting suicidal replication to enhance the safety of attenuated viral vaccines
P28	Kaitlyn Sarlo Davila	USDA NADC & ORISE	United States	Host response in the porcine lymph node to infection by PRRSV 2, Influenza B and their coinfection
P29	Chia-Ming Su	University of Illinois	United States	Mediation of type 1 IFN signaling by PRRSV nsp5-related protein
P30	Lihua Wang	Kansas State University	United States	Amplification of IgG genes from classical swine fever virus C-strain E2 glycoprotein specific single porcine B cells
P31	Xingyu Yan	University of Illinois	United States	Novel features of the PRF signal in the nsp2 region of emerging PRRSV variants
P32	Wannarat Yim-im	Iowa State University	United States	Characterization of PRRSV in clinical samples and the MARC-145 and/or ZMAC cell culture isolates
P33	Fangfeng Yuan	University of Illinois	United States	Development of a Blocking Enzyme-Linked Immunosorbent Assay for Detection of Antibodies against African Swine Fever Virus
P34	Fangfeng Yuan	University of Illinois	United States	Establish pregnant sow-fetus models to assess safety and efficacy of influenza vaccines
P35	Jeffrey Zimmerman	Iowa State University	United States	Control and elimination of PRRSV from a 38,000 sow system
P36	Federico Zuckermann	University of Illinois	United States	Effective protection induced by an experimental subunit DIVA vaccine against PRRS virus
P37	Alex Pasternak	Purdue University	United States	Genome wide association study of thyroid hormone levels in piglets and fetuses following challenge with PRRSV

Poster 01

Antiviral Roles of Interferon Induced Transmembrane 3 (IFITM3) Protein on Seneca Virus A (SVA) Replication

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Seneca Virus A (SVA) is a non-enveloped, positive sense, ssRNA virus belonging to the family *Picornaviridae*. SVA causes vesicular diseases in pigs with clinical signs like foot and mouth disease virus. Multiple outbreaks have been reported in the US, Brazil, and China resulting in significant economic losses. The role of interferon stimulated genes (ISGs) in SVA replication and pathogenesis remains largely unknown. Interferon-induced transmembrane 3 (IFITM3) is an ISG which have been reported to restrict the replication of many viruses including Influenza virus, Human metapneumovirus, West Nile virus, etc. The objective of this study is to investigate the antiviral roles of IFITM3 against SVA.

To achieve this objective, we have overexpressed IFITM3 protein in H1299 cells followed by SVA infection to check the virus titer and stained the cells with antibody against IFITM3 and VP2 protein of SVA. Furthermore, we have silenced endogenous IFITM3 in H1299 cells followed by SVA infection and performed TCID50/ml as well as western blot. We have also treated the H1299 cells with interferon-alpha followed by SVA infection to further verify the role of IFITM3 and other ISGs in restricting virus replication.

Results showed that over-expression of IFITM3 significantly reduced virus titers as compared to vector control. Additionally, colocalization of IFITM3 with the virus was observed. As expected, silencing of endogenous IFITM3 resulted in a significantly increased viral titer and viral protein expression compared to control silencing RNA as revealed by TCID50 assay and western blot. Furthermore, our preliminary data showed that interferon treatment reversed the silencing effect of IFITM3 on virus replication as compared to without interferon treatment. A negative correlation between the level of IFITM3 expression and viral protein expression was observed.

In summary, our preliminary data suggested that IFITM3 played an antiviral role in SVA replication. Further investigations are needed to evaluate the mechanisms by which IFITM3 partially restricts SVA replication.

Poster 02

Role of IFITM3 in PRRSV replication

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive sense, single-stranded RNA virus that belongs to the Arteriviridae. Interferon induced transmembrane protein 3 (IFITM3) is a member of the interferon induced antiviral genes (ISGs) and exhibits potent antiviral activity against many enveloped RNA viruses. The primary objective of the study is to examine the role of IFITM3 in PRRSV replication.

Marc145 cells were transfected with either vector control or plasmid containing HA-tagged IFITM3. At 48 h after transfection, cells were infected with 1 MOI of PRRSV 23983. Western blot, immunofluorescence staining and confocal microscopy, flow cytometry and TCID50 assays were performed to confirm the expression of the desired proteins and to determine the virus titer and the number of virus positive cells.

Our preliminary data have shown that over-expression of exogenous IFITM3 in MARC-145 cells reduced virus replication by an average of approximately 5.4-fold. Amphotericin B treatment only partially restore the replication of PRRSV in MARC-145 cells over-expressing IFITM3. Confocal microscopic analysis revealed that abundant colocalizations of PRRSV with early endosome marker EEA1s were observed at 3 h after virus infection. Only limited colocalization between PRRSV and late endosome/lysosome marker LAMP1 was observed at 3 h after virus infection. No significant difference between the percentage of PRRSV positive cells was observed between control vector transfected cells and IFITM3-expressing plasmid transfected cells ($p > 0.05$) at 3 h post infection. However, the percentage of PRRSV positive cells was significantly lower ($p < 0.05$) in IFITM3 transfected cells than the vector control transfected cells at 24 h post infection. Treatment of MARC-145 cells with Interferon alpha and gamma upregulates the expression of Mx1 and IFITM3, which is positively correlated with reduced virus replication. Overall, IFITM3 exhibits antiviral activities against PRRSV.

Poster 04

Use of a viral isolation assay for detection of Porcine Reproductive and Respiratory Syndrome Virus in feedstuffs

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Current methods for detection of virus in feed rely on PCR and the resulting Ct values. While these results hold some value for understanding if feed has been contaminated, they are not as useful when evaluating the amount of infectious virus being contained in the feed. This study aimed to develop a viral isolation assay for porcine reproductive and respiratory syndrome virus (PRRSV) in three different feedstuffs and relate PCR results to amounts of detectible infectious virus. For this work, ten grams of either solvent-extracted soybean meal (SBM), dried distillers grains with solubles (DDGS), complete swine feed (FEED), or 10 mL of media (DMEM) were inoculated with 105.4 TCID50/mL of type 2 PRRSV strain 129 and incubated at either 4°C, 23°C, or 37°C. At one hour, 24 hours, 48 hours, and 72 hours post-inoculation, one gram was removed and suspended in 5 mL of DMEM before centrifugation and filtration. The sample was then titrated and used to inoculate confluent monolayer of MARC-145 cells in a 96-well plate and TCID50/ml was determined. Each supernatant sample underwent RNA extraction which was then used for qRT-PCR to detect the change in detectible virus across matrix type, temperature, and time. A significant interaction ($P = 0.0278$) was observed for matrix × temperature × hour for live virus detected in the VI model. With this model, a decrease in infectious virus was observed in the SBM at 23 and 37°C compared to 4°C across all four time points. Amount of detectible infectious PRRSV in DDGS decreased from 1 h to 72 h post-inoculation when incubated at 4°C. Incubating inoculated DDGS at 37°C instead of 4°C causes a decrease in detectible infectious virus at each sampling time. Change in time or temperature did not result in observable differences in levels of infectious PRRSV in a FEED matrix. Compared to the PRRSV control at each sampling time, SBM had less infectious virus at 23°C and 37°C. The amount of detectible infectious virus was decreased in DDGS and FEED at 4°C and 23°C at each time point compared to the PRRSV control at the same temperatures as well. However, at 37°C for these two matrices, there was less infectious PRRSV observed at 1 and 24 hours post-inoculation than in the PRRSV control. Only matrix type had an effect on the amount of viral RNA detected through PCR analysis ($P = 0.0319$). The viral control had more viral RNA detected than DDGS (105.26 and 102.47 viral RNA/mL; respectively) with SBM and FEED being intermediate (104.18 and 103.86 viral RNA/mL, respectively). This work demonstrates the ability of SBM, DDGS, and feed to harbor detectible infectious virus, but also that storage time and temperature influences virus inactivation compared to viral control within the conducted VI model. Virus isolation was used to determine infection risk for various matrices; however, the observed interactive response within this study demonstrates that further research is needed to determine if PRRSV is being inactivated or if it is bound to organic material and therefore not being detected.

