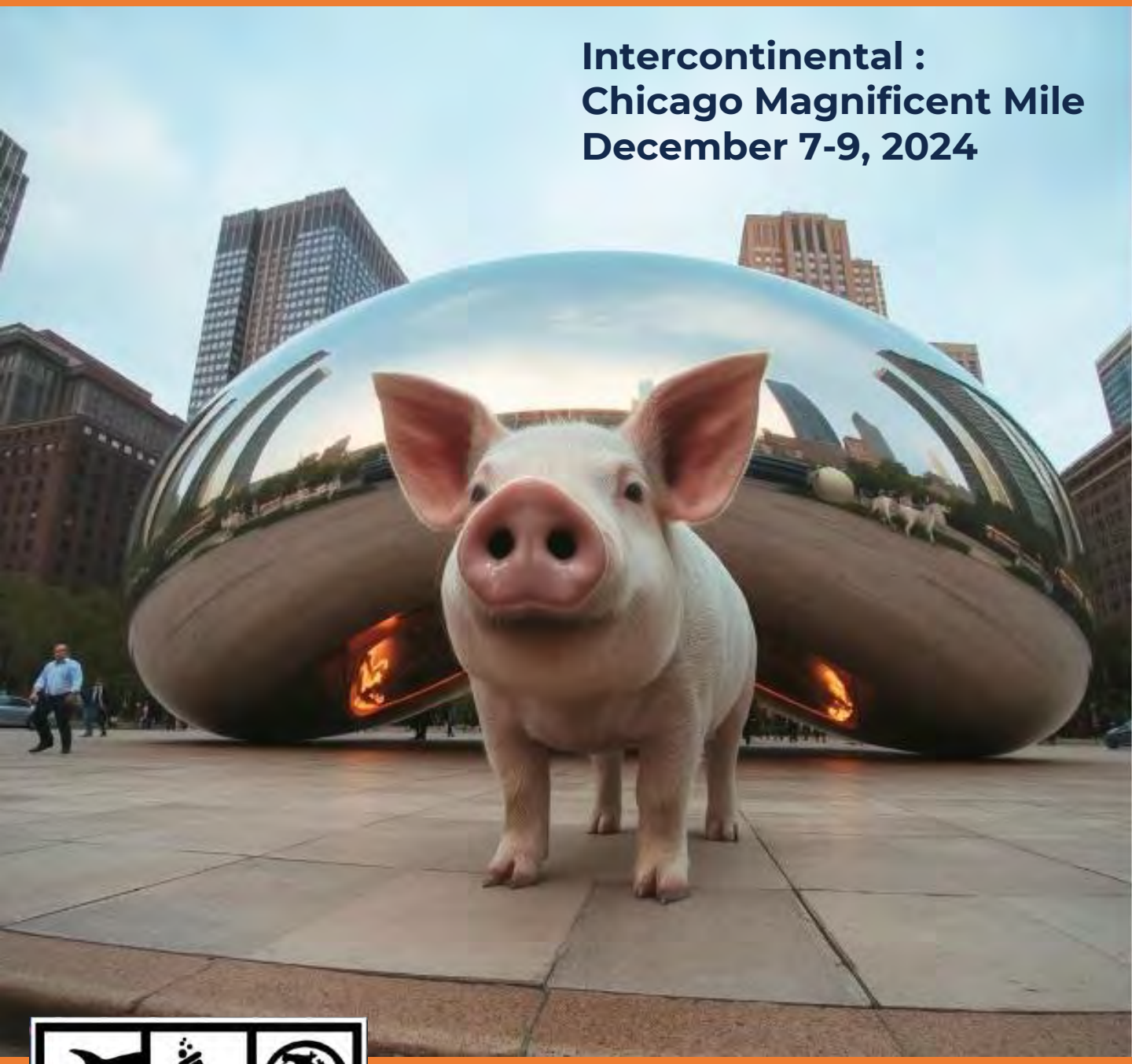
2024 NAPRRS/NC229: International Conference of Swine Viral Diseases



Intercontinental : Chicago Magnificent Mile December 7-9, 2024

NORTH AMERICAN PRRS SYMPOSIUM

Proceedings

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Proceeding editors: Pablo Pineyro, Mariana Kikuti, Ying Fang, Raymond (Bob) Rowland

Welcome to 2024 NAPRRS/NC229 ICSVD

Dear Friends and Colleagues,

Welcome to Chicago and the 21st annual NAPRRS/NC229 International Conference of Swine Viral Diseases (ICSVD)! Since its start in 2003 as the North American PRRS (NAPRRS) Symposium, this event has evolved into a premier global forum dedicated to advancing research and collaboration on swine viral diseases. We're thrilled to have you join us for this year's gathering.

The conference remains rooted in collaboration, with NAPRRS/NC229's early meetings paving the road for the inclusive and dynamic discussions we have today. What began as a small, closed session among NC229 representatives has grown into a fully open, international conference where industry, academia, and government join forces to tackle global emerging challenges in swine health.

This year's program futures with three keynote talks to spotlight the recent outbreaks of HPAI H5N1 virus and the emergence of highly virulent European PRRSV-1 in swine herds. The program contains five plenary sessions, a biosecurity session in partnership with the Swine Health Information Center, two industry-sponsored lunch sessions highlighting advances in swine health technologies. The poster session on Monday afternoon, offers a forum for discussing research findings and exploring new research collaborations. Our traditional evening banquet, now on Monday evening, will present graduate student awards and honor two prestigious scientists on their significant contributions to the swine health field.

We warmly welcome our attendees and are grateful for the excellent abstract submissions that make this event a success. We hope this conference not only provides you with valuable insights for your work but also fosters connections that will drive innovation in swine health. We also extend our sincere gratitude to our sponsors, whose support is essential to the success of this meeting.

Enjoy the conference and the beautiful city of Chicago!

Ying Fang, Executive Director
Mariana Kikuti & Pablo Pineyro, co-chair of Scientific Committee
Raymond (Bob) Rowland, NAPRRS/NC229 advisor

2024 NAPRRS/NC229 ICSVD Organizing Committees

NAPRRS/NC229 Advisor:

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University of Illinois

Executive Director:

Dr. Ying Fang
University of Illinois

Planning Committee:

Dr. Jose Angulo
Zoetis Animal Health

Dr. Lisa Becton
Swine Health Information

Dr. Jay Calvert
Zoetis Animal Health

Dr. John Harding
University of Saskatchewan

Dr. Enric Mateu
Autonomous University of Barcelona

Dr. Hans Nauwynck
Ghent University

Dr. Megan Niederwerder
Swine Health Information Center

Dr. Hanchun Yang
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Dr. Mariana Kikuti
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Dr. Pablo Pineyro
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Committee Members:

Dr. Andreia Arruda
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Dr. Hiep Vu
University of Nebraska

Dr. Laura Miller
Kansas State University

Dr. Alex Pasternak
Purdue University

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University of Illinois

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2024 NAPRRS/NC229 ICSVD
David Benfield Award Competition Judges

Dr. Alex Pasternak
Purdue University, USA

Dr. Kimberly VanderWaal
University of Minnesota, USA

Dr. Enric Mateu
Universitat Autònoma
de Barcelona, Spain

Dr. Laura Miller
Kansas State University, USA

Dr. Henry Osemeke
Iowa State University, USA

Dr. Mariana Kikuti
University of Minnesota, USA

Dr. Hiep Vu
University of
Nebraska-Lincoln, USA

Dr. Pablo Pineyro
Iowa State University, USA

Dr. Jianqiang Zhang
Iowa State University, USA

Dr. Reid Phillips
Boehringer Ingelheim
Vetmedica, USA

Dr. Jose Angulo
Zoetis Animal Health, USA

Dr. Yanhua Li
Yangzhou University, China

2024 NAPRRS/NC229 Fellow Award

Jay Calvert: An Industry Partner & Friend

Dr. Jay Calvert grew up in Northern Illinois on the banks of the Fox River, surrounded by forests and corn fields. At the age of 9, he watched the first men walk on the moon, inspiring a lifetime interest in space and rocketry to compliment his ongoing fascination with biology. He wanted to travel to Mars and characterize the indigenous life forms (or their fossil remains) when he grew up, but that goal has not (yet) been achieved. In 1977, Jay graduated from Barrington High School with a strong science background including advanced placement Physics and Chemistry, as well as more Biology. This is in spite of the fact that his gang of friends resembled the cast of *That 70's Show*. Jay moved downstate and earned a B.S. from Eastern Illinois University in 1981. He was the only graduate in his class with two majors (Zoology and Environmental Biology) and two minors (Chemistry and Botany). In 1988 he received a Ph.D. in Genetics from Purdue University where he fell in love with the genome structures and gene expression mechanisms employed by viruses. It was also during this period that he fell in love with and married his wife Wendy, 41 happy years so far.

He conducted his postdoc training at the University of Guelph in Ontario (1988-1990), where he characterized the covalent linkage between the 5' end of the birnavirus genomic RNA and the VPg protein. He completed a second postdoc (1990-1994) at the USDA-ARS Avian Disease and Oncology Laboratory in East Lansing Michigan.

In 1994, Jay accepted a research position with SmithKline Beecham Animal Health (SBAH) in Lincoln, Nebraska. A year later, Pfizer bought SBAH. In 2012, Pfizer spun off its animal health division to form Zoetis, where he still works today as a research director in Kalamazoo. Jay began his work on PRRS in 1994 and never stopped. In the late 1990's he determined one of the first full length sequences of a PRRSV-2 virus and completed the first full-length cDNA infectious clone of PRRSV-2 in 1998. The GFP-expressing version, affectionately named Kermit, was key in his characterization of CD163 as an important receptor for PRRSV. The distribution of "Kermit" and other reagents throughout the PRRS research community is responsible for the acceleration of several important discoveries.

Dr. Calvert was the Project Team Leader for the research phase of both Fostera PRRS (PRRSV-2) and Suvaxyn PRRS MLV (PRRSV-1). Both vaccines show safety and efficacy in pigs as young as one day of age.

Jay has been an active member of the PRRS research community since 1994, including participation in CAP-1 and CAP-2, NC-229, presentations at many PRRS and Nidovirus symposia, and managing the ongoing Zoetis sponsorship of the North American PRRS Symposium along with many successful research collaborations with universities.

He turned 65 this year and plans to retire in 2025. He's looking forward to spending more time with Wendy and his son Alex, fishing, building, and flying rockets, and tasting every new craft beer that comes out. He says that he will miss the wisdom and camaraderie of his friends in the PRRS research community. Don't forget to buy him a beer.

2024 NAPRRS/NC229 Fellow Award

The Journey of a Woman Scientist Role Model

Dr. Joan Lunney grew up in Philadelphia, PA, earning a B.S. in Chemistry from Chestnut Hill College. After teaching high school chemistry in Camden, NJ, she pursued her Ph.D. in Biochemistry at the Johns Hopkins University, Baltimore MD, performing her research on glycoprotein receptors at the National Institutes of Health (NIH). For her postdoctoral research, she pursued an immunology focus and selected the laboratory of Dr. David Sachs who studied swine as an organ transplantation model. There she characterized the swine major histocompatibility complex (MHC), the swine leukocyte antigen (SLA) complex, genes and proteins that regulate organ transplantation acceptance/rejection. She also produced the first monoclonal antibodies reactive with swine immune T cell subset. She continues to produce publicly available monoclonal antibodies against immune cells and cytokines. These efforts are instrumental in unraveling the mysteries of pig immunity.

In 1983 she was offered a position at the USDA ARS lab at the Beltsville Agricultural Research Center, Beltsville, MD, exploring genetic control of swine responses to the parasites, *Trichinella spiralis*, *Toxoplasma gondii*. Starting in 1989, she was selected to serve on the Animal Genome Mapping Committee and coauthored the 1991 report to Congress on "Implementation Plans for Joint Animal Germplasm Program and Animal Genome Research Program." This work set the stage for several swine genome mapping projects, including her leadership in the annotation of the porcine immunome.

In the early 2000s, Dr. Lunney changed her research to the study of PRRSV. She led the effort to establish the PRRS Host Genetics Consortium (PHGC), a first of its kind collaboration between swine breeding companies and researchers. The initial investment of $850K from the National Pork Board was followed by $15M combined funding from USDA and Genome Canada. This effort resulted in the identification of a genetic allele in *GBP5* that is associated with improved PRRSV resistance, including increased weight gain and decreased virus load. Dr. Lunney was an active participant of NC229 and the PRRS Coordinated Agricultural Project (PRRS CAP). She organized several symposia and edited 2 special journal issues on PRRS research.

In recognition of her research contributions, Dr. Lunney has received several awards, including Distinguished Veterinary Immunologist by American Assn of Veterinary Immunologists (1996), Fellow of the American Association for the Advancement of Science (1998), recognition by the International Society for Animal Genetics (2017), and the Conference of Research Workers in Animal Disease (2022). She was named a member of the USDA ARS Hall of Fame (2019), received the U.S. Presidential Rank Award as a Meritorious Senior Professional (2022), and the International Union of Immunological Societies, Veterinary Immunology Committee's Distinguished Veterinary Immunologist Award (2023).

Perhaps her greatest recognition comes from her dedication to the mentoring of young scientists, particularly women. Over the past 30 years, she has established and maintained several friendships within the PRRS community.

Dr. Lunney lives in Bethesda MD; her husband Will Idler passed away in 2018. She enjoys traveling, most recently to the Galapagos Islands, and spending time at her second home on the Choptank River on Maryland's Eastern shore.

2024 NAPRRS/NC229 ICSVD
Boehringer Ingelheim Travel Fellowship Awardees

Akhila Naru
University of Illinois

Chi Chen
University of Illinois

Fangfeng Yuan
Massachusetts Institute of Technology

Jing Huang
University of Minnesota

Joao Paulo Herrera da Silva
University of Minnesota

Julia Baker
University of Minnesota

Junyu Tang
University of Illinois

Kassandra Durazo Martinez
University of Nebraska-Lincoln

Kristen Walker
USDA ARS/Morgan State University

Laurie Touchard
University of Missouri

Lu Yen
Iowa State University

Marcello Melini
University of Minnesota

Mehak Kapoor
Iowa State University

Nakarin Pamornchainavakul
University of Minnesota

The Nguyen
University of Nebraska-Lincoln

Trevor Arunsiripate
Iowa State University

Xiaomei Yue
University of Minnesota

2024 NAPRRS/NC229 ICSVD
David Benfield Awardees

Best Oral Presentation

1st Place: Trevor Arunsiripate, Iowa State University
2nd Place: Molly Kroeger, Iowa State University
2nd Place: Ethan Aljets, Iowa State University
3rd Place: Julia Baker, University of Minnesota

Best Poster Presentation

1st Place: Bala Mounika Reddi, Iowa State University
1st Place: Marcello Melini, University of Minnesota
2nd Place: Akhila Naru, University of Illinois
3rd Place: Jing Huang, University of Minnesota

Keynote Speaker: Daniel Perez

College of Veterinary Medicine
UNIVERSITY OF GEORGIA

Wings of Worry: The Avian Flu Pandemic Potential

8:10 – 9:10 AM, December 8, 2024

Keynote Speaker: Enric Mateu

Autonomous University of Barcelona, Spain

Emergence of PRRSV-1 strains of enhanced virulence in Europe: *overview and evolution*

3:30 – 4:15 PM, December 9, 2024

Keynote Speaker: Andrew Bowman

College of Veterinary Medicine
The Ohio State University

Influenza A virus in swine: the expanding problem

7:15 – 8:00 PM, December 9, 2024

2024 NAPRRS/NC229 ICSVD PROGRAM

Saturday, December 7

1:00 - 6:00 pm Conference check-in/onsite registration (*2nd Floor Foyer*)

4:00 - 5:00 pm NAPRRS/NC229 organizing committee meeting (*Michigan, 5th Floor*)

Sunday, December 8

(Plenary sessions in Renaissance Ballroom, 5th Floor)

8:00 - 8:10 am: Ying Fang, Bob Rowland:
Opening remarks

8:10 - 9:10 am: Keynote talk: Daniel Perez
Wings of Worry: The Avian Flu Pandemic Potential

Session 1: From Entry to Viral Infection

Moderators: Joan Lunney, Dongwan Yoo

9:10 - 9:25 am: Alberto Brandariz Nuez
Role of N-glycosylation in PRRSV infection

9:25-9:40 am: Yanhua Li
The dimerization of PRRSV nsp1alpha is important for viral subgenomic RNA synthesis

Lightning Talks

9:40 - 9:45 am: Kassandra Durazo-Martinez
Cross-Species Analysis of CD163 Orthologs Reveals Key Domain for PRRSV Susceptibility

9:45 - 9:50 am: Chi Chen
An intra-family conserved high-order RNA structure within the M ORF is important for PRRSV subgenomic RNA accumulation and infectious virus production

9:50 - 9:55 am: Junyu Tang
A novel reverse genetics system for PRRSV and the generation of a transcription network-reprogrammed virus

9:55 - 10:00 am: Lu Yen
Segment-Specific Enteroids from Pig Small Intestine in Matrigel and Transwell Inserts: Susceptibility to Porcine Epidemic Diarrhea Virus (PEDV)

10:00- 10:30 am Break

Session 2: Insight into Viral Pathogenesis and Host Response

Moderators: Xiuqing Wang, Federico Zuckermann

10:30 - 10:45 am: Federico Zuckermann
A plausible mechanism responsible for the variation of PRRS virus virulence

10:45 - 11:00 am: Xufang Deng
Defining Interferon Antagonism Hierarchy of Porcine Epidemic Diarrhea Virus

11:00 - 11:15 am: Xiuqing Wang
CRISPR-Cas9 mediated identification of host factors for influenza infection and persistence

11:15 - 11:30 am: Daniel Ciobanu
Understanding Host X Pathogen Associations In Swine Infectious Diseases

Lightning Talks

11:30-11:35 am: Mehak Kapoor
scRNAseq analysis of PBMCs during acute PRRSV infection: searching for host cellular markers to predict persistent infection

11:35 - 11:40 am: Yuhan Wen
Single cell T cell receptor profiling in pig lungs using single-cell TCR sequencing analysis

11:40 - 11:45 am: Molly Kroeger
Evaluation of PCV3 humoral responses in experimentally infected pigs and dynamic of maternally derived antibodies in piglets from naturally infected sows

11:45 - 11:50 am: Kristen Walker
Investigation of fetal liver, heart, and thymus transcriptomes for prediction of reproductive failure

11:50 - 11:55 am: Laurie Touchard
Investigating MAIT cell contributions to anti-influenza virus immunity using MR1 knockout pigs

11:55 - 12:00 pm: Julia Baker
Computationally predicted T-cell epitope trends for 30 years of wild-type PRRSV-2 strains from the USA

Session 3: Zoetis Lunch Session: Applied research in the swine industry - swine disease control and elimination update

Moderators: Jose Angulo, Jay Calvert

12:00 - 12:30 pm: Food Service

12:30 - 12:40 pm: Jose Angulo
Welcome and Introduction

12:40 - 1:05 pm: Giovani Trevisan
Novel approaches to swine pathogen monitoring and surveillance: the cornerstone for effective disease control and elimination

1:05 - 1:30 pm: Bill Hollis
The art of controlling and eliminating swine diseases in the field using science-based methods and tools: A practitioner's perspective

1:30 – 2:00 pm: Q & A Session

Session 4: Novel Vaccines and Vaccination Strategies

Moderators: Shafiqul Chowdhury, Elisa Crisci

2:00 - 2:15 pm: Marti Cortey
Efficacy of an intranasal naturally attenuated live PRRSV-2 vaccine against a highly virulent PRRSV-1 strain

2:15 - 2:30 pm: Shafiqul Chowdhury
Novel Pseudorabies Virus (PRV) Vectored Subunit Vaccine Against African Swine Fever

2:30 - 2:45 pm: Hiep Vu
Lipid Nanoparticle-Encapsulated DNA Vaccines: A Versatile Platform for Rapid Development of Vaccines Against Influenza A Viruses of swine

2:45 - 3:00 pm: Elisa Crisci
PRRSV-2 immune biobank for vaccine efficacy prediction

Lightning Talks

3:00 - 3:05 pm: Nakarin Pamornchainavakul
Experimental evidence of vaccine-driven evolution of PRRSV-2 in pigs-to-pig infection chains

3:05- 3:10 pm: Fangfeng Yuan
Rational design and immunogenicity evaluation of mRNA-based vaccine for African Swine Fever virus

3:10 - 3:15 pm: The Nguyen
Lipid nanoparticle-encapsulated DNA vaccine encoding African swine fever virus p54 antigen elicits robust immune responses in pigs

3:15 - 3:20 pm: Molly Kroeger
Assessment of homologous and heterologous PCV2 vaccine efficacy in a PCV2d/PRRSV co-challenge model

3:20 - 4:00 pm Break

NC229 Special Session

Moderators: Pablo Pineyro, Andreia Arruda

4:00–4:05 pm: Pablo Pineyro
NC229 Session opening remarks

4:05–4:20 pm: Hiep Vu
NC229 multi-state program summary and future perspective

4:20–4:40 pm: Michelle Colby
USDA-NIFA Research Funding Opportunities for 2025

4:40–5:30 pm: NC229 station Representatives
Station accomplishments and opportunities for interdepartmental collaboration

Monday, December 9

(Plenary sessions in Renaissance Ballroom, 5th Floor)

Session 5: Rapid Sensing of Viral Infection and Disease Counter Measures

Moderators: Cesar Corzo, Scott Dee

8:00 - 8:15 am: Scott Dee
Further evidence that science-based biosecurity provides sustainable prevention of PRRS virus and improves productivity in swine breeding herd

8:15 - 8:30 am: Kimberly VanderWaal
PRRSV-2 variant classification: a dynamic nomenclature for enhanced monitoring and surveillance

8:30 - 8:45 am: Jianqiang Zhang
Refining genetic classification of global type 1 porcine reproductive and respiratory syndrome virus and characterization of their geographic distributions

8:45 - 9:00 am: Cesar Corzo
A Decadal Review of Porcine Deltacoronavirus Occurrence in U.S. Breeding Herds

Lightning Talks

9:00 - 9:05 am: Joao Paulo Herrera da Silva
Long-term evolutionary dynamics of Porcine Epidemic Diarrhea Virus (PEDV) in the U.S. a decade after introduction

9:05 - 9:10 am: Xiaomei Yue
Time-to-stability of porcine epidemic diarrhea virus (PEDV) and the associated factors in U.S. breeding herds

9:10 - 9:15 am: Igor Paploski
Characterizing dead animal disposal practices on sow farms and assessing PRRSV risks associated with rendering

9:15 - 9:20 am: Marcello Melini
Use of post-mortem samples from growing pigs to detect five swine pathogens

9:20 - 9:25 am: Ethan Aljets
Isolation and characterization of porcine sapovirus genogroup III

9:25 - 9:30 am: Trevor Arunsiripate
Comparison of historical and contemporary field isolates of porcine hemagglutinating encephalomyelitis virus

9:30 - 10:00 am Break

Session 6: SHIC Special Session - Novel Tools & Technologies to Address Emerging Diseases of Swine

Moderators: Megan Niederwerder, Lisa Becton

10:00 - 10:15 am: Derald Holtkamp
Development of a standardized outbreak investigation program web-based application

10:15 - 10:30 am: Onyekachukwu (Henry) Osemeke
Optimizing tongue-tip sampling protocols for enhanced PRRS virus isolation

10:30 - 10:45 am: Erin Kettelkamp
Evaluation of deployable fan coverings for biocontainment of airborne swine pathogens

10:45 - 11:00 am: Brett Ramirez
Evaluation of electrostatic precipitation for biocontainment of viral and bacterial pathogens emitted from finishing facilities

