# 2022 NAPRRS/NC229 International Conference of Swine Viral Diseases

NORTH AMERICAN PRRS SYMPOSIUM

## InterContinental: Chicago Magnificent Mile
## December 2-4

# Proceedings

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# Welcome to 2022 NAPRRS/NC229 International Conference of Swine Viral Diseases

Dear Friends,

We are delighted to gather together again at Chicago. The NAPRRS/NC229 International Conference of Swine Viral Diseases (ICSVD) was originated as the North American PRRS Symposium in 2003. In recent years, the emergence and spread of new swine viruses have expanded the focus to include emerging and transboundary swine diseases. Our 2022 meeting expands into a 3-day international conference, including nine plenary sessions and a special Zoetis lunch session as the means to deliver a much larger and more comprehensive meeting experience. Also, of note is the special Saturday afternoon sessions co-organized with Swine Health Information Center (SHIC) to emphasize modern technologies and translational research in emerging swine diseases. The Saturday evening reception and poster session provide a forum for attendees to discuss the latest research and explore collaborative relationships for new research initiatives.

We sincerely appreciate the interest and support of our community, as shown by our attendance of over 170 people, and the large number of excellent abstracts. We hope this symposium will provide new insights for your research and new opportunities for collaboration. We also express our appreciation for the generous donations from our sponsors, who are essential for the success of this meeting.

Enjoy the beautiful city of Chicago!

Ying Fang, Executive Director
Raymond (Bob) Rowland, NAPRRS/NC229 advisor

# NAPRRS/NC229 ICSVD Organizing Committee

## NA PRRSS/NC229 Advisor:

**Dr. Raymond (Bob) Rowland**
*University of Illinois*

## Planning Committee:

Executive Director
**Dr. Ying Fang**
*University of Illinois*

Internal Coordinators
**Jackie Sue Sturdyvin**
**Kristen Eignher**
*University of Illinois*

**Dr. Jose Angulo**
*Zoetis*

**Dr. Jay Calvert**
*Zoetis*

**Dr. Joseph F. Connor**
*Carthage Veterinary Service, Ltd*

**Dr. Megan Niederwerder**
*Swine Health Information Center*

**Dr. Paul Sundberg**
*Swine Health Information Center*

## Joint Scientific Committee:

### Co-Chairs

**Dr. Ying Fang**
*University of Illinois*

**Dr. Roman Pogranichniy**
*Kansas State University*

## Committee Members:

**Dr. Andreia Arruda**
*The Ohio State University*

**Dr. Diego G. Diel**
*Cornell University*

**Dr. Scott Kenney**
*The Ohio State University*

**Dr. Pablo Pineyro**
*Iowa State University*

**Dr. Hiep Vu**
*University of Nebraska*

**Dr. Zhengguo Xiao**
*University of Maryland*

# NAPRRS/NC229 ICSVD David Benfield Award Competition Judges

**Dr. Andreia Arruda**
*The Ohio State University*

**Dr. Fernando Osorio**
*University of Nebraska*

**Dr. Gyula Balka**
*University of Veterinary Medicine, Hungary*

**Dr. Pablo Pineyro**
*Iowa State University*

**Dr. Jay Calvert**
*Zoetis*

**Dr. Tomasz Stadejek**
*Warsaw University of Life Sciences, Poland*

**Dr. Diego G. Diel**
*Cornell University*

**Dr. Roman Pogranichniy**
*Kansas State University*

**Dr. Christopher Gaulke**
University of Illinois

**Dr. Hiep Vu**
*University of Nebraska*

**Dr. Luis Gimenez-Lirola**
Iowa State University

**Dr. Dongwan Yoo**
*University of Illinois*

**Dr. Scott Kenney**
The Ohio State University

**Dr. Jianqiang Zhang**
Iowa State University

# 2022 Travel Fellowship Recipients

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

This year the 2022 ICSVD welcomes a generous donation from:

## Boehringer Ingelheim Travel Fellowships

**Shamiq Aftab**
*South Dakota State University*

**Jing Huang**
*University of Minnesota*

**Carolyn Lee**
*Ohio State University*

**Mehdi Maury Laouedj**
*University of Montreal*

## Elanco Travel Fellowships

**Chi Chen**
*University of Illinois*

**Gaurav Rawal**
*Iowa State University*

**Yi-Fan Shen**
*The Ohio State University*

**Chia-Ming Su**
*University of Illinois*

**Lu Yen**
*Iowa State University*

## Swine Health Information Center Travel Fellowships

**Guilherme Arruda Cezar**
*Iowa State University*

**Kate Dion**
*Iowa State University*

# David Benfield Award Student Winners


**Best Oral Presentation**

1st place: Sushmita Kumari, University of Nebraska-Lincoln
2nd place: Gabriela do Nascimento, Cornell University
3rd place: Yi-Fan Shen, The Ohio State University

**Best Lightning + Poster Presentation**

1st place: Kristen Walker, USDA Animal Parasitic Diseases Laboratory
2nd place: Chia-Ming Su, University of Illinois Urbana-Champaign
3rd place: Kassandra Durazo Martinez, University of Nebraska-Lincoln

**Best Poster Presentation**

1st place: Marie-Jeanne Pesant, Université de Montréal, Canada
2nd place: Grzegorz Tarasiuk, Iowa State University
3rd place: Rachel Schambow, University of Minnesota

# NAPRRS/NC229 ICSVD
# Keynote Speakers

1:00-1:30 pm, December 2
**Sandra Blome, Friedrich-Loeffler-Institut, Germany**
African Swine Fever and its challenges in Europe

1:30-2:00 pm, December 2
**Jay Calvert, Zoetis, USA**
Vaccination strategies to reduce PRRSV recombination

2:00 - 2:30 pm, December 2
**Luc Dufresne, Seaboard Foods, USA**
PRRS update…Why are we losing the battle?

8:00 - 9:30 am, December 3
**Amy Vincent, USDA-ARS, USA**
Influenza A virus in US swine populations and the confluence between swine and human health

11:15 - 11:30 am, December 4
**David Benfield, The Ohio State University, USA**
35 years – What's next?

# Zoetis Special Lunch Session Invited Speakers

**December 3**

12:00 pm

**Will Lopez, PIC, USA**

Quality weaned pigs, implications for health and performance in growing pigs

12:20 pm

**Jordi Baliellas, GSP, Lleida, Spain**

PRRSV surveillance using tongue tips Fluids (TTF), practical implications dealing with PRRS outbreaks

12:40 pm

**Gustavo Machado, North Carolina State University, USA**

Major enhancement of U.S. swine industry biosecurity: what does this really mean for a production system to explain between-farm disease spread?

# NAPRRS/NC229 and SHIC Co-organized Session Invited Speakers

**December 3**

1:30 pm

**Paul Sundberg, Swine Health Information Center (SHIC), USA**

Emerging Swine Disease Investigation: SHIC update

1:45 pm

**Maria Sol Perez Aguirreburualde, University of Minnesota, USA**

Global disease monitoring for swine diseases

2:15 pm

**Leela Noronha, USDA-ARS, USA**

Japanese Encephalitis Virus: an Emerging Transboundary Pathogen

3:30 pm

**Luis Gimenez-Lirola, Iowa State University, USA**

Next generation of swine diagnostic laboratory platforms

3:45 pm

**Noelle Noyes, University of Minnesota, USA**

Development of a single, rapid workflow for simultaneous detection of >50 swine viruses from field samples

4:00 pm

**Liang Dong, Iowa State University, USA**

Low-cost biosensors for rapid, on-chip, on-site detection of swine respiratory viruses

4:15 pm

**Jianfa Bai, Kansas State University, USA**

Differentiation of PRRS vaccine strains using Luminex bead-based technology

**2022 NAPRRS/NC229 ICSVD Program**

## Friday, December 2, 2022

**9:00 am – 8:00 pm:** Conference Check-In/Onsite Registration (3rd Floor, King Arthur Court)

**12:45 – 8:00 pm:** Plenary Sessions (3rd Floor, King Arthur Court)

**12:45 pm**:
Opening Remarks: Welcome to 2022 ICSVD
*Ying Fang, University of Illinois, USA*

**1:00 – 3:00 pm:** Session 1: Field Challenges in Disease Control and Prevention
*Moderators: Andreia Arruda, Jeffrey Zimmerman*

- 1:00 pm: African Swine Fever and Its Challenges in Europe
  *Sandra Blome, Friedrich-Loeffler-Institut, Germany*
- 1:30 pm: Vaccination Strategies to Reduce PRRSV Recombination
  *Jay Calvert, Zoetis, USA*
- 2:00 pm: PRRS update…Why are we losing the battle?
  *Luc Dufresne, Seaboard Foods, USA*
- 2:30 pm: Eradication of PRRS from Hungarian Pig Herds from 2014 to 2022
  *Gyula Balka, University of Veterinary Medicine, Budapest, Hungary*
- 2:45 pm: National PRRS Reduction Strategy Launched in Denmark
  *Nicolai Weber, Danish Agriculture & Food Council, Copenhagen, Denmar*

**3:00 – 3:30 pm:** Break

**3:30 – 5:30 pm:** Session 2: NC229 Station Report and Business Meeting
*Moderators: Roman Pogranichniy, Pablo Pineyro-Pineiro*

- 3:30 pm: NC229-Business Meeting Opening and Introduction
  *Roman Pogranichniy, Kansas State University, USA*
- 3:45 pm: USDA-NIFA Update
  *Michelle Colby, USDA, USA*
- 4:15 pm: NC229 Performance Highlights in Year 2021
  *Pablo Pineyro-Pineiro, Iowa State University, USA*

- 4:30 pm: Station Representative Reports
- 5:30 pm: Closing Remarks and Renewal of NC229 Multistate grant

**6:00 – 8:00 pm:** ICSVD Welcome Dinner and Lightning Talk Session

**6:00 pm**: Start Food Service

**6:30 – 8:00 pm:** Session 3: Virus-Host Interaction and Pathogenesis (I)
*Moderators: Elisa Crisci, John Harding*

- 6:30 pm: Role of PSTII in PRRSV-CD163 Interaction
  *Raymond (Bob) Rowland, University of Illinois, USA*
- 6:35 pm: Deletion of CD163 Domain Five Protects Pigs from Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Infection
  *Brianna Salgado, University of Illinois, USA*
- 6:40 pm: Identification of Conserved Amino Acid Residue on PRRSV Glycoprotein 2 Critical for Infectivity in Macrophages
  *Jayeshbhai Chaudhari, University of Nebraska-Lincoln, USA*
- 6:45 pm: Furin Cleavage is Required for Swine Acute Diarrhea Syndrome Coronavirus Spike Protein-Mediated Cell–Cell Fusion
  *Jinman Kim, Chungnam National University, Republic of Korea*
- 6:50 pm: Changes in Thyroid Hormone Levels Following Porcine Circovirus-2, Mycoplasma, Salmonella or Brachyspira Inoculation
  *John Harding, University of Saskatchewan, Canada*
- 6:55 pm: Novel Experimental Design Reveals Additional Evidence of Natural Genetic Variation in Response to PRRS Challenge
  *Jenelle Dunkelberger, Topigs Norsvin Research Center, the Netherlands*
- 7:00 pm: Experimental Pig Study Comparing Pathogenicy of PRRSV 1-4-4 L1C Variant with Other Lineage 1 Strains
  *Gaurav Rawal, Iowa State University, USA*
- 7:05 pm: Characterization of the Subclinical Infection of Porcine Deltacoronavirus in Grower Pigs Under Experimental Conditions
  *Lu Yen, Iowa State University, USA*
- 7:10 pm: Transcriptomic Analysis of Air-Liquid Interface Porcine Respiratory Epithelial Cell Cultures Infected with Porcine Hemagglutinating Encephalomyelitis Virus
  *Rahul Nelli, Iowa State University, USA*

- 7:15 pm: Establish A Pregnant Sow-Neonatal Pig Model System to Study Influenza-Microbiome Interactions
  *Lufan Yang, University of Illinois, USA*
- 7:20 pm: Mitochondrial Dysfunction and Oxidative Stress in PRRSV-2 Infected Lung Macrophages
  *Elisa Crisci, North Carolina State University, USA*
- 7:25 pm: Identification and Characterization of Nidovirus-host Molecular Interactions
  *Mehdi Maury Laouedj, University of Montreal, USA*
- 7:30 pm: Porcine Reproductive and Respiratory Syndrome Virus Induces Degradation of the Promyelocytic Leukemia Protein and Promotes Viral Replication
  *Chia-Ming Su, University of Illinois, USA*
- 7:35 pm: Overexpression of IFITM3 Induces Autophagy in H1299 Cells and Enhances SVA Replication
  *Shamiq Aftab, South Dakota State University, USA*
- 7:40 pm: Investigation of Fetal Gene Expression Patterns in the Liver, Heart, and Kidney for Prediction of Reproductive Failure
  *Kristen Walker, USDA-ARS, USA*
- 7:45 pm: PRRSV Infection of Alveolar Macrophages Promotes Inflammation and Inhibits Apoptosis
  *Kassanddra Durazo-Martinez, University of Nebraska-Lincoln, USA*
- 7:50 pm: GP5-Specific Antibody Response to Porcine Reproductive and Respiratory Syndrome Virus Challenge in Vaccinated Swine
  *Jing Huang, University of Minnesota, USA*
- 7:55 pm: Switching Immune Target: Applying MJPRRS Classifications to Characterize How PRRSV GP5-Epitope C Changes Over Time
  *Julia Baker, University of Minnesota, USA*

## Saturday, December 3, 2022

**7:00 am – 6:00 pm:** Conference Check-In/Onsite Registration (3rd Floor, King Arthur Court)

**8:00 am – 5:00 pm**: Plenary Sessions (3rd Floor, King Arthur Court)

**8:00 – 9:30 am**: Session 4: Virus-Host Interaction and Pathogenesis (II)
Moderators: *Joan Lunney, Scott Kenney*

- 8:00 am: Influenza A Virus in US Swine Populations and the Confluence Between Swine and Human Health
  *Amy Vincent, USDA-ARS, USA*
- 8:30 am: Partnership Update: Functional Genomics Approach in Livestock to Delineate Host Factors Critical for Emerging Coronavirus Replication
  *Scott Kenney, The Ohio State University, USA*
- 8:45 am: The P1 Region is a Major Determinant of Pathogenicity and Virulence of Senecavirus A Infection
  *Diego Diel, Cornell University, USA*
- 9:00 am: IFN Suppression-Negative and NF-κB Activation-Negative PRRSV in Pigs During Coinfection With Streptococcus Suis
  *Dongwan Yoo, University of Illinois, USA*
- 9:15 am: Deconstructing the Role of SYNGR2 in Viral Disease Susceptibility in Swine
  *Daniel Ciobanu, University of Nebraska, USA*

**9:30 am – 10:00 am:** Break

**10:00 am – 11:30 am:** Session 5: Novel Vaccine Development and Vaccination Strategies
*Moderators: Laura Miller, Hiep Vu*

- 10:00 am: Development of a Broadly Protective Vaccine Against Swine Influenza Virus Based on the M2 Envelope Protein
  *Federico Zuckerman, University of Illinois, USA*
- 10:15 am: A Swine Influenza Candidate Vaccine Platform Based on a Consensus Sequence for Hemagglutinin of H1 Subtype
  *Gabriela do Nascimento, Cornell University, USA*
- 10:30 am: A trivalent Pichinde Virus Vectored Vaccine Expressing HA Proteins of H1N1, H1N2 and H3N2 Influenza Viruses Elicit a Balanced Protective Immunity Against Influenza Infection of Pigs
  *Sushmita Kumari, University of Nebraska-Lincoln, USA*
- 10:45 am: Effect of Killed PRRSV Vaccine on Gut Microbiota Diversity in Pigs
  *Christopher Gaulke, University of Illinois, USA*
- 11:00 am: Senecavirus a (SVA) and Foot-and-Mouth Disease Virus (FMDV) Viral-Like-Particle (VLP) Based Vaccines Induced Cellular and Humoral Immune Response in Pigs
  *Kepalee Saeng-chuto, Chulalongkorn University, Thailand*

- 11:15 am: Engineering an African Swine Fever Virus Multiepitope Protein for Use in an ASF Nanoparticle-based Subunit Vaccine
  *Carolyn Lee, The Ohio State University, USA*

**11:30 am – 1:30 pm:** Zoetis Special Lunch Session
Session 6: Swine Health and Production, the Inevitable Blend
*Moderators: Jose Angulo, Jay Calvert*

- 11: 45 am: Welcome to Zoetis Special Session
  *Jose Angulo, Zoetis, USA*
- 12:00 pm: Quality Weaned Pigs, Implications for Health and Performance in Growing Pigs
  *Will Lopez, PIC, USA*
- 12:20 pm: PRRSV Surveillance Using Tongue Tips Fluids (TTF), Practical Implications Dealing with PRRS Outbreaks
  *Jordi Baliellas, GSP, Lleida*, *Spain*
- 12:40 pm: Major Enhancement of U.S. Swine Industry Biosecurity: What Does This Really Mean for Production System to Explain Between-Farm Disease Spread?
  *Gustavo Machado, North Carolina State University, USA*
- 1:00 pm: Round Table Discussion