Poster 06

PRRSV detection in swine herds with different demographics and PRRSV management strategies

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Porcine reproductive and respiratory syndrome virus (PRRSV) has been one of the major health-related concerns in the swine production industry. Through its rapid transmission and mutation, the simultaneous circulation of multiple PRRSV strains can be a challenge in PRRSV diagnostic, control, and surveillance. The objective of this longitudinal study was to describe the temporal detection and genetic variability of PRRSV in swine farms with different production types and PRRS management strategies. Tonsil scraping samples (n = 343) were collected monthly from three breeding and two growing herds for one year. In addition, processing fluid samples (n = 204) were obtained from piglet processing batches within the three breeding farms while pen-based oral fluid samples (n = 111) were collected in the two growing pig farms. Viral RNA extraction and reverse-transcription quantitative PCR (RT-qPCR) were conducted for all samples, along with ORF5 sequencing on processing fluid samples. The sample positivity threshold was set at quantification cycle (Cq) of ≤ 37. The results suggested a higher probability of detection in processing fluids compared to tonsil scraping specimens (odds ratio (OR) = 3.86; P = 0.096) in breeding farms whereas oral fluids were outperformed by tonsil scrapings (OR = 0.26; P < 0.01) in growing pig farms. Phylogenic analyses on the PRRSV ORF5 gene in processing fluids identified the presence of multiple PRRSV strains (genetic identity > 98%) classified into different lineages in two breeding farms within and between sampling events. The results described herein may lead to an improvement in PRRSV diagnostic and surveillance by selecting proper specimens and supporting sequencing more PCR positive samples.

Poster 07

Elaboration and evaluation of an autogenous inoculum as a PRRS control tool: An experience in Peru

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Objective: Summarize the methodology to elaborate an inoculum from a homologous strain of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) and describe the post-inoculation impact in productive and reproductive parameters in 5 200 animals, after receiving a 104 viral copies/ml single-dose of the inoculum, as a part of a PRRS acclimatization program in a large-scale pig farm.

Methods: Blood samples were collected from piglets qPCR positives to North American PRRSv from a recent outbreak. To prepare an inoculum from a homologous strain, other diseases were discarded by qPCR, such as Porcine Circovirus type 2 (PCV-2), Porcine Parvovirus (PPV), Influenza A Virus (IAV) and Classical Swine Fever Virus (CSFV). Then the number of viral copies/ml was calculated. The first step was the cDNA synthesis from positive RNA samples and controls, the next step was the amplification of an ORF7 gene fragment of PRRSv by end-point PCR, which was purified from an agarose gel using the Wizard SV Gel and PCR Clean-Up System Kit (Promega, USA). The amplicon concentration was quantified using the QuantiFluor® dsDNA System kit in a Quantus Fluorometer (Promega). A standard curve was generated by qPCR based on serial dilutions of the quantified amplicon, which allowed the calculation of the number of viral copies/ml for each positive sample. Chosen samples were diluted with PBS 1X to a concentration of 104 viral copies/ml, Gentamycin was added to the inoculum and then it was stored at -80°C until use. After progressive thawing, 2 ml of inoculum was applied intramuscularly to 5 200 animals including replacement gilts, boars, and all sows of the farm, independently of the reproductive status (pregnant or lactating). Clinical signs were observed, and reproductive and productive parameters were measured at 3-, 7- and 10-weeks post-inoculation (pi). Seroconversion was measured by ELISA 21 days pi.

Results: Between days 1 and 2 pi, lack of appetite was observed in 0.5% of the animals. Neither fever nor other clinical signs were observed. Appetite was restored after 24 hours. Antibodies against PRRSv were detected 21 days pi by ELISA. Pre-weaning mortality (PWM) decreased 29.9%, 58% and 69% at 3-, 7- and 10-weeks pi, respectively. Wean-to-finish mortality increased in rearing (21-70 days), which is the group most affected by the outbreak, unlike fattening (71-150 days) whose average remains, so that the use of inoculum seems not having influenced these age groups. On the other hand, while no variation in total born piglets was observed, the stillbirth piglets decreased 34.7% and 56.8% at 7- and 10-weeks pi, respectively. Finally, although weaning weight increased by 28.1%, it is important to note that at 3 weeks pi there was an outbreak of PEDV that also influenced weaning weight gain, so it would not be appropriate to attribute this increase in value only to the use of the inoculum.

Conclusions: A single-dose intramuscular injection of 2 ml of PRRSv autogenous inoculum containing 104 viral copies/ml induces seroconversion without any considerable adverse effect. A short-term evaluation suggests an improvement in productive and reproductive parameters on the farm.

Poster 11

Whole Genome or Single Genes? A Phylodynamic and Bibliometric Analysis of PRRSV

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Diversity, ecology, and evolution of viruses are commonly determined through phylogenetics, an accurate tool for the identification and study of lineages with different pathological characteristics within the same species. In the case of PRRSV, evolutionary research has divided into two main branches based on the use of a specific gene (i.e., ORF5) or whole genome sequences as the input used to produce the phylogeny. In this study, we performed a review on PRRSV phylogenetic literature and characterized the spatiotemporal trends in research of single gene vs. whole genome evolutionary approaches. Finally, to determine the most accurate type of data to be applied to understand and predict the evolutionary trends of PRRSV, we used publicly available data to produce a Bayesian phylodynamic analysis following each research branch and compared the results to determine the pros and cons of each particular approach. This study provides an exploration of the two main phylogenetic research lines applied for PRRSV evolution, as well as an example of the differences found when both methods are applied to the same database. We expect that our results will serve as a guidance for future PRRSV phylogenetic research.