11:00 - 11:15 am: Lucas Spetic Da Selva
Self-vaccinating pigs to save labor, improve efficacy and enhance biosecurity

11:15 am – 11:30 pm: Rachael Schulte
Development of an online dashboard for near-real global swine disease surveillance

Session 7: Sponsor Lunch Session: Current & Future Technologies in Swine Health

Moderators: Rolf Rauh, Leyi Wang

11:30 - 12:30 pm Food Service

12:30 - 12:45 pm: Bob Rowland
History and future of virus receptor modification for the prevention of PRRS and other swine diseases

12:45 – 1:00 pm: Reid Phillip
Efficacy study evaluating INGELVAC PRRS MLV against a PRRSv '144' Lineage-1C.5 variant heterologous challenge

1:00 – 1:15 pm: Sineej Madathil
Think Big! Maximize your Samples with Power of Multiplexing

1:15 - 1:30 pm: Laura Miller
Spatial Transcriptomics: A New Frontier for Swine Viral Pathobiology Research

1:30 - 3:30 pm **Poster session** *(Streeterville and Avenue Ballroom, 1st Floor)*

Session 8: Emerging Swine Diseases and Global Disease Control

Moderators: Mariana Kikuti, Jishu Shi

3:30 - 4:15 pm : Enric Mateu
Emergence of PRRSV-1 strains of enhanced virulence in Europe: overview and evolution

4:15 - 4:30 pm: Jean-Pierre Frossard
Assessing the relative importance of geographic proximity and pig movement-related factors to the spread of PRRSV-1 in the United Kingdom

4:30 - 4:45 pm: Jishu Shi
Of pigs and men: the best-laid plans for prevention and control of African swine fever

4:45 - 5:00 pm: Mariana Kikuti
PRRS Virus Variant 1H.18: Occurrence and Classification Challenges

5:00 - 5:15 pm: Pablo Pineyro-Pineiro
Emerging porcine circoviruses

5:15 – 6:15 pm **Break**

Banquet and Award Ceremony

6:15 - 7:15 pm: Food service

7:15 - 8:00 pm: Andrew Bomen
Influenza A virus in swine: the expanding problem

8:00 - 9:00 pm: Award Ceremony

8:00 – 8:15 pm: Jay Calvert
Thirty years of PRRS research in my rearview mirror

8:15 – 8:30 pm: Joan Lunney
PRRS research – Importance of teamwork

8:30 - 9:00 pm: Graduate Student Award Announcement

2024 NAPRRS/NC229 ICSVD POSTER PRESENTATIONS

Section 1: Virus Structure and Gene Function

#01-01 Role of N-glycosylation in PRRSV Infection
Presenter: Alberto Brandariz Nunez

#01-02 An Intra-Family Conserved High-Order RNA Structure Within the M ORF is Important for Arterivirus Subgenomic RNA Accumulation and Infectious Virus Production
Presenter: Chi Chen

#01-03 Cross-Species Analysis of CD163 Orthologs Reveals Key Domain for PRRSV Susceptibility
Presenter: Kassandra Durazo-Martinez

#01-04 PRRSV nsp1α Differentially Regulates Viral RNA Synthesis
Presenter: Yanhua Li

#01-05 A Novel Reverse Genetics System for Porcine Reproductive and Respiratory Syndrome Virus and the Generation of a Transcription Network-Reprogrammed Virus
Presenter: Junyu Tang

#01-06 Segment-Specific Enteroids from Pig Small Intestine in Matrigel and Transwell Inserts: Susceptibility to Porcine Epidemic Diarrhea Virus (PEDV)
Presenter: Lu Yen

Section 2: Viral Infection and Host Responses

#02-01 Comparison of Historical and Contemporary Field Isolates of Porcine Hemagglutinating Encephalomyelitis Virus
Presenter: Trevor Arunsiripate

#02-02 Computationally Predicted T-cell Epitope Trends for 30 Years of Wild-Type PRRSV-2 Strains from the USA
Presenter: Julia Baker

#02-03 Coinfection with PEDV and BVDV Induces Inflammatory Bowel Disease Pathway Highly Enriched in PK-15 Cells
Presenter: Jinghua Cheng

#02-04 Deconstructing the Role of SYNGR2 in Viral Disease Susceptibility in Swine
Presenter: Daniel Ciobanu

#02-05 Understanding Host X Pathogen Associations In Swine Infectious Diseases
Presenter: Daniel Ciobanu

#02-06 Defining Interferon Antagonism Hierarchy of Porcine Epidemic Diarrhea Virus
Presenter: Xufang Deng

#02-07 Longitudinal Assessment of Associations Between Mucosal Microbial Communities and Porcine Respiratory and Reproductive Syndrome Virus Pathogenesis
Presenter: Luoyan Duan

#02-08 Porcine Antibody Response to Epitope A on PRRSV GP5 and Its Role in Virus Neutralization
Presenter: Jing Huang

#02-09 scRNAseq Analysis of PBMCs During Acute PRRSV Infection: Searching for Host Cellular Markers to Predict Persistent Infection
Presenter: Mehak Kapoor

#02-10 Evaluation of PCV3 Humoral Responses in Experimentally Infected Pigs and Dynamic of Maternally Derived Antibodies in Piglets from Naturally Infected Sows
Presenter: Molly Kroeger

#02-11 Single Cell T cell Receptor Profiling in Pig Lungs Using Single-Cell TCR Sequencing Analysis
Presenter: Darling Melany Madrid

#02-12 The Absence of CD163 Lessens the Pathogenicity of PCV2d in Pigs
Presenter: Raymond (Bob) Rowland

#02-13 Investigating MAIT Cell Contributions to Anti-Influenza Virus Immunity Using MR1 Knockout Pigs
Presenter: Laurie Touchard

#02-14 Investigation of Fetal Liver, Heart, and Thymus Transcriptomes for Prediction of Reproductive Failure
Presenter: Kristen Walker

#02-15 Generation of ANP32A Knock-out Cell Line for Functional Assessment of Novel Swine Isoform on Avian Influenza Virus Polymerase Activity
Presenter: Lianna Walker

Section 3: Vaccination Strategies and Therapeutics

#03-01 PRRSV-2 Immune Biobank for Vaccine Efficacy Prediction
Presenter: Elisa Crisci

#03-02 Evaluation of a Stable Oil-In-Water Adjuvant for Recombinant PRRSV Vaccine
Presenter: Marie-Eve Koziol

#03-03 Assessment of Homologous and Heterologous PCV2 Vaccine Efficacy in a PCV2d/PRRSV Co-Challenge Model
Presenter: Molly Kroeger

#03-04 Comparison of economic losses between status 3 and status 2vx farrow to wean farms equipped with air filtration facilities within 12 weeks following the introduction of PRRSV NADC30-like strain
Presenter: Reid Philips

#03-05 Efficacy of an Intranasal Naturally Attenuated Live PRRSV-2 Vaccine Against a Highly Virulent PRRSV-1 Strain
Presenter: Enric Mateu

#03-06 Development of a Foot-and-Mouth Disease Virus Vaccine Candidate: Utilizing Non-Toxic Enterotoxin and E. coli as an Adjuvant-Delivery System
Presenter: Akhila Naru

#03-07 Lipid Nanoparticle-Encapsulated DNA Vaccine Encoding African Swine Fever Virus p54 Antigen Elicits Robust Immune Responses in Pigs
Presenter: The Nguyen

#03-08 Experimental Evidence of Vaccine-Driven Evolution of PRRSV-2 in Pigs-To-Pig Infection Chains
Presenter: Nakarin Pamornchainavakul

#03-09 The Efficacy Study Evaluating INGELVAC PRRS® MLV Against a PRRSV '144' Lineage-1C.5 Variant Challenge
Presenter: Reid Philips

#03-10 Development and In Vitro Characterization of Messenger RNA-Based Vaccines Against Senecavirus A
Presenter: Kepalee Saeng-Chuto

#03-11 A Live-Attenuated Virus Vaccine Candidate Protects Pigs Against Contemporary Pandemic Genotype II African Swine Fever (ASF) Virus
Presenter: Jishu Shi

#03-12 Lipid Nanoparticle-Encapsulated DNA Vaccines: A Versatile Platform for Rapid Development of Vaccines Against Influenza A Viruses of Swine
Presenter: Hiep Vu

#03-13 Rational Design and Immunogenicity Evaluation of mRNA-based Vaccine for African Swine Fever Virus
Presenter: Fangfeng Yuan

#03-14 An Effective Vaccine Against Swine Influenza A Virus Based on the Matrix Protein 2 (M2)
Presenter: Federico Zuckermann

#03-15 Novel Pseudorabies Virus (PRV) Vectored Subunit Vaccine Against African Swine Fever
Presenter: Shafiqul Chowdhury

Section 4: Diagnostics, Surveillance, and Disease Control

#04-01 Isolation and Characterization of Porcine Sapovirus Genogroup III
Presenter: Ethan Aljets

#04-02 BioAerium: A Real-Time Detector of Airborne Animal Viruses
Presenter: Michael Caffrey

#04-03 Further Evidence that Science-Based Biosecurity Prevents Porcine Reproductive and Respiratory Syndrome Virus Infection and Improves Productivity in Breeding Herds
Presenter: Scott Dee

#04-04 Assessing the Relative Importance of Geographic Proximity and Pig Movement-Related Factors to the Spread of PRRSV-1 in the United Kingdom
Presenter: Jean-Pierre Frossard

#04-05 Long-Term Evolutionary Dynamics of Porcine Epidemic Diarrhea Virus (PEDV) in the U.S. a Decade After Introduction
Presenter: Joao Paulo Herrera da Silva

#04-06 A Decadal Review of Porcine Deltacoronavirus Occurrence in U.S. Breeding Herds
Presenter: Mariana Kikuti

2024 NAPRRS/NC229 ICSVD POSTER PRESENTATIONS

#04-07 PRRS Virus Variant 1H.18: Occurrence and Classification Challenges
Presenter: Mariana Kikuti

#04-08 Isolation and Characterization of Novel Reassortant Mammalian Orthoreovirus from Pigs in the United States
Presenter: Wenjun Ma

#04-09 Think Big! Maximize Your Samples with Power of Multiplexing
Presenter: Sineej Madathil

#04-10 Use of Post-Mortem Samples from Growing Pigs to Detect Five Swine Pathogens
Presenter: Marcello Melini

#04-11 Assessment of farm Surface PRRSV Contamination Through Viability RT-qPCR
Presenter: Marcello Melini

#04-12 Spatial Transcriptomics: A New Frontier for Swine Viral Pathobiology Research
Presenter: Laura Miller

#04-13 Characterizing Dead Animal Disposal Practices on Sow Farms and Assessing PRRSV Risks Associated with Rendering
Presenter: Igor Paploski

#04-14 Maximizing PRRSV Diagnostic Accuracy: Insights from Tongue Tip Testing
Presenter: Igor Paploski

#04-15 An Automated 384-Well RT-qPCR Assay for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Enhances High-Volume Testing Capabilities in the Veterinary Diagnostic Laboratory
Presenter: Bala Mounika Reddi

#04-16 Of Pigs and Men: The Best -Laid Plans for Prevention and Control of African Swine Fever
Presenter: Jishu Shi

#04-17 PRRSV-2 Variant Classification: A Dynamic Nomenclature for Enhanced Monitoring and Surveillance
Presenter: Kimberly VanderWaal

#04-18 Investigating the Current Practice in Oral Fluid Sampling in the U.S. Swine Industry: An Exploratory Study
Presenter: Xiaomei Yue

#04-19 Time-to-Stability of Porcine Epidemic Diarrhea Virus (PEDV) and the Associated Factors in U.S. Breeding Herds
Presenter: Xiaomei Yue

#04-20 Refining Genetic Classification of Global Type 1 Porcine Reproductive and Respiratory Syndrome Virus and Characterization of Their Geographic Distributions
Presenter: Jianqiang Zhang

#04-21 Comparison of Economic Losses Between Status 3 and Status 2vx Farrow to Wean Farms Equipped With Air Filtration Facilities Within 12 Weeks Following the Introduction of PRRSV NADC30-like Strain
Presenter: Kangning Zhao

ABSTRACTS
(Arranged in alphabetical order by presenting author's last name)

Isolation and characterization of porcine sapovirus genogroup III

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Sapoviruses have been classified into 19 genogroups (GI to GXIX) with a wide range of host species. Among them, eight genogroups (GIII and GV-GXI) have been detected in pigs with GIII SaV being the most frequently detected genogroup. Porcine sapovirus (PSaV) was first identified in the United States by electron microscopy in 1980 and the cell culture isolate Cowden strain (genogroup III) obtained in 1988 marks the sole available PSaV cell culture isolate to date. This study aimed to isolate contemporary PSaV GIII in cell culture and develop various diagnostic assays. We first attempted virus isolation from 58 PSaV GIII PCR-positive samples with Ct values in the range of 10.5–35.8 in LLC-PK1 cells. Virus isolation was positive on 10 samples and four selected isolates were serially passed in LLC-PK1 cells for 10 passages (P0-P9). These virus isolates demonstrated robust growth and maintained genetic stability for up to 10 passages. Whole genome sequencing revealed that these contemporary SaV isolates were genetically distinct from the Cowden isolate. Two PSaV GIII VP1 peptides were synthesized and used to immunize rabbits (two rabbits for each peptide) for a duration of 90 days to generate peptide-specific antisera. The VP1 peptide-specific rabbit antisera were successfully used for immunofluorescence staining of virus-infected cells to verify the virus isolation and titration outcomes. One antiserum was also used to develop an immunohistochemistry (IHC) assay for detecting PSaV in tissues; strong and clear IHC staining was observed in SaV-positive small intestine tissues. In addition, one contemporary PSaV isolate was used to develop an indirect fluorescent antibody (IFA) assay for detecting antibody in serum samples. The IFA antibody assay was validated using serum samples collected from 296 pigs previously used in four experimental PEDV or PRRSV studies. Notably, even in pigs categorized as having “high health” status for experimental studies, SaV antibody was detected from a considerable proportion of them. In summary, the contemporary PSaV isolates, the VP1 peptide-specific rabbit antisera and the diagnostic assays obtained or developed in the present study offer a valuable resource for further characterizing virus replication, sensitivity of the virus to disinfectants and other environmental factors, and viral pathogenicity in pigs, and for advancing vaccine development efforts.

Comparison of historical and contemporary field isolates of Porcine Hemagglutinating Encephalomyelitis Virus

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Porcine hemagglutinating encephalomyelitis virus (PHEV) has been historically associate with neurological disease in neonatal pigs. This betacoronavirus presents a neurologic tropism in pre-weaned piglets causing vomiting and wasting disease due to neuritis of the Meissner plexus and an encephalitis. Recently, PHEV has been detected in a case of influenza-like respiratory disease of show pigs in Michigan, US. However, the role of PHEV in respiratory disease has not yet been confirmed experimentally. During 2020, three diagnostic cases from pigs with reported respiratory clinical signs were received at ISU-VDL. The presence of the virus was confirmed by qPCR, *in-situ* hybridization and virus isolation in cases of necrotizing bronchitis and bronchiolitis. Therefore the objective of this study is to compare the pathogenic role of the historical PHEV_Mengeling strain and a contemporary PHEV field isolate. Twenty seven, 12-week-old pigs were inoculated intratracheal and intranasal with 5 and 2 ml respectively with 10^6 TCID50/ml of Mengeling strain (n=9), contemporary ISU-PHEV isolate strain (n=9) and growth media (n=9). Serum samples were collected on days 0, 2, 5, 10, and 14 for antibody detection, and three pigs per group were euthanized on days 2, 5, and 14 to evaluate gross and histological lesions and molecular detection of PHEV nucleic acid in turbinates, trachea, and lung. No significant clinical signs (sneezing, coughing, or dyspnea) were observed in the control group or in either of the challenged groups. The control group remained seronegative and no viral nucleic acid was detected by qPCR trough the study. The presence specific IgG PHEV antibodies was confirmed in both challenged groups by 5 DPI with the higher S/P by 10 DPI. Common gross findings in both groups were found in the lung characterized by mild multifocal depressions were suggestive of lobular atelectasis. PHEV nucleic acid was detected by qPCR nasal turbinates and trachea in the Mengeling group while animals in contemporary ISU-PHEV group were positive in the nasal turbinates and lung. Histological evaluation showed mild epithelial attenuation of the mucosal upper respiratory epithelium in both groups. These findings suggest that this model induced a subclinical upper respiratory infection, with the virus being shed in nasal secretions and causing mild gross and histological lesions. However, no significant differences were observed between the historical and contemporary strains. Additionally, PHEV tracheal and nasal inoculation can induce seroconversion. The importance of this finding is that it adds to the understanding of PHEV as a predisposing factor in the porcine respiratory disease complex, potentially allowing infection by other respiratory pathogens due to damage to the mucosal respiratory barriers.

Computationally predicted T-cell epitope trends for 30 years of wild-type PRRSV-2 strains from the USA

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Despite tremendous work on the adaptive immune responses to PRRSV-2 infection, the correlates of protection are not completely understood. The lack of research on cell-mediated immunity, especially as it relates to "immune-phenotype" of the virus, has left a knowledge gap in our understanding of PRRSV. Here, we begin to bridge this gap by investigating how T-cell epitope content maps onto viral diversity and evolution, with implications for cross-protection from the perspective of cell-mediated immunity. To achieve this, we utilize a bioinformatic pipeline developed by EpiVax to computationally predict MHC class I and class II epitopes in PRRSV-2 whole genomes.

The objective of this study was to quantify T-cell epitope content for wild-type PRRSV-2 variants and commercially available modified live vaccines (MLVs). Specifically, we investigate how T-cell epitope content has changed across decades and between different phylogenetic lineages and assess the extent to which predicted T-cell epitopes are shared between wild-type viruses and MLVs.
All structural proteins of a phylogenetically diverse set of 161 wildtype PRRSV-2 viruses from the USA spanning the past ~30 years, as well as the five common MLVs, were obtained from GenBank. T-cell epitope content was predicted for each virus using the PigMatrix tool, which screens potential epitopes predicted to bind both MHC class I and class II swine leukocyte antigens (SLA). Pairwise T-cell epitope content comparisons ("EpiCC scores") were used to generate metrics of epitope cross-conservation (% epitopes shared) between viral structural proteomes. These epitope cross-conservation metrics were used to group viruses into T-cell epitope "immune clusters" via classical multi-dimensional scaling (MDS) and K-means clustering.

General trends of wild-type viruses over the past 30 years demonstrate that T-cell epitope content has shifted away from the VR-2332 prototype virus (10~15% loss of shared epitopes) and all commercially available vaccines queried. Additionally, more T-cell epitopes are shared within lineage compared to between lineages, and clustering analysis correlated well with phylogenetic tree structure. These findings support the hypothesis that lineages are immunologically distinct, and that MLVs may not be well matched to current wild-type strains. Interestingly, more T-cell epitopes are shared amongst older lineages, regardless of genetic relatedness, whereas recent sub-lineages have greater diversity in their T-cell epitope content. These findings demonstrate a general expansion of the T-cell epitope landscape, though without obvious directional pressure within clusters through time.
This is the first attempt to computationally predict T-cell epitopes of PRRSV-2 structural proteins from viruses representing 30 years of PRRSV-2 in the USA. We systematically compared these epitope predictions to the evolutionary history of the viruses queried to reveal the interplay between the evolutionary and immunological processes. The general expansion of T-cell epitope content and the co-circulation of distinct T-cell epitope immune clusters suggests that T-cell epitope content likely contributes to relative fitness of different PRRSV-2 genetic variants in the multi-strain ecosystem in which PRRSV-2 evolves. This work lays the foundation for further investigation of PRRSV-2 T-cell epitopes and the role that host T-cell responses may play in viral evolution and adaptation.

Role of N-glycosylation in PRRSV infection

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the main viral pathogens that causes the most significant economic losses in the swine-producing countries. PRRSV is divided into two distinct species, PRRSV-1 and PRRSV-2. The PRRSV virion envelope contain four glycosylated membrane proteins and three non-glycosylated envelope proteins. Previous studies have suggested that PRRSV-linked glycans are critical structural components for virus assembly. In addition, it has been suggested that PRRSV glycans are involved in the interaction with host cells and critical for virus infection. In contrast, recent experiments showed that removal of N-glycans from PRRSV does not affect virus infection of permissive cells. Thus, there are not compelling evidences to strongly support that N-glycans in the PRRSV envelope directly contribute to viral infection. To analyze the role of N-glycosylation on PRRSV infection, we evaluated the specific contribution of the envelope protein-linked N-glycans to infection of permissive cells. To this end, we employed a novel strategy to modify envelope protein-linked N-glycans that consists of production of monoglycosylated PRRSV and viral glycoproteins with different glycan states. Our data demonstrated that removal or alteration of N-glycans from PRRSV affected virus infection. Specifically, we found that complex N-glycans are required for an efficient infection in cell cultures. Furthermore, we observed that presence of high mannose type glycans on PRRSV surface is the minimal requirement for a productive viral infection. Our results also showed that PRRSV-1 and PRRSV-2 have different requirements of N-glycan structure for an optimal infection. In addition, we showed that removal of N-glycans from PRRSV does not affect viral attachment, suggesting that these carbohydrates played a major role in regulating viral entry. In agreement with these results, immunoprecipitation assays and colocalization experiments indicated that N-glycans present in the viral envelope glycoproteins are not required to bind to the viral receptor CD163. Finally, we found that the presence of N-glycans in CD163 is not necessary for PRRSV infection.

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BioAerium: A Real-Time Detector of Airborne Animal Viruses

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Animal viruses continue to have devasting effects on the nation's food suppliers. For example, highly pathogenic bird flu costs to US suppliers are currently >1B USD/year. Moreover, the US swine industry suffers from endemic PRRSV with costs estimated to be >600M USD/year. In both cases, the viruses are thought to spread by airborne particles. In this work we describe our recent efforts to develop novel and robust nucleic acid amplification assays of PRRSV. Specifically, we will discuss the sensitivity and robustness of our LAMP-based assays. In addition, we will show a prototype device that samples airborne virus by electrostatic precipitation into a reaction well for subsequent real-time detection. Finally, we will discuss a recently developed model system based on Virus Like Particle (VLP) that enables rapid prototype validation and optimization. Our overall goal is to develop a portable, low-cost, autonomous sensor to detect airborne animal viruses in real-time, with an initial focus of PRRSV. Importantly, the system is designed to rapidly adapt to other swine viruses or other animal viruses in general. We envision that such a surveillance platform can be widely deployed to mitigate infection and secure the nation's food supply.

Thirty years of PRRS research in my rearview mirror

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Having just completed 30 years of service with a major animal health company, I'd like to briefly share some thoughts and observations on the exciting advancements in PRRS virology and vaccinology I've witnessed and in some cases contributed to. When I started working on PRRSV in 1994, the name "Porcine Reproductive and Respiratory Syndrome Virus" had only recently be proposed as a standard name for this new virus with many names, the first full length sequence (Lelystad virus) and the assignment to the Arterivirus family were new, and the first two commercial vaccines were entering the market (Cyblue©, an inactivated vaccine in Spain and RespPRRS™, a modified live vaccine in the US). We have made many advancements since then in our understanding of viral genomes, and the structure and function of the proteins they encode. Many PRRS proteins have a primary function in the viral replication cycle, but also have secondary functions that disrupt innate or acquired immunity in various ways. These functions are responsible for the characteristic immune dysfunction and immune evasion seen in PRRS virus infections, and are still being identified and characterized in labs today. Control of PRRS using commercial vaccines has improved only incrementally. Although there are more choices now, with some important differences in label claims, we are still largely dependent on MLV vaccines, and these are not approved or recommended for PRRS-free herds. The discovery of the CD163 PRRS receptor, and other cellular proteins that interact with viral proteins, allowed an expansion of the number of cell lines that can be used to grow and attenuate PRRS viruses. Inhibitors of these interactions are being investigated for the control of PRRS infections. And when I shift my gaze from the rearview mirror to the windshield, I see PRRS-resistant gene-edited pigs looming on the horizon.