**1:30 – 3:00 pm:** Session 7: Emerging Diseases and Field Detection
*Co-Organized by NAPRRS/NC229 and Swine Health Information Center (SHIC)*
*Moderators: Megan Niederwerder, Roman Pogranichniy*

- 1:30 pm: Emerging Swine Disease Investigation: SHIC Update
  *Paul Sundberg, Swine Health Information Center (SHIC), USA*
- 1:45 pm: Global Disease Monitoring for Swine Diseases
  *Maria Sol Perez Aguirreburualde, University of Minnesota, USA*
- 2:00 pm: Rapid Response Program and Outbreak Investigations for Field Detection of the Source of Pathogen Entry
  *Kate Dion, Iowa State University, USA*
- 2:15 pm: Japanese Encephalitis Virus: an Emerging Transboundary Pathogen
  *Leela Noronha, USDA-ARS, USA*
- 2:30 pm: Monitoring Clinical Progression and Sequencing Data of PRRSV2 L1C144 Variant Affected Breeding Herds
  *Mariana Kikuti, University of Minnesota, USA*

- 2:45 pm Swine Disease Reporting System: A tool for Emerging Swine Disease Investigation
  *Guilherme Arruda Cezar, Iowa State University, USA*

**3:00 – 3:30 pm:** Break

**3:30 – 5:00 pm:** Session 8: Modern Technologies in Swine Disease Diagnostics
*Co-Organized by NAPRRS/NC229 and Swine Health Information Center (SHIC)*
*Moderators: Paul Sundberg, Noelle Noyes*

- 3:30 pm: Next Generation of Swine Diagnostic Laboratory Platforms
  *Luis Gimenez-Lirola, Iowa State University, USA*
- 3:45 pm: Development of a Single, Rapid Workflow for Simultaneous Detection of >50 Swine Viruses from Field Samples
  *Noelle Noyes, University of Minnesota, USA*
- 4:00 pm: Low-Cost Biosensors for Rapid, On-Chip, On-Site Detection of Swine Respiratory Viruses
  *Liang Dong, Iowa State University, USA*
- 4:15 pm: Differentiation of PRRS Vaccine Strains Using Luminex Bead-Based Technology
  *Jianfa Bai, Kansas State University, USA*
- 4:30 pm: Predicting Antigenic Dissimilarity for PRRSV Type 1: Applications of Machine Learning
  *Kimberly VanderWaal, University of Minnesota, USA*
- 4:45 pm: PRRS View: An Analytical Platform for the Assessment of PRRSV ORF5 Genetic Sequences
  *Anugrah Saxena, Iowa State University, USA*

**6:00 – 8:00 pm**: Poster Session and Reception (1st Floor, Avenue and Streeterville Room)

## Sunday, December 4, 2022

**7:00 am – 12:00 pm** (7th Floor, Grand Ballroom)
Conference Check-In

**8:00 am – 12:00 pm**: Plenary Sessions (7th Floor, Grand Ballroom)

**8:00 – 9:30 am:** Session 9: Viral Detection, Diagnostics and Surveillance
*Moderators: Diego Diel, Phillip Gauger*

- 8:00 am: PRRSV-1 Strain with Increased Pathogenicity Isolated from a Severe Outbreak in England
  *Jean-Pierre Frossard, Animal and Plant Health Agency, UK*
- 8:15 am: Refining PRRSV-2 Genetic Classification Based on Global ORF5 Sequences and Investigation of Geographic Distributions and Temporal Changes
  *Jianqiang Zhang, Iowa State University, USA*
- 8:30 am: Historical Analysis of N-glycosylation Patterns in PRRSV-2 in the U.S.
  *Igor Paploski, University of Minnesota, USA*
- 8:45 am: Contrasting PRRSV Temporal Lineage Patterns at the Production System, State, and Regional Levels
  *Yi-Fan Shen, The Ohio State University, USA*
- 9:00 am: Incidence of Porcine Parainfluenza Virus 1 (PPIV1) Co-Infections with Influenza A Virus (IAV) and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in Herds with Respiratory Disease
  *Tomasz Stadejek, Warsaw University of Life Sciences, Poland*
- 9:15 am: Detection and Frequency of Potential Zoonotic Rotavirus in Suckling Pigs in the South of Peru
  *Milagros Lostaunau, San Marcos National University, Peru*

**9:30 – 10:00 am:** Break

**10:00 – 11:15 am: Session 10:** Virus Transmission, Biosecurity and Area Control
*Moderators: Scott Dee, James Lowe*

- 10:00 am: Detection of Senecavirus A in Pigs from a Historically Negative National Swine Herd and in Associated Feed Imports from an Endemically Infected Country
  *Scott Dee, Pipestone, USA*
- 10:15 am: Comparative Adsorption of Porcine Reproductive and Respiratory Syndrome Virus Strains to Minnesota Soils
  *Joaquin Alvarez-Norambuena, University of Minnesota, USA*
- 10:30 am: Addressing Biocontainment Through Environmental Contamination Assessment in Farms Housing PRRS Lineage 1C 1-4-4 Positive Pigs
  *Claudio Melini, University of Minnesota, USA*

- 10:45 am: Gilt Introduction in Danish PRRSV-Positive Farms
  *Elisabeth Okholm Nielsen, SEGES Innovation, Denmark*
- 11:00 am: Comparative Survival of Different Strains of Porcine Reproductive and Respiratory Syndrome Virus at Different Temperatures
  *Angie Quinonez-Munoz, University of Minnesota, USA*

**11:15 am – 12:00 pm:** Student Award Ceremony

- 11:15 am: 35 years – What's Next
  *David Benfield, The Ohio State University, USA.*
- 11:30 am: Award Announcement
  *NAPRRS/NC229 ICSVD scientific committee*
- 12:00 pm: Closing Remarks
  *Raymond (Bob) Rowland, University of Illinois, USA*

# NAPRRS/NC229 ICSVD Poster Presentations

| Virus-Host Interaction and Pathogenesis | | |
|---|---|---|
| **Poster #** | **Presenter Name** | **Title** |
| **01** | Shamiq Aftab | Overexpression of IFITM3 induces autophagy in H1299 cells and enhances SVA replication |
| **02** | Julia Baker | Switching immune target: applying MJPRRS classifications to characterize how PRRSV GP5-epitope C changes over time |
| **03** | Jayeshbhai Chaudhari | Identification of conserved amino acid residue on PRRSV glycoprotein 2 critical for infectivity in macrophages |
| **04** | Daniel Ciobanu | Deconstructing the Role of SYNGR2 in Viral Disease Susceptibility in Swine |
| **05** | Elisabeth Crisci | Mitochondrial dysfunction and oxidative stress in PRRSV-2 infected lung macrophages |
| **06** | Diego Diel | The P1 region is a major determinant of pathogenicity and virulence of Senecavirus A infection |
| **07** | Jenelle Dunkelberger | Novel experimental design reveals additional evidence of natural genetic variation in response to PRRS challenge |
| **08** | Kassanddra Durazo-Martinez | PRRSV Infection of alveolar macrophages Promotes Inflammation and Inhibits Apoptosis |
| **09** | John Harding | Changes in thyroid hormone levels following porcine circovirus-2, Mycoplasma, Salmonella or Brachyspira inoculation |
| **10** | Jing Huang | GP5-specific antibody response to porcine reproductive and respiratory syndrome virus challenge in vaccinated swine |
| **11** | Scott Kenney | Partnership update: Functional genomics approach in livestock to delineate host factors critical for emerging coronavirus replication |
| **12** | Jinman Kim | Furin cleavage is required for swine acute diarrhea syndrome coronavirus spike protein-mediated cell–cell fusion |
| **13** | Changhee Lee | Deletion of pentad residues in the N-terminal domain of spike protein fully attenuates porcine epidemic diarrhea virus in piglets |
| **14** | Mehdi Maury Laouedj | Identification and characterization of nidovirus-host molecular interactions |
| **15** | Rahul Nelli | Transcriptomic analysis of air-liquid interface porcine respiratory epithelial cell cultures infected with porcine hemagglutinating encephalomyelitis virus |
| **16** | Gaurav Rawal | Experimental pig study comparing contemporary and historical porcine respiratory coronavirus isolates in |

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|---|---|---|
| | | pigs with and without subsequent influenza A virus infection |
| **17** | Gaurav Rawal | Experimental pig study comparing pathogenicity of PRRSV 1-4-4 L1C variant with other Lineage 1 strains |
| **18** | Raymond (Bob) Rowland | Role of PSTII in PRRSV-CD163 interaction |
| **19** | Brianna Salgado | Deletion of CD163 domain five protects pigs from infection with porcine reproductive and respiratory syndrome virus (PRRSV) infection |
| **20** | Chia-Ming Su | Porcine reproductive and respiratory syndrome virus induces degradation of the promyelocytic leukemia protein and promotes viral replication |
| **21** | Hiep Vu | Partnership: Systematic screening of ASFV proteome to identify immunogenic antigens |
| **22** | Kristen Walker | Investigation of fetal gene expression patterns in the liver, heart, and kidney for prediction of reproductive failure |
| **23** | Lufan Yang | Establish a pregnant sow-neonatal pig model system to study influenza-microbiome interactions |
| **24** | Lu Yen | Characterization of the subclinical infection of porcine deltacoronavirus in grower pigs under experimental conditions |
| **25** | Fangfeng Yuan | Evaluation of the stability and immunogenicity of a novel PPIV1 vector for expression of ASFV p30/p54 antigens |
| **26** | Dongwan Yoo | IFN suppression-negative and NF-κB activation-negative PRRSV in pigs during coinfection with Streptococcus suis |
| | | |
| **Novel Vaccines, Vaccination Strategies, and therapeutics** | | |
| **Poster #** | **Presenter Name** | **Title** |
| **27** | Joel Miranda | Comparison of the immune response of a PRRS MLV vaccine administered at 3 days or 3 weeks of age by different routes in piglets with maternal antibodies |
| **28** | Joaquin Ivarez-Norambuena | Experimental in-vitro evaluation of PRRSv modified-live vaccine and wild-type virus sequenced detection in co-infections |
| **29** | Chi Chen | Establishing pregnant sow-fetus models to assess safety and efficacy of influenza vaccines |
| **30** | Gabriela do Nascimento | A swine influenza candidate vaccine platform based on a consensus sequence for hemagglutinin of H1 subtype |
| **31** | Christopher Gaulke | Effect of Killed PRRSV Vaccine on Gut Microbiota Diversity in Pigs |
| **32** | John Harding | Thyroid hormone supplementation following PRRSV-2 and PRRSV-MHP infection |

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| **33** | Marie-Eve Koziol | A CSF vaccine based on E2 recombinant glycoprotein and adjuvanted with oil-in-water emulsion induces a full protection in a pig field trial |
| **34** | Sushmita Kumari | A trivalent Pichinde virus vectored vaccine expressing HA proteins of H1N1, H1N2 and H3N2 influenza viruses elicit a balanced protective immunity against influenza infection of pigs |
| **35** | Horacio Lara | Field trial experience with an intranasal nonpathogenic novel vaccine against PRRS |
| **36** | Carolyn Lee | Engineering an African Swine Fever Virus Multiepitope Protein for Use in an ASF Nanoparticle-based Subunit Vaccine |
| **37** | Hongyao Lin | Preventing ASF transmission through intradermal vaccination |
| **38** | Marie-Jeanne Pesant | Tenofovir and pro-drug tenofovir disoproxil fumarate inhibit porcine reproductive and respiratory syndrome virus [PRRSv] in vitro |
| **39** | Kepalee Saeng-chuto | Codon and conditions optimization for ASFV protein expression in vitro by mRNA expression system: A rapid and adaptable vaccine platform |
| **40** | Kepalee Saeng-chuto | Senecavirus a (SVA) and foot-and-mouth disease virus (FMDV) viral-like-particle (VLP) based vaccines induced cellular and humoral immune response in pigs. |
| **41** | Federico Zuckermann | Development of a broadly protective vaccine against swine influenza virus based on the M2 envelope protein |
| | | |
| **Emerging Diseases and Field Investigation** | | |
| **Poster #** | **Presenter Name** | **Title** |
| **42** | Maria Sol Perez Aguirreburualde | Global disease monitoring for swine diseases |
| **43** | Guilherme Arruda Cezar | Swine Disease Reporting System: A tool for Emerging Swine Disease Investigation |
| **44** | Kate Dion | Rapid Response Program and Outbreak Investigations for Field Detection of the Source of Pathogen Entry |
| **45** | Mariana Kikuti | Monitoring clinical progression and sequencing data of PRRSV2 L1C144 variant affected breeding herds |
| **46** | Leela Noronha | Japanese Encephalitis Virus: an Emerging Transboundary Pathogen |
| | | |
| **Modern Technologies in Swine Disease Diagnostics** | | |
| **Poster #** | **Presenter Name** | **Title** |
| **47** | Luis Gimenez-Lirola | Next generation of swine diagnostic laboratory platforms |

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# ABSTRACTS

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**Overexpression of IFITM3 induces autophagy in H1299 cells and enhances SVA replication**

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Seneca Virus A (SVA) is a non-enveloped, positive sense, ssRNA virus belonging to the family *Picornaviridae*. SVA causes vesicular diseases in pigs with clinical signs like foot and mouth disease virus. Multiple outbreaks have been reported in the USA, Brazil, and China resulting in significant economic losses. SVA and several other viruses including influenza A virus, dengue virus, and classical swine fever virus have been shown to induce autophagy for increased virus replication. Interferon Induced Transmembrane Protein 3 (IFITM3) is an interferon stimulated gene (ISGs) that has broad antiviral activity. It has been reported that overexpression of IFITM3 induces autophagy. The objective of this study is to investigate the role of IFITM3 overexpression in SVA replication and induction of autophagy in SVA susceptible cells.

To achieve this objective, exogenous IFITM3 was overexpressed in H1299 cells and PK-15 cells followed by SVA infection to check both virus titer and viral protein expression by $TCID_{50}$ assay and Western Blot, respectively. To examine whether IFITM3 overexpression induces autophagy, we performed Western Blot to examine the expression of LC-3B and confocal microscopy to show co-localization between EGFP-LC3 and late endosomal marker LAMP-1. SVA infected cells and Torin1 were used as positive controls and the plasmid vector control was included as a negative control for confocal microscopy. To further confirm whether autophagy enhances SVA replication in H1299 cells, we treated cells with 3-methyl adenine (3MA), an inhibitor of autophagy, and compared supernatant virus titer with those of non-treated cells by $TCID_{50}$ assay.

Our results indicated that IFITM3 overexpression in H1299 cells significantly enhanced viral replication as compared to vector control. IFITM3 overexpression alone in H1299 cells, but not in PK-15 cells, induced autophagy as evidenced by the detection of LC3B using western blot and co-localization of EGFP-LC3 and LAMP-1 using confocal microscopy. We hypothesize that autophagy induced by IFITM3 overexpression in H1299 cells may contribute to the enhanced SVA replication. We have indeed observed a reduced viral titer in 3MA treated H1299 cells as compared to non-treated group. We also observed that the exogenous IFITM3 is very stable in SVA infected H1299 cells, but not in SVA infected PK-15 cells.

To summarize, our preliminary data suggested a possible role of IFITM3 overexpression in induction of autophagy in H1299 cells contributing to enhanced SVA replication. Further studies are required to evaluate the mechanisms by which IFITM3 overexpression enhances SVA replication in both the cell types.

**Comparison of the immune response of a PRRS MLV vaccine administered at 3 days or 3 weeks of age by different routes in piglets with maternal antibodies**

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The vaccination of piglets against PRRS seeks strategically to generate an active immunity against PRRS before they get infected. This approach is increasingly used in breeder sites of Europe that have been stabilized, or are close to stabilization, but where infection still persists in nurseries. In some farms, piglet vaccination is performed around weaning; in other farms, vaccination is administered earlier, usually in the first week of life. It is known that the maternal immunity might interfere with vaccination and so an early administration during lactation might increase this risk. Besides, some PRRS vaccines can be administered by intramuscular or by intradermal routes. While intramuscular vaccination is a well-established way of vaccination, it causes more stress to the pig than the intradermal route.

The objective of the present study was to evaluate the immune response of a genotype 1 commercial MLV PRRS vaccine administered at 3 days of age in presence of maternally derived antibodies. The trial was conducted on a PRRSV stable farm that applied a periodic mass vaccination program for sows. Four groups of piglets (n=18) were vaccinated at 3 days or 21 days of age by either the intramuscular (IM) or the intradermal route (ID). A placebo group was kept as a control. Animals were bled at 3, 21, 28, 56, and 70 days of age to assess seroconversion and the development of neutralizing antibodies.

Before vaccination (3 days of age), the homologous viral neutralization test (VNT) titers were 3.4 $\log_2$ and 4.0 $\log_2$ for the ID groups vaccinated at 3 and 21 days of age, respectively. On the other hand, the IM groups had homologous VNT titers of 2.9 $\log_2$ and 5.6 $\log_2$ for animals vaccinated at 3 and 21 days, respectively. For animals vaccinated IM at 3 days of age, neutralizing antibodies developed from day 21 of age, but the ID group was mostly negative at that age. At 56 days of age, the VNT titers of animals vaccinated at 3 days of age were similar regardless of the vaccination route and remained so at 70 days of age. Regarding animals vaccinated at 21 days of age, no differences in the development of antibodies were observed between the IM and ID groups, and by day 56 of age most animals had VNT titers of 3 $\log_2$ or higher.