Poster 13

Altered gene expression associated with thyroid hormone metabolism following in utero PRRSV infection

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Objective: Thyroid hormones, their metabolites, and their metabolic enzymes are necessary for proper maternal reproductive function as well as fetal development and maturation. We have previously demonstrated a suppression in circulating thyroid hormone levels (T3 and T4) following PRRSV infection, however the physiological mechanism(s) underlying this disruption remains unknown. We hypothesize that some portion of this suppression results from changes in thyroid hormone metabolism, either in key fetal organs or at the maternal fetal interface (MFI). The key components of this metabolic network include deiodinases that alter biological activity through the removal of iodines from thyroid hormone's inner and outer rings, sulfotransferases and sulfatase that conjugate or remove a sulfo group respectively, and solute carriers that act as thyroid hormone transporters. The overall objective was to investigate the expression of these components within the fetal kidney, liver, and the MFI.

Methods: Pregnant gilts (n=27) were either experimentally challenged with PRRSV2 (n=22, strain NVSL 97-7895) or sham inoculated (n=5) at gestation day 85. Fetal tissues were collected from all live fetuses (n=286) at 21 days post infection (DPI) and subsequently classified based on fetal preservation and viral load in thymus and serum into one of four phenotypic groups (n = 10/group): control from sham inoculated gilts (CON), uninfected with no detectable viral load (UNIF), high viral load viable (HV-VIA), or high viral load with severe meconium staining (HV-MEC) with both high viral groups showing >5 log10 of virus in serum and thymus. Tissues including liver (LVR), kidney (KID), maternal (END) and fetal (PLC) components of the MFI were evaluated by absolute quantification PCR for gene expression of three deiodinases (DIO1, 2, & 3), five sulfotransferases (SULT1A3, 1B1, 1C2, 1E1, & 2A1), sulfatase (STS), as well as two solute carriers (SLC16A2 & 16A10).

Results: Regardless of phenotypic group, expression of DIO1 and SULT1C2 were greater in fetal tissues in contrast to DIO2, DIO3, SULT1B1, and SULT2A1 which were more abundantly expressed in MFI tissues. While many significant alterations in gene expression were identified between phenotypic groups across the four tissues evaluated, DIO3 was the most intriguing. Expression of this inner ring deiodinase was significantly elevated in the LVR, END, and PLC of the more resilient (HV-VIA) relative to all other groups including the more susceptible HV-MEC group.

Conclusions: During fetal PRRSV infection, there's evidence that the degree of thyroid hormone suppression may differentiate between resilient and susceptible phenotypes. The differential upregulation of DIO3, which serves to metabolize T3 and T4, may be responsible for this effect. In addition, the alteration of DIO3 gene expression within maternal tissue (END) associated with the HV-VIA fetuses suggest a fetal signaling mechanism capable of crossing the MFI. In this context, increased expression of DIO3 may play a protective role and enhance fetal viability following fetal PRRS infection.

Poster 14

Modified-live virus vaccination induces heterologous immunity against different type-2 PRRSV strains.

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Objective: Cross-protection against heterologous strains is a major hurdle of vaccines against PRRSV – the porcine reproductive and respiratory syndrome virus. Heterologous vaccine efficacy relies on the induction of both humoral and cellular immunity that reacts against various PRRSV strains. Thus, this study investigated vaccine efficacy and immunogenicity of the Prevacent modified live virus (MLV) vaccine against four type-2 PRRSV strains.

Methods: Sixty weaners were divided into five MOCK- and five MLV-vaccinated groups. After four weeks, each of these groups were challenged for two weeks with MOCK or one of four PRRSV-2 strains – NC174, NADC20, NADC30, or VR2332. Heterologous vaccine efficacy was assessed by lung pathology and viremia. Heterologous vaccine immunogenicity was determined via nasal swab IgA, serum IgG and neutralizing antibody levels, and a detailed T-cell response analysis – proliferation, IFN-gamma production, and differentiation of CD4, CD8, and TCR-gamma/delta T cells.

Results: Vaccination showed heterologous efficacy against VR2332 (reduced viremia), and NADC20 and NADC30 (reduced lung pathology and viremia). Vaccination also induced a strong systemic IgG response and increased the number of animals with neutralizing antibody titers against VR2332, NADC20, and NADC30. Vaccination also improved the heterologous T-cell response: Vaccinated animals not only had a higher frequency of memory/effector CD4 T cells but also an improved heterologous CD4 and CD8 IFN-gamma response against NC174, NADC20, and NADC30.

Conclusions: Overall, the MLV vaccine Prevacent elicited various degrees of both vaccine efficacy and immunogenicity against different heterologous PRRSV-2 strains.

Poster 15

Newly emerged Lineage 1C porcine reproductive and respiratory syndrome virus (PRRSV2) variant

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Objective: Here, we describe the viruses associated with several farm-level porcine reproductive and respiratory syndrome (PRRS) outbreaks caused by unusually similar viruses at the ORF5 level was reported in December 2020 by swine producers to the Morrison Swine Health Monitoring Project (MSHMP, representative of 50% of the U.S. breeding herd).

Methods: The orf5 PRRSV sequences associated with these cases were compared to over 30,000 historical orf5 sequences (1998 – May 2021) from routine monitoring efforts in breeding, gilt developing units, growing and finishing herds amongst MSHMP participants. The emerging genetic cluster was defined by a nucleotide identity of ≥98% between samples and was classified into lineage/sub-lineage and RFLP patterns. Phylogenetic trees were constructed using NextStrain. ORF5 sequences of cases detected up to December 2020 were compared to 8,922 Lineage 1C ORF5 sequences. A subset of cases was submitted for whole genome sequencing and compared to 365 PRRSV2 whole genome sequences from North America publicly available at GenBank.

Results: All 192 cases were classified as Lineage 1C. Most cases (172/175; 98.29%) were RFLP 1-4-4 type, while two were 1-4-3 and one was 1-7-4 RFLP types. 154 breeding and grow-finishing sites in the Midwestern U.S were affected. Transmission seemed to have occurred in two waves, with the first peak of weekly cases occurring between October and December 2020 and the second starting in April 2021. Majority of cases occurred in a 120 km radius. ORF5 and whole genome sequencing results suggests that this represents the emergence of a new variant within Lineage 1C distinct from what has been previously circulating.

Conclusion: The occurrence of within farms PRRS outbreaks in several sites associated with such a highly similar virus at the ORF5 region is unprecedented. Sequences associated with this region-wide outbreak correspond to a unique clade within Lineage 1C.

Financial Support: Swine Health Information Center and NIFA-NSF-NIH.

Poster 16

Porcine reproductive and respiratory syndrome virus 2 (PRRSV-2) genetic diversity and occurrence of wild type and vaccine-like strains in the United States swine industry

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Objective: Here, we describe porcine reproductive and respiratory syndrome virus genotype 2 (PRRSV-2) genetic diversity in the U.S. over the course of 10 years'.