An intra-family conserved high-order RNA structure within the M ORF is important for arterivirus subgenomic RNA accumulation and infectious virus production

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Synthesis of subgenomic RNAs is a strategy commonly used by polycistronic positive sense single-stranded RNA viruses to express 3′-proximal genes. Members of the order of Nidovirales, including coronaviruses and arteriviruses, use a unique discontinuous transcription strategy to synthesize subgenomic RNAs. In this study, in silico synonymous site conservation analysis and RNA structure folding predicted the existence of intra-family conserved high-order RNA structure within the M ORF of arteriviral genomes, which was further determined to be important for the transcription/accumulation of subgenomic RNAs and production of infectious viral particles. Mutations disrupting the stability of the RNA structures significantly decreased the accumulation of multiple subgenomic RNAs. In contrast, the impact of mutagenesis on full-length genomic RNA accumulation was limited. The degree to which wild-type levels of subgenomic RNA accumulation were maintained was found to correlate with the efficiency of infectious virus production. Moreover, the thermo-stability of stems within the high-order RNA structure is also well correlated with viral replication capacity and the maintenance of subgenomic RNA accumulation. This study is the first to report an intra-Arteriviridae conserved high-order RNA structure that is located in a protein-coding region and functions as an important cis-acting element to control the accumulation/transcription of arteriviral subgenomic RNAs. This work suggests a complex regulation mechanism between genome replication and discontinuous transcription in nidoviruses.

Coinfection with PEDV and BVDV induces inflammatory bowel disease pathway highly enriched in PK-15 cells

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Piglet diarrhea is often caused by mixed infections and causes huge economic losses to the swine industry. From the 1078 diarrhea stools tested in our survey from 2017 to 2022 in local area of China, PEDV was the key pathogen that was closely related to the death of piglets with diarrhea. In addition, coinfection of PEDV-positive samples with BVDV reached 17.24%. Although BVDV infection in swine is typically subclinical, the effect of PEDV and BVDV coinfection on disease severity and the potential molecular mechanism of coinfection with these two viruses remain unknown. To better understand the pathogenesis mechanism of virus coinfection, we developed a model of coinfection with porcine epidemic diarrhea virus (PEDV) and bovine viral diarrhea virus (BVDV) in PK15 cells, and a tandem mass tag (TMT) combined with LC-MS/MS proteomic approach was used to identify differential protein expression profiles. Additionally, we performed drug experiments to explore the inflammatory response induced by PEDV or BVDV mono- or coinfection. A total of 1094, 1538, and 1482 differentially expressed proteins (DEPs) were identified upon PEDV monoinfection, BVDV monoinfection and PEDV/BVDV coinfection, respectively. KEGG pathway analysis revealed that PEDV and BVDV coinfection led to a highly significantly enrichment of the inflammatory bowel disease (IBD) pathway. In addition, the NF-kappa-B signaling pathway was more intensively activated by PEDV and BVDV coinfection, which induced higher production of inflammatory cytokines such as IL-6, IL-8, IL-18 and TNF-alpha, than PEDV or BVDV monoinfection. Our study indicated that cattle pathogens might play synergistic roles in the pathogenesis of porcine diarrhea, which might also improve our understanding of the pathogenesis of multiple infections in diarrhea.

Novel Pseudorabies Virus (PRV) Vectored Subunit Vaccine Against African Swine Fever

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African swine fever (ASF) is a highly contagious fatal disease of domestic and wild pigs caused by the ASF virus (ASFV). This has spread over three continents: Africa, Asia, and Europe. In 2021, the disease appeared in the Dominican Republic and Haiti. Therefore, ASF poses a substantial threat to the US swine industry. Currently, there are no commercial vaccines available to prevent and control ASF. Experimentally, live attenuated or gene-deleted ASFV vaccines have shown protective efficacy. However, these vaccines are not safe since there is the risk of reversion to virulence or recombination of vaccine viruses with field viruses. We recently developed a highly attenuated novel quadruple gene mutant pseudorabies virus (PRVqmv) vector lacking functional thymidine kinase (TK), glycoprotein G (gG), gE, and US9 genes. Subsequently, we generated three codon-optimized (pig) ASFV chimeric gene expression cassettes designated as PA-1, PA-2, and PA-3, that together included ten carefully chosen ASFV proteins: i.e., i) PA-1: ASFV MGF505-5R-Flag– P2A – B646L– F2A – F317L– GMCSF; ii) PA-2: ASFV B602L-HA – P2A – E183L-c-Myc – T2A – E199L-VSV-G – F2A – EP153R-S; iii) PA-3: ASFV M448R-V5 – P2A – MGF505-7R-His – E2A – CP204. The chimeric ASFV polyprotein gene cassettes, PA-1, PA-2, and PA-3, were designed to include different self-cleavable peptide 2A (P2A, E2A, F2A, and T2A) in between the proteins to yield individual proteins when expressed in the transfected infected cells. These three ASFV chimeric gene cassettes, PA-1, PA-2, and PA-3, were incorporated into PRV gG-deletion, gE-US9 deletion, and TK-deletion/insertion plasmid vectors, respectively, and validated the ten chimeric ASFV proteins' expression. To generate a PRVqmv recombinant virus expressing the 10 ASFV chimeric proteins (PRVqmv Sub ASFV-P-10) by homologous recombination, we performed co-transfection of the insertion vector plasmids PA-1, PA-2, and PA-3 DNA with the full-length PRVqmv genomic DNA. Three putative PRVqmv recombinants expressing all the 10 ASFV proteins (PRVqmv Sub-ASFV-P10) were isolated, plaque purified, and characterized for the individual chimeric ASFV protein expression in vitro by immunoblotting and confirmed by full-genome DNA sequencing. Further characterization of the PRVqmv Sub-ASFV-P10 by plaque size assay, one-step growth kinetics, and virus stability assays are in progress. Protective vaccine efficacy of the PRVqmv Sub-ASFV-P10 against a virulent ASFV challenge in pigs is planned at Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, Canada.

Deconstructing the Role of SYNGR2 in Viral Disease Susceptibility in Swine

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Viral diseases pose a constant threat to the economic sustainability of livestock producers due to detrimental effects on animal growth and welfare. Recently, by integrating experimental infections, genome-wide associations, gene annotation, transcriptome sequencing and in vitro models, we identified a gene (SYNGR2) and a missense DNA polymorphism (SYNGR p.Arg63Cys) associated with susceptibility to PCV2b, a prevalent swine DNA virus. The objectives of this study were to validate, characterize and provide direct evidence of the role of SYNGR2 and its alleles in susceptibility to PCV2 strains and other DNA (e.g., ASFV) and RNA viruses that infect swine (e.g. CSFV) and other livestock (e.g., BVDV).

Using CRISPR-Cas9 mediated gene editing, we generated predicted SYNGR2 knock-out (KO) clones for PK15, Vero and MDBK cells and allelic substitution (emSYNGR2+p.63Cys, 2emSYNGR2+p.63Cys) clones for PK15 cells. PK15 and MDBK are both kidney epithelial cell lines of porcine and bovine origin, respectively, while the Vero cell line is derived from African green monkey kidney epithelium. Following infection of wild-type (PK15, Vero) and the edited cell lines with a ASFV isolate (VNUA-ASF-L01, genotype II), only the emSYNGR2+p.63Cys PK15 clone was found to be permissible to infection. In addition, a predicted KO MDBK clone was generated to test the role of SYNGR2 in BVDV infection. DNA sequencing of this clone confirmed the truncation of SYNGR2 with a 253 bp deletion that removed most of exon 3, intron 3, and the beginning of exon 4, suggesting a lack of function. The wild type and edited MDBK cells will be infected with a cytopathic BVDV type 1 isolate (Singer). The isolate was sequenced using Oxford Nanopore Technology and the complete viral genome was assembled. This research will generate knowledge that could be applied to improve animal health and welfare as well as reduce economic losses to the livestock industry.

Understanding Host X Pathogen Associations In Swine Infectious Diseases

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Recent disease outbreaks emphasize the continued economic threat of infectious diseases to the global swine industry. These challenges highlight the need for efficient approaches to identify and characterize novel pathogens while furthering development of modern surveillance and management technologies that capture relevant disease phenotypes. Natural infections can be efficiently used to capture a multitude of relationships between host genetics and different viral and bacterial pathogens. The objective of this proposal is to evaluate relationships between full-spectrum microbial profiling, host genetics, animal growth and health status in a typical swine farm. Growth data and multiple tissue samples were collected from four batches of terminal crossbred pigs (4 x 250 pigs). During the grow/finish phase (~100 days), the pigs were allocated into a room with 25 pens, with 10 pigs/pen. Body weight was obtained at birth, weaning and 3 times during grow/finish phase. Individual blood samples, fecal and nasal swabs were collected at weaning and 3 times during grow/finish phase. During this phase, weekly pen-based oral fluids were collected using ropes (~ 15 time points/pen). Initial oral metagenomic data, based on Oxford Nanopore sequencing, reflected common nursery microbial profile including specific oral or upper-respiratory microbiota. Among classified reads there were 455 microbial species detected with a presence larger than 0.01%. Atypical porcine pestivirus (APPV), with 51 sequencing reads obtained, was the most abundant viral species observed. Other classified viruses included Porcine astrovirus 4, Porcine sapelovirus or Porcine bocavirus 5. The bacterial profile included opportunistic pathogens (e.g., Streptococcus suis), oral- (e.g., Prevotella dentalis, Rothia mucilaginosa) or fecal-specific (e.g., Clostridium sp.) and known pathogenic species (e.g., Glaesserella parasuis). Tail clips or ear notches were collected and used for DNA isolation using the Omega Mag-Bind Blood & Tissue DNA kit. Approximately 50% of samples (n ~ 500; Batch 1 and 2) were genotyped with the custom SowPro91 Affymetrix array (111,141 SNPs). SowPro91 was enriched with SNPs in the Swine Leukocyte Antigen complex region and include DNA polymorphisms in genes with ontologies related to innate and adaptive immunity and causal variants known to affect viral disease susceptibility (e.g. SYNGR2 p.Arg63Cys). High-quality genotypes were obtained by filtering the raw data using ≥ 93% sample and ≥ 97% SNP call rates. This genomic information will be integrated with microbial profiling and used in genome-wide associations to discover genetic markers associated with resilience to specific pathogens and improved health status. The long-term goal of this project is to develop molecular surveillance tools and management solutions that improve genetic resilience to infectious diseases, increase fitness, and reduce economic losses to swine producers.

PRRSV-2 immune biobank for vaccine efficacy prediction

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The high mutation rate of PRRSV represents a big challenge and raises an important question for swine producers: Which vaccine can best protect my herd against an emerging strain? Currently no technology can adequately answer that question. To overcome this issue, we have established at the North Carolina State Veterinary College a vaccine efficacy prediction system that consist of an immune biobank (cells and serum) from pigs which received PRRSV-2 vaccines that are commercially available in US. The immune biobank enables to evaluate in vitro which vaccine induces the strongest immune response against a circulating PRRSV-2 strain based on neutralizing antibodies (nAb) levels and interferon-gamma producing cells responses (ELISpot assay). The immune biobank was tested against four North Carolina (NC) PRRSV-2 lineage 1 isolates (NC134, NC18-9-7, NC20-1, NC23-11), two prototype viruses (VR2332, NADC-20) and five MLV vaccine strains: differences in vaccine interferon-gamma responses were observed against NC18-9-7, NC23-11, NC20-1 and VR2332, whereas all vaccines showed similar responses against NC134 and NADC-20. Sera from vaccinated animals showed different nAb titers against NADC-20 and VR2332, but no nAb titers were present against NC134 and NC18-9-7 strains. Additionally, a whole genome sequence homology approach was used to evaluate the evolutionary divergences between vaccine strains and field isolates tested in the immune biobank. The project aims to improve precision animal management for PRRSV-2 in North Carolina by decreasing the impact of the disease with a proactive outbreak mitigation approach.

Self-Vaccinating Pigs to Save Labor, Improve Efficacy and Enhance Biosecurity

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Environmental enrichment (EE) devices or programs are required in some countries and in some markets. Any EE device with secondary purpose would likely encourage adoption. We developed an EE device that allows pigs to self-administer liquids, designing the EE device to align with pig's rooting, investigation, and play behaviors. We have previously shown that this method of vaccine delivery was efficacious for pigs to self-delivery a *Salmonella* vaccine. In this study we sought to determine if EE self-administration device could deliver vaccines for four diseases common among growing pigs. A baseline sample determined the antibody status of subjects. Serum and Oral Fluids IgG and IgA assays determined efficacy across three treatment groups: (1) a Control group that was not vaccinated, (2) a Hand Vaccinated group in which pigs were individually vaccinated by oral gavage or intramuscular (i.m.) injection, and (3) a Self-vaccinated group. Each four studies used 36 pigs (12 per treatment) over a period of up to 59 days post-vaccination. When *Erysipelas* vaccine was tested self-administered pigs developed both oral and serum antibodies equal to those of hand-vaccinated pigs. Pigs that self-administered the Ileitis vaccine developed only oral fluid antibodies. Pigs receiving *Mycoplasma* or *Influenza* vaccines via self-administration did not develop antibodies equal to those in hand-vaccinated animals. We conclude that certain vaccines can work as effectively through self-administration as through labor-intensive individual vaccinations. Other vaccines did not readily induce antibody synthesis. Vaccine formulations or administration methods might need adjustment to facilitate self-vaccination for selected vaccines, or an EE device might need to accommodate alternative methods of administration (such as i.m. or s.c.). Using EE for self-vaccination of selected vaccines is feasible today, reducing labor needs, eliminating the need for needles, and enabling pen-level vaccinations or delivery of other animal health products (drugs, pheromones, etc.).

Further evidence that science-based biosecurity provides sustainable prevention of PRRS virus and improves productivity in swine breeding herd

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is a globally significant pathogen of pigs. Controlling the entry of PRRSV to swine breeding herds is critical to maximizing animal health, welfare, and productivity. The objective of this study was to evaluate the impact of improved biosecurity on the PRRSV incidence risk and to understand whether these practices influenced breeding herd productivity.

Methods: A retrospective cohort study evaluating PRRSV incidence risk was conducted over a 3-year period, from July 1, 2021 to June 30, 2024, across breeding herds from a commercial swine production system (Pipestone, MN). Over the course of the project, 69 herds/321,013 sows participated in year 1, 76 herds/381,404 sows in year 2, and 75 herds/384,207 sows in year 3. Across the database, 2 cohorts of herds which differed in their level of biosecurity practiced were classified as Next Generation Biosecurity (NGB) COMPLETE or NGB INCOMPLETE.1 PRRSV incidence risk was calculated using MSHMP guidelines for each disease year, and over the 3-year period. The difference in the proportion of PRRSV positive herds (# new PRRSV infections/# breeding herds) across the 3-year period was analyzed for significance by Chi square, the cumulative PRRSV incidence risk across all herds during the 3-year period was calculated, and the association between the level of biosecurity (COMPLETE vs INCOMPLETE) and disease burden (PRRSV incidence risk) was tested by Chi square. In addition, differences in key performance indicators (KPIs) between 43 NGB COMPLETE HERDS and 19 NGB INCOMPLETE herds over disease years 1 and 2 were analyzed for significance by T test. For assessment of KPIs, only herds that provided two complete years of data during disease year 1-2 and were controlled by genetics, nutrition, farm management, employee training programs, animal handling protocols, record keeping, and animal health were selected. Finally, neighboring swine density within an 8.3 km radius of each breeding herd was calculated over the 3 years.

Results: The proportion of positive herds was 6/69 (8.7%) in year 1, 7/76 (9.2%) in year 2, and 11/75 (14.6%) in year 3 ($p = 0.77$). The cumulative incidence risk was 8.0%, and significantly lower ($p < 0.0001$) PRRSV incidence risk was associated with NGB COMPLETE vs NGB INCOMPLETE herds. Regarding differences in KPIs, NGB COMPLETE herds had higher total born piglets/farrowing ($p = 0.047$), and pigs weaned/female ($p = 0.021$), lower preweaning mortality ($p = 0.013$) and shorter weaning to first service interval ($p = 0.007$) than NGB INCOMPLETE herds. In addition, while not statistically significant ($p = 0.15$), NGB COMPLETE herds also had an increase of 0.91 pigs weaned/mated female/year. Finally, no differences were observed in the area density surrounding NGB COMPLETE and NGB INCOMPLETE herds across the 3 years of the study.

Discussion: This retrospective cohort study provides evidence that NGB, while not perfect, provided sustainable control and prevention of PRRSV and improved breeding herd productivity. This is the first report of successful PRRS control in a large commercial production system for this extended period of time.

Defining Interferon Antagonism Hierarchy of Porcine Epidemic Diarrhea Virus

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Porcine epidemic diarrhea virus (PEDV) is a swine enteric coronavirus that causes severe diarrhea, dehydration, and death in neonatal pigs, with a 90-100% mortality rate. Currently available vaccines provide only partial protection due to their inability to elicit robust lactogenic immunity in dams that protect neonatal pigs. Without access to a safe, effective vaccine, PEDV continues to spread, with an average positivity rate of 10-15%, posing a significant threat to the pork industry. The overarching goal of this project is to develop live-attenuated vaccine prototypes with improved immunogenicity. Coronaviruses are infamous for their ability to suppress the host's innate immune responses by encoding multiple immune antagonists. We hypothesize that the suppression of innate immunity by viral antagonists can lead to enhanced viral infection, tissue damage, and compromised adaptive immunity. Our previous research, along with that of others, has identified several PEDV proteins that antagonize the antiviral interferon (IFN) pathway (termed IFN antagonists). It has been shown that simultaneously mutating multiple IFN antagonists can attenuate viral pathogenicity in vivo. The specific goal of this work is to define the hierarchy of PEDV IFN antagonists and identify which one plays the most antagonistic role. Using reverse genetic platforms, we generated recombinant PEDV mutants that express single or combinations of mutated antagonists. We also developed porcine cell lines expressing a luciferase reporter gene driven by the IFN-beta promoter or the ISRE promoter, which can be used to assess type I IFN activation and IFN signaling, respectively. After analyzing type I IFN activation and IFN signaling in cells infected by single (Nsp1, Nsp15, and Nsp16) and double mutants (Nsp1+15, Nsp1+16, and Nsp15+16), we found that cells infected with the Nsp15 mutant exhibited the earliest and highest reporter activity among the tested single mutants. The double mutants Nsp1+15 and Nsp15+16 induced similar IFN activation or signaling levels as the single Nsp15 mutant, indicating that a weak combinatorial effect cannot be detected by this method. The relative elevation of IFN activation and signaling in cells infected with the Nsp1+16 mutant was barely detected at 24 hours post-infection (hpi), but reached similar levels to those of the other double mutants at 36 hpi and were markedly higher than in cells infected by the single Nsp1 or Nsp16 mutant. This suggests a combinatorial effect between the Nsp1 and Nsp16 antagonism. Our further growth kinetic analysis suggests that the differences in IFN activation and signaling are not likely due to replication differences among these mutants but are attributable to their capability to antagonize the IFN pathway. Taken together, our results show that PEDV Nsp15 is the most potent IFN antagonist among the tested proteins. The reporter cell lines generated in this study are useful tools for evaluating IFN antagonist candidates of swine viruses. Next, we will examine a few other IFN antagonist candidates and determine the sensitivity of these mutant viruses to type I and III IFN treatment. It is our goal to define and translate the antagonism hierarchy to PEDV pathogenicity and immunogenicity in animals.

Longitudinal assessment of associations between mucosal microbial communities and porcine respiratory and reproductive syndrome virus pathogenesis

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A growing body of work implicates mucosal microbial communities as important factors in mediating host-viral interactions. Porcine respiratory and reproductive syndrome virus (PRRSV) infection causes substantial disruption of microbial communities which has been linked to differential viral outcomes. However, most work to date has focused on early infection timepoints and utilized cross sectional, single timepoint experimental design making it unclear how long lived these impacts on microbial communities are and if they result in lasting impact on host physiology or viral infection dynamics. Here we longitudinally profiled gut and nasal microbial communities of PRRSV infected (N = 31) and uninfected control pigs (N = 7) from challenge to viral extinction using 16S rRNA gene sequencing. Associations between microbial diversity and infection were assessed collectively across all timepoints and individually at each site and timepoint using generalized linear mixed models and permutational multivariate analysis of variance. Overall PRRSV infection associated with altered microbial community profiles, however, the initiation and magnitude of these effects varied by sampling site and timepoint examined. To determine if this microbiome disruption correspond to viral outcomes, we correlated microbial diversity with markers of viral pathogenesis at timepoints where we observed microbiome disruption. Consistent with our prior work, microbiome diversity was also associated with viral load early in PRSSV infection and this effect waned as infection resolved. Collectively, this work clarifies the longitudinal crosstalk between PRRSV infection and mucosal microbial communities, and ongoing work seeks to develop microbiome-based strategies to mitigate the impacts of viral infection on swine health.

Cross-Species Analysis of CD163 Orthologs Reveals Key Domain for PRRSV Susceptibility

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CD163 serves as the primary receptor for viral entry, and CD163 orthologs from various species, including humans, mice, dogs, and monkeys, can confer susceptibility to the virus when ectopically expressed in non-susceptible cells. However, pigs are the only known natural host for PRRSV. This study reexamined the susceptibility of cells expressing different CD163 orthologs to understand why only pigs, but not other species, are naturally susceptible to PRRSV infection. We infected HEK-293T cells expressing CD163 from humans, mice, monkeys, and pigs with a PRRSV isolate and observed varying degrees of infection. Notably, cells expressing porcine and monkey CD163 were highly susceptible, while those expressing human and mouse CD163 showed minimal susceptibility to PRRSV infection. The profound difference in susceptibility between human and monkey CD163 is striking, given that these two receptor molecules share 97% amino acid sequence identity. To pinpoint the domains responsible for this difference, we performed domain exchanges between human and monkey CD163. We discovered that human CD163 bearing the monkey PSTII domain supported PRRSV infection to levels similar to wild-type monkey CD163. Conversely, monkey CD163 with the human PSTII domain exhibited infection levels similar to wild-type human CD163. These findings indicate that while CD163 orthologs from different species can confer susceptibility to PRRSV infection, the intensity varies significantly and that the PSTII domain is crucial in supporting PRRSV infection.

Long-term evolutionary dynamics of Porcine Epidemic Diarrhea Virus (PEDV) in the U.S. a decade after introduction

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Introduction: Porcine Epidemic Diarrhea Virus (PEDV) (Coronaviridae family) is responsible for significant losses in the swine industry worldwide. PEDV was introduced to the United States (U.S.) in 2013 and quickly spread across the country. Evolutionary dynamics studies of this virus since its emergence have focused on the early stages of the epidemic, identifying two strains (indel and the more virulent non-indel strains). However, our understanding of its evolution and molecular epidemiology in the current phase of endemic circulation in the country remains limited. Here, we explore the temporal dynamics of PEDV in the U.S. and provide an overview of its evolutionary status a decade since its introduction.