In conclusion, IM vaccination at 3 days or 21 days did not show any significant difference. Vaccination using the ID route resulted in a slightly delayed development of neutralizing antibodies, although these differences disappeared later.

## Comparative adsorption of Porcine Reproductive and Respiratory Syndrome Virus strains to Minnesota soils

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### Introduction

Porcine reproductive and respiratory syndrome (PRRS) has become the most significant disease in the US (1). Data from the Morrison Swine Health Monitoring Project (MSHMP) indicate an increasing trend of PRRS cases between October and December (2), a time when manure is pumped and spread on soils (3). It is known that PRRSV can be found in feces and manure pits (4,5) but there is scarce data on whether PRRSV present in the manure could reach groundwater (6-8). Therefore, the aim of this study was to compare different strains of PRRSV for their percolating ability through different soil types.

### Materials and Methods

For the purpose of this study, we used three different PRRS viruses (RFLP 1-7-4 L1A, 1-4-4 L1C, and 1-26-2 LXX), which were grown and titrated in MARC-145 cells. A total of 13 soils were used; 6 from sites surrounding pig farms and 7 obtained from the UMN Agronomy Department. A vertical glass column model was used in which 5g, 10g and 20g of each soil was placed inside the glass columns with a filter paper in the bottom end. Enough water was added to soil columns until the soil was moist and the air bubbles were removed. Then a solution containing virus and water (1:1 v/v) were added to the glass column and percolated water was collected and subsequently titrated in MARC-145 cells. Results were analyzed through ANOVA and multiple linear regression.

### Results

For all PRRSv strains, we were able to isolate virus in the percolates regardless of the amount of soil added (5g, 10g, & 20g). With the 1-7-4 strain, the initial titter decreased from 6.50 to 3.83 $\log_{10}TCID_{50}$/100 µL after being diluted with water (positive control). The virus was isolated from all 5g soil samples, 12 out of 13 10g soil samples and from 6 out of 13 samples. In the case of the 1-4-4 strain, the titter decreased from 5.50 to 3.17 $\log_{10}TCID_{50}$/100 µL after being diluted with water. The virus was isolated from all the 5g soil samples, 6 out of 13 10g soil samples and 3 out of 13 20 g soil samples. As for the 1-26-2, the titter decreased from 5.17 to 3.50 $\log_{10}TCID_{50}$/100 µL. We were able to isolate the virus from all samples at 5g and 10g, and in 11 out of 13 20g soil samples (Fig. 1).

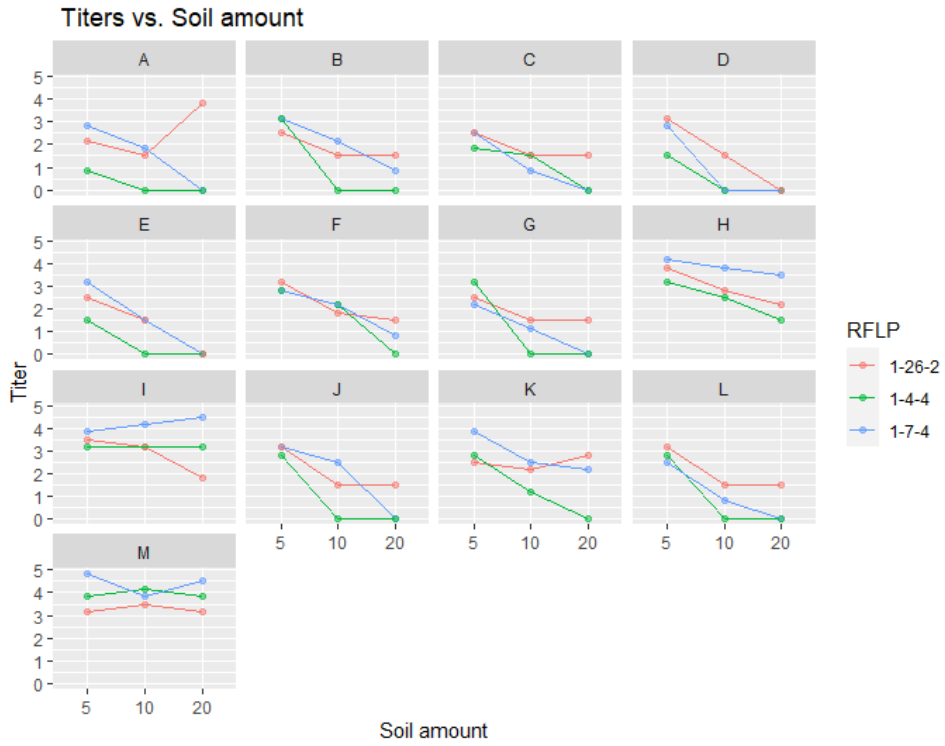


**Figure 1.** Dot chart of the $TCID_{50}$ results by type of soil and virus.

## Conclusions

1. All virus strains were able to percolate through all amounts and types of soil.
2. There is an inversely proportional relationship between viral titer and amount of soil.

## References


1. Holtkamp DJ, Kliebenstein JB, Neumann EJ, et al. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *J Swine Health*
2. Sanhueza, J. M., Stevenson, M. A., Vilalta, C., Kikuti, M., & Corzo, C. A. (2020). Spatial relative risk and factors associated with porcine reproductive and respiratory syndrome outbreaks in United States breeding herds. *Preventive veterinary medicine*, *183*, 105128.
3. Arruda, A. G., Vilalta, C., Perez, A., & Morrison, R. (2017). Land altitude, slope, and coverage as risk factors for Porcine Reproductive and Respiratory Syndrome (PRRS) outbreaks in the United States. *PloS one*, *12*(4), e0172638.
4. Linhares DCL, Torremorell M, Joo HS, Morrison RB. Infectivity of PRRS virus in pig manure at different temperatures. *Vet Microbiol*. 2012; 160(1-2):23-28.
5. Montoya J, Vilalta C, Sanhueza J, Corzo CA. Prevalence of PRRSv, PEDv, PDCoV and TGE in pig farm manure pits. Allen D. Leman Conference. 2020.
6. Goyal SM, Gerba CP. Comparative adsorption of human enteroviruses, simian rotavirus and selected bacteriophages to soils. *Appl Environ Microbiol*. 1979; 38(2): 241-247.

7. Gerba CP, Goyal SM, Hurst CJ, LaBelle RL. Type and strain dependence of enterovirus adsorption to activated sludge, sopild and esturine sediments. *Water Res*. 1980. 14:1197-1198.
8. Goyal SM, Amundson D, Robinson RA, Gerba CP. Viruses and drug-resistant bacteria in groundwater of southeastern Minnesota. *J Minn Acad Sci*. 1989. 55:58-62.

**Experimental *in-vitro* evaluation of PRRSv modified-live vaccine and wild-type virus sequenced detection in co-infections**

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## Introduction

Porcine reproductive and respiratory syndrome (PRRS) virus is known to cause great economic losses in the swine industry (1). Modified-live vaccines (MLV) have been used in the field to mitigate clinical impact and reduced wild-type virus shedding before wild-type (WT) exposure (2). Vaccines have also been used during an outbreak as a control strategy (3, 4). The main diagnostic test to confirm a new viral introduction is PCR together with ORF5 sequencing. However, interpretation of these results can be complex when two strains in the same sample since the sequencing of one strain may be favored (5). Therefore, the aim of this study is to assess the detection of wild-type or vaccine-like sequences in *in-vitro* samples spiked with both strains at different concentrations.

## Materials and Methods

Two viruses were used for this study, a wild-type strain RFLP 1-7-4 lineage 1A and a vaccine strain RFLP 2-5-2 lineage 5 from Ingelvac PRRS MLV (Boehringer Ingelheim Animal Health, Duluth, Georgia, USA). Each strain was subjected quantitative PCR (qPCR) at the University of Minnesota Veterinary Diagnostic Laboratory (UMNVDL), and subsequently diluted to obtain three different concentrations of RNA copies per mL ($10^6$, $10^5$, $10^4$). Samples comprised a mix of all possible concentration combinations of WT and MLV to create 9 different groups with 3 replicates each (Table 1). Samples were then RT-PCR tested and ORF5 sequenced.

## Results

An MLV-like sequence (RFLP 2-5-2) was most frequently obtained when the vaccine had a concentration equal or higher to the wild-type strain (in 5 out of 9 groups, 55.6%). A wild-type sequence (RFLP 1-7-4) was most frequently obtained in 1 out of 9 (11.1%) groups, when concentration of wild-type was 2 logs higher than the concentration of vaccine. In group 4, one sequence was MLV-like (RFLP 2-5-2) and 2 sequences were wild-type (RFLP 1-7-4 and 1-10-4). Only one sequence was generated in group 8 (vaccine at $10^4$ and wild-type at $10^5$), which was classified as wild-type (RFLP 1-7-4), and none of the replicated for group 9 yielded a sequence. One to three nucleotide differences from either the original MLV or wild-type sequences were observed in 8 samples, as shown in the percent nucleotide identity in Table 1. This resulted in changes in the RFLP pattern of the consensus sequence for two samples.

**Table 1.** PRRS ORF5 sequencing results by group according to RFLP classification and percent nucleotide identity to the original vaccine and wild-type strain

| Group | vaccine concentration | Wild-type concentration | RFLP | MLV percent nucleotide identity | Wild-type percent nucleotide identity |
|---|---|---|---|---|---|
| 1 | 10^6 | 10^6 | 2-5-2 | 100.0 | 87.6 |
| | | | 2-5-2 | 100.0 | 87.6 |
| | | | 2-5-2 | 100.0 | 87.6 |
| 2 | 10^6 | 10^5 | 2-5-2 | 100.0 | 87.6 |
| | | | 2-5-2 | 100.0 | 87.6 |
| | | | 2-5-2 | 100.0 | 87.6 |
| 3 | 10^6 | 10^4 | 2-5-2 | 100.0 | 87.6 |
| | | | 2-5-2 | 100.0 | 87.6 |
| | | | 2-5-2 | 100.0 | 87.6 |
| 4 | 10^5 | 10^6 | 2-5-2 | 100.0 | 87.6 |
| | | | 1-10-4 | 88.0 | 99.6 |
| | | | 1-7-4 | 87.9 | 99.7 |
| 5 | 10^5 | 10^5 | 2-5-2 | 100.0 | 87.6 |
| | | | 1-5-2 | 99.7 | 87.6 |
| | | | 2-5-2 | 99.8 | 87.4 |
| 6 | 10^5 | 10^4 | 2-5-2 | 100.0 | 87.6 |
| | | | 2-5-2 | 100.0 | 87.6 |
| | | | 2-5-2 | 99.8 | 87.4 |
| 7 | 10^4 | 10^6 | 1-7-4 | 87.8 | 99.8 |
| | | | 1-7-4 | 87.7 | 99.8 |
| | | | 1-7-4 | 87.7 | 99.8 |
| 8 | 10^4 | 10^5 | NA | NA | NA |
| | | | 1-7-4 | 87.7 | 99.8 |
| | | | NA | NA | NA |
| 9 | 10^4 | 10^4 | NA | NA | NA |
| | | | NA | NA | NA |
| | | | NA | NA | NA |

## Conclusions

We observed a tendency to sequence the vaccine strain when present at same or higher than wild-type strain concentrations in the same sample. When vaccinated herds are undergoing WT elimination, it is important to consider that the vaccine strain may be masking wild-type infections.

## Limitations

- Only one vaccine was evaluated
- Only one PRRS strain was tested
- Primers used for sequencing

**References**


1. Holtkamp DJ, Kliebenstein JB, Neumann EJ, et al. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *J Swine Health*
2. Linhares, D. C., Cano, J. P., Wetzell, T., Nerem, J., Torremorell, M., & Dee, S. A. (2012). Effect of modified-live porcine reproductive and respiratory syndrome virus (PRRSv) vaccine on the shedding of wild-type virus from an infected population of growing pigs. *Vaccine*, *30*(2), 407-413.
3. Dee, S. A., Joo, H. S., Henry, S., Tokach, L., Park, B. K., Molitor, T., & Pijoan, C. (1996). Detecting subpopulations after PRRS virus infection in large breeding herds using multiple serologic tests. *Swine Health and Production*, *4*(4), 181.
4. Linhares, D. C. L., Cano, J. P., Torremorell, M., & Morrison, R. B. (2014). Comparison of time to PRRSv-stability and production losses between two exposure programs to control PRRSv in sow herds. *Preventive veterinary medicine*, *116*(1-2), 111-119.
5. Kikuti, M., Sanhueza, J., Vilalta, C., Paploski, I. A. D., VanderWaal, K., & Corzo, C. A. (2021). Porcine reproductive and respiratory syndrome virus 2 (PRRSV-2) genetic diversity and occurrence of wild type and vaccine-like strains in the United States swine industry. *PloS one*, *16*(11), e025953.

**Effect of heating or diluting swine oral fluid samples on qPCR detection**

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Reports in the literature state that detection of nucleic acids (NA) in human oral fluids (OF) is improved by heating and/or diluting the sample. With heat, for example, Ranoa et al. (2020) described direct PCR for SARS-CoV-2 RNA on samples heated at 95°C for 30 minutes; lower temperatures and/or shorter incubation times did not achieve the same results. In this study, we tested the effect of heating (95°C × 30 m) or diluting (tris borate EDTA; TBE) swine oral fluid samples on the detection of RNA, i.e., PRRSV, influenza A virus (IAV), PEDV, or DNA, i.e., *Mycoplasma hyopneumoniae* (*MHP*).

In Experiment 1, OF samples containing PRRSV (n = 8), IAV (n = 8), PEDV (n = 8) or *MHP* (n = 8) were 2-fold serially diluted (neat, 1:2, 1:4, 1:8) using PRRSV-, IAV-, PEDV-, and *MHP*-free OF as diluent (n = 32 aliquots per pathogen). Each aliquot was split into 4 and randomized to one of 4 procedures: (P1) heat (95°C × 30 m) and direct qPCR; (P2) heat, cool (25°C × 20 m) and direct qPCR; (P3) heat, cool, nucleic acid extraction, and direct qPCR; (P4, i.e., control) extraction and qPCR.

In Experiment 2, OF samples containing PRRSV (n = 9), IAV (n = 10), PEDV (n = 10), or *MHP* (n = 10) were split into three aliquots: (D1) undiluted; (D2) diluted 1:2 with OF free of PRRSV, IAV, PEDV, and *MHP*; (D3) diluted 1:2 with TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA). Samples were randomly ordered and then tested.
Nucleic acid extraction and qPCRs were done using commercial products (IDEXX Laboratories, Inc.).

In Experiment 1, standard procedures (P4, control) produced 32/32 aliquots positive for PRRSV, 32/32 for IAV, 32/32 for PEDV, and 31/32 for *MHP*. In contrast, the aggregated results for P1, P2, and P3, produced 1/96 aliquots positive for PRRSV, 5/96 for IAV, 15/96 for PEDV, and 47/96 for *MHP*, i.e., multiple false negatives. For all pathogens, standard procedures produced lower Cqs than positives in procedures P1, P2, or P3. The results (means) from Experiment 2 showed no gain with D2 or D3:

| | | | |
|---|---|---|---|
| PRRSV | - undiluted Cq = 32.3; | D2 Cq = 34.2; | D3 Cq = 36.8. |
| IAV | - undiluted Cq = 29.0; | D2 Cq = 30.0; | D3 Cq = 29.9. |
| PEDV | - undiluted Cq = 25.5; | D2 Cq = 26.0; | D3 Cq = 25.5. |
| *MHP* | - undiluted Cq = 33.0; | D2 Cq = 33.6; | D3 Cq = 33.9. |

In conclusion, the heat and dilution treatments described in the literature were detrimental to the detection of PRRSV, IAV, PEDV, and *MHP* nucleic acids in oral fluid samples by qPCR. Interestingly, examination of the literature showed that these reports often did not include comparisons with standard methods. That is, quantitative measures of the gain or loss in performance achieved by alternative methods was typically lacking. In this study, the inclusion of comparisons showed that optimum results were obtained using standard extraction and amplification methods.

**Efficiency standardized PRRSV serum RT-qPCR results**

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Over time, consistency of diagnostic PCRs has been improved by the widespread use of commercial extraction and amplification reagents, but further improvement may be achieved by expressing results relative to a reference standard (RS). In fact, this is typically done in basic research by expressing PCR results as "efficiency standardized quantification cycles" (ECqs): $ECq = E^{-delta\ Cq}$ where E = amplification efficiency and delta Cq = (sample Cq - RS Cq).