Methods: A database comprising 10 years' worth of sequence data obtained from swine production systems routine monitoring and outbreak investigations was used. A total of 26,831 ORF5 PRRSV-2 sequences from 34 production systems were included in this analysis. Within group mean genetic distance (i.e. mean proportion of nucleotide differences within ORF5) per year according to herd type was calculated. The percent nucleotide difference between each sequence and the ORF5 sequences from four commercially available PRRSV-2 vaccines (Ingelvac PRRS MLV, Ingelvac PRRS ATP, Fostera PRRS, and Prevacent PRRS) within the same lineage over time was used to classify sequences in wild-type or vaccine-like.

Results: The mean ORF5 genetic distance fluctuated from 0.09 to 0.13, being generally smaller in years in which there was a relative higher frequency of dominant lineage. Vaccine-like sequences comprised about one fourth of sequences obtained through routine monitoring of PRRS. We found that lineage 5 sequences were mostly Ingelvac PRRS MLV-like. Lineage 8 sequences up to 2011 were 62.9% Ingelvac PRRS ATP-like while the remaining were wild-type viruses. From 2012 onwards, 51.9% of lineage 8 sequences were Ingelvac PRRS ATP-like, 45.0% were Fostera PRRS-like, and only 3.2% were wild-type. For lineage 1 sequences, 0.1% and 1.7% of the sequences were Prevacent PRRS-like in 2009-2018 and 2019, respectively.

Conclusion: These results suggest that repeated introductions of vaccine-like viruses through use of modified live vaccines might decrease within-lineage viral diversity as vaccine-like strains become more prevalent. Overall, this compilation of private data from routine monitoring provides valuable information on PRRSV viral diversity.

Financial support: Swine Health Information Center and NIFA-NSF-NIH.

Poster 18

A post-genome-wide association study testing the effect of a missense mutation in the DIO2 gene on fetal response following maternal PRRSV-2 infection

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Variation in the fetal pig genome may influence the adaptive responses to in utero constraints such as maternal malnutrition or host response to disease challenges such as PRRS virus (PPRSV) infection. Previously, we identified an intergenic single nucleotide polymorphism (SNP) (DRGA0008048 on SSC7) significantly associated with fetal viability in response to maternal PRRSV infection. The *iodothyronine deiodinase 2* (DIO2) gene was located ~14 kb distal to the SNP. As thyroid hormone disruption has been previously associated with fetal response to infection, and DIO2 encodes an outer ring deiodinase enzyme responsible for regulating the bioactivity of thyroid hormone, this gene was selected as a priority candidate for further studies related to fetal viability. Sanger sequencing was used to try to identify possible causative mutations within the exons of the DIO2 gene. The samples were from boars (n=6) bred to gilts (n=22) inoculated with PRRSV-2 (1×10^5 $TCID_{50}$ NVSL 97–7895 total dose) on mean gestation day 86 and euthanized after 21 days and litters characterized for fetal phenotypes. A missense mutation was identified changing asparagine to serine at position 91 of the DIO2 protein in two out of three sires homozygous for the beneficial DRGA allele and was selected for fetal genotyping. This SNP was not found in the gilts. Fetuses (n=153) were genotyped by TaqMan SNP assay, including some fetuses from a previous PRRSV challenge of pregnant gilts (n=78). To test the association of the Asn91Ser (reference homozygotes versus heterozygotes carrying the mutant allele) mutation with fetal phenotypes from PRRSV infected gilts, fetal viability and death, viral load in serum and thymus, serum thyroid hormone levels (T4, T3), body weight and organ weights were determined using univariate linear or logistic mixed-effects regression models. Fetal PRRS disease outcomes were not related to the Asn91Ser mutation, including probabilities of fetuses being viable or dead, fetal viral load in serum or thymus, serum thyroid hormone levels, or fetal growth. Thus, these results did not provide any evidence for the effect of the missense mutation on fetal PRRS outcome in response to maternal PRRSV infection. In conclusion, this suggests that although a missense mutation in DIO2 in partial linkage with the DRGA SNP was identified, it does not affect fetal viability, or the effect was too small to be detected in these studies.

Poster 19

Montanide™ Gel 01 PR, An Adjuvant For Safe and Efficacious Porcine Epidemic Diarrhea and Transmissible Gastroenteritis Bivalent Inactivated Vaccines

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Introduction: Porcine epidemic diarrhea (PED) and Transmissible Gastroenteritis (TGE) are highly contagious, intestinal infectious diseases caused respectively by PED virus (PEDV) and TGE virus (TGEV). These two diseases are characterized by severe diarrhea, vomiting and dehydration. PEDV and TGEV infections can occur in pigs of all ages, infections are most serious in piglets, with high morbidity and mortality. Vaccination is an effective strategy to control PED and TGE diseases. Montanide™ GEL 01 PR (GEL 01) is an innovative adjuvant based on a dispersion of a high molecular weight polyacrylic polymer in water. In this study, we evaluated the immunopotentiating performance and safety of GEL 01 in a PEDV-TGEV bivalent inactivated vaccine.

Materials and Methods:

1- SAFETY TRIAL

- Animals: 3 pregnant sows / group, 3 three-days-old piglets / group
- Antigen: Inactivated PEDV (PEDV/AH2012/12) and Inactivated TGEV (TGEV/JS2012)
- Groups:
    - GEL 01: MONTANIDE™ Gel 01 (10%) + Antigen
    - OIL: Water-in-oil-in-water adjuvant + Antigen
    - PBS: No adjuvant (PBS + Antigen)
    - Control: DMEM (antigen medium without antigen)
- Vaccination: Pregnant sows: One shot, 4 ml, IM, at 4 weeks before delivery, at Houhai acupoint; Piglets: One shot, 1 ml, IM
- Data Collected:
    - On Pregnant Sows: Pyrogenicity, general reactions and local reactions, Effects on farrowing and offspring piglets health
    - On Piglets: Pyrogenicity, clinical signs, and local reaction

2- EFFICACY TRIAL

- Animals: 3 pregnant sows / group and their piglets
- Antigen: Same as in Safety trial
- Groups: Same as in Safety trial
- Vaccination: Two shots, 4 ml each, IM, 6 weeks and 3 weeks before farrowing, at Houhai acupoint
- Data Collected (Duration: 9 weeks)
    - Humoral response assessment: Lactic IgA titer (PED) from sows at farrow, 2 and 4 weeks post farrow. Serum neutralization titer from sows before each injection, at 6 and 3 weeks pre farrow, and at farrow.
    - Challenge procedure: piglets protection through maternal milk. Suckling piglets were challenged at 5 days old via oral administration with 100 LD50 of either TGEV (15/group) or PEDV (15/group). Measurements: Hyperthermia duration, intestinal lesions, and viremia.

Poster 19 (Continued)

Montanide™ Gel 01 PR, An Adjuvant For Safe and Efficacious Porcine Epidemic Diarrhea and Transmissible Gastroenteritis Bivalent Inactivated Vaccines

Results:

1- SAFETY TRIAL

- Safety of single shot in PREGNANT SOWS:
    - Sows body temperature increase was ≤1°C
    - No other systemic reaction observed.
    - No inflammatory reaction at inoculation site
    - No abortion, stillbirth or weak offsprings
- Safety of single shot in PIGLETS:
    - Piglets body temperature increase was ≤1°C
    - Normal breathing, appetite and behaviors
    - No swelling and inflammatory reaction at the injection site
- MONTANIDE™ GEL 01 based vaccine is safe for pregnant sows and for piglets.