Methods: The incidence of PED was assessed based on the number of positive sow farms reported by the Morrison Swine Health Monitoring Project (MSHMP). Furthermore, we analyzed a total of 556 Spike protein subunit 1 sequences, 328 from MSHMP and 228 from GenBank (2013-2024). 313 of these were nearly complete genomes. For the spike protein, we employed Bayesian methods to reconstruct time-scaled phylogenetic trees and estimate the effective population sizes (Ne) using BEAST v1.10.4. Recombination analyses were performed using RDP5 software. Analyses for both PEDV strains were conducted separately.

Results and Conclusion: Sequence availability was proportionate to incidence data, with more sequences available during the epidemic period (May 2013 - December 2014), followed by a reduction in the number of both cases and sequences in subsequent years. The indel and non-indel strains showed a low Ne at the beginning of the PEDV epidemic, reflecting a population founder effect. Subsequently, both entered a period of expansion. Reliable Ne estimates for the indel strain (99.7% nucleotide identity) could only be obtained until 2017 due to data availability. The non-indel strain (n=499) exhibited high nucleotide identity (mean: 99.7 ± 0.004). All recent sequences (2017 onwards) grouped into two small and distinct clades (99.8% identity within clades, 98.4% between clades), each occurring in specific regions in the U.S. This suggests that there is strong regional structuring or even compartmentalization of PEDV spread, which has implications of disease elimination efforts. Other clades existing during the early epidemic period appeared to have gone displaced by 2019. Fluctuations in the effective population size for the non-indel strain reveal cycles of population contractions and expansions, perhaps associated with seasonality. The indel ORF-S was under negative selection, while the non-indel strain evolved neutrally with several sites that were under positive selection; these substitutions appear randomly distributed across the phylogeny, suggesting they may be recurrent mutations. One site under positive selection was in an epitope of the spike protein. Preliminary analysis detected thirteen recombination events in complete genome sequences, two of which left numerous descendants. The ORF-S of the non-indel strain showed high genetic similarity, patterns of temporal structuring, and several sites under positive selection. The possible displacement of older clades by more recent ones, may reflect fitness differences, highlights the need for continued molecular surveillance. In addition, more representative sampling across regions is needed to confirm observed patterns.

Assessing the relative importance of geographic proximity and pig movement-related factors to the spread of PRRSV-1 in the United Kingdom

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PRRS control strategies are best informed by the correct identification of the highest risk pathways for PRRSV introduction, allowing the most appropriate measures to be prioritised. Transmission mechanisms for PRRSV have been established, but mainly in areas where PRRSV-2 predominates (such as North America) rather than PRRSV-1. The structure and scale of the pig industry and farm practices differs in the United Kingdom (UK) compared to North America, with smaller farms, lower pig densities and more outdoor units in the UK. Only PRRSV-1 has been detected in UK pigs to date, and few data are available to accurately inform the relative importance of different transmission routes for the spread of PRRSV in the UK. The objective of this study was to evaluate the contributions of geographic proximity, and links between farms through pig movements, to the spread of PRRSV, based on the genetic similarity of PRRSV found in UK farms.

A dataset of 567 complete ORF5 sequences of PRRSV-1 from diagnostic submissions in the UK between 2014 and 2020 was compiled. Pairwise comparisons of the sequences were produced detailing the distance between pairs and the percentage similarity. Each sequence was assigned to their holding identifier and its geographic origin. This was used to link holdings with Livestock Demographic Data Group pig movements data from a two-year period relevant to the submission date. These data were used to extract information on the estimated herd size and holding type, the number of pigs moved on during the two-year period, the number of holdings from which pigs were received and whether the holding could be identified as part of a large pig company.

From the 567 sequences, 265 unique holdings were identified, with 1 to 21 sequences per holding. The farms were generally representative of the geographical distribution of pigs in the UK, and the majority (88.7%) were of the largest size category and were either feeder or breeder-finisher farms. The proportion of similar sequences was affected by the number of suppliers and pig movements a holding had; those with more movements (on and off) showed significantly greater sequence diversity, suggesting greater transmission of new strains onto the holding. Holdings with fewer unique suppliers generally had more similar sequences. When sequences from the same company were analysed, only 4 of 17 holdings were significantly more likely to have similar sequences, suggesting that simply being within a company or production pyramid did not influence the sequence diversity as much as the direct movements of pigs and use of suppliers. Investigating the potential for local spread, 10 of the 14 counties showed greater similarity within than outwith the area. The results suggested that the number of sources and degree of pig movements affected sequence diversity on a holding. The results also highlighted large-scale regional effects, with greater similarity from sequences within regions than in other regions. This suggests that reducing the number of suppliers to a holding and reducing movements outside of geographical regions could help reduce the spread of PRRSV-1 strains in the UK.

Development of a standardized outbreak investigation program web-based application

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Objective: To protect the United States swine industry from substantial economic losses caused by an emerging or transboundary disease outbreak, producers and veterinarians need to be prepared to rapidly identify, control, and eliminate the pathogen. In response to events following the introduction of the porcine epidemic diarrhea virus (PEDV) in 2013, the Swine Health Information Center (SHIC) funded the development of the Rapid Response to Emerging Disease Program (RRP) in August 2016. The aim of the RRP was to respond rapidly to conduct outbreak investigations using a standardized approach when a new transboundary or emerging disease threat occurs. The objective of this study was to develop a web-based application for a Standardized Outbreak Investigation Program (SOIP) to easily capture, and retain data from investigations in a secure industry-wide database.

Methods: The web-based program was developed by the Department of Veterinary Diagnostic and Production Animal Medicine at Iowa State University using common programming languages, databases and deployment servers. The application was launched in December 2023.

Results: The web-based application splits the SOIP form into two main sections. The first section is the pre-investigation survey. It is intended to capture information before the investigation is conducted on-farm. The second section is the SOIP investigation form, captures information from the investigation conducted on-farm and any follow-up. The pre-investigation survey includes a subsection to enter the dates and frequency of entry events. Diagnostic reports, dendrograms, homology tables, animal movement information, or other documentation related to the investigation may be uploaded. Maps of the site and surrounding area and daily local weather data for the investigation period are automatically pulled from commercially available sources. The investigation form section includes sub-sections to enter information about the characteristics of the herd, premises, and surrounding area. The remainder of the investigation form includes questions organized by entry event. Each entry event may be ranked as high, medium, or low for the likelihood that the event caused the outbreak. Space to summarize key findings and justify the rankings is provided.

Conclusions: The new web-based application will advance the aim of the RRP and make it easier to conduct investigations of endemic diseases to help producers and veterinarians identify and prioritize farm and production system-specific biosecurity hazards to improve biosecurity. The industry-wide database will facilitate research to identify industry-wide gaps in biosecurity and help the industry identify solutions to improve biosecurity faster.

Porcine antibody response to epitope A on PRRSV GP5 and its role in virus neutralization

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Immune selection pressure on the glycoprotein 5 (GP5) of porcine reproductive and respiratory syndrome virus (PRRSV) has been proposed to be the key factor driving the emergence of novel genetic variants. The GP5 ectodomain includes a linear, immunodominant epitope known as epitope A, situated adjacent to a conserved neutralizing epitope designated as epitope B. The retention of epitope A on mature viral particles depends on a shift in the signal peptide cleavage site on GP5. This study investigates the presence of epitope A in selected vaccine and field strains and examines whether epitope A-specific antibodies prevent neutralizing antibodies from accessing epitope B through steric hindrance.

Serum samples collected from pigs inoculated with either of two modified live virus (MLV) vaccines or three field isolates of PRRSV were subjected to indirect ELISA to determine antibody responses to linear peptides representing different epitopes, including epitope A, on the GP5 ectodomain. Post-challenge serum from pigs previously vaccinated with an MLV vaccine identical in epitope A sequence to the challenge field strain IA/2014 was used to measure the anamnestic antibody response to epitope A using ELISA. In addition, GP5 sequences (amino acids 1-61) from different stains were analyzed in silico for potential signal peptide cleavage sites with SignalP 4.1. Antibodies to epitope A and epitope B were isolated from the post-challenge serum using affinity chromatography, and the presence of epitope A on two PRRSV strains was validated by Western blot with respective peptides. The neutralization activity of epitope-specific antibodies and antibody-depleted serum flow-through was determined against the FL12 virus strain. The binding avidity to GP5 ectodomain and the neutralization activity against FL12 of epitope B-specific antibodies was measured by competitive binding and neutralizing assay with epitope A antibodies.

Epitope A-specific antibodies were detected in pigs vaccinated with either of the two MLV vaccines but not in pigs infected with various field isolates. Virus challenge did not induce an anamnestic antibody response to epitope A, suggesting the absence of epitope A on the challenge virus. Western blot analysis confirmed the absence of epitope A in the field strain 46/2020, while the lab-adapted FL12 strain retained it. In-silico analysis of signal peptide cleavage sites suggested that the three selected field strains lacked epitope A, whereas all five commercial MLV strains harbored it. However, depleting antibodies to either epitope A or epitope B did not affect overall virus neutralization by PRRSV immune serum, indicating that antibodies to other neutralizing epitopes may compensate for the depletion, retaining the total polyclonal serum neutralization activity. Epitope A antibodies did not reach 50% neutralization activity for IgG concentrations up to 4.3 µg/mL, while epitope B antibodies exhibited 50% neutralization activity at 0.867 µg/mL. Moreover, epitope A antibodies significantly inhibit the binding of epitope B antibodies in a dosedependent manner. The neutralization activity of epitope B antibodies remained unchanged in the presence of epitope A antibodies. In summary, results from this study demonstrate that antibody binding to epitope A competitively abrogates epitope B binding in a competitive ELISA assay but does not inhibit the neutralization activity of epitope B-specific antibodies. Further studies are needed to understand the role of conformational epitopes to fully elucidate the interference of antibodies to GP5 as regards virus neutralization.

scRNAseq analysis of PBMCs during acute PRRSV infection: searching for host cellular markers to predict persistent infection

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The lack of knowledge of the molecular events occurring during PRRSV infection impedes our ability to use genetics to improve disease resilience. Understanding the cellular heterogeneity, molecular mechanisms, and dynamics of immune response is crucial for developing better strategies for disease surveillance and prevention. This study aims to measure differential gene expression in specific cells circulating in blood at day 14 post-infection (DPI), a crucial time of PRRSV acute infection, as an initial step in finding predictive markers for persistently infected pigs.

A total of 36 specific-pathogen-free 4 weeks old gilts from 6 litters were obtained, of which 30 were infected by PRRSV-2 isolate SD95-21, and 6 were mock-infected (MI) as controls. Peripheral blood mononuclear cells (PBMCs) were collected weekly from all pigs from 0-84 days post infection (DPI). Real time qPCR on PBMCs and lymphoid tissue samples (collected at necropsy at 84 DPI) allowed infected pigs to be differentiated into persistently infected (PI) and virus extinction (VE) phenotypes. Single-cell RNA sequencing (scRNA-seq) using the 10X Genomics Chromium controller of PBMCs from 14 DPI from 2 MI, 2 VE, and 5 PI pigs from a total of 4 litters was used to characterize differential abundance (DA) of specific cell types across the three phenotypes. We performed computational analysis using the 10X Genomics Cell ranger and Scanpy toolkits. Data preprocessing included removal of ambient RNA and doublets and standard quality control filters. Using dimensionality reduction visualization (UMAP), 30 distinct clusters of cells were identified. DA analysis of cell "neighborhoods" within specific clusters was performed using GLM in MiloR to distinguish biological differences (sow as random and infection status as fixed effects) from nuisance variation.

DA analysis results showed that abundance of cells was statistically different (adj $P<0.01$) for MI compared to either PI or VE for approximately 75% of all clusters. This indicates a large effect due to infection at 14 DPI. To focus on the largest differences, visual inspection of the UMAP plots showed major differences between MI and PI or VE for two clusters that were annotated as monocytes. These clusters (named 10 and 14) were lower in abundance in MI pigs compared to PI or VE pigs; in fact, cluster 10 was completely missing in MI pigs and contained high levels of TCF4+ and CD163+ cells and low levels of CD14+ cells. A similar DA analysis comparing PI and VE pigs showed no statistical significance for any cluster, indicating that at this early stage, no cell types are highly DA between pigs that eventually became PI versus VE.

This study highlights significant differences in abundance of PBMC cell types between infected and uninfected pigs at 14 DPI, including very large differences in specific monocyte clusters. One cluster, absent in MI pigs, contained activated monocytes that were likely responding to PRRSV infection. While we did not detect significant differences in cell abundances between PI and VE pigs, differential gene expression analysis within clusters may provide additional clues as to changes in cell function or state between these eventual infection states at this early age. This study was funded by USDA-NIFA #2023-67015-39079.

Evaluation of deployable fan coverings for biocontainment of airborne swine pathogens

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Introduction: With rising disease outbreaks across the Midwest, controlling swine respiratory disease pathogens has become crucial. While air filtration reduces pathogen introduction via aerosols, biocontainment remains underexplored. Pathogens such as porcine reproductive and respiratory syndrome virus (PRRSv), porcine epidemic diarrhea virus (PEDv), and influenza A virus (IAV) are spread by respiratory secretions and other microscopic particles. Studies have shown virus detectability and viability to be influenced by particle size, with PRRSv and IAV isolated from particles larger than 2.1 µm. There is demand for ready-to-use biocontainment tools that can be quickly implemented on swine farms to reduce aerosol transmission pressure. This study aimed to evaluate the efficacy of rapidly deployable exhaust fan coverings for aerosol biocontainment by measuring total air particle quantity reductions.

Materials and Methods: One wean-to-finish site stocked with mid-finishing pigs was selected, featuring various fan sizes (24" pit fans, 36" wall fans, 50" wall fans). Three materials were compared to a fan with no covering (negative control): PolyKlean™ synthetic media (Blue Poly), Nylon, tear-resistant fan sock (Fan Sock) staked to the ground to redirect airflow, and Polyethylene privacy screen (Black Screen). Airborne particle counts were collected using an optical particle counter (OPC) measuring particles from 0.3 to <5 µm. Sampling points were mapped based on the diameter of the fan cone. Five air particle measurements were collected 1 meter from the interior of the fan shutters and 1, 2, and 3 meters from the exterior of the fan. Three consecutive air particle measurements were performed per distance and location to calculate an average particle count, with three replicates completed for each treatment and fan. Minimum ventilation pit fans and stage two pit fans ran continuously at 100% power during sample collection. Each enrolled fan ran individually at 100% power while treatments were applied. Weather conditions were recorded at all sample locations using a portable weather station. Data were analyzed using a two-way repeated measures ANOVA to evaluate the effects of fan cover treatment and fan size on air particle quantity measurements with log transformation. Analysis was conducted using the lmer function in R studio, with fan identification as a random effect. Results were considered significant with $p \leq 0.05$.

Results and Discussion: At 1 meter outside the fan, the fan sock treatment had a lower average 5.0 µm particle count compared to no cover or the blue poly treatments ($p=0.046$). At 1 meter in front of the fan on the inside of the barn, the 50" fan had significantly lower particle counts of particles measuring 1.0 µm ($p=0.002$) and 5.0 µm ($p=0.003$), and overall ($p<0.001$). Differences in air particle counts between the 24", 36" and 50" fan size were not observed ($p>0.10$). No effects were observed based on weather conditions during the sampling period. Under the conditions of this study, the fan sock treatment had the greatest impact of reducing air particles at 1 meter outside of a fan compared to the other fan cover treatments, especially for 5.0 µm particles. However, as the distance from the fan increased, there was no difference in air particle quantity across the fan cover treatments. As a result, significant differences in the total number of air particles detected were not observed across the fan cover treatments. This pilot study suggests that rapidly deployable fan coverings may reduce air particle quantity, thus questioning its role in improving the biocontainment of aerosolized pathogens.

A Decadal Review of Porcine Deltacoronavirus Occurrence in U.S. Breeding Herds

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Objective: Porcine Deltacoronavirus (PDCoV), emerged in the United States (US) in 2014. Studies at the time contributed to our understanding of its emergence but the current situation of PDCoV in the US is largely unknown. Therefore, we characterized the PDCoV occurrence in the US breeding herd population since its emergence.

Methods: Breeding herd data from the Morrison Swine Health Monitoring Project (MSHMP) between January 2015 and December 2023 were analyzed, representing approximately 60% of the US breeding herd. Participating production systems voluntarily report herds' PDCoV outbreaks. Yearly cumulative incidence was calculated using the number of breeding herds reporting either PRRS, PEDv, or PDCoV statuses (i.e., sites that were sharing information for at least one of the main diseases monitored by MSHMP) as the denominator and reported cases as the numerator.

Results: Throughout 2015–2023, the median number of sites monitored through MSHMP was 1166, ranging from 1062 in 2015 to 1187 in 2020. In total, 244 PDCoV outbreaks occurred in 186 sites from 22 production systems across 16 states. During the entire period, 140 herds reported one PDCoV outbreak, 36 herds reported PDCoV twice, 8 reported it three times, and 2 herds reported it four times. For sites that experienced more than one PDCoV outbreak, the interval between outbreaks had a median of 2.11 years. Cases were more frequently reported during the fall and winter seasons. Most cases occurred in the South (69.9%) and the Midwest (24.7%), with the remaining cases located in the West (1.6%) and Northeast (0.5%). In 2017, a shift in the spatial distribution occurred with the majority of cases originating from the South. The yearly cumulative incidence ranged from 0.44% in 2017 to 4.28% in 2023.

Conclusion: The potential seasonality of PDCoV transmission in colder months was also observed in other countries. Even though PDCoV occurs at a much lower frequency than other important swine diseases in the U.S., such as PRRS and PED, it is still very much present in the U.S. breeding herd as cases continue to be reported each year. In fact, PDCoV incidence has increased when compared to earlier years. These results show that PDCoV monitoring is still important, and control measures need to be strengthened to limit the spread and impact of the disease.

PRRS Virus Variant 1H.18: Occurrence and Classification Challenges

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Restriction Fragment Length Polymorphism (RFLP) may erroneously group genetically dissimilar PRRSV into the same type while segregating closely related ones. Phylogenetic methods to organize PRRSV into lineages and sub-lineages tend to form groups too broad for many epidemiological investigations. A PRRSV variant classification is being proposed by an AASV-funded working group to address finer-scale classification across all sub-lineages. Here, we describe the occurrence of a novel PRRSV variant and discuss classification challenges. In December 2023/January 2024, outbreaks associates with PRRSV classified as a relatively rare RFLP pattern (1-12-2) were reported to the Morrison Swine Health Monitoring Project (MSHMP) PRRSV sequence database. The obtained sequence was used as a seed and a case was defined by nucleotide identity of ≥98% between samples with any additional case identified used as seeds for subsequent rounds (snowball method) as our standard case definition. Cases were described by their RFLP pattern, lineage/sub-lineage, AASV variant classification, and occurrence in time and space. A total of 61 sequences belonging to this variant (1H.18) have been identified as of May of 2024 on the MSHMP database, most of them classified as either RFLP 1-8-4 (n=32), 1-12-2 (n=20), or 1-12-1 (n=1). Sequences belonging to this group were assigned to sub-lineages L1C or L1H, with classification varying according to the method employed (PRRSView or distance to the nearest reference). Both the snowball method and AASV variant classification (1H.18) had 100% agreement in case ascertainment, while the combination of RFLP and sub-lineage (L1H RFLP 1-12-2) had only 60% positive predictive value. The 1H.18 variant has been identified in nine production systems located mostly in IA (n=23) and MN (n=22), although one sequence was detected in IL. Detections date back to 2018, with a slight uptick in sequence counts in 2020 and in both sequences and individual sites in late 2023. The 61 1H.18 sequences originated from 41 unique sites (5 breeding, 10 grow-finish, 11 others, and 20 unknown). The production impact of this variant has not yet been formally assessed, although both mild and more severe clinical presentation have been reported. The uptick in 1H.18 sequences in 2020 likely went undetected due to all of the sequences at the time being classified as RFLP 1-8-4, a common RFLP type that occurs in many different lineages and sub-lineages, and many of the sequences originated from a single site. The challenges in classifying this variant through classical methods such as RFLP and sub-lineages highlight the need for finer-scale PRRS classification.

Evaluation of a stable oil-in-water adjuvant for recombinant PRRSV vaccine

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Background and objectives: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is the causative agent of PRRS, a viral disease widespread globally. The swine industry reports large economic losses due to variable morbidity and mortality with reproductive disorders in sows and respiratory symptoms often with co-infection in growing and finishing pigs. Oil-in-water (O/W) based vaccines are commonly used against PRRSV, known as a destabilizing antigen hence the increased need for highly stable vaccines. This study aims to compare a recombinant gp 5 protein and recombinant M protein with inactivated cultural PRRSV based vaccines formulated with a "resistant" O/W adjuvant (MONTANIDE™ ISA 28 R VG), or carbomer.

Materials and Methods: The safety and efficacy of the vaccines were evaluated in production conditions by injecting intramuscularly 42-45 days healthy pigs with a negative serology for PRRS, with 2 ml of the vaccines at D0 and D32, 10 pigs per group. Safety was assessed by palpation, in vivo and post mortem visual assessment of the site of injection and thermometry of all animals before vaccination, 24, 48 and 72 hours after vaccination. In order to evaluate efficacy, blood sampling was performed at D0, 32, 64 and 115 to assess specific antigen antibodies and virus-neutralizing antibodies by ELISA

Results: For the safety trial, the body temperature monitoring showed a slight increase (less than 1°C) 24 hours after vaccination for the two adjuvants. Then, all vaccine groups returned to normal body temperature. Otherwise, no behavior changes were recorded in both groups. Finally, Post-mortem autopsy and visual examination of the injection site did not reveal any tissue changes in piglets in the two groups. The results showed that the O/W based vaccine demonstrated a good safety profile.

Regarding efficacy assessment, from D30, the O/W based vaccine induced statistically higher antibody titers than carbomer and presented 100% of sero-conversion. On the other hand, a higher level of neutralizing antibodies against PRRSV was registered after the boost with O/W adjuvant vaccine compared to the carbomer vaccine.

Discussion & Conclusion: Taken together, these results showed that MONTANIDE™ ISA 28 R VG adjuvant is well suited for formulating highly destabilizing antigens, providing a balanced safety/efficacy profile compared to carbomer.

Assessment of homologous and heterologous PCV2 vaccine efficacy in a PCV2d/PRRSV co-challenge model

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PCV2 is an economically significant, highly prevalent pathogen and is the etiological agent of porcine circovirus-associated disease (PCVAD). Vaccination does not prevent viral replication and infection, potentially leading to gaps in immunity caused by heterologous infection and coinfection. While cross-protection amongst different PCV2 subtypes has been demonstrated, questions remain regarding the clinical, pathological, and immunological efficacy of commercial vaccines against homologous and heterologous challenges. Therefore, this study assessed differences in clinical, pathological, and immunological parameters in heterologous and homologous PCV2 vaccinated pigs under a PCV2d/PRRSV co-challenge model.

On study day 0 (D0), 60 three-week-old commercial pigs were vaccinated with Ingelvac PRRSV MLV. Additionally, pigs received a PCV2a vaccine (n=20), PCV2d vaccine (n=20), or no PCV2 vaccine (n=20). On D28, all 60 pigs were inoculated with 1 mL IM and 1 mL IN of PCV2d (5 log10/2ml dose) and 2 mL IM of 1-7-4 PRRSV (4.0 TCID50/mL). Mortality was recorded throughout the study duration. All remaining pigs were euthanized on D56, and fresh and fixed sections of lymph node, tonsil, and lung were collected to determine viral load, microscopic lesion severity, and amount of PCV2 antigen detected within lesions. Serum samples were collected on D0, 28, 42, and 56 to measure levels of PCV2 viremia, total antibodies by ELISA, and neutralizing antibodies (NA) by PCV1-2 chimeric virus neutralization assay.