The objective of this study was to explore the application of the ECq methodology to routine diagnostic testing.
1. Target specific (PRRSV RNA) and matrix-specific (serum) reference standards (RS) were created by rehydrating and then diluting ($1 \times 10^4$) a PRRSV MLV vaccine (Ingelvac® PRRS MLV) with PRRSV-free serum. Four reference standards were run on each plate.
2. Serum samples (n = 44) to be tested were collected from 4 pigs vaccinated with a PRRSV MLV (Ingelvac® PRRSV MLV) on days post vaccination (DPV) 0, 5, 8, 11, 14, 17, 21 and 28.
3. Testing was performed in 48-well plates using commercial reagents (RealPCR* DNA/RNA Spin Column Kit, RealPCR*RNA Master Mix and RealPCR*NA PRRS Types 1-2 RNA Mix, IDEXX Laboratories, Inc.) and the MIC PCR™ Cycler (Bio Molecular Systems, Australia).
4. Sample results and plate RS efficiencies were calculated by commercial software (MIC PCR™, v2.10.4). The "E" for each plate was then calculated as the mean of the 4 RSs on the plate and the delta Cq was for each sample calculated as (sample Cq – mean RS Cq). Across 8 plates, the mean plate RS Cq and E responses were 30.6 and 90%, respectively. All serum samples were negative on DPV 0 (ECq 0, Cq ≥ 40) and all were positive on DPVs 5 - 28 (mean ECq = 8.4, mean Cq = 31.1).

In this study, ECq represents the PRRSV RNA fold change in a sample relative to the plate reference standards. For example, an ECq of 8.4 indicated that the concentration of PRRSV RNA was 8.4 times the concentration in the RS. Accounting for amplification efficiency (E) improves test consistency because sample Cqs are directly related to E. Assuming 100% E will lead to the over estimation of target concentration. Moreover, expressing Cqs in the context of an agreed-upon reference standard could improve comparability across laboratories. In addition, all results have an ECq numeric value, i.e., "indeterminate" Cq results become zeros. Therefore, statistical analyses are valid with ECqs and it is possible to calculate cutoffs and evaluate diagnostic performance using receiver operating characteristic analysis.

## Switching immune target: applying MJPRRS classifications to characterize how PRRSV GP5-epitope C changes over time

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PRRSV is the most economically significant disease of swine in the US, with industry-wide losses estimated at $664 million annually. PRRSV evolves rapidly, which poses a major barrier to effective immunization by generating genetic and antigenic diversity leading to poor cross-protection between different strains. Currently, PRRSV strains are classified into phylogenetic lineage based upon their ORF5 sequence. Lineage prevalence changes through time, possibly due to immune-mediated competition, as any evolution that occurs in antigenic regions of the virus could contribute to immune escape. The surface protein GP5 is of particular interest for immunity because it contains several epitopes, including epitope C, which is thought to be a target for homologous neutralization and overlaps with a hypervariable region (HVR2) in the ORF5 gene. Using epitope C as an exemplar, our objective was to quantify how epitopes vary across time and assess the extent to which changes in epitope C were associated with PRRSV strain emergence. Using the MJPRRS immune groups (developed by Phibro Animal Health), which characterize different amino acid patterns present in epitope C, we investigated whether these patterns clustered according to viral lineage, which would support the hypothesis that lineages have different immunological phenotypes. We found that most non-lineage 1 clades were classified as MJPRRS pattern D1, which was the predominant epitope pattern of PRRSV strains circulating prior to the introduction and expansion of lineage 1 in the US. In contrast, within the currently prevalent lineage 1, MJPRRS epitope pattern D4 was the most common, though other patterns are more common in newer sub-lineages, such as D7 in sub-lineage L1A (including many RFLP-pattern 1-7-4 viruses) and D5 in sub-lineage L1H (including many contemporary 1-8-4 viruses). Because hypervariable regions of GP5 are subject to rapid evolutionary change, a virus's epitope MJPRRS group can switch. We investigated the rates of such switching events between different MJPRRS groups using Bayesian Evolutionary Analysis Sampling Trees (BEAST) discrete trait analysis. This analysis showed that pattern D1 was the most likely ancestral epitope pattern for non-lineage 1 PRRSV strains. Subsequently, other MJPRRS epitope patterns began to emerge within each non-lineage 1 clade. For example, lineage 9 contains several switches to D2 and D4, with subsequent switches to D5 and D6. Overall, the rates of switching epitope patterns were highest from D1 to D2, D1 to D4, and from D5 to D4. In contrast, pattern D4 was the most likely ancestral epitope for lineage 1. Early epitope pattern switches were associated with the emergence of sub-lineages, e.g., pattern D7 emerged with sub-lineage 1A and pattern S1 emerged with sub-lineage 1F. Within each sub-lineage, there are additional micro-emergences associated with pattern switching, e.g., separate clades within sub-lineage 1C switching to pattern D5 or to pattern S2 and on to S1. Overall, the rates of switching MJPRRS groups within Lineage 1 were highest from pattern D4 to D5, D4 to D6, and from D7 to D4 and D6. Characterizing PRRSV evolution through the lens of MJPRRS epitope pattern classifications can provide insight into how the virus has historically been changing from an antigenic perspective. This may allow for more precise immunization strategies in the field by targeting the circulating epitope pattern and those that it is most likely to transition to. Investigating other regions of the PRRSV genome, particularly those that are likely antigenic, may highlight patterns of emergence of potentially immunologically distinct strains.

**PRRSV Surveillance Using Tongue Tips Fluids (TTF), Practical Implications Dealing with PRRS Outbreaks**

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Monitoring the Porcine Reproductive and Respiratory Syndrome virus (PRRSv) has become a key element for surveillance and decision making in breeding herds. The evolution of the PRRSV positivity correlates with the efficacy of the measures we have applied to control the disease.

The use of processing fluids has emerged as an effective technique for monitoring PRRSV presence in a cost-effective way. However, in the countries where castration is not practiced, we need other techniques to evaluate the presence of PRRSV during the first days of life.

The drained fluid from removed parts of cadavers such as tongue tips fluid (TTF), is a good alternative even in a low prevalence scenario. One of the main advantages of TTF is that we are targeting in a group of animals (dead animals) where the likelihood of detecting the viruses would be higher. It also doesn't require any difficult training; we only need to take a tip from the tongue of dead animals and store it in frozen conditions.

We collect the tongue tips as an aggregated sample in bags based on the age of the pigs, the batch, the site, etc. During the thawing of these samples, we obtain a drained fluid which contains saliva, blood and amniotic liquid traces in stillborn piglets.

The quality of the TTF is better compared with other aggregated samples such as oral fluids because the liquid obtained from tongue tips is similar to a serum. Therefore, the cT values of TTF are lower the first weeks after an outbreak and it helps the sequencing process. We can also use the evolution of the cT values in stillborn piglets to predict if we are close to stability at the birth moment, in clinical cases where the cT value and the percentage of negative PCR results increases after consecutive weeks or batches.

Active surveillance of PRRSV and other diseases from TTF is possible in breeding, nursery and finishing farms. TTF is a feasible technique to put into practice for monitoring swine diseases in an easy, economic and non-invasive way.

**Eradication of PRRS from Hungarian pig herds from 2014 to 2022**

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According to recent calculations, the estimated losses due to PRRS in Hungary were close to 5 billion HUF (≈12.5 M USD) per year, thus the Pig and Food Chain Safety Strategy of the Hungarian Government (2013–2022) highlighted the need for the eradication of PRRS virus (PRRSv) from the Hungarian domestic swine population.

Therefore, in Hungary (first among the EU Member States), a National PRRS Eradication Program was introduced in 2014 in order to reach a more efficient, and more competitive international market position. The veterinary authorities carried out the implementation of the program with consent and continuous monitoring by the organizations of the pig industry. The legal background of the eradication program was set out in Decree 3/2014 (16 January) of the Minister of Rural Development, that has been approved by the competent committee of the European Union.

The National PRRS Eradication Plan specified the establishment of separate PRRS eradication plans for each large-scale pig herd, including large breeding farms. The tests required for the certifications were carried out at the state's expense. Based on these results, the herds were certified by the local veterinary authority. The definitions have been updated in 2017 according to the World Organization for Animal Health (OIE). The international PRRS regulations in the Terrestrial Animal Health Code, including the concept and category of a "vaccinated free" (VF) large-scale breeding herd, was introduced.

The National PRRS eradication program in Hungary is based on a territorial principle, and it is obligatory for each swine farm. PRRS eradication was carried out mainly by the depopulation-repopulation method.

Altogether, 165 PRRSv infected large-scale breeding farms had to create their own eradication plan, which was individually evaluated and approved by the local authorities. Finally, 94 farms (with 69,111 sows) out of the 165, successfully eradicated PRRS by depopulation/repopulation with a certain level of compensation from the authorities, six reached VF status.
Although its significance has decreased in recent decades, 20% of the Hungarian pig population still consists of small-scale (backyard) farms (< 100 animals). As a result of the program, PRRS-free status of the small-scale herds was achieved by the end of 2015, and this status was maintained from 2016–2022.

By March 31, 2022, the total pig population of Hungary, including fatteners, reached wild type PRRSv-free status. To date, five herds use modified live virus vaccination of the breeding herd and rear PRRSv-free piglets.

**African swine fever and its challenges in Europe**

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It is not without reason that African swine fever has recently been called a forgotten pandemic that has transformed from an exotic disease to one of the greatest threats to domestic and wild pigs world-wide.

Europe is currently facing different scenarios with front and point introductions, disease in wild boar and domestic pigs, and areas with long-lasting endemicities. This diversity calls for tailored approaches to surveillance, diagnosis, and control. While our proven, traditional control strategies work well for industrial pig farms, we quickly reach our limits when we have to control the disease with the means available to date in the abundant wild boar population (now also including wild boar in cities) or in regions with a majority of backyard farms. In addition, we face conflicts of interest that arise between disease control and conservation or between hunting and farming, respectively. To turn the tide, vaccines could be the missing tool. Here, benefit-risk-analyses will have to precede the decisions to implement vaccination strategies with the vaccines available to date (that are not licensed so far). Furthermore, we have to stay open-minded when it comes to alternative approaches.

Beyond the aforementioned challenges, the virus itself also holds surprises. With the introduction into Germany in 2020, viral variants were observed that showed local distribution and could be followed by genomic epidemiology.

To combat the disease, we will have to tie strong bonds between countries and disciplines. Furthermore, we have to work on closing critical knowledge gaps.

**Rapid detection of African swine fever virus DNA using a colorimetric LAMP PCR**

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African swine fever virus (ASFV) currently represents the biggest threat to the porcine industry worldwide, with high economic impact and severe animal health and welfare concerns. Since its reintroduction into the Eurasian continent in 2007, ASFV has spread both east and west-ward, leading to a series of outbreaks during the 2010s, that culminated in introduction of the virus in large pork-producing countries, such as China and Germany. Notably, in 2021, ASFV was detected in the Dominican Republic and Haiti, raising concern over the reemergence of the virus in the Americas. Given the lack of approved vaccines against ASFV, control of the virus relies heavily on molecular surveillance. While real time PCR is the gold standard diagnostic technique, LAMP-PCR has become more enticing due to its potential for point-of-care use and minimal expense, equipment and training required.

The present study led to development of two LAMP assays for the detection of ASFV with potential to be used in production or slaughter facilities, with results comparable in sensitivity and specificity to current reference qPCR methods. Initial screening of four different primer sets (6 oligomers each) targeting the p72 gene was carried out in a fluorometric LAMP detection assay using a synthetic plasmid containing the cloned ASFV p72 gene sequence as a positive control. Two of the primer sets, named ASFV-LAMP-BG2 and ASFV-LAMP-BG3, were selected for validation given their faster detection time. Both primer sets showed good thermal stability, amplifying the ASFV DNA at temperatures between 60-70°C in 5-6 minutes. These primer sets also proved to be highly sensitive, detecting as few as one ASFV-plasmid DNA copy/µL, in both fluorometric and colorimetric reactions. ASFV-LAMP primer sets were also able to detect genotype I and II ASFV virus strains, though at a slightly higher detection limit than the reference qPCR assay. LAMP amplification was not affected by the nature of the matrices, including oral fluids, tonsils, blood, or rectal swabs. It also showed high specificity when tested against DNA and RNA from 25 different pathogens affecting swine, or genetically related to those of relevance in swine health. Taken together, the results show that ASFV-LAMP-BG2 and ASFV-LAMP-BG3 would be a useful tool for rapid, highly sensitive on-site diagnostic testing.

## Vaccination Strategies to Reduce PRRSV Recombination

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With the increase in whole genome sequencing, it has become abundantly clear that many "new" PRRS viruses are actually the result of recombination between two or more existing strains PRRSV-1 or PRRSV-2. Recombination between PRRSV-1 and PRRSV-2 has not been reported to occur in nature. It is well known that both species of PRRS viruses have a high mutation rate, but less well understood that they also have a very high rate of homologous recombination. This is due in part to the distinctive mechanism employed by PRRSV and other Nidoviruses to express structural proteins from subgenomic RNAs. These sgRNAs are generated when the RNA polymerase, with a nascent strand of newly synthesized RNA, detaches from one position on its genome-length RNA template and reattaches to a distant site on the same (or different) RNA template to continue RNA synthesis. This requirement for the polymerase to hop from one location to another makes Nidoviruses particularly prone to homologous recombination by the template-switching mechanism. Modified live vaccine viruses are not exempt from recombination, and it is not uncommon for whole genome sequencing to reveal that "vaccine-like" isolates from the field (based on ORF 5 sequencing) are actually recombinant viruses containing a vaccine-derived ORF 5 in the context of wild-type sequences. In several well documented cases, two vaccine strains have recombined with each other to produce a vaccine-like virus that is less attenuated than either vaccine parent. Fortunately, recombination between strains of PRRS virus can *only* occur when both viruses infect the same cell (in the same pig), at the same time. Using this as a guiding principle, there are a variety of vaccine management strategies that can be used to reduce the opportunity for recombination between vaccine viruses and field strains, and between vaccine strains. These strategies will be discussed in the presentation.

**Confirmed porcine reproductive and respiratory syndrome virus (PRRSV) tissue diagnosis and pathogen interactions over time and age category**

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes losses of over $664 million annually in the US, affecting the whole swine industry. In addition, the virus is part of the porcine respiratory disease complex, occurring in many cases associated with other pathogens, which can increase production losses. However, PRRSV coinfections with other pathogens are not often described because monitoring several agents on a farm could be costly and laborious. A feasible method to quantify the frequency of submissions with multiple etiologies/disease diagnoses is to utilize the disease diagnostic system (Dx code) implemented at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). The Dx code system facilitates recording, standardization, and data aggregation of disease diagnosis from tissue cases. Based on the Dx code database, this research analyzed the most common pathogens occurring simultaneously by age category and year, clarifying the co-diagnosis of PRRSV dynamics and interactions.

Diagnostic code data from 2013 to 2022 were retrieved from ISU-VDL and aggregated by submission level, using the accession ID as the unit of analysis (case). Using SAS (v.9.4; SAS Institute), the data was cleaned, organized, and set in a collated format using the Dx codes components, i.e., system, insult, lesion, etiology. In cases with two or more Dx codes, the etiology, system affected, pathogens involved, and types of lesions were assigned to one row as one case. For example, a case with two Dx codes: respiratory, viral, pneumonia, PRRSV and respiratory, bacterial, pleuritis, *Glaesserella parasuis* were merged in one row as respiratory, insult 1 (viral), insult 2 (bacterial), lesion 1 (pneumonia), lesion 2 (pleuritis), etiology 1 (PRRSV), and etiology 2 (*Glaesserella parasuis*). Each case with a Dx code assigned to PRRSV and other associated pathogens was counted as co-diagnosis. Final aggregated data were analyzed by the system affected, age category, and year.

The final dataset comprised 26,596 cases assigned with the PRRSV Dx code, representing 37.8% of total respiratory cases (15,643 of 41,410) and 34.4% of systemic cases (9,035 of 26,368). 10,237 PRRSV cases had other pathogens diagnosed. The percentage of PRRSV co-diagnosis cases had continuously increased since 2014, when 565 of 1,906 (29.6%) cases had co-diagnosis, compared to 2020, when 2,162 of 3,982 (54.9%) cases had a co-diagnosis with PRRSV. In 2021, the cases reached the largest absolute number, with 2,681 of 5,361 having a PRRSV co-diagnosis. Regarding cases with a co-diagnosis, the age category grow-to-finish represented 5,361 cases (52.4%), followed by nursery at 4,369 (42.75%). Overall, *Pasteurella multocida* (24.1%), *Glaesserella parasuis* (15%), *Streptococcus suis* (14.8%), Influenza A virus (IAV) (14.7%), and *Mycoplasma hyorhinis* (9.5%) were the main pathogens co-diagnosed with PRRSV. Not surprisingly, *Pasteurella multocida*, *Glaesserella parasuis*, *Streptococcus suis*, and IAV are pathogens in the porcine respiratory disease complex. Breaking down these data by systems, PRRSV with IAV

leads the respiratory category (2,830 of 8,301 – 34.1%), and PRRSV with *Glaesserella parasuis* leads the systemic cases (943 of 2,863 - 32.9%). These results highlight the increasing number of cases with pathogen interactions and generate awareness of the importance of monitoring other pathogens in addition to PRRSV that may impact swine production systems.