Conclusions:

MONTANIDE™ GEL 01:

- Allows the formulation of a protective vaccine against PED and TGE.
- This PED-TEG vaccine is safe for pregnant sows and confers immunity to offspring through milk IgA after birth.
- The vaccine is safe in piglets. A boost vaccine to prolong immunity in piglets could be investigated.

Poster 20

Variation analysis of Swine influenza virus (SIV) H1N1 sequences in experimentally infected vaccinated and non-vaccinated pigs

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Swine influenza is a highly contagious and widely distributed disease that generates important economic losses in the pig industry. Nowadays, one of the most extended strategy used to control Swine influenza viruses (SIVs) is the trivalent vaccine application, which formulation contains the most frequently circulating SIV subtypes H1N1, H1N2 and H3N2. These vaccines do not provide sterilizing immunity against the virus, potentially favoring viral evolutionary dynamics. To better understand the main mechanisms that shape viral evolution, in this work, the SIV intra-host diversity was analyzed in samples collected from both, vaccinated and non-vaccinated animals challenged with H1N1 influenza A virus. In the present study 276 single nucleotide variants were found within 28 whole SIV genomes obtained by next generation sequencing. Differences in nucleotide variants between groups were established and the impact of each substitution found was hypothesized according to previous literature. Substitutions were allocated along all influenza genetic segments, while the most relevant non-synonymous substitutions were allocated in the NS1 protein on samples collected only from vaccinated animals. These substitutions could affect both, mRNA viral translation and pathogenesis. Moreover, new viral variants were found in both vaccinated and non-vaccinated pigs, showing relevant substitutions in the HA, NA and NP proteins that may be contributing to evasion of host immune system, virulence and host adaptation. Overall, results of the present study suggest that SIV is continuously evolving despite vaccine application, therefore new substitutions may increase viral fitness under field conditions.

Poster 21

R2™ for mitigating the risk of PEDV, PRRSV and SVA -contaminated feed using an animal challenge model

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Introduction: Contaminated feed has been confirmed as a vehicle for transmission of several viruses such as PEDV, ASV, SVA, PRRSV etc., These viruses could maintain their infectivity in feed ingredients even after 30-37-day transport, emphasizing the importance to reduce their potential transmission via contaminated feed. Feed Energy's R2 is a patent pending, natural low pKa lipid-based line of products that provides a source of essential fatty acids along with proven feed biosecurity benefits. The objective of this study was to evaluate the efficacy of Feed Energy's R2 product to mitigate or inactivate PEDV, PRRSV and SVA in an infected feed using an animal challenge model, employing controlled field conditions and multiple metrics.

Methods: The study was conducted at the Pipestone Applied Research Biosafety level-2 research facility. One hundred pigs (15kg) were placed per room (6 pens/room) per treatment group during a 15-day trial period. The treatments consisted of a positive control group with no mitigant, and in the treatment group the animals were fed Feed Energy-R2 product. Viral challenge was performed by using a 454g block of ice (-80□C) were dropped into each feed bin on days 0 and 6 of the study containing equal concentration (5 logs TCID50/mL) of PEDV, PRRSV-174 and SVA. Post-mortem samples (from 30 pigs/room at 15dpi) included rectal swabs for PEDV, serum for PRRSV and tonsil for SVA were tested by RT-qPCR and ORF5. In addition, pigs were scored daily for the presence of the following clinical signs: Diarrhea (PEDV), Dyspnea/weight loss/rough hair coat (PRRSV), Lameness/vesicles (SVA). Differences in growth performance between groups were analyzed for significance ($p < 0.05$) using ANOVA.

Results and Discussion: RNA from all three viruses was detected in samples from the positive control group along with evidence of clinical signs suggestive of all three diseases, poor performance (0.14 kg ADG) and elevated mortality (10%). None of the pigs fed Feed Energy R2 showed signs of disease. Despite the presence of low SVA genomic copies in post-mortem samples from R2 group, all pigs demonstrated improved health and performance as compared to controls. Average daily gain improved by > 380% as compared to controls (0.54 kg vs. 0.14 kg, $p < 0.05$), along with no mortality and clinical signs.

Conclusions: The data provided in this study support the risk of contaminated feed as a means of viral entry to farms. Pre-treatment of feed with the Feed Energy-R2 product can effectively mitigate infection in pigs that were exposed to feed contaminated with PEDV, PRRSV and SVA.

Citation: Dee et al. An evaluation of additives for mitigating the risk of virus-contaminated feed using an ice-block challenge model. Transboundary and Emerging Diseases. 2021; 68:833-845. https://doi.org/10.1111/tbed.13749

Poster 22

Impact of fetal PRRSV2 infection on the organ-specific regulation of cell division

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Objective: Infection with porcine reproductive and respiratory syndrome virus (PRRSV) during late gestation is known to negatively affect fetal cell division. However, the relative scale of this effect across a wider array of fetal organs and the underlying regulatory mechanism are unknown. The objective of this study was two fold: first, to identify the fetal organs most severely impacted following infection, and second, to evaluate the relationship between organ specific gene expression and phenotypic factors such as fetal viability, viral load, endocrine status and morphometrics.

Methods: Twenty-seven pregnant Yorkshire gilts were bred to Landrace sires and either experimentally challenged with PRRSV2 strain NVSL 97-7895 (n=22) or sham inoculated (n=5) at gestation day 85. Gilts and fetuses were humanly euthanized at 21 days post infection and a wide array of fetal organs including heart (HRT), liver (LVR), lung (LNG), thymus (THY), kidney (KID), spleen (SPN) and loin muscle (MUS) collected. In experiment 1, gene expression for three cell cycle promoters (CDK1, CDK2, CDK4) and one inhibitor (CDKN1A) were evaluated in a previously established model of biologically extreme phenotypic subsets including control (CON n=10), uninfected (UNIF n=10), high viral load viable (HV-VIA n=10) and high viral load meconium-stained (HV-MEC n=10) fetuses. In experiment 2, total RNA was extracted from an additional HRT (n=90) and KID (n=84) samples from fetuses showing at least some level of systemic infection (serum viral load >0.74 log10). The normalized expression of CDKN1A was then evaluated relative to existing phenotypic data including serum thyroid hormones (T3 & T4) which are depressed following infection, viral load in serum and thymus, and specific organ and fetal weights.

Results: In experiment 1, no significant differences were found between CON and UNIF groups for any of the four genes of interest. All tissues except LVR showed significant down-regulation of at least one CDK gene in high-viral load fetuses relative CON, with all three significantly down-regulated in the HRT. Significant up-regulation in CDKN1A was observed in high viral load fetuses relative to controls in all tissues except MUS, with the largest up-regulation in the HRT and the lowest significant up-regulation found in the LNG. In experiment 2, expression of CDKN1A in the HRT and KID were significantly correlated with one another. CDKN1A expression was also highly correlated with serum and thymic viral load as well as fetal T4, but there was no significant relationship with T3, fetal weight or specific organ weight.