The mortality rate was numerically higher in the PCV2a vaccinates (15%) compared to the PCV2d vaccinates (5%). PCV2 viral load and viremia, lesions associated with lymphoid depletion, and amount of PCV2 antigen detected in tissues was significantly lower in the PCV2d vaccinates, correlating to subclinical infection. In contrast, approximately 20-40% of the PCV2a vaccinates had moderate lymphoid depletion with moderate amounts of antigen in tissues and significantly higher levels of viremia, corresponding to clinical disease. While total levels of PCV2-specific antibodies measured by ELISA were similar between the PCV2a and PCV2d vaccinates, PCV1-2 chimeric virus neutralization assays revealed differential subtype-specific NA titers among the PCV2a and PCV2d vaccinates. Prior to challenge on D28, PCV2d vaccinates had significantly higher NA titers against the PCV1-2d vaccine and challenge chimeric viruses, while the PCV2a vaccinates had significantly higher NA titers against the PCV1-2a vaccine chimeric virus.

Homologous vaccination may provide greater protection in virulent co-infection scenarios in the field, partially due to the earlier, enhanced development of homologous NAs. Future studies should evaluate differences in the cell-mediated response following homologous/heterologous vaccination and virulent challenge. While PCV2 vaccines are widely implemented in the swine industry, each farm should consider local or regional disease epidemiology when implementing PCV2 vaccine strategies to maximize protection achieved from homologous vaccination. Information gained in this study should aid in the development of vaccine design and optimal strategies to better control PCVAD under field conditions.

Evaluation of PCV3 humoral responses in experimentally infected pigs and dynamic of maternally derived antibodies in piglets from naturally infected sows

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Porcine circovirus type 3 (PCV3) was first identified in the United States in 2016 and has been associated with reproductive failure and multisystemic inflammation. PCV3 is a circular, single-stranded DNA virus of approximately 2000 bp with two major identified structural proteins, the capsid (Cap) and replicase (Rep). Information regarding PCV3 humoral response is scarce. Therefore, the objectives are to evaluate the Cap and Rep humoral response following experimental PCV3 infection and transfer of maternal antibodies in piglets from naturally infected sows by IFA and ELISA.

Eighteen, 5-week-old CD/CD pigs were randomly assigned to 4 treatment groups: PCV3, PCV3+KLH (keyhole limpet haemocyanin), Negative Control, and KLH control. PCV3 and PCV3+KLH groups were intramuscularly and intranasally inoculated with 2mL of PCV3 tissue homogenate on days 0 and 7. PCV3+KLH and KLH control groups were administered 1mL of KLH in the hip and shoulder on days 3 and 7. Serum was collected weekly until day 42 post inoculation (dpi). Piglet serum from three PCV3 seropositive sows was collected weekly until 9 weeks post-farrowing (pf). For the IFA, PCV3 Cap and Rep genes were cloned into a plasmid expression vector and transfected into HEK 293T cells. Serially diluted swine serum was added to transfected cells followed by FITC-conjugated goat anti-swine IgG secondary antibodies. For the ELISA, the Cap and Rep plasmid expression vectors were transformed in BL21 E. coli. Proteins were purified from cell lysate by nickel column affinity chromatography and used as coating antigen.

Experimentally infected pigs developed Cap IgG antibodies at 14 dpi, reaching significantly higher levels between 28-42 dpi ($p<0.05$). Rep IgG antibodies were detectable at 28 dpi with significantly higher levels between 35-42 dpi ($p<0.05$). Antibody titers detected by IFA against the Cap were 16-fold higher than the Rep. Commercial piglets showed high levels of IgG against the Cap during week 1 pf declining to undetectable levels by 7-9 weeks pf. Rep IgG detection was variable between litters with highest levels occurring 1-week pf and becoming nondetectable 5 weeks pf. Cap antibodies titers were 9-fold higher compared to Rep antibodies by IFA and Cap.

Similar to PCV2 infection, PCV3 induces a delayed humoral response detectable several weeks post-infection with higher titers against the Cap protein compared to the Rep protein. Both Cap and Rep antibodies were detected in neonatal pigs, suggesting maternal passive transfer. Given the prolonged PCV3 viremia reported in the literature, the protective role of Cap or Rep antibodies warrants further investigation. PCV2 maternally derived antibodies confer passive protection. Thus, future studies should evaluate the protective role of PCV3 maternal antibodies.

PRRSV nsp1α differentially regulates viral RNA synthesis

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PRRSV nsp1α is the first viral protein translated in virus-infected cells and released from viral polyprotein 1a via auto-cleavage. It plays important roles in viral replication, suppression of host innate immune response, and cell-mediated immune responses. Nsp1α was demonstrated to form a homodimer in vitro based on its crystal structure. In this study, we aimed to characterize the role of dimerization in the functions of nsp1α. Through alanine scanning, valine132 and proline134 were identified as key residues for the dimerization of nsp1α. A recombinant HP-PRRSV expressing an additional nsp1α with a Flag-tag was generated to confirm the homodimer confirmation of nsp1α in PRRSV-infected cells. Using the recombinant viruses expressing an additional Flag-nsp1α mutant of V132A or P134A, we confirmed that V132A and P134A disrupted the dimerization of nsp1α in PRRSV-infected cells. The critical role of valine132 and proline134 in the dimerization of nsp1α was also confirmed in a PRRSV-1 strain. When ectopically expressed, the mutants containing a substitution of V132A or P134A have no obvious effect on the function of nsp1α in antagonism host type I IFN production and degrading SLA-I molecules. The mutations of V132A and P134A introduced in a replicon system of HP-PRRSV significantly attenuated the expression of the Gaussia luciferase reporter, suggesting the dimerization of nsp1α is critical for viral replication. To confirm this, the mutations of V132A and P134A were respectively introduced into the PRRSV genome. A recombinant virus (vV132A) carrying the V132A mutation was rescued, while the P134A mutant was lethal. In comparison with the WT virus, the growth of vV132A in MARC-145 cells was significantly attenuated. Viral RNA synthesis in BHK-21 cells transfected with the cDNA clones was evaluated by RT-qPCR. V132A upregulated the relative abundance of all subgenomic RNAs of minus-strand and positive-strand, especially sgRNA6, while V134A downregulated the minus-strand sgRNA2, sgRNA4 and sgRNA5 and all positive-strand subgenomic RNAs but upregulated sgRNA6. Taken together, our results proved that nsp1α dimerization plays an important role in regulating viral RNA synthesis but is not related to nsp1α's immunosuppression activity.

PRRS Research – Importance of Teamwork

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Porcine reproductive and respiratory syndrome (PRRS) was first reported as “mystery swine disease” in Lelystad, the Netherlands, 23 years ago, and in North America in 1992. We now know this disease as being due to infections with type 1 and 2 PRRSV, a single stranded RNA virus. Since then, major research efforts have resulted in vaccines with moderate to poor efficacy in preventing infection, particularly with heterologous viral strains. As a result of NC229 there has been better coordination to address vaccine efforts.

Our genomic resistance efforts, termed the PRRS Host Genetics Consortium, were complementary to the PRRSV vaccinology research. We assembled a diverse team of researchers: virologists, immunologists, geneticists, computational biologists, to probe underlying mechanisms that could result in PRRS resistant pigs. With National Pork Board funding and cooperation from 6 different swine breeding companies we performed 15 trials analyzing samples collected from PRRSV2 infected nursery pigs. We discovered a genetic allele of GBP5 that resulted in pigs with lower viral load and increased weight gain despite the presence of PRRSV infection. This allele is now being used for genetic selection by breeding companies. More detailed gene and protein expression work has pinpointed gene expression pathways and alternate anti-viral response mechanisms.

More recently, we have focused on the pregnant gilt model (PGM) of PRRSV infection working with a team of Canadian and US researchers including those with reproductive expertise. We probed critical maternal and fetal tissues and genes to forecast fetal resistance (a fetus with no viral load despite maternal infection), resilience (a fetus which survives despite PRRSV RNA in fetal thymus and serum following maternal infection), or susceptibility (high viral load and/or meconium staining) to congenital PRRSV infection. Data supports the hypothesis that placental transfer of the virus is a rate limiting step and that specific genes associated with innate immunity help protect the fetus.

All of this research could not have been accomplished without well-organized teams, starting with the detailed planning of experiment designs and sample collection, the organized PRRSV infections in BSL2 facilities, the pig breeding companies providing pigs from current genetic lines, and the dedicated teams carefully collecting valuable blood and tissue samples. That work set the basis for data production and analysis by students and postdocs mentored by established scientists, the secure database storage of results for continued evaluation and the final submission of manuscripts for peer review. Moreover, none of this could have been accomplished without the backing and funding by the National Pork Board, USDA NIFA and ARS, Genome Canada and affiliated universities and the NC229 viral and NRSP8 genome projects.

All led to major team accomplishments!

Isolation and characterization of novel reassortant mammalian orthoreovirus from pigs in the United States

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Mammalian orthoreovirus (MRV) infects multiple mammalian species including humans. A United States Midwest swine farm with approximately one thousand 3-month-old pigs experienced an event, in which more than 300 pigs showed neurological signs, like “down and peddling”, with approximately 40% mortality. A novel MRV was isolated from the diseased pigs. Sequence and phylogenetic analysis revealed that the isolate was a reassortant virus containing viral gene segments from three MRV serotypes that infect human, bovine and swine. The M2 and S1 segment of the isolate showed 94% and 92% nucleotide similarity to the M2 of the MRV2 D5/Jones and the S1 of the MRV1 C/bovine/Indiana/MRV00304/2014, respectively; the remaining eight segments displayed 93%–95% nucleotide similarity to those of the MRV3 FS-03/Porcine/USA/2014. Pig studies showed that both MRV-infected and native contact pigs displayed fever, diarrhea and nasal discharge. MRV RNA was detected in different intestinal locations of both infected and contact pigs, indicating that the MRV isolate is pathogenic and transmissible in pigs. Seroconversion was also observed in experimentally infected pigs. A prevalence study on more than 180 swine serum samples collected from two states without disease revealed 40%–52% positive to MRV. All results warrant the necessity to monitor MRV epidemiology and reassortment as the MRV could be an important pathogen for the swine industry and a novel MRV might emerge to threaten animal and public health.

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Single cell T cell receptor profiling in pig lungs using single-cell TCR sequencing analysis

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Respiratory diseases are a leading cause of mortality and morbidity in growing pigs. Influenza A viruses (IAV) are among the most common swine respiratory viral agents and often give rise to co-infections from opportunistic bacteria. Lung T cells are essential to robust immunity against IAV re-infection. However, current understanding of pig lung T cell subsets and phenotypes remains incomplete due in part to limited swine reagent availability. To address this gap in knowledge, we profiled T cells within pig lung samples using paired single-cell RNA sequencing (scRNAseq) and T-cell receptor (TCR) sequencing (scTCRseq). We accomplished this by developing swine TCR alpha and beta primers compatible with the 10x Genomics Chromium Next GEM Single Cell 5' kit. Six four-week-old mixed breed pigs were intratracheally infected either once, or twice, 15 days apart, with 2009 pandemic H1N1 IAV strain A/California/04/2009 (CA04). Lung samples were collected and processed into single cells five days after infection. The final dataset consisted of 27,000 lung cells, approximately 9,000 of which were alpha-beta T cells. Among our findings were that in pigs exposed to IAV twice compared to once, we observed a higher proportion of tissue-resident memory (Trm), cytotoxic, and proliferative T cells. CD4+ and CD8+ Trms and cytotoxic T cells had the highest number of expanded clones and some of the re-infected pigs harbored T cells with the same TCR sequence. Our results show that that we can perform high-efficiency T cell immune repertoire profiling in pigs at the single cell level, which should be useful for understanding swine cellular immune responses and for designing more effective swine vaccines.

Emergence of PRRSV-1 strains of enhanced virulence in Europe: overview and evolution

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One of the most remarkable characteristics of PRRSV is its great genetic diversity that is generated from two main mechanisms, mutation and recombination. This genetic diversity gives rise to a constant appearance of variants, some of which can become predominant if they are the fittest for a given environment. In some cases, these variants can have a virulence higher than the average; when this happens, the consequences for the affected pig population can be dramatic. The emergence of strains of enhanced virulence has been a constant since the outbreaks of so-called atypical PRRS in the 1990s, through the appearance of hypervirulent strains in China in 2006 to the current 1-4-4 L1C.5 in the USA. PRRSV-1 strains were traditionally considered to be less virulent than PRRSV-2 until the isolation of the Lena strain of PRRSV-1.3. However, this subtype 1.3. had very limited spread in Europe, so this finding did not generate the attention it deserved. In 2014, Italian researchers reported the emergence of a strain of virulence equal to or even greater than Lena, which they named PR-40. Subsequently, a strain of increased virulence was also reported in Austria, although in neither case did the spread of the disease outside the affected areas occur. At the beginning of 2020, PRRS outbreaks of unusual virulence began to be reported in Spain with increased abortion rates (>70% in the onset of the outbreak in sows at last third of gestation) and very high mortalities in sows and growing piglets, up to 10% and 50%, respectively. The first sequencing data indicated that the causative strain (which was named Rosalía) belonged to the clade derived from PR-40, but also showed that Rosalía was the result of different recombination events, some of them with local Spanish strains. Rosalía spread firstly among the areas with the highest pig-density during 2020 and 2021 with devastating effects, and from 2022 it began to be reported in other areas. To date, Rosalía's progeny is already widely predominant in new PRRS outbreaks and although some outbreaks appear to be less virulent, large numbers of casualties continue to occur, because of the disease, especially in weaners where mortalities mayrange between 15-25% in average, even after the strain becoming endemic. The most common clinical features are high fever, severe pulmonary involvement with a diversity of lesions including interstitial pneumonia, proliferative necrotizing pneumonia, and strong perivascular and bronchial infiltration, as well as suppurative bronchopneumonia in cases of bacterial complications. Pulmonary edema is a common finding among dead pregnant sows. The rapid spread of the virus and the fact that it has infected millions of animals in a very short period of time has led to a very large diversification and, at this time, there is no longer a single Rosalía strain, but a constellation of variants generated again by mutation and recombination that predominate over other clades.

Efficacy of an intranasal naturally attenuated live PRRSV-2 vaccine against a highly virulent PRRSV-1 strain

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The use of mucosally-delivered vaccines for PRRSV has been scarcely explored. The purpose of the present study was to test the efficacy of an intranasal PRRSV-2 vaccine (Innovac L-PRRS) in a challenge model with an emerging highly virulent PRRSV-1 strain. Sixty-eight PRRSV-free crossbred pigs were assigned to 4 groups (G1-G4). G1 animals were intranasally vaccinated with Innovac L-PRRS; G2 animals received a commercial PRRSV-1 attenuated IM vaccine. G3 and G4 were kept as unvaccinated controls. At 28 days after vaccination, 3 animals per group were euthanized to assess lung lesions after the vaccination and the remaining 14 pigs in G1, G2 and G3 were intranasally challenged with the highly virulent PRRSV-1 strain Rosalia (R1, 2ml at 105.5 TCID50/ml). G4 animals received a placebo. Half of the pigs were necropsied at 10 days post-challenge (DPC) and the remaining animals were kept until day 28 DPC, when the experiment ended. Temperatures and clinical signs were recorded from -2 to 14 DPC and weights were recorded weekly. Viremia and nasal shedding were assessed by RT-qPCR. The humoral response was assessed by means of ELISA and the cell-mediated immune response was determined by means of the interferon-gamma (IFN-g) ELISPOT. In the unvaccinated animals, fever was observed from 3 to 10 DPC with temperatures above 41-41.5ºC. In contrast, in G1 and G2 fever was of shorter duration (6-8 DPC for G1 and 3-7 DPC for G2) with no animals >41ºC. Similarly, respiratory signs were less severe in the vaccinated animals. At 10 DPC severe pneumonia was observed in the unvaccinated pigs (up to 70.5% of pneumonic lung areas) while in vaccinated pigs, lung lesions ranged from 0.5 to 48%, being similar for G1 and G2. In the unvaccinated group, viremic animals were detected for the whole challenge period. Similarly, in G1 and G2 significant reduction of viremia or nasal shedding was not observed. Weight gains of non-vaccinated/challenged animals were poor (635 gr/day in G3 vs 989 gr/day in G4) while vaccinated groups performed better (724 and 778 gr/day for G1 and G2, respectively). Both vaccines induced rapid seroconversion with higher S/P values in G2 after challenge. R1-specific IFN-g secreting cells were detected since 7 DPC, when G1 animals showed the higher frequencies; however afterwards G2 animals showed the highest increase. In summary, vaccination with Innovac L-PRRS showed some beneficial effect even in a worst-case scenario of a heterologous challenge and suggest the potential of intranasal vaccines for PRRSV.

Assessment of farm surface PRRSV contamination through viability RT-qPCR

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Introduction: Farm biosecurity measures and the level of compliance play a crucial role, especially in an industry where one individual oversees large populations of growing pigs across different sites. Growing pig caretakers move between sites to conduct daily chores such as ensuring barns are well ventilated and pigs have proper access to feed and water. As most growing pig sites lack shower-in/shower-out facilities, there is a probability of fomite contamination and virus dissemination across sites of different PRRSV statuses. Understanding whether frequently touched surfaces by personnel contain viable PRRSV is crucial for raising awareness of the transmission risk and encouraging the industry to reconsider and enforce biocontainment procedures. We conducted a surface contamination assessment sampling project in PRRSV-positive growing pig farms using a novel viability quantitative RT-qPCR (v-RT-qPCR) assay, to 1) determine whether viable PRRSV can be detected on frequently touched surfaces by farm personnel; 2) assess whether the level of PRRSV population shedding and the probability of detecting viable PRRSV are related; 3) explore the relationship between standard and v-RT-qPCR for PRRSV detection under field conditions.

Methods: Ten PRRSV-positive growing pig farms located in the Midwest, USA, from one production system were enrolled in this cross-sectional study. Given the scarcity of PRRSV environmental detection data and standardized protocols for sampling, we decided to base our sampling strategy on our previously conducted PRRSV environmental study. Therefore, we conveniently collected a total of 20 samples per farm from different surfaces. Investigators wore clean Tyvek coveralls, plastic cover boots, and clean pair of gloves during sample collection, changing gloves between each sampling event. Selected surfaces were swiped with a DMEM moistened Swiffer pad, and placed in a resealable bag. The pad was squeezed inside the bag and the liquid was poured into 20 ml sterile Falcon tubes. During the farm visit, a set of 8 ropes (16 pens) per barn were hung to collect oral fluids to characterize the level of population shedding in the barn. Samples were refrigerated and transported to the University of Minnesota for processing, and submitted to the Veterinary Diagnostic Laboratory for individual PRRSV screening through RT-PCR. Positive samples were then sent for further testing via viability RT-qPCR testing.

Results: PRRSV was detected through oral fluids in all farms, with a proportion of positive ropes ranging from 50 to 100%. Overall, 20 out of 200 (10%) environmental samples were positive on the screening RT-PCR with Ct values ranging from 32.8 to 35.9. Positive samples originated from pig pen penning housing sick pigs, general pig pen penning housing, mortality handling equipment, exhaust pit fan cone dust, sorting board handle, and main entry floor close to bench or line of separation. At the time of writing, RT-PCR positive samples had been recently submitted for v-RT-qPCR and results were not available.

Use of post-mortem samples from growing pigs to detect five swine pathogens

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Introduction: Most of the post-weaning sampling relies on oral fluid samples since jugular venipuncture can be time-consuming and requires skilled personnel. Assessing whether easy to collect post-mortem samples (i.e., tongue tip fluid [TTF], intracardiac blood [IC], oral/nasal swabs [ONS], rectal swabs [RS], superficial inguinal lymph nodes [SILN]) can provide value for diagnosis and monitoring in the post-weaning stages is necessary as this methodology can be adopted by the industry when resources are scarce, even though they are not the gold standard specimen. Therefore, the objectives of this study are 1) to assess the sensitivity and specificity of TTF, ONS, and SILN in growing pigs when compared to IC, in presence of porcine reproductive and respiratory virus (PRRSV); and, 2) describe detection of Porcine Circovirus type 2 and 3 (PCV-2, PCV-3), Porcine Parvovirus type 1 and 2 (PPV-1, PPV-2), Lawsonia intracellularis (Li), and Influenza A virus (IAV), through RT-PCR of collected post-mortem samples (e.g., TTF, ONS, RS, SILN).

Methods: One wean-to-finish farm group of pigs undergoing a PRRS outbreak was sampled when animals were 5 weeks of age (WOA) and 11 WOA. A second group of growing pigs undergoing a similar health challenge was sampled at the grow-finish farm at 15 WOA at a different location. During each sampling event, 30 dead pigs were included in the study for a total sample of 90 pigs. Besides TTF, the other collected post-mortem specimens were IC (for PRRSV), ONS (for IAV and PRRSV), RS (for PPV and Li), SILN (for PCV and PRRSV), all samples were tested individually through RT-PCR. The sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated for PRRSV. Proportion of RT-PCR positive results from samples types tested for other pathogens were compared by descriptive statistics.

Results: All pathogens were detected at least once in TTF with Ct values ranging from 11.6 to 39.8. PRRSV, PCV2/PCV3, PPV1/PPV2, Li, and IAV were detected in 96%, 22%, 49%, 6%, and 38% of the TTF samples, respectively. For PPRSV the best results for all sample type comparison were at 11 WOA, TTF had Se=84%, Sp=9%, PPV=62%, NPV=25%, ONS had Se=74%, Sp=73%, PPV=82%, NPV=62%, and SILN had Se=100%, Sp=9%, PPV=66%, NPV=100%. PCV2/PCV3 was detected in 6% of SILN samples. PPV1/PPV2 was detected in 31% of the RS samples. Li was detected in 0% of the RS samples. IAV was detected in 38% of the ONS samples.

Conclusion: We detected different pathogens using TTF samples at three different ages during the growth and finishing phases of the pigs, as well as in other sample types such as ONS, RS, SILN, and IC. Detection can vary according to the assessed pathogen and the age of the pigs. However, the overall diagnostic performance of the used specimens still requires further investigation. Indeed, complete and exhaustive collection of multiple clinical specimens from different body systems remains the standard in diagnostic investigations.

Spatial Transcriptomics: A New Frontier for Swine Viral Pathobiology Research

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Objective: Porcine Respiratory and Reproductive Syndrome (PRRS) is the most economically damaging illness for the swine industry, costing over 1 billion per year in lost production in the United States. Our research aims to provide a greater understanding of PRRS host-immune response by identifying genes involved in the host response to infection and determining how infection with PRRS alters the host immune response pathways. We aim to answer previously unanswered questions about infected and bystander cells in the intrinsic and extrinsic apoptotic pathways during PRRS infection.

Methods: A lung tissue sample from an adolescent swine infected with PRRS was analyzed through spatial molecular imaging, providing quantification of 1,000 RNAs through in situ hybridization chemistry at single-cell and sub-cellular resolution. Spatial imaging and data were analyzed using Python and R code packages to provide analysis and deeper understanding of gene expression across the tissue sample in the infected state.

Results: The spatial molecular imaging and spatial informatics data provided 71 transcripts per cell of new information regarding gene expression in PRRS infection. Gene activity involved in the intrinsic and extrinsic apoptosis pathways, including ATF-3, p53, Caspase-8, and Caspase-3 is measured and analyzed to show the connection between PRRS infection and induction of apoptosis.