**Swine Disease Reporting System: A Tool for Emerging Swine Disease Investigation**

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The Swine Disease Reporting System (SDRS) is a Swine Health and Information Center-funded initiative to aggregate and report veterinary diagnostic data collected from veterinary diagnostic laboratories (VDLs). The SDRS database is one of the largest diagnostic databases in the US, including polymerase chain reaction (PCR) data. Results from 5 VDLs (Iowa State University, University of Minnesota, South Dakota State University, Kansas State University, and Ohio Animal Disease Diagnostic Laboratory) are included in the database. Seven swine pathogens: porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV), transmissible gastroenteritis virus (TGEV), *Mycoplasma hyopneumoniae*, porcine circovirus type 2 (PCV2), and influenza A virus (IAV) are included. The objective of this study is to demonstrate the capability of the SDRS database to inform the US swine industry about emerging and re-emerging animal health threats on a real-time basis.

Data is collected from VDLs using Health Level Seven International (HL7), application programming interface (API) connection, and comma-separated values (CSV) files. Data standardization, modeling, and visualization are performed using commercially available softwares, e.g., SAS, R, RMarkdown, and Power B.I. Investigators review all the analyses to ensure the accuracy of the data. Preliminary results are shared with an advisory group composed of veterinarians/producers distributed across different U.S. regions and representing independent and integrated production systems to gain additional input about the results before releasing reports. Monthly reports in a variety of formats (PDF, audio, video, and dashboards) are made available at www.fieldepi.org/SDRS for public access.

The database contains more than 1 million cases (> 2.6 million samples) that revealed epidemiological megatrends for the pathogens monitored in the project. Examples of SDRS contributions to stakeholders are the detection of cyclic patterns with higher detection of PRRSV, PEDV, and PDCoV during colder months throughout the years. Also demonstrated, in 2020, an increased activity of PRRSV in October and November was associated with the emergence of a new highly pathogenic PRRSV strain (RFLP 1-4-4 Lineage 1C variant). TGEV has not been detected since April of 2021, suggesting that this virus might be circulating at a low prevalence in the United States. In September of 2022, the SDRS also reported the lowest percentage of positive detection of PDCoV since 2018 (3478 submissions, and 0.40% positive). At a state-level monitoring, an increased detection of PEDV RT-rtPCR positive submissions was observed initially in February of 2022. Monthly monitoring of virus detection revealed a substantial increase in positive submissions from Missouri, Minnesota, Iowa, Kansas, Nebraska, and North Carolina,

generating awareness for the swine industry to take the appropriate biosecurity and biocontainment measures.

Furthermore, the SDRS also demonstrated that higher levels of PRRSV, IAV, PEDV, and PDCoV detection occur in the wean-to-market age category compared to sow farms. In addition, the increased activity of PRRSV and IAV in the wean-to-market age category precedes the increase in detection for adult/sow farms. In response to SDRS disease data and field data, the Swine Health Information Center, National Pork Board, and the Foundation for Food & Agriculture research allocated $2.3 million for research focusing on wean-to-harvest biosecurity (https://www.swinehealth.org/shic-sets-focus-on-wean-to-harvest-biosecurity/). These findings highlight the importance of SDRS to monitor endemic, emerging, and re-emerging pathogens affecting the US swine population, assisting veterinarians and producers in making informed animal health decisions.

**Identification of conserved amino acid residue on PRRSV glycoprotein 2 critical for infectivity in macrophages**

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Porcine reproductive and respiratory syndrome virus (PRRSV) has a strict tropism for cells of monocyte/macrophage linage of swine. The viral minor glycoproteins 2 (GP2), GP3, and GP4 have been shown to interact with CD163, a key receptor for virus entry. MARC-145, a monkey kidney cell line, has been routinely used for the propagation of PRRSV *in vitro*. In this study, the PRRSV strain NCV1 was passaged on MARC-145 for 95 passages, and two plaque clones (C-1 and C-2) were randomly selected for further analysis. The C-1 virus nearly lost the ability to replicate in porcine alveolar macrophages (PAMs), the natural *in vivo* cellular targets of PRRSV infection, as well as porcine kidney cells expressing porcine CD163 (PK15-pCD163), while the C-2 virus replicates well in these two cell types. The objective of this study was to identify viral genetics that are determinants for the virus infectivity in PAMs.

The complete genome of NCV1 at different passage levels was sequenced and analyzed to identify potential mutations responsible for the loss of infectivity of C-1 in PAMs. Reverse genetics was employed to manipulate these mutation sites to demonstrate their association with the virus infectivity in PAMs and PK15-pCD163.

The C-1 virus carried four unique amino acid mutations that were not found in the C-2 virus or viruses at other passage levels: three in the nonstructural proteins and a K160I in GP2. Alignment of GP2 sequences available in GenBank revealed that K160 residue was highly conserved among PRRSV-2 isolates. The introduction of an I160K substitution in GP2 of the C-1 virus restored its infectivity in PAMs and PK15-pCD163 cells. On the other hand, the introduction of a K160I mutation in GP2 of the virulent PRRSV-2 strain NCV13, which replicates efficiently in PAMs, significantly impaired its infectivity in PAMs and PK15-pCD163 cells. Importantly, pigs inoculated with the NCV13-K160I mutant virus exhibited lower viremia levels and lung lesions than those infected with the wild-type NCV13.

Collectively, these results demonstrate that the K160 residue in GP2 of PRRSV is one of the determinants of cellular tropisms.

**Transcriptomic analysis of air-liquid interface porcine respiratory epithelial cell cultures infected with porcine hemagglutinating encephalomyelitis virus**

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Porcine hemagglutinating encephalomyelitis virus (PHEV) is a neurotropic porcine betacoronavirus that replicates initially in the upper respiratory tract. The present study is a continuation of our previous studies based on an organotypic air-liquid interface porcine respiratory epithelial cells (ALI-PRECs) culture system, in which we demonstrated that PHEV disrupts the homeostasis of porcine respiratory epithelia by altering the ciliary function and induce antiviral, proinflammatory cytokine and chemokine responses. Here, we further investigate the mechanisms driving the early innate immune modulation in ALI-PRECs in response to PHEV infection. Following inoculation with PHEV, total RNA was collected from ALI-PRECs at 24 hours post inoculation (hpi), 36 hpi, and 48 hpi. After determining the RNA quality (Agilent bioanalyzer), RNA-seq analysis, including library preparation and sequencing, was performed at the Iowa State University Genomics center using the Illumina Hiseq 6000 to generate 100 bp paired-end reads. The sequences were checked for quality with FastQC, trimmed with Trimmomatic to remove adapters, and aligned on the Sscrofa 11.1 genome with HiSat2. Differential gene expression (DEG) was performed using DeSeq2 utilizing a parametric fit type and poscounts to account for genes with zero counts. DEG analysis was based on the model treatment + hpi + treatment:hpi + E. Statistically significant gene modulation reported for the interaction effect of treatment:hpi. The number of significant (FDR < 0.15) differentially expressed genes (DEGs) increased with time post-infection, with 112 DEGs at 24 hpi, 163 DEG at 36 hpi, and 179 DEGs at 48 hpi. As expected, GO terms associated to the Type-1 interferon response were significantly enriched in all three time points. GO terms related to chemokines were observed at 36 hpi and 48 hpi, while DEGs in GO terms associated to ciliary function were expressed at 48 hpi. Using Qiagen Ingenuity Pathway Analysis (IPA), significant networks, top functions, and canonical pathways associated with DEGs were identified. Among several predicted networks, hypercytokinemia/ hyperchemokinemia in the pathogenesis of influenza pathway was the most significant canonical pathway at all three time points. Predicted dysregulation of DEGs involved with RIG-I-like receptors (RLRs), IRF-3/IRF-7, and transcription factors such as STAT1, AP1 were noticed as early as 24 hpi. Their activation is predicted to activate several DEGs involved in the proinflammatory response, including CCL5 and CXCL10, via the NFkB signaling pathway. In summary, the RNA-seq analysis performed in this study further supports our previous results that the ALI-PRECs culture system is a suitable *ex vivo* infection model to study PHEV and other porcine respiratory pathogens.

**Mitochondrial dysfunction and oxidative stress in PRRSV-2 infected lung macrophages**

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is one of the most economically impacting viruses for the global swine industry. PRRSV has a known tropism for lung macrophages, where it causes impaired immune responses and altered mitochondrial function. This study evaluated the metabolic and immune profiles of primary porcine alveolar macrophages (PAM) and lung parenchymal macrophages (PIM) infected with different strains of PRRSV-2, a highly pathogenic North Carolina (NC)-174 and a lowly pathogenic NC-134 isolate. Primary enriched mononuclear phagocytes were infected *ex vivo*, sorted, and the total RNA was used for a transcriptomic approach; additionally, gene expression was further validated by RT-qPCR and NanoString technology. Complementary functional assays were used to further investigate the mitochondrial dysfunction and oxidative stress induced by PRRSV-2 infection. PAM and PIM showed different transcriptomic profiles during the early stage of infection, with downregulation of genes involved in the oxidative phosphorylation and electron transport chain pathways. This genetic reprogramming matched the mitochondrial impairment observed in functional assays. ROS production was reduced upon NC-134, and stimulated during NC-174, infection in cultured lung macrophages. Mitochondrial respiration showed different profiles between infected PIM and PAM: PAM reduced their spare respiratory capacity and ATP production upon both NC-134 and NC-174 strain infections, whereas NC-174 infected PIM increased their respiratory capacity and proton leak levels. These results provide valuable insights into the pathogenetic mechanism of different virulent PRRSV-2 strains, focusing on the alteration of mitochondrial function in lung macrophages during early infection.

**Detection of Senecavirus A in pigs from a historically negative national swine herd and in associated feed imports from an endemically infected country**

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**Introduction:** The purpose of this report is to describe the initial clinical diagnosis of SVA in a swine farm from a historically naïve national herd, and the results of a diagnostic investigation designed to identify potential routes of entry of SVA to the country and the farm. For confidentiality, names of all countries and companies will not be disclosed. In July 2022, vesicular lesions were observed on the snouts and feet of pigs in the case pork production system. Diagnostic testing indicated the presence of SVA in vesicular fluids and ruled out FMDV. Prior to the onset of clinical signs, feed ingredients had been imported from other countries, several known to be endemically infected with SVA, and formulated diets contain these imported ingredients were being fed to pigs prior to and during the onset of clinical signs. All feed ingredients had been stored at a warehouse at the feed mill, which was located on a separate site from the farm, and different personnel worked at the mill and the farm.

**Methods:** A diagnostic investigation was conducted to identify potential sources of viral entry. A total of 39 samples (dust and grain probe) were collected, including samples from facilities and equipment, i.e., floor surfaces from storage warehouses and associated driveways on affected farms and mills, along with feed mixers, from bulk-feed ingredients, including soybean meal imported from SVA-positive and SVA-negative countries, along with raw soybeans and corn gluten meal from SVA-negative countries, and micronutrients from SVA-positive countries, including valine, lysine, methionine, vitamin C, threonine, and tryptophan, were sampled. In addition, one tote bag containing lysine from an SVA-positive country was sampled, due to visible debris, i.e., feed dust and dirt observed on its external surface. Finally, dust samples of poultry feed and dust from associated feed storage areas, along with samples of dust from plant food, were collected. Samples were tested for the presence of SVA RNA by PCR. To validate the ability of the PCR assay to detect SVA RNA, six feed samples from an unaffected farm were spiked with vesicular fluid from affected pigs with clinical signs.

**Results:** Across all samples tested, two of the five samples of dust from soybean meal imported from an SVA-positive country, and the sample from the external surface of the tote bag, from a different SVA-positive country, were positive for SVA RNA. All other samples were PCR-negative. All positive control samples were PCR-positive for SVA RNA.

**Discussion:** As this was the initial incursion of SVA to the country, the fact that SVA RNA was present only in specific types of samples, i.e., 40% of the samples from dust samples from imported soybean meal and the one sample from surface debris obtained from an imported feed tote, is significant. The fact that clinical signs of SVA were noted in pigs being fed diets formulated with these imported ingredients suggests that infectious SVA could have been present in the feed. In conclusion, this case describes the first potential link between the entry of a novel viral agent to a naïve national swine herd through the prior importation of feed ingredients from an endemically infected county.

**Rapid Response Program and Outbreak Investigations for Field Detection of the Source of Pathogen Entry**

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In 2016, the Swine Health Information Center (SHIC) funded a project to develop a Rapid Response Corp (RRC) to develop a network of trained, prepared, and committed veterinarians, state health animal officials, epidemiologists, and, when needed, federal animal health officials to conduct epidemiologic investigations when a new transboundary or emerging disease threat occurred.

Resources developed for the Rapid Response Program, including the outbreak investigation form, are available at www.swinehealth.org. To view these resources, click on "Rapid Response Program" under the "Emerging Disease" tab and then on "Rapid Response Program Resources."

The objective of an outbreak investigation is to identify how a new emerging or transboundary pathogen is transmitted from one herd to another. An outbreak investigation form is utilized to collect epidemiologic information systematically, comprehensively, and consistently. The investigator subjectively assigns findings from the epidemiologic investigation for each category of events as high, medium, or low likelihood as the source of the pathogen introduction and is included in a report along with the information collected during the investigation.

The SHIC RRC has completed outbreak investigations for regional outbreaks of porcine epidemic diarrhea virus (PEDV) and Seneca virus A. Additionally, the Rapid Response program tools have been utilized by swine veterinarians to investigate the introduction of pathogens endemic to the United States into farms that were previously negative for porcine reproductive and respiratory syndrome virus (PRRSV) and PEDV in sow herds and a regional outbreak of Actinobacillus pleuropneumonia in growing pigs. It has also served as a tool to perform a biosecurity hazard analysis to identify areas where a farm may want to focus on improving biosecurity processes to mitigate the potential risk of pathogen entry.
In 2022, the outbreak investigation form was updated to cover new technologies, diagnostic tests or sample types, biosecurity procedures, and areas of potential pathogen introduction that were not covered or required more detail compared to the previous form by a group of practicing swine veterinarians, researchers, and epidemiologists. A components document to define the scope, methodology, reporting, and terminology was developed to further explain how to standardize outbreak investigations.

This presentation will review the process of performing an outbreak investigation, examples of how outbreak investigations have assisted in field detection of the source of PRRSV and PEDV introductions into farms, and a review of the project to update and standardize the outbreak investigation form and components document.

**A swine influenza candidate vaccine platform based on a consensus sequence for hemagglutinin of H1 subtype**

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Influenza A viruses (IAV-S) belonging to H1 subtype are endemic in swine worldwide. The evolution of swine influenza viruses (IAV-S) reflects several introductions of IAVs from birds and humans into the swine population. Antigenic drift and antigenic shift lead to a substantial antigenic diversity of circulating IAV-S strains. As a result, the most used vaccines based on whole inactivated viruses (WIV) provide low protection against divergent H1 strains due to the mismatch between the vaccine virus strain and the circulating one. The design of sequences based on contemporary virus isolates may minimize this sequence diversity and protect against heterologous strains by centralizing the immunogenicity of the vaccine antigen. Here, the consensus coding sequence of the full-length of HA from H1 subtype was generated *in silico* after careful analysis and alignment of the sequences from IAV-S isolates obtained from public databases by early 2018. The DNA fragment containing this consensus coding sequence was subcloned into a poxviral transfer vector by enzymatic digestion and ligation to finally be inserted into the ORFV121 locus of Orf virus by homologous recombination. The immunogenicity and protective efficacy of the resulting ORFVΔ121conH1 recombinant virus was evaluated against divergent strains in piglets. Virus shedding after intranasal challenge with two IAV-S strains was assessed by real-time RT-PCR and virus titration in permissive cells. Viral genome copies and infectious virus were reduced in nasal secretions of immunized animals. Flow cytometry analysis showed the percentage of T helper/memory cells secreting IL-17A and IFN-γ, as well as cytotoxic T lymphocytes (CTLs) secreting IFN-γ, were significantly higher in the peripheral blood mononuclear cells (PBMCs) of the vaccinated groups compared to unvaccinated animals when they were challenged with a pandemic strain of H1N1 (H1N1pdm). Interestingly, the percentage of IL-17A induced T cells was higher in the bronchoalveolar lavage of vaccinated animals in relation to unvaccinated animals in the groups challenged with a H1N1 from the gamma clade. In summary, the delivery of the consensus HA from H1 IAV-S subtype by Orf virus, a poxvirus vector, decreased the shedding of infectious virus and the viral load of IAV-S in nasal secretions and induced cellular protective immunity against divergent influenza viruses in swine.

**Novel experimental design reveals additional evidence of natural genetic variation in response to PRRS challenge**

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Results of a previous multifactorial PRRS challenge trial provide evidence of substantial phenotypic and genetic variation in host response to challenge, where both growth rate and mortality under challenge were heritable traits. The trial described below was conducted as a follow-up study with the objective of estimating genetic parameters for host response to experimental PRRS challenge, in an attempt to validate these results. Compared to the previous study, efforts were made to improve upon the experimental design to maximize genetic variation in host response to challenge.

Three-way commercial crossbred finishing pigs ($n$ = 1,971) were used for this study. Landrace x Large White females were mated to either a Synthetic or Duroc sire line and pigs were farrowed at a commercial sow farm. In total, 90 boars were selected and used for breeding. Boars were selected based on extreme breeding values (BV) for mortality under challenge, derived using the previous PRRS challenge trial as the training population. Further, pooled semen doses, consisting of two boars per pool, were used to avoid confounding of dam by sire.