Conclusions: Evidence of supressed cell division was entirely restricted to the infected fetuses. While HRT was the most severely impacted, altered gene expression was detected in all tissues evaluated. This, in combination with the high correlation between CDKN1A expression in the KID and HRT, is evidence for a central regulatory mechanism controlling post infection cell division. T4 and viral load are highly correlated with the expression of CDKN1A but further research is required to differentiate between cause and effect.

Poster 23

Primary porcine respiratory epithelial cells self-limit SARS-CoV-2 replication possibly by undergoing rapid apoptosis

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The ability of SARS-CoV to infect different species, including humans, dogs, cats, minks, ferrets, hamsters, tigers, and deer, pose a continuous threat to human and animal health. Pigs, though closely related to humans, seem to be less susceptible to SARS-CoV-2. Former in vivo studies failed to demonstrate clinical signs and transmission between pigs, while later attempts using a higher infectious dose reported viral shedding and seroconversion. This study investigated species-specific cell susceptibility, virus dose-dependent infectivity, and infection kinetics, using primary human (HRECs) and porcine (PRECs) respiratory epithelial cells. Despite higher ACE2 expression in HRECs compared to PRECs, SARS-CoV-2 infected, and replicated in both PRECs and HRECs in a dose-dependent manner. Cytopathic effect was particularly more evident in PRECs than HRECs, showing the hallmark morphological signs of apoptosis. Further analysis confirmed an early and enhanced apoptotic mechanism driven through caspase 3/7 activation, limiting SARS-CoV-2 propagation in PRECs compared to HRECs. Our findings shed light on a possible mechanism of resistance of pigs to SARS-CoV-2 infection, and it may hold therapeutic value for the treatment of COVID-19.

Poster 25

Inactivation of two swine viruses on shoes by BioSec, a shoe-sanitizing station

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Enhancement of biosecurity measures is critical to prevent and control pathogen transmission in swine farms. Soles of shoe are known as a significant source of pathogenic microorganisms. Indirect transmission of high economic impact diseases such as those caused by Porcine Reproductive and Respiratory Syndrome virus (PRRSv) and Porcine Epidemic Diarrhea virus (PEDv) by shoes and other fomites, has been described. BioSec is a shoe sanitizing station that combines Ozone + UVC (UVZone) technology and has been found effective against bacterial pathogens. The objective of this study was to assess the inactivation rate of PRRSv and PEDv on rubber soles and polyblend boot material by BioSec.

The PRRSv and PEDv were propagated and titrated in Marc-145 and Vero-81 cells, respectively. Approximately 1cm2 coupons of rubber sole and polyblend boot material were cut. The coupons were placed in a sterile 24-well tissue culture plate and were contaminated with 40 µl of PEDv or PRRSv per coupon. The virus inoculum was air-dried for about 1 hour in a biosafety cabinet (BSC). Subsequently, four of the virus-spiked coupons were placed on the left side of the BioSec machine. An operator stepped on the right side of the machine to activate it for a standard time of eight seconds as specified by the manufacturer. After treatment, coupons were removed aseptically, and the surviving virus was eluted from them using an eluent solution (3% beef extract-0.05M glycine). Four virus-spiked non-BioSec treated coupons were used as negative control. Serial 10-fold dilutions of eluates from treated and non-treated coupons were prepared in MEM. All dilutions were inoculated in monolayers of appropriate cells contained in 96-well microtiter plates using 3 wells per dilution. Plates were examined daily for the appearance of virus-induced cytopathic effects (CPE). After 7 days of incubation, virus titers were calculated (Karber, 1931) and expressed as log10 TCID50/mL.

On an average, ¬>99% of the PRRSv was inactivated on both rubber sole and polyblend boot material. In addition, an average of 98.55 % and ¬>99% of PEDv was inactivated on rubber sole and polyblend material, respectively. These findings demonstrate the efficacy of BioSec inactivating swine pathogens in shoe materials and its potential use to enhance biosecurity practices in swine farms.

Poster 26

Oral delivery system for a rapid-response porcine epidemic diarrhea virus (PEDV) vaccine

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The recent increase in the number of emerging infectious diseases, a majority of which are caused by RNA viruses, necessitate improved approaches for rapid response vaccine development and delivery. A novel rapid attenuation method for RNA viruses was previously developed by reversibly denaturing the viral capsid by exposure to gentle heat, followed by fragmentation of the genome by RNAse treatment. Virions with attenuating mutations produced as result of RNA damage and repair were rescued by serial passage in cell culture. The heat and RNAse treated porcine epidemic diarrhea virus (PEDV) vaccine was highly effective and safe in weanling piglets. To address the challenges with inducing strong lactogenic immunity in sows, which is critical for neonatal protection, a novel, biodegradable oral vaccine delivery system designed to protect the vaccine antigen against the acidic gastric environment and targeting delivery of the cargo to enterocytes was developed. Following optimization with various molar ratios of the ingredients, an antigen loading capacity of 80% with negligible cytotoxicity in Vero cells and vesicle sizes of 400-1000nm were observed. The efficacy and safety of the vaccine and oral delivery system is currently under test in a pregnant sow model. The developed rapid-attenuation strategy and oral delivery platforms have potentially broad applications to other newly emerging RNA viruses and enteric pathogens.

Poster 28

Host response in the porcine lymph node to infection by PRRSV 2, Influenza B and their coinfection

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Both Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and influenza B (IBV) cause natural infections in pigs. PRRSV is the more common swine infection and can develop coinfections with IBV, a zoonotic virus able to infect humans. As a primary infection PRRSV can suppress the host immune system, leaving pigs susceptible to secondary infections such as IBV and contributing to the enormous economic impact of the disease. This study investigates the host transcriptomic response in the lymph node following PRRSV and IBV infections as well as their coinfection. Seronegative pigs 3 to 4 weeks old were split into four treatment groups: control; intranasally infected with Type 2 PRRSV NPB strain; intranasally infected with B/Brisbane/60/2008 virus; or intranasally coinfected with both viruses. Three pigs from each of the four treatment groups were necropsied 5 days post-infection(dpi) and lymph node samples were collected for transcriptomic analysis. Differentially expressed gene (DEG) analysis was carried out using DeSeq2 based on the model treatment + E. The coinfected group had 13 DEGs which were not significant in either independent infection. Many of these genes play a role in the glycerolipid metabolism, membrane transport, and MAPK pathways which suggests the creation of a suitable environment for viral replication which may allow the lymph nodes to function as a reservoir and establish a persistent infection.