Conclusions: Previously, it has been unanswered whether PRRS infection directly induces apoptosis and apoptotic gene expression. Spatial transcriptomics provides a new platform to answer questions that have previously not been answered about viruses and immune response pathways by analyzing infected and bystander cells in a positional and regional context.

Development of a Foot-and-Mouth Disease Virus Vaccine Candidate: Utilizing Non-Toxic Enterotoxin and E. coli as an Adjuvant-Delivery System

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Foot-and-Mouth Disease Virus (FMDV) induces vesicular lesions in cloven-hoofed animals, causing significant economic losses in livestock industry worldwide. Among all FMDV serotypes, serotype O is highly prevalent globally, causing annual economic losses of $6 billion to $21 billion in livestock industries due to reduced productivity, increased control costs, and trade restrictions. Current available vaccines face challenges with safety concerns, lack of cross-protection, thermal instability, and complications in differentiating infected from vaccinated animals (DIVA). In this study, we adapted a novel Multi-Epitope Fusion Antigen (MEFA) platform for FMDV vaccine development. It integrates consensus B- and T-cell epitopes from all four structural proteins (VP1, VP2, VP3, VP4) and the non-structural protein 3A, which was expressed using mutant E. coli heat-labile toxin as the backbone. Three distinct subunit vaccine constructs were developed (VC1, VC2, and VC3), each contains different epitope composition. VC1 contains five epitopes from the VP1 and 3A regions, selected from previous research. VC2 comprises eight epitopes, including five from VC1 and three predicted by IEDB. VC3 also includes eight epitopes, with four from VC1 and additional epitopes predicted from the VP2, VP3, and VP4 regions. The candidate vaccine antigens were first characterized by SDS-PAGE and Western blot analysis and subsequently tested in animals. Four groups of rabbits were obtained. The group 1-3 rabbits were vaccinated with a vaccine construct, while group 4 rabbits were mock vaccinated with PBS as a control. We observed a significant increase in serum IgG titers in the VC1-vaccinated group of rabbits compared to those in the control group. Flow cytometry analysis of PBMCs isolated from the VC1-vaccinated group revealed significant CD4+ T cell responses, with the highest responses observed in PBMCs stimulated with epitope T3 (AAIEFFEGMVHDSIK) compared to epitopes T1 (ENYGGETQVQRRQHT) and T2 (TDVSFILDRFVKVTP). In contrast, control animals exhibited minimal CD4+ T cell activation, indicating the vaccine's effectiveness in eliciting a specific immune response. As expected, the CD8+ T cell response in immunized animals was non-significant, as the CD8+ T cell epitopes were only included in VC2 and VC3 constructs. Testing the immune responses in VC2 and VC3-vaccinated rabbits are in progress. The results of these analyses will guide the development of an optimal vaccine construct for subsequent vaccine efficacy test in pigs. This study is expected to generate a novel MEFA vaccine to provide a broadly protective immunity against FMDV infection.

Lipid nanoparticle-encapsulated DNA vaccine encoding African swine fever virus p54 antigen elicits robust immune responses in pigs

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African swine fever virus (ASFV) is one of the most significant viral pathogens affecting swine production worldwide. Although several live attenuated ASFV vaccines have been approved for clinical use in certain countries, there is concern that these vaccine viruses might revert to virulence. Subunit vaccines containing one or a few viral immunogens offer a safer alternative. Our research focuses on developing a DNA-based vaccine against ASFV. A major challenge in developing a DNA-based vaccine is the knowledge gaps regarding viral proteins that can induce protective immunity. Additionally, unencapsulated DNA vaccines often exhibit low immunogenicity due to the inefficient cellular entry of plasmid DNA, leading to low protein expression. In this study, we utilized ASFV p54 as a model antigen to explore the feasibility of using lipid nanoparticles (LNPs) as nanocarriers to enhance the immunogenicity of DNA vaccines. Pigs immunized with the p54 LNP-DNA vaccine elicited high titers of p54-specific antibodies and T-cell responses after the second immunization. Using ELISAs based on an overlapping peptide library, we identified three antigenic areas within p54. Additionally, we noted that pigs vaccinated with the p54 LNP-DNA vaccine exhibited a similar antibody profile as those vaccinated with an experimental live attenuated vaccine or infected with a wild-type ASFV strain. Overall, our findings highlight the promising potential of LNP-DNA as an effective platform for developing gene-based vaccines against ASFV.

Optimizing Tongue-Tip Sampling Protocols for Enhanced PRRS Virus Isolation

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) poses an ongoing challenge to swine health worldwide. While postmortem tongue tissue fluids (TF) have proven to be a cost-effective method for detecting PRRSV in swine herds via RT-qPCR, confirming infectious virus presence through isolation remains crucial. This study examined the potential of isolating PRRSV from perinatal piglet mortalities' TF using various sample collection protocols and cell lines.

Methods: The study evaluated four TF collection protocols: 1) extraction of TF from freshy collected tissues using Phosphate Buffered Saline (PBS), 2) extraction of TF from freshy collected tissues using Virus Transportation Medium (VTM), 3) extraction of TF from freeze-thawed tissues using PBS (Freeze-thaw), and 4) extraction of TF from homogenized tissues (Homogenate). Additionally, two cell lines (ZMAC and MARC-145) and a batch of primary alveolar macrophages (PAM) were assessed for the probability of successful PRRSV isolation from the TF samples. Samples were taken from 597 perinatal mortalities in a 5000-head PRRSV-positive breeding herd over four days. Tongue tissues were collected in 20 batches (approximately 30 mortalities per batch) and each tongue was divided into four quarters, with each quarter assigned randomly to one of the four collection protocols. A total of 80 sample bags (20 batches of ~30 mortalities each × 4 protocols) underwent RT-qPCR analysis. For virus isolation (VI), the 10 batches with the lowest average Ct values were selected. Statistical analysis using R software assessed the significance of differences in RT-qPCR Ct across protocols and statistical differences in the likelihood of successful PRRSV isolation across protocols and cell types.

Results: The mean Ct values for PBS, VTM, Freeze-thaw, and Homogenate groups were 21.9, 21.8, 22.6, and 24.8, respectively, with the Homogenate group showing a statistically higher mean Ct than the others. VI success probabilities (and 95% CI) were 22.6% (8.1%, 49.1%) for PBS, 12.1% (3.4%, 39.1%) for VTM, and 2.8% (0.4%, 16.4%) for both Homogenate and Freeze-thaw groups, with no significant differences ($p > 0.1$) between protocols. Among cell types, MARC-145, ZMAC, and PAM cells had VI success rates of 3.1% (0.59%, 14.9%), 21.0% (8.4%, 43.5%), and 4.8% (1.1%, 19.1%), respectively, with ZMAC showing significantly higher VI success ($p < 0.1$). PRRSV ORF-5 sequencing on the VI samples and isolates revealed PRRSV 1-3-4 L1C.5 strain with 99-100% nucleotide identity to each other.

Experimental evidence of vaccine-driven evolution of PRRSV-2 in pigs-to-pig infection chains

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Immune escape mechanisms of porcine reproductive and respiratory syndrome virus type 2 (PRRSV-2) are believed to drive the emergence of genetic variants despite extensive vaccination in the United States. The direct effect of vaccine-induced immune pressure on PRRSV-2 evolution is rarely demonstrated. This study analyzed PRRSV-2 genomes from pig-to-pig infection chains with varying immune backgrounds to investigate this in a controlled environment. We hypothesized that genomes from vaccinated pigs would show greater genetic divergence from the challenge virus than those from non-vaccinated groups, with changes more frequent in antigenic regions. An in vivo experiment compared PRRSV-2 micro-evolution across pig-to-pig transmission chains. Weaned pigs were divided into two vaccinated groups and one unvaccinated group. Each group had seven 3-month-old pigs challenged 64 days post-vaccination with PRRSV-2 sub-lineage 1A (L1A). Serum samples were collected over 14 days post-challenge, and samples with the highest virus titer were used as inoculum for the next batch of pigs within the same treatment, repeated for six batches, involving 126 pigs over ~84 days. Serum samples with a virus PCR Ct-value of <30 (n=184) were sequenced using Oxford Nanopore technology. Genetic relationships were analyzed via pairwise nucleotide distance comparison and phylogenetic approaches. Deep sequencing data were used to quantify and compare viral quasispecies dynamics across samples. Over the 84-day pig-to-pig infection chain, genomes from unvaccinated pigs remained genetically similar to the challenge virus (mean distance to inoculum = 0.2%) and to each other (mean within-group distance = 0.1%). In contrast, vaccinated groups showed greater genetic variation (mean within-group distance = 3.8-4.0%) and were more genetically different from the initial inoculum (mean distance to inoculum = 2.6-3.5%). This genetic distinctiveness in vaccinated groups was also seen at the protein level, with non-synonymous mutations altering amino acids. 9.09-30.77% of these mutations occurred in immunologic epitopes, including the gp5-M protein ectodomain. Phylogenetic analysis showed samples from unvaccinated pigs clustered near the tree's root (the original inoculum), while those from vaccinated groups formed ladder-like structures that were progressively more distant from the inoculum, indicating directional evolution under selection pressure. This selection was also clear in vaccinated groups at the viral quasispecies level, where a particular haplotype dominated over others at the end of the experiment. In summary, these preliminary findings confirm that immune pressure influences PRRSV-2 evolution. Viruses in vaccinated animals were more diversified and genetically distinct from the parent virus, with many changes occurring in immunogenic regions. Further research is needed to understand the phenotypic consequences of these differences and the complexity of virus population dynamics within the host.

Characterizing Dead Animal Disposal Practices on Sow Farms and Assessing PRRSV Risks Associated with Rendering

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Pigs on sow farms often die from various causes, including contagious diseases. An association between using rendering for dead animal disposal and higher risk or PRRSV outbreaks has been reported on the literature. However, a detailed characterization of disposal practices and facilities used in the U.S. is needed to understand and mitigate associated risks. This study aims to characterize the facilities, structures, and procedures for dead animal disposal on sow farms, as well as test if environmental samples collected in and around dead animal disposal structures contain detectable quantities of PRRSV and PEDV.

Ten standard operating procedures (SOPs) from different systems were obtained. Data from SOPs were categorized into sections: biosecurity, dead animal gathering space in the barn, removal from the barn, transport out of the barn, and final destination and used to create a questionnaire for on-site visits. Five farms with 2 to 5 thousand sows on each from three systems were visited to apply the questionnaire and collect environmental samples for PRRSV and PEDV testing.

The SOPs varied significantly in detail, with the most comprehensive being 12 pages and the shortest with 1 page containing 11 lines. Biosecurity measures were generally outlined but inconsistent, particularly regarding personnel roles. Dead animal gathering spaces were mentioned in most SOPs, often cleaned and disinfected, but methods varied. Dead sow removal methods were usually detailed, while dead piglet removal and afterbirth was less consistently addressed. Transport methods of carcasses between the barn and the compost pile or rendering truck pick up site was done primarily using tractors or pickups and was cited in most SOPs, but cleaning protocols were often not mentioned. Final disposal destinations were inconsistently mentioned. Farm visits revealed that dead animal removal was typically the last task of the day, regardless of PRRS status. Environmental conditions, especially wind direction, influenced if removal happened daily. Wildlife exposure to disposal areas was commonly reported.

Three finisher sites (all PRRSV positive, one PEDV positive) were visited and a total of 25 environmental samples were collected from roads, machines, and structures involved in moving dead animals. All samples from one farm tested negative for PRRSV and PEDV by RT-PCR. From the remaining two farms, a total of 12 and 5 samples tested RT-PCR positive for PRRSV-2 and PEDV, respectively. Positive samples were found on dead animals, rendering boxes, skid loader buckets, and nearby ground. One PRRSV isolate was obtained from a sample collected on the ground, while no viable PEDV was isolated.

Creating SOPs for dead animal removal on farms is challenging due to the need for specific yet adaptable instructions. SOPs should include detailed biosecurity protocols, procedures for removing dead animals, and cleaning and disinfection guidelines. This study shows that samples collected from or near rendering boxes can test positive for farm-specific viruses and may contain viable viruses. Positive samples on roads suggest potential PRRSV transmission pathways between farms. Further studies on different disposal methods, such as composting or incineration, are warranted to ensure effective biocontainment of viruses on farms.

Maximizing PRRSV Diagnostic Accuracy: Insights from Tongue Tip Testing

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a major pathogen in swine, causing significant economic losses globally, with the U.S. alone losing an estimated $664 million annually. These losses stem from reproductive issues, high pre-weaning mortality, and reduced production efficiency. Annually, up to 40% of the U.S. breeding herd experiences PRRSV outbreaks, requiring efforts to achieve farm stability by eliminating detectable viremia in weaning-age pigs. Recent research highlights the potential of using tongue tips from dead piglets to monitor PRRSV. However, factors such as testing strategy, sample processing, and storage conditions can impact RT-PCR accuracy. This study aims to test different processing and testing protocols to identify the most effective procedures for optimizing PRRSV detection in tongue tips.

A total of four sow farms with approximately 2 thousand sows each were visited so far, with 80 dead piglets being sampled (20 animals on each farm). Tongue tips were sectioned craniocaudally into three portions prior to submission for diagnostic, each portion being allocated to different sampling testing protocol (individual versus in pools of n=5 and n=20 for each farm). Samples were frozen overnight, thawed, and 0.5mL of PBS was added to tongue fragments which were then massaged for 15 seconds. Tongue tip fluid (TTF) was collected and both TTF and tissues were submitted for PCR for PRRSV at the University of Minnesota Veterinary Diagnostic Laboratory. The sensitivity of tissue was calculated using TTF as the reference test. Agreement of pools with individual testing was described.

The testing of TTF produced a higher number of positive samples compared to tongue tissue samples. Ct values of TTF in individual samples ranged from 29 to 35, while those for individual tongue tissues ranged from 32 to 35. The sensitivity of detecting PRRSV in tongue tissues compared to TTF was 23%. When TTF was pooled in groups of 5, the agreement with individual TTF results was 81%, with three false negatives identified. TTF pooled in groups of 20 showed a 66% agreement with individual TTF, with one false negative. In contrast, tongue tissue pooling in groups of 5 showed a 94% agreement with individual results, with one false positive. Tongue tissue pooled in groups of 20 had a 100% agreement with individual tissue results.

These findings indicate that processing tongue tissues does not significantly improve PRRSV detection compared to using TTF. While pooling generally showed good agreement with individual testing, it is important to interpret the results with caution, particularly due to the risk of false negatives. Further sampling to investigate the effect of temperature and time a sample is kept frozen on the ability to detect viruses on the samples is ongoing.

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Wings of Worry: The Avian Flu Pandemic Potential

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Influenza A viruses (IAVs) pose a constant threat to public health due to their ability to cross species barriers and cause pandemics. This presentation focuses on two subtypes of particular concern: H9N2 and H5N1. H9N2 avian influenza viruses are prevalent in poultry and have been identified as a potential pandemic threat. Our research investigates the evolution of H9N2 and molecular features that allow them to cause respiratory infections in mammals, including humans. Highly pathogenic avian influenza (HPAI) H5N1 viruses also pose a significant threat. The recent spread of HPAI H5N1 to various mammalian species, including marine mammals and dairy cattle, underscores the need for continued surveillance and risk assessment. Overall, this presentation highlights the importance of monitoring the evolution of IAVs at the animal-human interface and emphasizes the need for effective vaccines and other alternative strategies to mitigate the threat of future pandemics.

Efficacy study evaluating INGELVAC PRRS® MLV against a PRRSV '144' Lineage-1C.5 variant challenge

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is a major swine viral pathogen that affects the pig industry worldwide. Vaccination with modified live vaccine (MLV) can mitigate the clinical impact of PRRSV infection and subsequently improve health and performance. The objective of this study was to evaluate the efficacy of INGELVAC PRRS® MLV vaccine against challenge from the emergent PRRSV '144' L1C.5 variant.

Materials and Methods: At three weeks of age (Day 0 of the study), 92 PRRSV naïve piglets were randomized into groups, and intramuscularly vaccinated with 2 ml of either a placebo (challenge controls n=46) or INGELVAC PRRS® MLV (n=46). Pigs were housed in rooms by group during the vaccination period. At day 28 of the study (D28), all pigs were comingled and challenged with 2.0 mL intramuscularly and 2.0 mL intranasally (1 mL per nostril) with 104.99 Log10TCID50/dose of PRRSV 1-4-4 L1C.5 variant. Serum samples and weights were collected periodically from D0 through termination of the study on D42. On day 42 (14 days post-challenge), all pigs were necropsied, and lungs were scored for the presence of macroscopic lesions. Serum samples were tested by RT-PCR for the presence of viremia. Data were analyzed using Generalized Linear Mixed Models. Pairwise comparisons between groups were conducted as appropriate using a level of confidence of 0.05 to indicate statistical significance.

Results: The lung lesion assessment found the INGELVAC PRRS® MLV vaccinated group to have significantly lower percent median gross lung lesion scores compared to the non-vaccinated (Placebo) challenged treatment group; 14.13% and 28.10% respectively. The INGELVAC PRRS® MLV vaccinated group demonstrated a significant increase in post-challenge ADWG (D28-D42), compared to the Placebo challenged treatment group; 0.35 lbs per day and 0.07 lbs per day respectively. Following challenge, the Placebo challenged pigs became viremic within 24 hrs of infection (D29), and all animals remained viremic during the post-challenge period. In comparison to the Placebo challenged group, the INGELVAC PRRS® MLV vaccinated group had significantly lower levels of viremia as measured and analyzed by Log10 genomic copies at D29 and D42 of the post-challenge period of the study.

Conclusions and Discussion: This study demonstrates that INGELVAC PRRS® MLV provides protection against the PRRSV '144' L1C.5 variant by significantly reducing lung lesions, significantly improving ADWG and significantly reducing viremia compared to Placebo challenged controls. The study further confirms the ability of INGELVAC PRRS® MLV to protect against relevant and contemporary PRRSV lineage-1 challenges.

Electrostatic precipitation for biocontainment from finishing facilities exhaust fans

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Aerosol transmission of infectious pathogens from finishing facilities poses a major biosecurity risk to surrounding sites since many facilities do not use aerosol biocontainment practices. Without biocontainment practices, emitted pathogens are dispersed and can potentially infect pigs housed in nearby facilities. This project's overall goal is to determine the feasibility of an electrostatic precipitation (ESP) system to contain, mitigate, and reduce the transmission of airborne pathogens from potentially infected finishing facilities. The objectives of this research project are to: (1) verify the effectiveness of airborne pathogen inactivation for PRRS and reduction in total bacterial count in a realistic finishing barn scenario for biocontainment and (2) perform a techno-economic analysis of incorporating an ESP system on finishing facilities to demonstrate scalability and risk reduction to surrounding facilities. This is a preliminary update on the status of this project and future work.

The absence of CD163 lessens the pathogenicity of PCV2d in pigs

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CD163 is a scavenger receptor on macrophages, which plays important roles in homeostasis, immunity, and resistance to PRRSV infection. Perhaps the most important biological function for CD163 is the binding and breakdown of hemoglobin-haptoglobin (Hb-Hp) complexes by macrophages, produced in response to inflammation. The hemoglobin breakdown products possess potent anti-inflammatory properties, establishing CD163 as an important anti-inflammatory mediator. However, there are some published reports supporting a pro-inflammatory role for CD163. For example, the crosslinking of CD163 by anti-CD163 antibodies or bacteria results in the release of pro-inflammatory cytokines, such as TNFα. The purpose of this study was to determine if the absence of CD163 suppressed or potentiated inflammation in response to infection with another macrophage-tropic virus, PCV2d. The design of the experiment was to infect and then co-mingle five WT with five CD163 KO pigs. The pigs, provided by Randy Prather at the University of Missouri, were infected with a PCV2d field isolate. The pigs were evaluated daily, and the study concluded at 21 days after infection. Lung scores for the WT pigs ranged from 4 to 12 (maximum of 12) with a mean of 7.8 +/- 3.5. For the KO group gross pathology scores ranged between 1 and 3, with a mean of 2.6+/- 1.1. A similar increase in the histopath scores for WT pigs was observed following evaluation of microscopic lung histology. PCR was used to measure virus nucleic acid in serum at 21 days after infection. The mean concentration for the WT pigs was 5.8+/-1.6 log virus templates/reaction versus 2.5+/-1.0 for the KO group. There was no difference in the PCV2d antibody response. Since CD163 is not a receptor for PCV2d, the results indicate that presence of CD163 can potentiate the virulence and inflammatory response of pigs during infection with PCV2d, thus challenging the notion that CD163 is an anti-inflammatory protein. In a larger picture, selective modifications of CD163 may help pigs to better adapt to the pro-inflammatory stimuli of the modern production system. This working is being supported by pending USDA NIFA Grant 13957049.

An automated 384-well RT-qPCR assay for porcine reproductive and respiratory syndrome virus (PRRSV) enhances high-volume testing capabilities in the Veterinary Diagnostic Laboratory

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Porcine reproductive and respiratory syndrome virus (PRRSV) remains one of the top three pathogens affecting the US swine industry, according to the Swine Health Improvement Plan (US-SHIP), with estimated losses of around $1.2 billion annually. Early detection of PRRSV allows for a rapid response in developing biosecurity measures and preventing virus spread. While reverse transcriptase real-time polymerase chain reaction (RT-qPCR) is a widely used method for detecting PRRSV, the number of tests performed at Iowa State University-Veterinary Diagnostic Laboratory (ISU-VDL) has increased close to 0.4 million in 2023, necessitating the need for high-volume testing. Our study aims to establish a high-volume RT-qPCR assay automation workflow using 384-well QuantStudio-7Pro system (QS7Pro; ThermoFisher) and compare its efficiency with the conventional 96-well ABI Fast 7500 system (ABI; ThermoFisher).

For the initial evaluation, viral RNA extractions were performed using the MagMAX Pathogen RNA/DNA kit on the KingFisher Apex system (ThermoFisher) using two PRRSV isolates (MN184, V1 and 1-7-4 L1A, V2) and 2158 diagnostic samples submitted for PRRSV testing at ISU-VDL. VetMAX™ PRRSV 3.0 reagents (PRRSV 3.0; ThermoFisher) with a total reaction volume of 20 µL (12 µL reaction mix + 8 µL of sample; ABI) for 96-well assays and 10 µL for 384-well assay (6 µL reaction mix + 4 µL of sample; QS7Pro) were performed using recommended thermal cycling conditions. For the high-volume workflow, an Automated Liquid Handler Instrument (ALHI; Bravo, Agilent Technologies) dispensed both reaction mix and samples onto the 384-well plates, followed by heat-sealing, centrifugation, and loading the plates into the automated 4°C incubator Cytomat-2-C-LiN (iCL; ThermoFisher). The 384-well plates were sequentially transferred from the incubator to the QS7pro by Orbitor-RS2 microplate mover (ORM; ThermoFisher) using plate scheduling Momentum software (ThermoFisher). After the RT-qPCR run, the ORM unloads the 384-well plates onto the reserve stacking nests, loads the next plate in the queue, and repeats until all RT-qPCR plates are run in iCL.

The findings from this study demonstrate that the PRRSV 3.0 assay performed comparably on both the 96-well ABI and 384-well QS7Pro platforms, with a Cq cutoff value set at 37. Intra- and inter-assay variability on 384-well assays was low, with coefficients of variation (%CV) of 1.4% and 0.4%, respectively. The variability between the 96-well and 384-well assays was consistently between 2.2% and 2.6% across multiple runs. Positive controls on the 384-well plates showed consistent results, with a Cq of 28.5 ± 0.5 across eight plates, tested in three seperate high-volume automation workflow runs. All plates stacked in the iCL were reliably loaded onto the QS7Pro by the ORM without lag or delay during the time periods tested.