Pigs were placed in a commercial research facility at weaning and tissue was collected for genotyping. Five days post placement, each pig was vaccinated for PRRS using a commercial modified live virus vaccine. Four weeks later, each pig was experimentally inoculated with 2 x $10^{3.5}$ TCID50 of PRRSV 1-7-4 (0 days post-infection [dpi]) and followed until marketing. Multiple secondary pathogens were detected post-challenge, including porcine circovirus, *Escherichia coli*, and *Lawsonia intracellularis*. Data was recorded at the individual level, including body weights at multiple timepoints to calculate average daily gain (ADG) from challenge to 21 dpi (ADG_0_to_21), 21 dpi to market (ADG_21_to_market), and 0 dpi to market (ADG_0_to_market). Mortality events and dates were recorded throughout the trial.

Genotypes across 25K single nucleotide polymorphisms were available for all 1,971 individuals and used to construct a genomic relationship matrix to facilitate genetic parameter estimation using an animal model with ASReml 4.0. Heritability was estimated for each ADG trait using a linear model and mortality traits were analyzed using a LOGIT model.

Consistent with results obtained for the previous trial, results from this study showed that ADG under challenge was moderately heritable at 0.22 (0.04), 0.16 (0.04), and 0.17 (0.04) for ADG_0_to_21, ADG_21_to_market, and ADG_0_to_market, respectively. Heritability of mortality, using records collected between 0 and 28 dpi, was estimated at 0.26 (0.09) (genetic variance = 1.32) for this study, compared to 0.09 (0.09) (genetic variance = 0.33) for the previous study.

In conclusion, results from this study validate the existence of substantial, naturally occurring genetic variation in host response to a multifactorial PRRS challenge, as detected in the previous trial. Further, with attention to the number and type of sires selected for breeding, including sires with extreme BVs for mortality under challenge, it is possible to reveal even more genetic variation in mortality following PRRS challenge than previously realized.

**PRRSV infection of alveolar macrophages promotes inflammation and inhibits apoptosis**

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Porcine reproductive and respiratory syndrome (PRRSV) is a devastating pathogen for the pig industry worldwide. PRRSV mainly infects porcine alveolar macrophage (PAM), and during the acute phase of infection no more than 2% of PAMs are infected with the virus. Therefore, conventional, bulk RNA-sequencing approach does not accurately reflect host transcriptional responses directly caused by virus infection. In the present study, single-cell RNA sequencing (scRNA-seq) was employed to characterize the host transcriptome responses of PAMs infected with a virulent PRRSV strain in vivo and ex vivo. For the ex vivo study, PAMs collected from healthy pigs were infected with the FL12-GFP and samples were collected at 0-, 6- and 12- hours post-infection for scRNA-seq. For the in vivo study, weaned pigs were infected with the PRRSV strain expressing green fluorescent protein (FL12-GPF) and PAMs were collected at 4- and 7- days post infection for scRNA-seq. Pro-inflammatory genes including interleukin 1A (IL-1A), chemokines 8 and 10 (CXCL8 and CXCL10), tumor necrosis factor (TNF), and interferon regulatory factor 1 (IRF1) were upregulated in cells directly infected with the virus (expressing the virus ORF7 transcript). Furthermore, anti-apoptotic genes, such as NF-KB inhibitors (NFKBIZ, NFKBIA, TNFAIP3), were also highly upregulated in the infected cells. Collectively the results demonstrate that PRRSV can modulate apoptosis and inflammatory signaling pathways. This study provides additional insights on the host responses to PRRSV infection.

**Next Generation Sequencing (NGS) for Comprehensive Genetic, Phylogenetic and Variant Analysis of *Betaarterivirus suid 2* (PRRSV-2) in an Integrated US Swine Producer**

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Viral metagenomics was applied to investigate the genetic and geographic distribution of *Betaarterivirus suid 2* (PRRSV-2), the causative agent of porcine reproductive and respiratory syndrome (PRRS). Historically, PRRSV-2 identification has occurred via detection of the glycoprotein 5 (GP5), although the remaining PRSSV-2 genes likely also contain genetic diversity which previous studies have not investigated. NGS allows a comprehensive genetic understanding of the swine virome in symptomatic and asymptomatic animals, including PRRSV-2 infections, while also providing the opportunity to assemble the complete genome of PRRSV-2 and other viruses of interest. Through NGS, scientists can study disease patterns, such as disease severity and geographic associations, of PRRSV-2 variants. In this study, 91 serum samples were collected from US swine herds across three states: Oklahoma, Kansas, and Texas. Samples underwent processing, extraction and PRRSV-2 qRT-PCR where only samples with a Ct value ≤ 26 progressed into library preparation and Illumina NextSeq sequencing. Kraken was utilized to assign viral taxonomy to sequencing reads. While NGS has aided in other PRRSV-2 studies, the present study determined that current PRRSV-2 analytic NGS methods seem insufficient in successfully identifying PRRSV-2 reads and assembling PRRSV-2 in qRT-PCR positive samples. Interestingly, PRRSV-2 was nearly always (89%) identified as a viral coinfection, indicating that PRRSV-2 appeared to cause viral co-infections with other swine pathogens. PRRSV-2 was found most often associated with a porcine endogenous retrovirus (57%) and a suspected novel porcine lentivirus (27%). While the PRRSV-2 glycoprotein 5 (GP5) has been used historically for phylogenetic analysis, five of the ten analyzed PRRSV-2 genes showed increased nucleotide diversity when compared to the GP5. This diversity outside of GP5 indicated that other PRRSV-2 genes could be suitable candidates to demonstrate PRRSV-2 diversity, which could be useful information for epidemiological analyses. Interestingly, both the whole genome- and GP5-based phylogenetic trees lacked clustering patterns relative to temporal or geographical distribution. The use of a PRSSV-2 gene exhibiting more diversity than GP5 for detailed genetic analysis could allow temporal and geographical patterns which are currently lacking when using GP5. While NGS can provide insights into the PRRSV-2 genome, this study indicates that different screening methods and bioinformatic approaches should be evaluated for assembling complete PRRSV-2 genomes. Future studies should evaluate PRRVS-2 genes s beyond GP5, as variation found in different genomic regions likely influence PRRSV disease presentation and transmission of PRRSV through swine herds. In surveying genetic diversity outside GP5, scientists and swine farmers will have an enhanced ability to monitor PRRSV-2 distribution and target genetic variants of concern. It will also be crucial in future studies to determine implications of different viral co-infections for PRRSV-2 disease.

**The P1 region is a major determinant of pathogenicity and virulence of Senecavirus A infection**

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Senecavirus A (SVA) is an emerging picornavirus that causes vesicular disease (VD) in pigs that is clinically indistinguishable from high-consequence foreign animal diseases (FAD), including foot-and-mouth disease. By comparing the pathogenicity of a historical and a contemporary SVA strains (SVV-001 and SD15-26, respectively), our group showed that these two viral strains present significant differences in virulence and pathogenicity in pigs. Notably, while pigs inoculated with the SVA SD15-26 strain presented characteristic clinical signs and lesions of SVA, none of the animals inoculated with the SVV-001 strain developed VD. SVV-001-inoculated pigs also presented lower viremia and significantly lower levels of virus shedding. Based on these observations, we hypothesized that the genetic differences between these two strains may account for their distinct virulence phenotype and pathogenicity in pigs. To address this hypothesis, we used a virulent rSVA SD15-26 infectious clone to construct recombinant chimeric viruses containing genome swaps of the 5'UTR, P1, P2, P3 and the 3'UTR genomic regions from the nonpathogenic SVA strain SVV-001. Resultant chimeric viruses ($rSVA^{SD15-26}$/SVV001-5'UTR, $rSVA^{SD15-26}$/SVV001-P1, $rSVA^{SD15-26}$/SVV001-P2, and $rSVA^{SD15-26}$/SVV001-P3; and $rSVA^{SD15-26}$/SVV001-3'UTR) were sequenced to confirm the identity and integrity of genome sequences. To evaluate the potential role of such genomic regions on SVA pathogenicity and infection dynamics, 35 fifteen-week-old SVA negative finishing pigs were randomly allocated into seven experimental groups (n = 5/group) and inoculated upon arrival with a virus suspension containing $10^8$ $TCID_{50}$ via the oronasal route. Most inoculated groups presented characteristics SVA clinical signs and lesions, except for $rSVA^{SD15-26}$/SVV001-P1-inoculated group which remained subclinical with no apparent clinical signs nor lesions. Remarkably, from day 5 to 14 pi, $rSVA^{SD15-26}$/SVV001-P1-inoculated group presented significant lower amounts of virus shedding in nasal secretions as determined by r-RT-PCR. The levels of virus shedding in oral secretions and feces were significantly reduced in this group on days 1, 10 and 14 pi. Viral load and tissue distribution of the chimeric viruses were similar to the parental rSVA SD15-26 virus. Interestingly, in both groups, 1 of 5 pigs did not present SVA RNA in the tonsil, an important organ associated with primary replication and persistence of SVA. The serological responses to both wt SVA strains, SVV 001 and SD15-26, were evaluated by virus neutralization assay (VN). All inoculated animals seroconverted and developed neutralizing antibodies (NA) starting on day 5 pi. $rSVA^{SD15-26}$/SVV001-P1-inoculated group presented reduced levels of NA compared to rSVA SD15-26-inoculated group. To confirm the results obtained in this study, we constructed two revertant viruses in which the parental SVA SD15-26 sequences of P1 and P2 were re-introduced into the backbone of the corresponding chimeric viruses. A pathogenesis study was performed with three experimental groups (rSVA SD15-26, $RvSVA^{SD15-26/SVV001-P1}$/SD15-26-P1 and $RvSVA^{SD15-26/SVV001-P2}$/SD15-26-P2), in which all groups presented characteristic clinical signs and lesions, comparable to wild type SVA SD15-26. Together these results demonstrate that the P1 region of SVA SD15-26 is a major determinant of the virus virulence and pathogenicity.

**PRRSV-1 strain with increased pathogenicity isolated from a severe outbreak in England**

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A severe outbreak of disease due to porcine reproductive and respiratory syndrome virus (PRRSV) was diagnosed in a breeding herd in East Anglia in June 2019. Initial suspicion of swine fevers was ruled out by official investigation and testing, and disease due to PRRSV-1 was diagnosed. The virus strain, 21301-19, was isolated, characterised by full genome sequencing and used in experimental infection of young piglets, resulting in more prominent lung lesions compared to a well characterised endemic PRRSV-1 strain from England.

Laboratory diagnoses (PCR and IHC) were carried out to characterize the potential pathogen(s) involved in the disease outbreak. Virus isolation was carried out on serum from an affected sow, using primary porcine alveolar macrophages. Culture supernatant was used to generate the full genome sequence of the virus strain, which was analysed and compared to corresponding sequences of other PRRSV-1 strains from England. Nine 16-week-old Piglets were challenged experimentally with $5.9x10^5$ $TCID_{50}$ of virus isolate intranasally, and sampled at regular intervals up to day 21, as well as having tissues collected at post-mortem on days 7 and 21 post infection. The data were compared to those from an equivalent study using another PRRSV-1 strain from England (215-06).

A pig breeding herd in England described widespread lethargy and inappetence in recently farrowed sows, with eight dying from a group of 90, and high mortality of piglets in the litters born to this group. Following negation of African and Classical swine fevers by testing, PRRSV antigen was detected in association with lesions in the spleen and lung of the affected sow by immunohistochemistry. A PRRSV-1 strain was isolated from the serum of the sow, and its full genome sequence obtained. The genome sequence was 88.4% similar to the published Lelystad virus sequence, and clustered with other British PRRSV-1 strain sequences in phylogenetic analyses. Experimental intranasal infection of piglets with the virus isolate resulted in viremia and shedding in nasal swabs, with similar virus loads as for strain 215-06. Higher virus loads were seen at 7 dpi in lung lavage with strain 21301-19, while they were similar for both viruses at 21 dpi. Gross lung pathology scores at 7 dpi and 21 dpi were significantly higher with strain 21301-19 than 215-06. Clinical scores and temperature profiles were unremarkable for both strains.

Virus strain 21301-19 was found to be a subtype 1 strain of PRRSV-1 which is likely to have evolved from previously circulating strains in England. Experimental infection of piglets, while not reproducing the severity of disease seen in the field, did result in higher lung lesion scores than another subtype 1 PRRSV-1 strain from England, indicating a higher pathogenic potential. Further studies comparing this virus strain to other higher pathogenicity isolates of PRRSV-1 for which experimental infection data is available are required to characterize the potential determinants of virulence.

**Effect of Killed PRRSV Vaccine on Gut Microbiota Diversity in Pigs**

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important pathogens affecting the global swine industry. Vaccination is still a main strategy for PRRSV control; however, host factors associated with vaccine efficacy remain poorly understood. Growing evidence suggests that mucosal microbe may influence host responses to vaccination. In this study, we investigated the effects of a killed virus vaccine on the gut microbiome diversity in pigs. Fecal microbial communities were longitudinally assessed in three groups of pigs (vaccinated/challenged with PRRSV, unvaccinated/challenged with PRRSV, and unvaccinated/unchallenged) before and after vaccination and after viral challenge. We observed significant interaction effects between viral challenge and vaccination on both taxonomic richness and community diversity of the gut microbiota. While some specific taxonomic alterations appear to be enhanced in vaccinated/challenged pigs, others appeared to be more consistent with the levels in control animals (unvaccinated/unchallenged), indicating that vaccination in-completely protects against viral impacts on the microbiome. The abundances of several microbial taxa were further determined to be correlated with the level of viral load and the amount of PRRSV reactive CD4+ and CD8+ T-cells. This study highlights the potential roles of gut microbiota in the response of pigs to vaccination, which may pave the road for the development of novel strategies to enhance vaccine efficacy.

**Next generation of swine diagnostic laboratory platforms**

LG Giménez-Lirola

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The ability of emerging and re-emerging pathogen agents to establish new niches or undergo genetic mutations led to the appearance of new diseases that pose several challenges to diagnosis, treatment, and surveillance. The identification of an emerging pathogen by conventional methods can be particularly difficult and time-consuming due to the novel nature of the agent, requiring different techniques for direct (e.g., isolation, PCR, histopathology) or indirect (antibody-based) detection of the pathogen. The ongoing challenge to the field of diagnostics is to assess current needs and apply contemporary knowledge to facilitate infectious agents' detection, identification, and characterization of both endemic and emerging pathogens. Looking back at past outbreaks we realize that the identification and characterization of a new infectious agent could take years or even decades. Such time frames have been decreased to weeks or months through the development of powerful molecular and immunological techniques. For example, when porcine epidemic diarrhea virus emerged in the U.S. in 2013, researchers across University's Veterinary Diagnostic Laboratories (VDLs) led the development of molecular and immunological techniques from scratch. Unlike large diagnostic corporations, VDLs are now seen as hubs of technological innovation and much more geared to face the challenge of translating an evolving idea into technology development towards the rapid response to emerging diseases. Indeed, the swine diagnostic technology landscape has dramatically evolved in recent years looking for more analytically sensitive, rapid and simple, yet high-throughput screening methods. The objective of this presentation is to review the basis of the next generation of diagnostic platforms currently available or on development. Particular emphasis will be put on the detection of anti-viral immune responses in pigs, providing specific examples of their real application on important viral infections of swine. Among others, we will discuss different microsphere-based liquid suspension (flow-based Luminex, and proximity-based AlphaLISA) and planar (multiplex dot-blot ELISA) arrays, which allow for multiplexing, higher dynamic range and analytical sensitivity, and/or reduction in assay steps. Moreover, the potential of the imaging and flow cytometry–based systems to visualize and measure cells, quantify reactions, and capture and store data or images will be also discussed.

**Changes in thyroid hormone levels following porcine circovirus-2, Mycoplasma, Salmonella or Brachyspira inoculation**

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We have previously shown that PRRSV-2 infection in any stage results in a profound decrease in thyroid hormone (TH), triiodothyronine (T3) and thyroxin (T4) levels, in circulation that is transient but associated with lower ADG in post-natal animals. Our objective was to determine if T3 or T4 are similarly deceased in pigs infected with other common swine pathogens.

Archived serum samples from a variety of single pathogen challenge studies were obtained from researchers in Canada and the USA (Table 1). Following shipment to University of Saskatchewan, TH levels were quantified by radioimmunoassay (RIA; MP Biomedical). Non-parametric tests were used to evaluate changes in T3 and T4 levels over time and between infected/non-infected groups. In datasets with repeated measures, overall difference by day were first verified using the Friedman's test before assessing pairwise differences by day.