Poster 31

Novel features of the PRF signal in the nsp2 region of emerging PRRSV variants

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There has been a recent emergence of PRRSV variants causing increased mortality in growing pigs and reproductive failure in sows. Our previous studies demonstrated that a -2/-1 programmed ribosomal frameshifting (PRF) signal located within the nsp2 region directs the production of two frameshifting products, nsp2TF and nsp2N. In historical PRRSV strains, ribosomes that make a -1 PRF immediately encounter a stop codon, terminating translation of the -1 reading frame to produce the truncated nsp2 product, named nsp2N. In this study, we analyzed the -2/-1 PRF sequence region of 74 PRRSV field isolates from 2015 to 2021 in the US. Sequencing revealed that 83.8% (62 of 74) of the strains contain substitutions that disrupt the -1 PRF stop codon, thus extending the nsp2N protein by an additional 14, 16 or 23 amino acids at the C-terminus. Further sequence analysis of the global database in GenBank demonstrated that the emergence of -1 PRF stop codon variants could be traced back in PRRSV sequences as far as 1992 and that the percentage of these variants quickly increased after 2011 (up to 59.5% of the total sequences analyzed). Ten different -1 PRF stop codon variant patterns were identified and two of them are predominantly circulating in the field. More importantly, these dominant variants were mostly reported from swine farms experiencing PRRSV outbreaks with increased mortality/morbidity, including the PRRSV RFLP 1-4-4 lineage 1C variant that has recently outbroken in several swine farms. To determine whether -1 PRF stop codon variation correlates with viral fitness, recombinant viruses containing different mutation patterns were constructed by reverse genetics, using PRRSV-2 isolate SD95-21 as the backbone virus. Compared with the parental SD95-21 virus, two mutants representing the two dominant variant patterns showed similar growth kinetics, while the rest of the mutants showed significantly reduced growth kinetics, suggesting that the most dominant -1 PRF stop codon variant patterns provide the best growth ability for the virus. This study provides novel insight into the genetic features contributing to PRRSV evolution with potential links to field virulence.

Poster 32

Characterization of PRRSV in clinical samples and the MARC-145 and/or ZMAC cell culture isolates

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Isolation of porcine reproductive and respiratory syndrome virus (PRRSV) is a primary means of obtaining isolates for autogenous vaccine production and other applications. However, it is unknown if cell culture isolates and virus in the clinical sample are equivalent. This study evaluated the consistency of RFLP patterns, genetic lineages, and sequences of PRRSV detected in clinical samples and cell culture isolates. This study compared PRRSV ORF5 sequences from 1,023 clinical samples (994 PRRSV-2, 26 PRRSV-1, and 3 PRRSV-1 and PRRSV-2 PCR-positive) and their isolates in MARC-145 and/or ZMAC cells. All 26 PRRSV-1 PCR-positive clinical samples and their isolates had 99.3-100% nucleotide (nt) identity, indicating the same PRRSV in the clinical sample and the corresponding cell culture isolate. For 3 clinical samples that were PCR positive for PRRSV-1 and PRRSV-2, both strains were isolated in ZMAC cells with 99.7-100% ORF5 nt identity to the respective viruses in clinical samples. Either PRRSV-1 or PRRSV-2, but not both, was obtained in MARC-145 cells with 99.8-100% ORF5 nt identity between the clinical samples and isolates. Comparison of sequences between PRRSV-2 PCR-positive clinical samples (n=994) and their isolates resulted in three categories. In category 1 (957/994=96.3%), ORF5 sequences had the same RFLP patterns and genetic lineages with 98-100% nt identity, suggesting the same virus strain present in clinical samples and isolates. In category 2 (24/994=2.4%), ORF5 sequences from clinical samples and their isolates had the same genetic lineages and 98.6-99.8% nt identity but different RFLP due to nucleotide mismatches at HincII or SacII cut sites although PRRSV were considered similar. In category 3 (13/994=1.3%), ORF5 sequences from clinical samples and their MARC-145 isolates had different RFLP patterns, different genetic lineages, and 84.4-88.7% nt identity, indicating different virus strains. In contrast, ORF5 sequences from clinical samples and ZMAC isolates had same RFLP patterns, same genetic lineages, and 99.5-100% nt identity, suggesting the same virus strains. The data suggested the possibility of a mixed infection of ≥2 PRRSV strains in these category 3 clinical samples. Vaccine-specific PCR and NGS performed on the selected five of these clinical samples and isolates indicated a mixed infection. In summary, PRRSV ORF5 sequences from clinical samples and isolates matched each other for majority of the cases. However, PRRSV between clinical samples and isolates and between the MARC-145 and ZMAC isolates could occasionally have different sequences. Characterizing PRRSV sequences from clinical samples and cell culture isolates should be conducted before using isolates for autogenous vaccines or other applications.

Poster 34

Establish pregnant sow-fetus models to assess safety and efficacy of influenza vaccines

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The ability of maternally derived immunity to provide fetal/neonatal protection has increased attention to maternal immunization. In our previous studies, we constructed a candidate influenza vaccine, in which a chimeric HA construct, HA-129, was expressed in the context of a whole virus backbone of A/swine/Texas/4199-2/98-H3N2 as a recombinant virus (TX98-129). When evaluated in a nursery pig model, the TX98-129 had ability to induce broadly protective immune response against genetically diversified influenza viruses [McCormick et al., 2015; PLoS One, 10(6):e0127649]. In the current study, we established pregnant sow-fetus models to evaluate the safety and efficacy of this candidate vaccine. In pregnant sows, the results consistently showed that TX98-129 induced an immune response against the TX98-129 virus and the parental viruses that used to construct the chimeric HA antigen. After challenge with a virulent IAV, a significant increase in antibody titers was observed in vaccinated sows at 5 and 22 days post challenge (dpc), and challenge virus was detected in nasal swab of only one vaccinated sow with low titer (10E+1.6 TCID50/mL) at 5 dpc. Challenge virus was not detected in the fetuses. A panel of immune cytokine genes was analyzed in blood and tissue (lung) samples collected from sows. The results showed that expression levels of IFN-α and IL-1β were higher in the lung of vaccinated sows than those of un-vaccinated pigs at 5 dpc. T cell subpopulation analysis conducted in PBMCs showed a relatively higher ratio of CD4CD8 and CD8 cells in vaccinated sows at 22 dpc after stimulation of PBMCs with either challenge virus or vaccine. Next, we evaluated vaccine-induced maternal passive immunity. Neonatal piglets from both immunized and unimmunized sows were challenged at 3 days post-farrowing and results showed that immune cytokines including IFN-α, IFN-γ, IL-15, and TNF-α were upregulated in newborn piglets from immunized sows. Accordingly, an increased antibody titer and deceased viral shedding were observed from neonates born from immunized sows. These results indicate that the challenge virus was cleared due to high level expression of inflammatory cytokines and relatively higher cell-mediated immune response in vaccinated sows. In addition, vaccine-induced maternal passive immunity was able to protect neonatal pigs from influenza virus infection. Taken together, this study provides a comparative swine model system to study the effect of influenza vaccine in host (maternal) immunity and fetal development.