In conclusion, the newly established high-volume automation workflow using the QS7Pro platform demonstrates comparable analytical sensitivity to the existing ABI platform. This system has the potential to conduct ~ 4000 RT-qPCR assays overnight. Future studies are in progress to increase the number of strains tested, including both vaccine and wild-type strains, to evaluate repeatability across diverse sample matrices and to perform a cost-benefit analysis for routine PRRSV surveillance and monitoring.

Development and in vitro characterization of messenger RNA-based vaccines against Senecavirus A

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Senecavirus A (SVA) causes vesicular disease in swine characterized by stomatitis, vesicles on the snout, and coronary bands, leading to economic losses in the global swine industry. Currently there are no commercially available vaccines against SVA. Messenger RNA (mRNA) has garnered increased attention in vaccine development research. The co-expression of P1-2A and 3C SVA genes induces capsid protein self-assembly, generating virus-like particles (VLP). While VLP shares similar morphology with natural viruses, it offers advantages in immunogenicity. Therefore, we hypothesize that mRNA-based vaccines translating VLP will elicit a robust neutralizing antibody and T-cell response, providing a safe and effective vaccine platform to control and prevent SVA infection. In this study, 11 SVA mRNA constructs including P1-2A-3C, P1-2A-IRES-3B, P1-2A and 3C, P1-2A-3BC, P1-2A-3BCM23P, P1-2A-3BCV24P, P1-2A-3BCG123P, P1-2A-3BCN124P, P1-2A-3BCG125P, P1-2A-3BCG126P, and P1-2A-3BCG127P mRNAs were designed. Plasmids were constructed with codon optimization. Kozak sequence was added together with 5'- and 3' untranslated regions. The mRNAs were produced and purified using commercial kits. These mRNAs were transfected using Lipofectamine MessengerMax, and the successful transfection was confirmed through fluorescent RNA in situ hybridization (FISH) with in-house probes. Immunofluorescence assay (IFA) measured by corrected total cell fluorescence (CTCF) and western blot confirmed the expression of structural proteins on PK-15, BHK-21, HEK-293T, and ST cell lines. In addition, the mRNA cell cytotoxicity was assessed. Our results demonstrate the successful transfection of all mRNAs and expression of structural proteins in all tested cell lines. Having 3B and point mutations on the 3C gene in mRNAs reduced cell toxicity while significantly enhancing VP2 expression. However, only five SVA mRNA constructs, including P1-2A-3C, P1-2A and 3C, P1-2A-3BC, P1-2A-3BCV24P, and P1-2A-3BCN124P, expressed all cleaved main proteins (VP1, VP2, VP3, and 3C) according to western blot analysis. The in vitro evaluation of various SVA mRNA candidates has demonstrated promising results for a potential mRNA vaccine candidate that can be utilized in further in vivo immunogenicity and challenge studies.

Development of an Online Dashboard for Near-Real Time Global Swine Disease Surveillance

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The Center for Animal Health and Food Safety, in collaboration with the Swine Health Information Center, has developed a near-real-time event-based surveillance system to support informed decision-making for U.S. pork producers. Our team produces monthly reports on the status of transboundary diseases outside the United States relevant to swine production. To further enhance disease monitoring, we are expanding our focus to include production diseases, and have begun development of a global swine disease dashboard. This dashboard will serve as an efficient tool for monitoring global swine disease trends and evaluating risks based on the availability of reliable information from reporting and surveillance systems, supporting informed decision-making for pork producers and stakeholders worldwide. This presentation will discuss the development and utility of the dashboard and its role in improving swine health management.

Of pigs and men: the best -laid plans for prevention and control of African swine fever

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The spread of high-risk human and animal diseases across borders in the last five years has clearly demonstrated that the best-laid plan can go wrong in infectious disease control and prevention. Outbreaks of African swine fever (ASF) have high consequential social and economic impacts in endemic countries. Without an available safe and efficacious vaccine, swine producers quickly realized that they have to significantly improve biosecurity measures on farms to minimize the risks connected with many factors associated with swine production. These risk factors include culled pigs, lagoons, pigs and feed purchased from outside suppliers, selling pigs to others (trucks and personnel from outside vendors), drinking water, boots and coveralls, insects, rodents and pets on farms, swine semen, and use of veterinary pharmaceuticals and vaccines. Implementing policies to encourage employees to follow biosecurity roles and remodeling the current facility for better biosecurity control are also common practices for many swine operations worldwide. However, many of these changes are very costly and can only be effectively managed by well-funded large operations. Unfortunately, swine production security is a "weakest-link in the chain" issue, where ASF outbreaks still occur, even on farms with the highest level of biosecurity that owners can provide.

Despite recent devastating outbreaks of ASF in Asia, Europe, and the Caribbean, North American and European countries proved decades ago that swine diseases like ASF and classical swine fever (CSF) can be eradicated through effective government policies, even without ideal vaccines. However, the world has since changed, with the rise of high-density swine farming and increasing globalization. These changes require more innovative solutions to tackle new challenges, such as finding the best methods to cull and dispose of thousands of pigs quickly and efficiently in a restricted area, while considering animal welfare, economic and environmental impacts, and technical feasibility. Additionally, what should be done if the government is unable to compensate for the economic losses resulting from preventive culling?

Successful prevention and control of ASF requires not only safe and efficacious vaccines and fast and accurate diagnostic tools but also science-based government policies that ensure the cooperation of all stakeholders of the swine industry. In this presentation, I will discuss the contributing factors behind the global successes and failures in the prevention and control of ASF based on what I have learned recently in ASF endemic countries in Asia and Europe.

A live-attenuated virus vaccine candidate protects pigs against contemporary pandemic genotype II African swine fever (ASF) virus

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African swine fever (ASF) is a highly contagious and severe hemorrhagic transboundary swine viral disease with up to 100% mortality rate, which leading to a tremendous socio-economic loss worldwide. The lack of safe and efficacious ASF vaccine is the greatest challenge in the prevention and control of ASF. In this study, we generated a safe and effective live-attenuated virus (LAV) vaccine candidate VNUA-ASFV-LAVL3 by serially passaging a virulent genotype II strain (VNUA-ASFV-L2) in an immortalized porcine alveolar macrophage cell line (3D4/21, 50 passages). VNUA-ASFV-LAVL3 lost hemadsorption ability but maintained comparable growth kinetics in 3D4/21 cells to that of the parental strain. Notably, it exhibited significant attenuation of virulence in pigs across different doses (103, 104, and 105 TCID50). All vaccinated pigs remained healthy with no clinical signs of African swine fever virus (ASFV) infection throughout the 28-day observation period of immunization. VNUA-ASFV-LAVL3 was efficiently cleared from the blood by 14-17 days post-infection, even at the highest dose (105 TCID50). Importantly, the attenuation observed in vivo did not compromise the ability of VNUA-ASFV-LAVL3 to induce protective immunity. Vaccination with VNUA-ASFV-LAVL3 elicited robust humoral and cellular immune responses in pigs, achieving 100% protection against a lethal wild-type ASFV (genotype II) challenge at all tested doses (103, 104, and 105 TCID50). Furthermore, a single vaccination (104 TCID50) provided protection for up to 2 months. These findings suggest that VNUA-ASFV-LAVL3 can be utilized as a promising safe and efficacious LAV candidate against the contemporary pandemic genotype II ASFV.

A novel reverse genetics system for porcine reproductive and respiratory syndrome virus and the generation of a transcription network-reprogrammed virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded, positive-sense RNA virus that belongs to the Arteriviridae family. Vaccines are available but pose a risk of reverting to virulence and recombining their genomic RNA with field strains. PRRSV synthesizes subgenomic mRNAs (sgmRNAs) through discontinuous transcription, and this process is regulated by base-pairing between the leader and body transcriptional regulatory sequences, designated TRS-L and TRS-B, respectively. We hypothesize that reprogramming the TRS network will create lethal incompatibilities with field PRRSV, thus preventing genome recombination. In the present study, we determined the leader-body junction for each sgmRNA species by RT-PCR and sequencing. To generate TRS-reprogrammed PRRSV, we first developed a novel reverse genetics system termed the infectious subgenomic amplicons (ISA). Five overlapping genomic fragments, F1 to F5, were individually amplified to enclose the full-length viral genome of 15 Kb. F1 was coupled with the CMV promoter, and F5 was linked with the SV40 polyadenylation signal. All five fragments were simultaneously transfected into BHK-21 cells, and the progeny was collected and amplified in PRRSV-susceptible cells. The production of infectious virus was verified through distinct cytopathic effects, RT-PCR, immunostaining, and Western blot. The HDV-like self-cleaving ribozyme element was unnecessary to rescue the virus in this reverse genetics system. Subsequently, we remodeled the TRS-L and TRS-B transcription networks by analyzing the RNA secondary structure adjacent to the TRSs. Using a double mutant PRRSV as a backbone, which is type I interferon-suppression negative and NF-kB-activation negative in its phenotype, specific mutations were sequentially introduced to individual TRSs via site-directed mutagenesis. This virus is expected to be viable and resistant to RNA recombination. Our reverse genetics system is straightforward, fast, and stably devoid of amplification in E. coli, and thus offers a rapid alternative to conventional reverse genetics systems for PRRSV. Our study paves the way to create a TRS-network reprogrammed PRRSV that is resistant to recombination.

Investigating MAIT cell contributions to anti-influenza virus immunity using MR1 knockout pigs

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Mucosal-associated invariant T (MAIT) cells are a recently discovered subset of unconventional T cells, which are defined by their innate-like phenotype. Unlike conventional T cells, MAIT cells do not recognize classical MHC molecules, but instead recognize antigens presented by the MHC class I-like molecule MR1, which is required for MAIT cell development. Several MR1-knockout (KO) mouse studies have reported that MAIT cells play a protective role in influenza virus infections. Although MAIT cells have been well characterized in humans and mice, very little is known about their contribution to immunity in livestock species, including pigs. Thus, we set out to determine whether MAIT cells contribute to swine anti-influenza immune responses using our newly generated MR1 KO pigs. To investigate MAIT cell contributions to innate immune responses, we necropsied MR1-deficient and -intact pigs 5 days post infection with A/swine/Kansas/10-91088/2010 H3N2. To assess acquired immune responses, we challenged MR1-deficient and -intact pigs with A/swine/Kansas/10-91088/2010 H3N2 and rechallenged the same pigs 2 weeks later with the heterologous A/swine/Texas/4199-2/98 H3N2 virus. Pigs were necropsied 5 days after re-challenge. In both studies, MR1-intact and MR1-deficient pigs presented similar body weights, body temperatures, viral titers, and lung pathology after influenza virus infection. However, after the heterologous virus challenge, we observed notable differences in pulmonary concentrations of several immune cells important for anti-influenza immune responses. These include that MR1-deficient pigs had higher concentrations of NK and SWC5+ gamma delta T cells and decreased concentrations of cytotoxic CD8 T cell compared to intact pigs. In summary, our finding that MR1-deficient pigs are equally susceptible to influenza virus infection compared to MR1-intact pigs contradicts prior mouse studies which have reported that MAIT cells make a substantial contribution of anti-influenza virus immunity. However, further investigations are warranted to determine if MAIT cells contribute to the size, scope, and durability of anti-influenza immune responses in pigs.

PRRSV-2 variant classification: a dynamic nomenclature for enhanced monitoring and surveillance

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Existing genetic classification systems for PRRSV-2 are insufficient for epidemiological monitoring that is routinely conducted by animal health professionals. Here, we introduce a fine-scale classification system for PRRSV-2 genetic variants in the U.S. that can dynamically accommodate new genetic diversity arising from virus evolution. This system was developed by an AASV-funded working group, beta-tested using national-scale datasets, and has begun to be utilized by diagnostic labs this year. Based on >25,000 U.S. PRRSV-2 ORF5 sequences, sub-lineages were divided into genetic variants by identifying well-supported clades using a clustering algorithm. By classifying new sequences every three months and systemically identifying new variants across eight years, we demonstrated that the prospective implementation of the variant classification system produced robust, reproducible results across time and is expandable to accommodate new genetic diversity. From 2015 and 2023, 118 variants were identified, with ~48 active variants per year, of which 26 were “common” (detected >50 times). A median of four new “common” variants were detected per year. Mean within-variant genetic distance was 2.4% (max: 4.8%). The mean distance to the closest related variant was 4.9%. A routinely updated webtool (https://stemma.shinyapps.io/PRRSLoom-variants/) was developed to enable end-users to assign new sequences to a variant ID. However, it is important to note that the current variant classification system relies on U.S. PRRSV-2 ORF5 sequences from 2015 onwards. Further efforts would be required to extend this variant classification system to include earlier sequences from the U.S., as well as sequences from other countries. As compared to RFLPs, we demonstrate how variant classification can better discriminate between previous and new wild-type strains on the same farm, determine possible source of new introductions into a farm/system, and track emerging variants regionally. In conclusion, a uniform variant classification system will support the findability and reusability of additional information or research related to a particular variant, thus increasing synergy between lab and field. Adoption of a unified classification system will enhance PRRSV-2 epidemiological monitoring, research, and communication, and improve industry responses to emerging genetic variants.

Lipid Nanoparticle-Encapsulated DNA Vaccines: A Versatile Platform for Rapid Development of Vaccines Against Influenza A Viruses of swine

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There is an urgent and compelling need for a novel vaccine platform that can be exploited to frequently update vaccines against influenza A virus of swine (IAV-S), a viral pathogen that not only causes significant economic losses to swine producers but also poses a significant public health concern due to its zoonotic potential. Currently, commercial IAV-S vaccines contain multiple whole-inactivated virus (WIV) strains emulsified in adjuvants. However, these WIV vaccines do not confer heterologous protection primarily due to the rapid evolution of the viral genome, leading to the constant emergence of new virus strains/variants. Recognizing the need to streamline the procedure for updating veterinary vaccines, the Center for Veterinary Biologics has issued a memorandum that provides the regulatory framework to facilitate the timely updating of veterinary vaccine antigens. According to these guidelines, if a veterinary vaccine is developed using a well-established nonreplicating, nonviable production platform and the initial vaccine product has already been fully licensed, the licensure process for new vaccines containing sequence variants of the same vaccine antigens can be expedited. Our laboratory has been dedicated to developing a versatile platform for the rapid development and updating of vaccines against IAV-S. Our platform utilizes a DNA plasmid to deliver the vaccine antigens because the DNA plasmid is noninfectious, nonreplicating, and safe for animal use. Additionally, DNA plasmids are highly stable at room temperature and can be produced in large quantities at low cost, making them especially suitable for veterinary usage. The innovation of our approach is that we encapsulate the DNA plasmid with a unique lipid nanoparticle (LNP) formulation to enhance cellular uptake and immunogenicity of the DNA plasmids. We demonstrated that pigs vaccinated with a single dose of our lipid nanoparticle encapsulated DNA (LNP-DNA) vaccine containing the HA gene of either H3N2, H1N1, or H1N2 subtype developed high titers of hemagglutination inhibition (HI) antibodies 7 - 14 days post-vaccination and were fully protected against challenge infection with the respective homologous IAV-s strains. Moreover, the LNP-DNA vaccine did not induce vaccine-associated enhanced respiratory diseases (VAERD), a phenomenon often observed when WIV-vaccinated pigs are challenged with a heterologous IAV-S strain. These results prove that LNP-DNA vaccines can be an attractive platform for the rapid development of vaccines against IAV-S and other viruses.

Investigation of fetal liver, heart, and thymus transcriptomes for prediction of reproductive failure

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the costliest diseases to pork producers worldwide. The pregnant gilt PRRSV infection model has emerged as an essential design for facilitating research into vertical viral transmission and evaluation of the fetal response to infection. The goal of this study is to identify critical tissues and genes that forecast fetal resistance (a fetus with no viral load despite maternal infection), resilience (a fetus which survives despite PRRSV RNA in fetal thymus and serum following maternal infection), or susceptibility (>5 log PRRSV RNA and/or meconium staining). We hypothesize that specific genes associated with innate immunity may provide some protection to foster the resilience observed in fetuses which contract no or maintain low levels of virus.

In this study pregnant gilts (N=27) were infected with PRRSV at day 85 +/- 0.9 of gestation. At 21 days post infection, the gilts and fetuses were euthanized, and fetal tissues collected for further investigation. The fetuses in this study were selected based on PRRS viral load (VL) in fetal serum (SER), placenta (PLC), and thymus (THY). We compared six distinct fetal groups; control (CTRL) (mock infected/no viral load), uninfected (UNIF) (<1 log SER or per mg PLC or THY), PLC-Only VL (>0.5 log virus in PLC; <1 log in SER and THY), PLC+SER VL (>0.5 log in PLC, 0.5-2.0 log in SER; <1 log THY), THY LOW VL (>0.5 log in PLC, 0.5-2.0 log in SER and THY), and THY MED VL (>0.5 log in PLC, >5.0 log in SER and 2.5-4.99 log THY). Total tissue RNA was extracted and purified from fetal liver, heart, and thymus. These samples were analyzed using Qubit for RNA concentration and overall quality (RIN #). Expression of genes, based on 12 pathways hypothesized to be involved in fetal resilience or susceptibility including innate immune, and nidovirus associated pathways. The gene codeset included 179 gene targets with 7 housekeeping genes and was analyzed using NanoString transcriptomics.

The purposeful selection of these gene pathways will provide insight into mechanisms of PRRSV resistance and susceptibility, focusing on differential gene expression between fetuses with low or medium levels of PRRSV infection versus those which successfully prevent infection (CTRL and UNIF). These results will also provide insight to targeted tissue responses and clues to their mechanism of fetal protection following maternal infection, to inform treatment options and swine facility management strategies.

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Generation of ANP32A Knock-out Cell Line for Functional Assessment of Novel Swine Isoform on Avian Influenza Virus Polymerase Activity

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Host acidic leucine-rich nuclear phosphoprotein 32 (ANP32) proteins have been demonstrated as important factors in avian influenza virus (AIV) zoonosis. Compared to other mammalian orthologues, swine ANP32A has a unique pro-viral capacity by enhancing AIV polymerase activity. While interspecific differences have been extensively characterized, no reports have been provided that assess intraspecific variation in swine ANP32 genes and impact on AIV permissiveness. Domestic swine are referred to as “mixing vessels” for influenza A strains and intermediate hosts in the generation of mammalian-adapted AIV. Therefore, identification of ANP32 variants that affect host permissiveness would provide a plausible avenue for limiting the emergence of novel influenza strains. The overall goal of this research is to identify variants within swine ANP32A/B that alter AIV pro-viral capacity using high-throughput parallel sequencing and in vitro functional assays. Sequence comparison has detected limited variation within the coding regions of either gene across wild and domestic pigs. However, multiple ANP32A isoforms have been identified, one of which has not been previously reported. Dual transfection of CRISPR-Cas9 guide RNAs targeting an exon common across all ANP32A isoforms expressed in the parental Newborn Pig Trachea (NPTr) cell line was utilized to generate a predicted knock-out clone for subsequent in vitro functional assessment of ANP32A variants and isoforms. Five edited NPTr clones were selected for further characterization based on preliminary screening for large homozygous deletions (>100bp) within the ANP32A target region. The outcomes of this research have the potential to benefit swine producers directly by improving animal welfare, but also human health by limiting the ability of swine to serve as AIV reservoirs and emergence of novel pathogenic strains with potential for human pandemic transmission.

CRISPR-Cas9 mediated identification of host factors for influenza infection and persistence -Progress Report

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Swine influenza viruses cause acute respiratory infection in pigs, which results in significant economic losses to the swine industry. Swine can serve as a mixing vessel and genetic reassortment of avian, human, and/or swine influenza A virus occasionally gives rise to new emerging viruses. Although vaccines against influenza viruses are the most effective prevention method, antiviral drugs are important in controlling new emerging viruses before vaccines are made available. Since all viruses including swine influenza virus depend on host cells for their replication, new anti-influenza therapeutic strategies could potentially be developed if we identify host's restriction factors for influenza virus replication. Towards this goal, we intended to use CRISPR-Cas9 genetic screening technology to identify host factors that are important in swine influenza virus replication in swine bronchial epithelial cells (NSBE). We will provide an update on the research progress we have made so far.

Transduction efficiency of HIV-based lentiviral vector in three different porcine cell lines

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Lentiviral vector has many advantages for use as a gene delivery vehicle. Human immunodeficiency virus (HIV)-based lentiviral vector is commonly used for such purpose. However, it has been reported previously that HIV-based lentiviral vector exhibits a relatively low transduction efficiency in porcine cells compared to human cells. To investigate this further, we compared transduction efficiency of three established porcine cell lines with HIV-based lentiviral vector. Human embryonic kidney cells (HEK-293T) are included as a control. Results showed that all three porcine cell lines had a significantly lower transduction efficiency compared to HEK-293T cells. Among the three porcine cell lines, pig kidney cells (PK15) showed the highest transduction efficiency. Porcine alveolar macrophages cell line (CRL-2843) had a significant lower transduction efficiency compared to the other two porcine cell lines. Swine bronchial epithelial cells (NSBE) fall between PK15 and CRL-2843 cells. Overall, HIV-based lentiviral vector showed a relatively low transduction efficiency in porcine cells. The mechanisms by which porcine cells restrict efficient transduction of HIV-based lentivirus remains to be determined in future studies.

Functional Mechanism of Porcine Epidemic Diarrhea Virus Nucleocapsid Protein in Evading from Host Interferon Immune Responses

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Porcine epidemic diarrhea (PED) is a highly contagious acute disease of pigs caused by porcine epidemic diarrhea virus (PEDV) that imposes significant threat to the swine industry. Recent studies suggest that PEDV has evolved to deploy complicated antagonistic strategies to escape from interferon (IFN) immune responses by using the viral proteins. The nucleocapsid (N) protein, a highly conserved structural protein of PEDV, is crucial for viral RNA packaging and viral particle assembling. It is also a multifunctional viral protein in mediating immune evasion of PEDV, but the specific mechanism in disrupting the IFN signaling remains to be further explored. We found that PEDV N protein could be distributed in both the cytoplasm and the nucleus of IPEC-J2 cells. By interacting with the immune-related host effectors, PEDV N suppresses the antiviral IFN-λ signaling via different mechanisms for its replication.

We first demonstrated that histone deacetylase HDAC1 acted as a negative regulator of PEDV replication in IPEC-J2 cells. PEDV N protein could be localized in the nucleus through the putative nuclear localization sequence 261-PKKNKSR-267 and competitively bind to the HDAC1-targeted transcription factor Sp1 in the nucleus. Consequently, PEDV N protein impaired HDAC1 transcription and induced enhanced protein acetylation. Signal transducer and activator of transcription 1 (STAT1) is one of the acetylated proteins as a result of HDAC1 inhibition by PEDV N protein. The PEDV N protein-induced STAT1 acetylation negatively regulated its phosphorylation and nuclear localization. Hence, PEDV inhibited transcription of ISG15 and OAS1, in favor of its replication.

Protein kinase A (PKA), another binding target of PEDV N, also participates in the anti-PEDV immune responses. Here, we observed that the cAMP level and PKA C-α (the active form of PKA) phosphorylation changed significantly upon PEDV infection and overexpression of different N proteins. Furthermore, we illustrated that PKA C-α could be hijacked by PEDV N proteins into the nucleus via direct interaction. The nucleus-localized PKA C-α failed to facilitate STAT1 phosphorylation regulated by JAK1. Thus, PEDV might utilize this mechanism to evade from the host antiviral IFN responses.

Overall, the above described novel immune evasion mechanisms are realized by the suppression of STAT1 activation through PEDV N protein. Our findings contribute to a better understanding of the PEDV-host interaction as part of the pathogenetic mechanisms of coronaviruses.