Table 1. Summary of experimental sera obtained for assessment of T3 and T4 levels by RIA

| **Institution (researcher)** | **Disease** | **No. samples** | **Groups/pigs** | **Time points (DPI)** |
|---|---|---|---|---|
| University Nebraska Lincoln (Ciobanu) | PVC2 | 21 | 12 high ADG, 9 low ADG | 0, 7, 14, 21, 28 |
| Iowa State University (ISU) (MHP-Arruda, Clavijo, Poeta; Salm-Kreuder, Naberhaus) | *Mycoplasma hyopneumoniae* (MHP) | 351 | 171 inoculated, 153 in-contact, 27 ctrl | Wkly (-3 to 59) |
| | *Salmonella* (various *enterica* subspecies) | 143 | 20 Derby, 20 Typhimurium, 20 serovar 4,[5]12:i.-, 12 ctrl | -13, plus 2, 4 or 28 |
| Prairie Swine Center (PSC) (Columbus, Rodrigues) | *Salmonella enterica* Typhimurium | 190 | 32 Typhimurium, 31 ctrl | -1, 4, 7 |
| University of Saskatchewan (Harding) | *Brachyspira* colitis (multiple trials) | 129 | *45 B. hyodysenteriae,* 60 *B. hampsonii* | 0 and term (< d28) |

Legend: ctrl=control, ADG=average daily gain, wkly=weekly, DPI=days post inoculation

PCV2: T3 and T4 were decreased in low ADG pigs at 21 and 28 DPI. T3 was lower at 14 DPI. MHP: T4 was lower in inoculated vs. ctrl at 14 and 49 DPI; T3 and T4 were higher at 7 DPI. Salm (ISU): T3 was lower in *S.* Typhimurium and serovar 4,[5]12:i.- inoculated pigs vs. ctrl and S. Derby at 2 DPI. There was no evidence of T3 or T4 suppression in pigs challenged with *B. hyodysenteriae* or *B. hampsonii* or S. Typhimurium by the PSC.

There is weak evidence of TH suppression following infection by MHP and some *Salmonella* subspecies but it is inconsistent by day and experiment. TH suppression following PCV2 infection is more definitive and associated with lower ADG.

We thank the researchers for their generous donation of archived sera.

## Thyroid hormone supplementation following PRRSV-2 and PRRSV-MHP infection

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Supplemental thyroxine (T4) alone or in combination with triiodothyronine (T3) is used to treat hypothyroidism in humans and dogs. In pigs, PRRSV-2 + *Mycoplasma hyopneumoniae* (MHP) co-infection causes a profound decrease in circulating thyroid hormone (TH) levels that is associated with lower ADG. Our objective was to determine if oral supplementation with synthetic T4 or a T3+T4 combination improves the growth rate of pigs infected with PRRSV-2 alone or in combination with MHP.

High-health 5-6 week old pigs were assigned to pens blocked by weigh (4 or 5/pen) and inoculated following a 7 or 14 day acclimation period as detailed in Table 1. PRRSV 97-7895 was administered IM:IN, MHP was administered intratracheal while under sedation. TH supplementation (Table 1) started on 1 or 2 DPI and continued until termination when organ weights and lung lesion severity was assessed (data not included). Levels of T3 and T4 were measured in sera using commercial RIA kits (MP Biomedicals).

Table 1. Experimental groups and treatments used to evaluate TH supplementation

| Trial | Groups (n) | TH supplementation | TH Dose | Bleeding/weight time points |
|---|---|---|---|---|
| 1-PRRSV | INOC (16)<br>Ctrl (8) | LT4 in drinking water, 50% of pens | 50 µg/kg with concentration in water adjusted daily based on previous 3-day water intake | 2-3 x/wk<br>(term 21 DPI) |
| 2- PRRSV+MHP | INOC (20)<br>Ctrl (10) | LT4 in drinking water (50% of pens) | 100 µg/kg (wk 1) and 75 µg/kg (wk 2-5) with concentration in water adjusted daily based on previous 3-day water intake | 2 x/wk<br>(term 34 DPI) |
| 3-PRRSV+MHP | INOC (24)<br>Ctrl (8) | LT3+LT4 oral bolus twice daily to 50% of pigs in each pen) | 30 µg/kg T4 plus 4.2 µg/kg T3 per day in split doses | 2 x/wk<br>(term 30 DPI) |

Legend: INOC=inoculated, Ctrl=control, LT3=liothyronine, LT4=levothyroxine. wk=week

In all trials, average daily gain (ADG) was suppressed following PRRS (± MHP) infection, however, supplementation with LT4 (trials 1 & 2) or LT3+LT4 (trial 3) did not improve ADG. T3 and T4 levels were suppressed following inoculation in non-supplemented pigs. T4 levels increased following supplementation in all trials, however, T3 levels did not, even in trial 3 pigs that were LT3+LT4 supplemented with doses previously shown to be physiologically effective in thyroidectomized pigs (data not shown).

There is no evidence that TH supplementation mitigates the reduction in growth rate following PRRSV and PRRSV+MHP infection. These results suggest PRRSV prevents the conversion of exogenous T4 to T3, reduces the intestinal absorption of exogenous T3, and/or increases the conversion of T3 into inactive metabolites.

**GP5-specific antibody response to porcine reproductive and respiratory syndrome virus challenge in vaccinated swine**

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped RNA virus that brings about great economic losses to the swine industry. Despite widespread use of modified live virus (MLV) vaccines, PRRSV outbreaks are common and is indicative of inconsistent protection of the vaccines against an evolving population of the virus. Glycoprotein 5 (GP5) is the major viral envelope protein that harbors neutralizing antibody epitopes within its ectodomain. Mutations in the GP5 ectodomain have been observed amongst different viral sub-lineages that have evolved over the past few years in vaccinated herds, indicating the possibility that changes in this surface glycoprotein may lead to immune escape from the host response. Neutralizing antibodies (NAbs) are crucial in providing protective immunity to the virus, but they develop these antibodies only after 28 days post exposure. This NAb response overlaps with the generation of anti-GP5 response, which also develops later in infection. The aim of this present study is to evaluate the contribution of GP5-specific epitopes to NAbs generated by the host. Weaned piglets were vaccinated with two types of MLV vaccines, each containing viruses of diverged lineages L5 and L1, respectively. At 64 days post-vaccination (d pv), pigs were challenged with a heterologous PRRSV L1A (RFLP 1-7-4) strain. Kinetics of GP5-specific antibody production was assessed by ELISA using recombinant GP5 (L5 lineage), and GP5 ectodomain peptides with consensus amino acid sequence from specific viral variants. Neutralizing ability of serum antibodies was determined by a focus-reduction microneutralization assay. Notably, GP5-specific antibodies were generated between 18 and 35 d pv, compared to antibodies to viral nucleocapsid proteins that were present in serum as early as 7 d pv. Though at 64 d pv similar levels of GP5-specific antibodies were observed in both vaccinated groups (vaccinated with different viral lineages), recognition of lineage-specific GP5 peptides at this time point was variable. Interestingly, little to no recognition of variant GP5 peptides, except the L5-specific peptide, was observed at 64 d pv. There was a rise in antibody response to all variant peptides in both vaccinated groups at 14 days post challenge, though not consistently significant. Meanwhile, in peripheral blood mononuclear cells (PBMCs) antibody-secreting cells (ASC) recognizing homologous full length viral GP5 protein were not detected until 35 d pv, in comparison to those recognizing the nucleocapsid protein that were observed at 7 d pv, which was consistent with the serological results. It is worth noting that the frequency of total GP5-specific ASC in circulating PBMCs decreased at 64 d pv, while the frequency of GP5 ectodomain-specific ASC remained higher than that in naïve controls. Studies are underway to evaluate the neutralizing function of serum obtained from vaccinated animals and to assess if variant-specific GP5 peptides competitively inhibit viral neutralization. Findings from this study will provide insights into the role of GP5-specific antibody response in protecting pigs against novel viral challenge. These data can be used to further improve development of vaccine strategies for PRRSV.

**Deletion of pentad residues in the N-terminal domain of spike protein fully attenuates porcine epidemic diarrhea virus in piglets**

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Porcine epidemic diarrhea virus (PEDV) is a highly contagious and deadly swine enteric coronavirus that poses a substantial financial threat to the global swine industry. Our previous study revealed that tissue culture-adapted PEDV strains, KNU-141112-S DEL2/ORF3 and -S DEL5/ORF3, were completely attenuated *in vivo*, suggesting that a common 46-nucleotide (nt) deletion (DEL) in an intergenic region (IGR) between spike (S) and ORF3 (S-ORF3 IGR) of both strains may contribute to the reduced virulence of PEDV. However, in addition to the 46-nt DEL in S-ORF3 IGR, the attenuated strains also independently contain 2-amino acid (aa; residues 56–57) or 5-aa (residues 56–60) DEL in the N-terminal domain (NTD) of S1, which might influence the pathogenicity. To investigate whether the duad or pentad DEL in the NTD of S1 is the determinant for the attenuation of PEDV, we generated mutant viruses, named icS DEL2 and icS DEL5, by introducing the same 2-aa and 5-aa DEL into S-NTD, respectively, using the reverse genetics platform for a highly pathogenic PEDV KNU-141112 strain (infectious cDNA clone KNU-141112, icKNU-141112). These reconstituted viruses displayed phenotypic characteristics comparable to the parental icKNU-141112 virus *in vitro*. We then orally inoculated neonatal, conventional suckling piglets with icDEL5, icDEL2, or icKNU-141112 to compare their pathogenicities. Although the virulence of both icDEL5 and icDEL2 viruses was significantly diminished when compared to that of icKNU-141112 causing severe clinical signs and 100% mortality, the degree of attenuation differed between two DEL mutant viruses: icDEL5 caused neither diarrhea nor mortality, whereas icDEL2 caused mild to moderate diarrhea, higher viral titers in feces and intestine tissue, and 25% mortality. Furthermore, the icDEL5-infected piglets showed no remarkable macroscopic and microscopic intestinal lesions, whilst the icDEL2-infected piglets showed extensive and serious pathological lesions in small intestine tissues. Our data indicate that deletion of pentad residues (GENQG) at positions 56–60 in S can be sufficient to give rise to an attenuated phenotype of PEDV.

**Monitoring clinical progression and sequencing data of PRRSV2 L1C144 variant affected breeding herds**

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Here, we describe how the time to stability of the PRRSV2 L1C144 variant affected monitored breeding herds, as well as describe within farm sequences identified over time and production profile of sites that reported a clinical re-break.

The Morrison Swine Health Monitoring Project (MSHMP, representative of 50% of the U.S. breeding herd) participating swine producers were monitored. Date of the first detected L1C144 variant sequence was linked to the site's reported PRRS status if the reported change to a new break occurred within ±30 days of the sequence detection. Amongst sites affected, changes in status as well as time to stability (TTS) and percentage of sites that reached stability within one year were described. Production data and sequences from sites that reported a re-break associated with a L1C144 variant were also described.

As of August 2022, L1C144 variant sequences were identified in 64 different breeding sites from 13 production systems, 49 of which were linked to a change in the reported PRRS status. For 14 sites that reached stability, the median TTS was 43 weeks (min. 18, max. 61, IQR: 29-58). However, 40.8% (20/49) sites remained as status 1 throughout the studied period, with median follow-up time of 55 weeks (min. 4, max. 86, IQR: 32-63). 8 (16.3%) reported a new viral introduction (i.e. 1.2 status). 7 herds depopulated and 8 herds reported a new viral introduction (i.e. 1.2 status). Of the latter, other L1C 144 variant viruses were identified in two sites. Both presented clinical re-breaks characterized by a second increase in total abortions, pre-weaning mortality and sow loss ~35 weeks after the initial L1C144 detection. Within farm L1C144 ORF5 nucleotide identity varied from 97.5% to 98.5%, with non-synonymous differences found in the principle neutralizing epitope, hypervariable region 2, and transmembrane regions 2 and 3.

Many herds affected by this variant remain positive. Sites in which a clinical re-break was detected had similar production losses. Active investigation of additional cases that might have gone undetected are warranted to identify patterns of strain evolution that might help explain the clinical re-break.

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**Furin cleavage is required for swine acute diarrhea syndrome coronavirus spike protein-mediated cell–cell fusion**

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Swine acute diarrhea syndrome coronavirus (SADS-CoV) was reported in China in 2017 and is a causative agent of porcine enteric disease. Recent studies indicate that cells from various hosts are susceptible to SADS-CoV, suggesting the zoonotic potential of this virus. However, little is known about the mechanisms through which this virus enters cells. In this study, we investigated the role of furin in SADS-CoV spike (S)-mediated cell–cell fusion and entry. We found that the SADS-CoV S protein induced the fusion of various cells. Cell–cell fusion was inhibited by the proprotein convertase inhibitor dec-RVKR-cmk, and between cells transfected with mutant S proteins resistant to furin cleavage. These findings revealed that furin-induced cleavage of the SADS-CoV S protein is required for cell–cell fusion. Using mutagenesis analysis, we demonstrated that furin cleaves the SADS-CoV S protein near the S1/S2 cleavage site, $_{446}$RYVR$_{449}$ and $_{543}$AVRR$_{546}$. We used pseudotyped viruses to determine whether furin-induced S cleavage is also required for viral entry. Pseudotyped viruses expressing S proteins with a mutated furin cleavage site could be transduced into target cells, indicating that furin-induced cleavage is not required for pseudotyped virus entry. Our data indicate that S cleavage is critical for SADS-CoV S-mediated cell–cell fusion and suggest that furin might be a host target for SADS-CoV antivirals.

**Keywords:** cell–cell fusion; cleavage; furin; spike; swine acute diarrhea syndrome coronavirus.

**A CSF vaccine based on E2 recombinant glycoprotein and adjuvanted with oil-in-water emulsion induces a full protection in a pig field trial**

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Classical swine fever (CSF) is a highly contagious viral disease which causes important economic losses in the pig industry. Even if many countries have succeeded in eliminating CSF, it remains endemic in South and Central America, Eastern Europe and Asia. Systematic prophylactic vaccination is the most effective strategy to control CSF in the endemic zones. In this aim, live attenuated vaccines have been widely used to control the disease but these conventional vaccines fail to identify infected from vaccinated animals (Differentiating Infected from Vaccinated Animals). Thus, a new generation of CSF DIVA vaccines are needed. In this study, a recombinant vaccine, based on the highly immunogenic structural glycoprotein E2 of CSF virus, is assessed in a pig trial.

Two groups of 7 pigs are intramuscularly injected in the neck with 2 ml of the E2 recombinant CSF vaccine at day 0 (D0) and D21. The vaccines are either adjuvanted with a carbomer or with an oil-in-water emulsion (Montanide™ ISA 28R VG ; 15/85 w/w). A third control group is left unvaccinated. After each vaccination, the body temperature and the local reactions at the injection site are monitored. The body weight gain is also controlled at D21 and D35. Moreover, blood samples are taken at different dates in order to assess the E2 specific antibodies by ELISA. At D35, a lethal dose of Shi-Myn strain of the CSF virus is intramuscularly injected into the posterior femoral muscle. After challenge, the vaccine protection is evaluated by calculating the survival rate and by PCR detection of CSF virus in the blood samples and in the spleens and lymph nodes.

In terms of safety, the 2 adjuvanted vaccines were very well tolerated: no abnormality was noticed in the injection site, the body temperatures did not exceed one celsius degree after injections and the body weight gains were normal. Regarding the efficacy, vaccine groups showed a similar antibody profile with a positive threshold reached after the boost at D21. After challenge, only the vaccine adjuvanted with the Montanide™ ISA 28R VG induced a survival rate of 100% while that based on carbomer failed to fully protect the pigs (57%). The monitoring of the viremia also underlined an early and total clearance of the virus with the emulsion based vaccine compared to the carbomer vaccine.

Taken together, these results showed that E2 recombinant CSF vaccine associated with an oily adjuvant as Montanide™ ISA 28R VG is a good vaccine candidate and adapted to protect pigs from the disease while maintaining a good safety profile.

**A trivalent Pichinde virus vectored vaccine expressing HA proteins of H1N1, H1N2 and H3N2 influenza viruses elicit a balanced protective immunity against influenza infection of pigs**

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Influenza A virus of swine (IAV-S) is an economically important viral pathogen. We investigated the use of the recombinant pichinde virus (rPICV) as a new viral vector for vaccine development against IAV-S. PICV is a non-pathogenic arenavirus that can infect a wide range of cell types from diverse host animal species. Our recently published study demonstrated that pigs infected with rPICV do not horizontally transmit the virus to sentinel pigs. Additionally, pigs vaccinated with a rPICV vectored vaccine expressing the HA antigen of IAV-S H3N2 strain could elicit high neutralizing antibody titers against HA and were protected against challenge infection with the homologous H3N2 strain. Due to the highly variable nature of the IAV-S genome, an effective vaccine must contain antigens from multiple IAV-S subtypes to ensure a high level of vaccine coverage. A major concern with multivalent IAV-S vaccines is that interference between different HAs antigens may occur when multiple vaccine immunogens are mixed in the same formulation. The objective of the present study was to evaluate the immunogenicity and protective efficacy of a trivalent rPICV vectored vaccine that expresses three different HA antigens: H1-pdm09, H1-δ1a, and H3-TX98. Pigs vaccinated with this trivalent rPICV vaccine developed neutralizing antibodies and IFN-g secreting T cells against all three parental IAV-S strains from which the HA antigens were derived to construct the trivalent rPICV vaccine. These new data suggest that vaccine interference did not noticeably occur when pigs were vaccinated with the trivalent rPICV vaccine. Significantly, upon challenge with the H1N1-pdm09 homologous strain, the vaccinated pigs showed no evidence of IAV-S RNA shedding in the nasal swabs or bronchial alveolar lavage (BAL) fluid and minimal lesions in the lung tissue. Collectively, our results indicate that a trivalent rPICV vectored vaccine can elicit a balanced protective immunity against all three tested IAV-S strains in vaccinated pigs.