Poster 35

Control and elimination of PRRSV from a 38,000 sow system

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Herein, we describe a strategy that successfully detected and eliminated PRRSV from a large PRRSV-negative production system in 12 weeks. The strategy had One Key Rule: one PRRSV positive test result led to the immediate depopulation of the barn.

Week 0: Presumed PRRSV-negative gilts (1,200) from a GDU were delivered to 7 farrowing sites
To assure PRRSV-negative gilts, one oral fluid (OF) sample from each pen of gilts was collected 24 hours prior to delivery and tested by PRRSV RT-qPCR.

Week 1: Suspect test results in 2 of 6 OFs from the GDU were observed and gilt deliveries were stopped. Gilts that had already been delivered were sampled at the rate of one OF and 5 serum samples per pen. Five sites were determined to have received PRRSV-positive gilts and 5 barns were depopulated (~2,000 gilts).

Week 2-8: Sows held in single crates were surveilled over 3 wks by sampling subgroups at the rate of 1 OF per 2 sows (pool of 3, 6 sows/sample). The 5 emptied barns were repopulated (~5,000 gilts total) at wk 8 and surveilled by sampling serum from subgroups of gilts and 1 OF from all source site pens, at 7d and 24h (PCR), and 72h (PCR) and 7d (ELISA) post arrival.

Week 9-10: The 19,000 replacement gilts to repopulate the GDU were sampled following the same protocol than above, except for testing on ELISA 10d post arrival instead.

Week 10-11: The GDU resumed normal operations, receiving weaned pigs and delivering PRRSV-negative gilts.

Summary:

- Among 14 farrowing sites (~67,000 sows) in the system, PRRSV was detected in 7 sites (~38,000 sows) and eliminated from 6 of these sites within 1 week (2,000 gilts were removed). Operations returned to normal in 6 of 7 sites within 8 weeks of initiating the control protocol.
- Gilts delivered to the smallest site (1,800 sows) had been mixed with weaned sows and the barn depopulation strategy was not effective. Therefore, an elimination plan was implemented.
- A total of ~8,000 PRRSV PCRs and ~5,000 ELISAs were performed in the company-owned laboratory. This strategy avoided the infection of 38,000 sows in an additional 7 farrowing sites and avoided losses estimated at ~200,000 pigs to market.
- In response to these events, surveillance in the GDU was increased; 1) 4 OF per barn one week prior to delivery and then 2) one OF plus 5 serum samples per pen 24 hours prior to delivery.

Conclusions:

- Including aggregate samples in the surveillance program provided early PRRSV detection.
- The on-site laboratory provided rapid diagnostic results and facilitated timely decisions.
- Monitoring quarantine and transfer facilities, plus segmented uploading practices, is essential to prevent PRRSV spread.

Poster 37

Genome wide association study of thyroid hormone levels in piglets and fetuses following challenge with PRRSV

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Thyroid hormones affect a wide range of biological processes, such as growth, metabolism, and development, and the link between thyroid hormones and innate and adaptive immunity is well established. Previous research has shown that piglet and fetal serum thyroid hormone (i.e., T3 and T4) levels decrease rapidly in response to PRRSV infection. However, the genetic control of T3/T4 homeostasis during infection is not completely understood. We hypothesized that thyroid hormone levels have potential as novel biomarkers for animal resilience. Our objective was to estimate genetic parameters and identify quantitative trait loci (QTL) for T3 and/or T4 levels of piglets and fetuses challenged with PRRSV. Sera from 5-week-old pigs (N=1792) at 11 days post inoculation (dpi) with PRRSV were assayed for T3 levels (piglet_T3). Sera from fetuses (N=1267) at 12 or 21 days post maternal inoculation (dpmi) with PRRSV of sows (N=150) in late gestation were assayed for T3 (fetal_T3) and T4 (fetal_T4) levels. Animals were genotyped using 60K Illumina or 650K Affymetrix SNPs panels. Heritabilities and phenotypic and genetic correlations were estimated using ASREML; genome wide association studies were performed for each trait separately using JWAS. All three traits were low to moderately heritable (10-18%). For piglet_T3 levels, phenotypic and genetic correlations with weight gain (0-42 dpi) were 0.26±0.03 and 0.67±0.14, respectively. Nine significant QTL were identified for piglet_T3, on SSC3, 4, 5, 6, 7, 14, 15, and 17, and collectively explained 30% of the genetic variation (GV), with the largest QTL, on SSC5, explaining 15% of the GV. For fetal_T3 levels three significant QTL were identified, on SSC1 and SSC4, and collectively explained 10% of the GV. For fetal_T4 levels five significant QTL were identified on SSC1, 6, 10, 13, and 15, collectively explaining 14% of the GV. Overlapping QTL for ≥ 2 traits were identified on SSC4, 6, and 15. Several putative immune-related positional candidate genes (i.e., within 200 Kb of QTL) were found including ADORA3, CD53, CD247, CSF1, IRF8, and MAPK8. Overall, thyroid hormone levels in PRRSV challenged animals were low to moderately heritable; piglet_T3 levels had a high genetic correlation with weight gain; several QTL that explained a substantial amount of the GV were identified, as were several pleiotropic QTL; and positional candidate genes with biological relevance were identified. Collectively, our results suggest thyroid hormone levels may be promising biomarkers for genetic improvement of resilience during PRRSV challenge.

David Benfield Award Student Winners

Best Oral Presentation:

1st place: **Fangfeng Yuan, University of Illinois**
Development of a Blocking Enzyme-Linked Immunosorbent Assay for Detection of Antibodies against African Swine Fever Virus

2nd place: **Chia-Ming Su, University of Illinois**
Mediation of Type 1 IFN Signaling by PRRSV nsp5-related Protein

Best Poster Presentation in Section of Vaccine and Diagnostics:

1st place: **Wannarat Yim-im, Iowa State University**
Characterization of PRRSV in clinical samples and the MARC-145 and/or ZMAC cell culture isolates

2nd place: **Allison Blomme, Kansas State University**
Use of a viral isolation assay for detection of Porcine Reproductive and Respiratory Syndrome Virus in feedstuffs

Best Poster Presentation in Section of Virus and Host Interaction:

1st place: **Xingyu Yan, University of Illinois**
Novel features of the PRF signal in the nsp2 region of emerging PRRSV variants

2nd place: **Shamiq Aftab, South Dakota State University**
Antiviral Roles of Interferon Induced Transmembrane 3 (IFITM3) Protein on Seneca Virus A (SVA) Replication

Pictured above are the symposium organizing committee members and student winners of David Benfield Award Competition for their oral and poster presentations.

From left to right: Dr. Ying Fang, Dr. Hiep Vu, Dr. Diego Diel, Dr. Roman Pogranichniy, Dr. Andreia Arruda, Dr. Scott Kenney, Chia-Ming Su, Fangfeng Yuan, Wannarat Yim-im, Allison Blomme, Xingyu Yan, and Shamiq Aftab.

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