Segment-Specific Enteroids from Pig Small Intestine in Matrigel and Transwell Inserts: Susceptibility to Porcine Epidemic Diarrhea Virus (PEDV)

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The initial stages of porcine epidemic diarrhea virus (PEDV) infection and the corresponding innate immune responses at the intestinal epithelium are not well understood, primarily due to the constraints of conventional cell culture and animal models. This research aimed to develop a porcine enteroid culture system to investigate potential variations in infection susceptibility across different sections of the porcine small intestine (duodenum, jejunum, and ileum). Intestinal crypt cells from nursery pigs were cultured in Matrigel to form porcine enteroid monolayer cultures (PEMCs). After characterization, these PEMCs were enzymatically dissociated and subcultured on transwell inserts (PETCs) to facilitate apical surface exposure for infection studies. The characterization of PEMCs and PETCs from different intestinal regions involved the assessment of morphology, proliferation, viability, and cellular phenotyping through immunohistochemistry/immunocytochemistry and gene expression analyses. Morphological and phenotypical assessment reveal no significant differences among PEMCs and PETCs across intestinal segments, indicating a close resemblance to the porcine intestinal epithelium. PETCs were then inoculated with 105 TCID50 /mL of a highly pathogenic PEDV non-S INDEL strain and incubated for 24 h. The infection outcomes were evaluated by observing cytopathic effect, PEDV N protein expression via immunofluorescence assay (IFA), and PEDV N-gene detection using quantitative reverse transcription polymerase chain reaction (RT-qPCR). Although PETCs derived from different segments of the small intestine were susceptible to PEDV infection, those from the jejunum showed a higher PEDV replication rate of the virus, as confirmed by IFA and RT-qPCR. This segment-specific enteroid culture model offers a reliable platform for virological studies, providing a controlled environment that addresses the limitations of in vivo and traditional cell culture approaches. Establishing standardized culture conditions and thoroughly characterizing the model are crucial for the advancement of enteroid-based infection studies.

Rational design and immunogenicity evaluation of mRNA-based vaccine for African Swine Fever virus

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The outbreaks African swine fever (ASF) in Eurasia have caused tremendous economic losses and the incursion to US would be disastrous to swine industry. Safe and effective vaccines have not been developed so far. Here, we report a novel ASFV mRNA vaccine designed with multiple rationalities to induce robust humoral and cellular immunities. Candidate vaccine antigens are selected by referring to homologs of protective antigens from the closely related vaccinia virus, known antigens eliciting strong host immune response, and viral capsid engineering for membrane-anchoring for optimal B cell engagement. To specifically induce strong T cell response, a T cell-directed vaccine antigen is designed by fusing multiple T cell epitopes (MTE) that are experimentally determined previously or predicted MHC-I high binders. Candidate antigens are formulated into lipid nanoparticle (LNP)- mRNA and further immunogenicity assessment in both mice and pigs reveals that different antigens elicits very distinct immune profiles including total antibody response, antibody effector functionality, and T cell response. Notably, the T cell-directed antigen induced robust cellular immunity. Furthermore, we demonstrated that multiple candidate cocktail vaccines based on distinct antigen immune profiles induced robust B cell and T cell immunities. Overall, The novel-designed vaccines coupled with mRNA technology shed light for an effective vaccine development against ASFV and strategies reported here can be utilized for developing vaccines against other large complex DNA viruses, such as monkeypox virus.

Keywords: ASFV, vaccine, antigen selection, antibody response, effector function, cellular immunity

Investigating the Current Practice in Oral Fluid Sampling in the U.S. Swine Industry: An Exploratory Study

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Introduction: Oral fluid (OF) sampling has been widely used in the United States (U.S.) swine industry for at least a decade. This sample type has been rapidly adopted by the industry for endemic disease monitoring (e.g., PRRS, PCV2, SIV) as it readily optimizes time and resources when conducting population-based sampling. This study characterizes the current use of OF sampling in pig production systems in the U.S. swine industry.

Methods: Two online questionnaires, conducted between June and October 2023, targeted field personnel and swine veterinarians to gather data on OF implementation, primary use, sampling protocols, handling, and processing procedures. Field personnel from a convenient sample of production systems was invited to participate, and swine veterinarians were invited through individual email invitations and the AASV newsletter.

Results: We received 67 valid responses from 42 companies and clinics, representing an estimated studied population of 58M growing pigs and 3.9M sows. The studied population might be overestimated due to overlapping between company and clinic responses. Widespread adoption of OF sampling was evident, with 99% of respondents familiar with or actively using it for diagnosis. Most respondents (69%) use one rope for every two pens, 21% use one rope per pen, while the remaining respondents employ alternative approaches, such as using three ropes per barn, two ropes per barn, or making determinations based on airspace/rooms. The median number of ropes hung per barn was 3 (Interquartile range IQR: 1.75; 4). The median number of pens sampled per barn was 6 (IQR: 4.25; 9), and the median number of pigs that are considered to be represented by a single OF-rope sample was 150 (IQR: 60; 275). Pigs typically have access to the rope for a median of 20 minutes (IQR: 17.5; 30). Sampling frequency for routine surveillance varies across farm types, with gilt-development units (GDU) collecting OF more frequently than sow and growing pig farms. Half of GDU respondents collect OF samples monthly whereas 33% do it weekly. Notably, 64% of respondents who used OF for routine surveillance increased sampling frequency in the face of clinical symptoms. Most respondents reported having written protocols for sample handling, with 50% making these protocols available to farm personnel. Once oral fluids samples are collected, 84% of the responses specified that the samples were stored in refrigeration or cooler with ice. When OF samples are submitted to the VDL, the pig age was included in the submission form most of the time (91%), followed by the Premises Identification Number (83%) and whether clinical signs were observed (64%).

Conclusion: Although a popular sampling methodology, OF sampling strategies vary widely. Efforts to standardize these procedures are necessary for interpretation across production systems.

Time-to-Stability of Porcine Epidemic Diarrhea Virus (PEDV) and the Associated Factors in U.S. Breeding Herds

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Introduction: Porcine epidemic diarrhea virus (PEDV) was first identified in the United States (U.S.) in April 2013. The epidemic period lasted for approximately 1.5 years, transitioning to endemic levels in 2015. Since then, little work has been published on how long it takes a sow herd to achieve a stable status (i.e., time-to-stability or TTS). The objective of this study was to estimate the PEDV TTS in the U.S. breeding herds during the epidemic and endemic periods and assess associated factors.

Materials and Methods: PEDV weekly monitoring data originating from 1,028 U.S. breeding herds that report PEDV status regularly between May 1, 2013 and June 30, 2023 were obtained from the Morrison Swine Health Monitoring Project. The TTS was calculated as the number of weeks in positive unstable (Status 1, from outbreak detection through end of shedding) before changing to positive stable status (Status 2 - absence of clinical signs and no detectable virus for at least four weeks, or Status 2fvi – positive stable with ongoing gilt field virus exposure). Mixed-effects Cox proportional hazards models were used to assess the association between TTS and the recorded factors, including season, filtration status, herd size, PEDV status of the herd before the break, whether the site experienced a PEDV outbreak within the previous 6 months, and the total number of prior breaks. Herd ID nested within the production system was added as the random effect in the models.

Results: In total, 384 PEDV breaks from 16 production systems were included in this study, with 203 occurring during the epidemic period (May 1, 2013 – December 31, 2014) and 181 during the endemic period (January 1, 2015 – June 30, 2023). Overall, the median TTS was 24 weeks (IQR: 18 - 31) during the epidemic and 14 weeks (IQR: 9 - 22) during the endemic period. A log-rank test showed a significant difference (p-value < 0.001) in TTS between the epidemic and endemic period. Factors significantly associated with TTS include the PEDV status of the herd before the outbreak and herd size. Herds breaking from status 2fvi reached stability faster (hazard ratio or HR, 8.6 and 3.6 for epidemic and endemic periods) than herds breaking from status 4 (negative). Larger herds (> 5,000 sows) took longer to reach stability (HR: 0.41 for epidemic and 0.45 for endemic periods) than small herds (<2,500 sows).

Conclusion: This study provides valuable and objective insights into TTS of PEDV breaks in the U.S. breeding herds, especially at a time when the industry is considering whether this disease could be eradicated from the U.S. swine population. These support decision-making in PEDV control and elimination strategies (e.g., forecasting PEDV elimination timeline, herd closure plan) and farm management such as pig flow management and investment timing.

Refining genetic classification of global type 1 porcine reproductive and respiratory syndrome virus and characterization of their geographic distributions

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Type 1 porcine reproductive and respiratory syndrome virus (PRRSV-1) primarily circulates in Europe but it is also detected in North America and Asia. Previous investigations, utilizing ORF5 sequences, categorized PRRSV-1 into four subtypes. Subtype 1 was further classified into 12 clades (A-L) or into three lineages with lineage 1 encompassing clades 1A–1G and lineage 3 including clades 3A–3G. However, neither system has been widely accepted for use. In this study, we introduce a statistically supported PRRSV-1 genetic classification system based on 10,446 global PRRSV-1 ORF5 sequences spanning the years 1991–2023. We proposed to replace “subtype” with “lineage”, aligning with the nomenclature used for PRRSV-2. Consequently, PRRSV-1 was classified into four lineages (L1–L4), with L1 comprising 18 sublineages (L1.1 to L1.18). The proposed classification system is flexible for expansion if additional lineages, sublineages, or more granular classifications are needed. Geographic distributions of global PRRSV-1 at lineage and sublineage levels were investigated. A small number of PRRSV-1 sequences were classified in lineages L2, L3, and L4 and were exclusively found in Eastern Europe. In contrast, PRRSV-1 in lineage L1 had widespread distributions, circulating in North America, Asia, and across Europe. In Europe, 17 out of 18 sublineages (excluding L1.13) within L1 were identified. In North America, only sublineage L1.1 was detected. In Asia, sublineages L1.1, L1.2, L1.6, L1.10, L1.11, L1.13, and L1.17 were identified, with L1.13 restricted to China thus far. This study also determined the classification and ORF5 nucleotide identity of six commercial PRRSV-1 vaccines (Porcilis L1.1, Unistrain L1.2, Pyrsvac-183 L1.2, Ingelvac PRRSFlex EU L1.3, ReproCyc PRRS EU L1.3, and Suvaxyn L1.11) to each lineage and sublineage. Moreover, the detection frequency of vaccine-like viruses were assessed. The phylogeny based on whole-genome sequences demonstrated a slightly different tree topology compared to that based on ORF5 sequences due to recombination. Recombination of PRRSV-1 was observed at intra-sublineage and inter-sublineage levels. A set of PRRSV-1 ORF5 reference sequences representing global diversity and these refined classifications are available for future diagnostic and epidemiological applications. This study serves as a benchmark, delineating the current genetic diversity of PRRSV-1, and the revised ORF5-based classification system offers a valuable tool for characterizing the genetic and phenotypic evolution of PRRSV-1

Comparison of economic losses between status 3 and status 2vx farrow to wean farms equipped with air filtration facilities within 12 weeks following the introduction of PRRSV NADC30-like strain

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is a major swine viral pathogen that affects the pig industry worldwide. Mass vaccination with modified live vaccine (MLV) can mitigate the clinical impact of PRRSV introduction and subsequently minimize economic losses. This retrospective study aims to compare the economic impact of two farrow-to-wean (FTW) farms equipped with air filtration facilities at status 3 and 2vx following the introduction of NADC30-like strain.

Materials and Methods: In Shandong Province, China, two large-scale FTW farms operated by a single group using a weekly batch-based production system. In June 2022, approximately 4,000 PRRS naïve crossbred gilts were introduced into Farm A. Subsequently, in August 2022, Ingelvac PRRS® MLV was actively used for herd immunization, and weekly processing fluid (PF) samples were collected for PRRSV monitoring. The NADC30-like strain was first detected in March 2023. Farm B consisted of a crossbred population with 8000 sows and was classified as status 3 according to AASV PRRS classification in April 2022. PRRSV PCR turned positive in January 2023 and was sequenced as NADC30-like strain. Economic losses were assessed using the partial budget algorithm through firstly comparison within the farms before and after detection of PRRSV over a period of twelve weeks following between these two farms with different PRRS statuses.

Results: In the case of Farm A with a status of 2vx, in comparison to the pre-detection period, weaned piglets per 1000 sows (WPTHS) decreased by 81, resulting in a reduction of ¥203,707 in weekly revenue. Additionally, there was an increase in sow costs by ¥676 per sow and weaner costs by ¥122 per piglet. For Farm B with a status of 3. Compared to the 12-week period prior to PRRSV detection, WPTHS decreased by 136, resulting in a weekly revenue decrease of ¥698,944. Additionally, there was an increase in the cost of sows by ¥1177 per sow and an increase in the cost of weaner raised by ¥278.

Compared to B Farm, within 12 weeks following the introduction of PRRSV, A Farm exhibited a significant decrease in the losses of 55 in WPTHS, a reduction of ¥501 in sow cost, and a decrease of ¥156 in piglet weaning cost. The annual immunization cost was assumed to be ¥36, and an ROI of 14 was attained through the implementation of a mass vaccination scheme for PRRSV MLV.

Conclusions and Discussion: This study provides evidence that prophylactic vaccination using MLV for the entire herd has been shown to mitigate economic losses associated with the introduction of PRRSV NADC30-like strain.

A plausible mechanism responsible for the variation of PRRS virus virulence

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It has become increasingly clear that since PRRS virus first appeared as a pathogen of swine in the late 1980s, there have been repeated cycles of emergence of virus strains with an alarming gradual increase in virus virulence. Possible mechanisms responsible for the increased levels of PRRS virus virulence have been proposed, including the reported ability of highly virulent PRRS virus to replicate intensely in macrophages, and/or trigger extensive macrophage death via apoptosis. However, there has been no assessment of these notions. Until recently, the mechanism responsible for the PRRS virus-infection induced death of macrophages via apoptosis was unknown. We reported (Chen et al., 2018), that the replication of PRRS virus in porcine macrophages triggers the activation of the unfolded protein response (UPR), which is a homeostatic pro-survival signaling network that orchestrates the recovery of endoplasmic reticulum (ER) function to restore homeostasis. However, under sustained and irremediable ER stress, as it occurs during a viral infection, a switch from pro-survival to pro-apoptotic signaling events of the UPR occur resulting in cell death by apoptosis via the intrinsic apoptotic pathway. This pathway includes the activation of pro-Caspase-9, which activates the terminal caspases 3 and 7 (3/7), resulting in the exposure of phosphatidylserine (PtdSer) to the cell surface, and the development of the characteristic apoptotic blebs. Notably, the threshold of ER stress that triggers the activation of the UPR stress sensors is determined by specific interactomes regulating the UPR signal amplitude, its kinetics, and its impact on cell physiology (Hetz et al., 2020). Hence, variation in the intensity of the UPR, which would have a direct effect on the extent and intensity of apoptosis that ensues, is expected. Noting that apoptosis is pro-inflammatory, we reasoned that PRRS viruses that exhibit markedly different levels of virulence might differ in their rate of growth in macrophages which, in turn, would differ in the intensity of the apoptosis elicited and, consequently, the intensity of the pro-inflammatory response they promote. We postulate that by replicating at a faster rate, highly virulent PRRS viruses, as compared to less virulent viruses, would trigger extreme levels of apoptosis, thus promoting severe inflammation resulting in greater virus pathogenicity. To test out hypothesis, we compared PRRS viruses isolated from several sow farms that exhibited different levels reproductive losses due to a PRRS outbreak ranging from high to low levels of abortion, sow and pre-weaning mortality, and total piglet loss. We characterized the biotype of these isolates based on their rate of growth in macrophages, caspase 3/7 activation and rate of apoptosis. Our results indicate a strong association between the severity of PRRS outbreak in a sow farm, as manifested by a high level of reproductive losses, and a relatively high rate of virus replication in macrophages, caspase 3/7 activation and rate of apoptosis, as compared to isolates exhibiting lower levels of virulence in the field.

An effective vaccine against swine influenza A virus based on the matrix protein 2 (M2)

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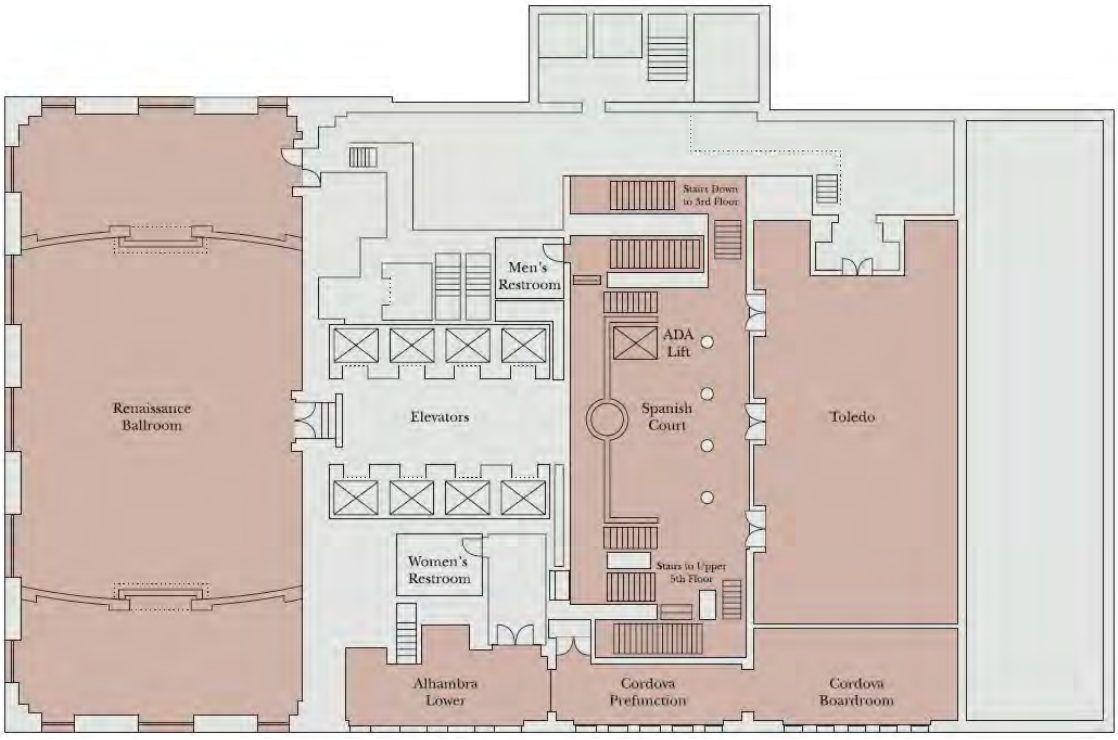
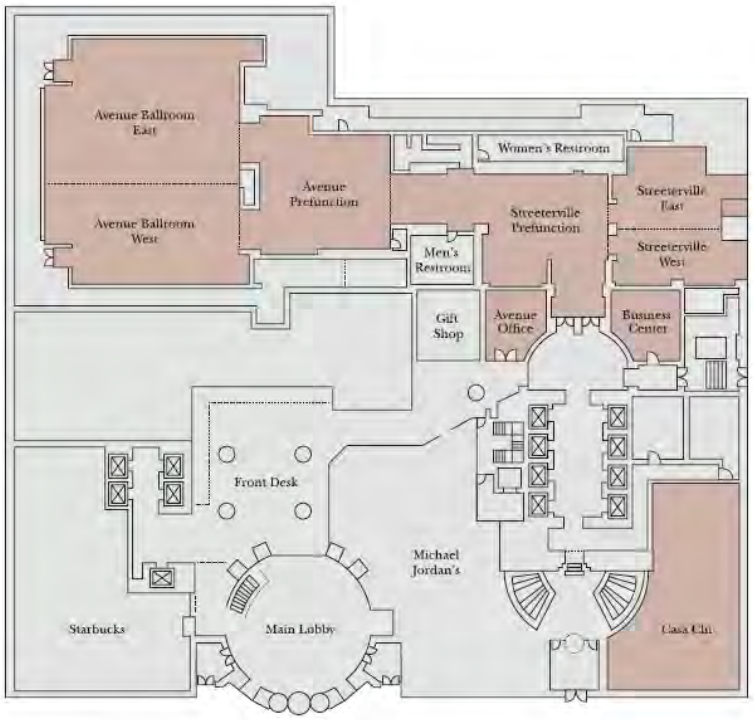
The syndrome resulting from the infection of swine with influenza A virus (IAV) presents a major economic burden to the pork industry and is one of the top three diseases affecting pigs in all phases of pork production. Effective vaccines are the cornerstone of defenses against acute influenza virus infections. Whole inactivated virus (WIV) vaccines are the only type of biologic used in the U.S. to protect pigs against IAV. Experimental data indicates that the protection provided to swine by commercial WIV influenza vaccines against contemporary strains of swine IAV (swIAV) is limited. This is due in part to the heterologous antigenic nature of the constantly increasing diversity of the hemagglutinin (HA) of this virus. The substantial diversity of the HA among co-circulating IAV viruses in swine herds poses a significant challenge for effective vaccine development. Notably, the matrix protein 2 (M2) of IAV is a highly conserved protein present in the virus envelope. More than 98% of swIAV strains circulating in U.S. swine herds share the identical pandemic (pdm) isoform of M2. M2 is a 97 amino acid long transmembrane protein that forms a tetramer on the virus envelope and acts as a viroporin. M2 consists of an intracellular C-terminal domain (positions 47 to 97), a transmembrane domain (positions 24 to 46), and an extracellular N-terminal domain (positions 1 to 23). The ectodomain of M2 (M2e) has been pursued for many years as candidate for a potential universal influenza vaccine for humans, primarily due to the difficulty of assembling the entire M2 protein in its natural transmembrane configuration. For this project, we examined the immunogenicity and protective efficacy of a novel IAV vaccine for swine consisting of recombinant full-length M2 protein displayed in its natural transmembrane configuration in soluble nanoscale membrane assemblies called nanodiscs (NDs). As an adjuvant, synthetic oligonucleotides (ODN) containing immunostimulatory unmethylated CpG dinucleotides (CpG motifs) were incorporated into the NDs structures displaying M2 (M2NDs). We determined that M2:NDs elicit the production of antibodies capable of recognizing IAV virions as well as virus-specific interferon-□-producing T cells. To assess the ability of M2NDs to confer protective immunity against swIAV, groups of pigs (n=9) were immunized at 5 weeks of age via both intranasal and intramuscular routes twice at a 3 week interval with either: M2NDs; empty NDs; or empty NDs mixed with M2. The animals were challenged intranasally with a H3N2 swIAV two weeks after the booster vaccination and monitored for 5 days. Five days after being challenged, the animals were euthanized and their lungs scored for the extent of pneumonia. Lung lavage samples were collected to measure the viral load. Pigs vaccinated with the M2NDs exhibited the presence of strong protective immunity as determined by the lack of pneumonic lesions in the lungs in 8 of the 9 pigs in this group, as well as a reduced viral load in nasal secretions and lung lavage. In contrast, animals immunized with empty NDs or empty NDs mixed with M2 showed no evidence of protection as indicated by exhibiting extensive pneumonia and a significant viral load in nasal secretions. This study demonstrates that the immunization of swine with M2 displayed in NDs provides strong levels of protective immunity against swIAV. Future work will aim to ascertain the minimal antigen dose and vaccination schedule necessary to elicit strong protective immunity. This novel vaccine offers great promise as a universal IAV vaccine to solve the challenges presented by the great diversity of swIAV present in the field.

Intercontinental Hotel Map



FIFTH FLOOR



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