**Identification and characterization of nidovirus-host molecular interactions**

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Two nidoviruses, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Epidemic Diarrhea Virus (PEDV, a coronavirus) are responsible for major economic losses in the worldwide swine industry. Our project aims to understand the molecular mechanisms of interactions between animal nidoviruses and their hosts. We hypothesize that close interactions between host and viral proteins define the fate of infection and pathogenesis of nidoviruses. Accordingly, the dynamics of virus-host interactions should lead to important changes in the intracellular levels of host proteins. Consequently, quantitative comparative proteomic profiling of the nidovirus-infected cells in a time-resolved manner would provide dynamic and global mapping of virus-host interactions. Thus, we analyzed proteomic profiles of host cells during nidoviral infection and characterized the protein composition of virions and exosomes produced by PEDV- or PRRSV-infected cells. Porcine intestinal cells, IPEC-J2, and simian Vero cells have been used for the PEDV studies, while PRRSV infection were studied on simian MARC-145 cells. We found that PRRSV and PEDV infections affected the abundance of many host proteins associated with exosomes. Similarly, the nidoviral infections resulted in significant alterations in the global host cell proteome. Both viruses were shown to induce temporal changes specific to their molecular pathogenesis: i.e., PEDV significantly modulated the abundance of proteins in biological pathways such as immune response, host cell entry, type I IFN signaling, cytoskeletal system, and cell cycling. We concluded that both PEDV and PRRSV infections modulate the intracellular environment, suggesting that host proteins may influence viral replication and pathogenesis.

**Field trial experience with an intranasal nonpathogenic novel vaccine against PRRS**

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The objective of this field trial was to evaluate the efficacy of a novel intranasal (IN) vaccine containing a natural nonpathogenic PRRSV strain (GX16) in a commercial farm located in Mexico.

The farm is in the state of Puebla, Mexico, being a multi-site farm with an inventory of 1,400 sows with an average birth of 850 piglets per week.

7,700 piglets were vaccinated at the third day of age (DA) by the IN route with a dose of 2.0 mL each, as recommended by manufacturer. Records of clinical signs, mortality and body weight were kept, and sera samples of 20 piglets per group were taken every 3 weeks to perform ELISA and RT-qPCR tests for PRRSV; sequencing of positive cases were performed.

Clinical inspection 5 hours post-vaccination, nursery and at weaning stages revealed no adverse effects induced by vaccination. RT-qPCR of umbilical cords resulted negative in 100% of the samples, meaning no transplacental infection of PRRSV. RT-qPCR in serum samples at 3 weeks of age (WA) resulted 100% positive for PRRSV, indicating replication of the vaccine virus strain, as confirmed by the genetic sequencing and RFLP (1-6-2). At 6 WA, RT-qPCR performed revealed that 75% of the animals were positive to a field PRRSV, and at 12 WA, 100% of the samples were positive, which indicates field virus exposure; it was confirmed that the field PRRSV had an RFLP 1-37-2. The ELISA tests resulted in positive and increasing S/P ratios at 3 and 6 WA, meaning immune response to vaccination. S/P ratios found at 9 and 12 WA were related to the natural exposure to field PRRSV. The productive parameters of the IN vaccinated piglets showed an average mortality of 1.88% during the weaning period vs 3.32% of those that received the MLV vaccine in previous groups, and an improvement in the ADG of 65 g/day in the same period, resulting in 447.5 g additional gain in body weight at the end of the weaning stage.

The IN naturally nonpathogenic vaccine strain G16X proved to be a safe and efficacious immunoprophylactic tool in the prevention of clinical manifestations and productive damage induced by heterologous field PRRSV challenge, which is indicative that the vaccine does not damage the innate nor the adaptative immune response of the pigs, preparing them to resist the field challenge with PRRSV.

**Engineering an African Swine Fever Virus Multiepitope Protein for Use in an ASF Nanoparticle-based Subunit Vaccine**

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African swine fever virus (ASFV), the virus that causes African swine fever (ASF), is a highly contagious and deadly virus affecting both domestic and feral pig populations with mortality rates approaching one hundred percent within seven days of infection. To date, there is no treatment or vaccine available for this disease. Although endemic in sub-Saharan Africa, ASF has also spread to parts of the European Union, Russia, China, southeastern Asia, the Dominican Republic, Haiti, and, most recently, Nepal. If ASF were to jump to the United States (U.S.), it has the potential to result in dire consequences to U.S. livestock producers, as well as the U.S. pork economy. Therefore, there is an increasing need for the development of vaccine interventions to treat ongoing outbreaks abroad before the disease makes its way to the U.S and if ASF were to be detected in the U.S. Recently, epitopes of ASFV have been identified and used in vaccine trials but have provided only limited protective immune responses and cross-protection has not been reported. Here, we use in-silico modeling and prediction tools to engineer a synthetic multiepitope ASFV protein, containing key immunogenic epitopes. The aim being to induce robust protective immune responses utilizing a single protein when coupled with existing nanoparticle intranasal vaccine technology. The nanoparticle intranasal vaccine platform has been shown to induce increased virus-specific mucosal immune responses and enhanced T cell immunity in comparison to standard intramuscular delivery platforms. The multiepitope was subsequently expressed using bacterial and mammalian cell expression systems and purified for analysis. Utilizing an intranasal nanoparticle-based subunit vaccine combined with established epitopes from critical ASFV proteins should prove effective at eliciting a protective immune response against circulating and emerging ASFV strains.

**Prevalence and pathogenic assessment of co-infection of porcine epidemic diarrhea virus and *Clostridium perfringens* type A**

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*Clostridium perfringens (C. perfringens)* is part of the normal gut microbiome in pigs but has has the potential to cause pre- and post-weaning diarrhea. However, there is poor understanding of the importance of this bacterium as a primary pathogen of diarrhea in piglets, and the prevalence and types of *C. perfringens* present in Korean pig populations is unknown. To study the prevalence and typing of *C. perfringens*, a total of 269 diarrheic samples were collected from suckling and weaning pigs suffering from diarrhea in 61 commercial farms during 2020–2022 and examined for *C. perfringens* type A (CPA), B (CPB), and C (CPC) by PCR. We also performed virus-specific RT-PCR for porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV), and porcine rotaviruses (PRV). We found that the most frequently identified *Clostridium* was CPA (19.7%) followed by CPC (0.4%). Among them, the mono-infection of CPA (47.2%) and co-infection of CPA (52.8%) with PEDV were predominant in diarrheal samples. Next, we conducted animal experiments to investigate the clinical outcomes of the mono-infection of CPA and sequential co-infection of CPA with highly pathogenic (HP) PEDV using 3-week-old conventional piglets. Our results indicated that the piglets mono-infected with CPA or HP-PEDV underwent no or mild diarrhea without mortality. However, the animals co-inoculated with CPA and HP-PEDV showed more serious diarrheal symptoms when compared to those of the mono-infection piglets. Furthermore, we observed that CPA could promote PEDV replication in the co-infected piglets with evidence of high viral titers in feces. Histopathological examination revealed more severe villous atrophy in the small intestine of the co-infected pigs than the mono-infection pigs. Collectively, although CPA alone did not cause diarrheal disease in weaning piglets, there existed obvious synergistic clinical presentations between CPA and PEDV co-infection in older piglets. Our data will advance the understanding of the synergistic pathogenic mechanism triggered by these porcine enteric pathogens and provide knowledge leading to control PED in affected or endemic farms.

**Developing DNA-nanopore Sensors for Direct Detection and Differentiation of Infectious and Noninfectious Porcine Viruses**

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Pen-side detection of viral infections is important to prevent the spread within herds; however, no current method can rapidly detect intact viruses on site with ability to tell whether the viruses are infectious or not, as the presence of virial nucleic acids and antigens does not mean the viruses are infectious. The lack of accurate testing causes misdiagnosis and spread of these viruses, leading to harsh economic effects. To overcome limitations of current methods, we are developing a method for direct detection and differentiation of infectious and noninfectious porcine reproductive and respiratory virus (PRRSV) and porcine epidemic diarrhea virus (PEDV), without sample pretreatment. DNA aptamers are selected from a DNA library with infectious virus and against noninfectious virus. The aptamers are being incorporated into a solid-state nanopore and tested to differentiate infectious virus from noninfectious virus in porcine saliva and serum samples, enabling a sensor to help control the spread of porcine viruses.

**Quality weaned pigs, implications for health and performance in growing pigs**

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Introduction/Objective: Wean to finish production systems constantly strive to get pigs to market in a profitable way. Essential for the execution of this production process is the adequate quality of raw materials, which in this case, it can be said are the quality weaned pigs. Age and weight of the weaned pigs are often used as a proxy of the quality of these groups of pigs. The objective of this ongoing study is to characterize the age variation among weaned pig litters at weaning and investigate the relationship between this variation and the growing pig's health and performance.

Methods: Collection production records from pig flows including weaning age of individual litters, health status at weaning and wean to finish production phase closeout information. Inclusion criteria for pig flows include having source farms that have different weaning ages and farms with different numbers of weaning events. The information will be analyzed by weaning groups to characterize the variation at group level and compare with growing pig's health and performance results.

Results: The results of this study will help us to understand and address the role of weaning age variation in post-weaning performance, thus allowing swine producers and swine health professionals to evaluate the quality of weaned pig batches by the weaning age dispersion. Moreover, it will show the value of management practices aimed to reduce the age variation of weaned pigs and improve health status.

Conclusion: The quality-weaned pig represents the baseline for growing pig's results. Age, weight and/or health status of weaned pigs cannot be modified in the post-weaning, therefore, the growing pig production phase is dependent on the volume and quality of raw materials fed into the system, the weaned pigs. There is a close relationship between weaning weight/weaning age and the response of growing pig groups to the various growing conditions and challenges they encounter in the post-weaning phase; this affects overall pig performance that impacts productive and economic decisions in swine operations.

**Partnership: Systematic screening of ASFV proteome to identify immunogenic antigens**

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African swine fever virus (ASFV) is widespread in many swine producing countries, causing large economic losses and affecting global food security. There are no effective vaccines or treatments available for ASFV. Although there are strong evidence suggesting that antibody is an important component of the protective immunity, the identity of viral proteins capable of eliciting protective antibody responses in pigs remain elusive. This knowledge gap severely hinders the development of effective ASFV vaccines. It is observed that pigs surviving an infection with an ASFV strain or those immunized with a live-attenuated vaccine strain develop solid homologous protective immunity. On the other hand, pigs vaccinated with experimental inactivated ASF vaccines do not. We hypothesize that pigs infected with a live ASFV strain develop a different antibody profile as compared to those vaccinated with an inactivated vaccine. The primary goal of this research is to identify viral proteins capable of eliciting a protective immune response in pigs. We recently developed high-throughput assay to systematically screen the whole ASFV proteome for identification of immunogenic antigens. In a pilot study, twenty-three serum samples were collected from pigs surviving a natural ASFV infection which included 17 samples from finishing pigs (~7 months old) and 6 samples from sows (between 12 and 36 months old). Additionally, 23 serum samples were collected from ASFV-naïve pigs to serve as negative controls. All sera from ASFV-surviving pigs tested positive while all sera from control pigs tested negative by two different commercial ELISA kits. Antibody reactivity of each serum sample was simultaneously measured against six ASFV structural proteins that are associated with the induction of putative protective antibody responses including including p12, p32, p54, pp62, C-type lectin and CD2v. All ASFV-surviving pigs had antibody against p32, p54 and pp62 while 91.3% surviving pigs had antibody against p12. Only small portions of ASFV-surviving pigs exhibited antibodies against C-type lectin (34.8%) and CD2v (26.1%). While antibodies against p12, p32, p54 and pp62 were similarly detected in both finishing pigs and sows, antibodies against C-type lectin and CD2v were mainly detected in sows but not in finishing pigs. These results suggest a differential humoral immune response to ASFV infection in sows and finishing pigs. Further studies are needed to better understand the nature of immune responses to ASFV infection in different pig populations.

**Detection and frequency of potential zoonotic Rotavirus in suckling pigs in the south of Peru**

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Porcine rotavirus (PoRV) belongs to the genus *Rotavirus* in the Family *Reoviridae*. Rotaviruses (RVs) are divided into ten groups (A-J) base on the antigenicity or the sequence of VP6, one of the capsid proteins. PoRV not only causes gastroenteritis in piglets, but is also considered, along with bovine rotavirus, a reservoir of emerging human strains. The aim of the study was to detect and quantify the frequency of RV A, C, and H in piglets from a farm located in the south of Peru, as well to determine the probable co-circulation of different RV species of zoonotic potential in the area of study. Five hundred and ninety-eight fecal samples were collected from piglets between 3 to 10 days of age, and pooled by litter (47 litters or pools), and were tested by reverse transcription PCR (RT-PCR) based on specific primers and probes for each RV species. The fecal samples were collected directly from the rectum of the animals using a sterile swab and put inside a corning tube with 300 µl of 10% PBS at 7.2 pH, virus transport media, and stored at -20°C. Specific RT-PCR primers for each RV species, RVA (NSP5), RVC (VP6), and RVH (VP6), were design. Of the 47 pools analyzed, 100% were positive for at least one of the viruses tested. Of the positive samples, 24 (51%) were monoinfected, while 21 (44.7%) had coinfections with two (89.5%) or three (4.3%) viruses. RVA was the most commonly detected virus (100%) followed by RVC (27.7%) and RVH (25.5%), respectively. This study confirmed the high prevalence of RVA. Most importantly, RVA was codetected with other RVs in a significant number of samples highlighting the complexity of the epidemiological situation and possible implications of synergistic or additive effect of co-infections with other enteric viral and bacterial pathogens on clinical presentations or pathology. Moreover, this is the first study confirming the presence of RVC and RVH in swine herds of Peru. Knowing that RVs infections are frequent in pig populations, further studies are necessary to better understand the pathogenesis and pathogenicity of different RVs genotypes, and the frequency of detection across age group towards devising better intervention strategies.

**Major Enhancement of U.S. swine industry biosecurity: what does this really mean for a production system and how on-farm biosecurity, pig movement data, can help explain the between-farm disease dissemination?**

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The well-established U.S. Secure Pork Supply (SPS) Plan for Continuity of Business serves as a guideline for enhancing the biosecurity features of swine farms to improve preparedness for outbreaks of foreign animal diseases. Although the role of biosecurity is well understood in preventing disease spread, the U. S. swine industry lacks knowledge of individual farm biosecurity plans and the efficacy of existing biosecurity measures. The Rapid Access Biosecurity (RAB) app (RABapp™) consortium aimed to 1) reduce ambiguity regarding the construction of on-farm enhancement biosecurity plans; 2) provide rapid access to standardized biosecurity plans at a national level; and 3) track movements between farms allows for the ability to efficiently develop disease spread transmission model and transform these models into decision support tools for animal health official and swine industry. As a result, the RABapp™ project was developed by an assembled consortium of the swine industry, government officials, and academic scholars' members. This study focuses on demonstrating the steps to enhance the on-farm biosecurity of individual swine farms and implement effective contact tracing by tracking between-farm movement data to help prevent the spread of infection to other farms.

Enhance the on-farm biosecurity: Although SPS plans help swine farms enhance their biosecurity features in preparation for infectious disease outbreaks, updating and accessing these plans in a timely manner before and during an emergency, is critical for swine industry resilience. Here, RABapp™ serves as a vital tool by providing producers, veterinarians, and government officials.
Movement data for animals and related products: The integration of movement data with SPS plans is a unique feature of RABapp™. RABapp™ calculates the transportation network of farms and displays this network within an interactive easy-to-use plot. Utilizing information extracted from SPS plans, RABapp™ also calculates the risk level of individual farms based on each farm's biosecurity infrastructure and the volume of animals moving through specific sites.
Disease surveillance and transmission models: Thanks to the increasingly broad participation of the swine industry in the RABapp™project, the team has utilized extensive movement and farm location data from swine companies to develop three state-of-the-art transmission models. The first two models enable the reconstruction of weekly outbreaks for two endemic diseases, PRRS and PEDV, while also providing short forecasts. In addition to the PRRS and PEDV models, the third and arguably most critical model is a transmission model for African swine fever (ASF). Tracking the spread of this major infectious disease threat as soon as the first case is detected on U.S. soil is a prominent goal of the RABapp™ project.

**Predicting antigenic dissimilarity for PRRSV type 1: Applications of Machine learning**

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The control of Porcine Reproductive and Respiratory Syndrome (PRRS) remains a significant challenge due to the genetic and antigenic variability of the causative virus (PRRSV). Predominantly, PRRS management includes using vaccines and live virus inoculations to confer immunity against PRRSV on farms. While understanding cross-protection amongst strains is crucial for the continued success of these interventions, understanding how genetic diversity translates to antigenic diversity amongst PRRSV strains remains elusive. In this study, we aimed to estimate antigenic dissimilarity *in silico* and identify differences in specific amino acids associated with the antigenic differences between viruses. We used mixed-effects machine learning models on antigenic distance estimates derived from serum neutralization assays cross-reacting sera against PRRSV with PRRSV isolates using 27 PRRSV1 viruses circulating in Europe. We categorized the cross-reaction assay results into two categories, referred to herein as low and high antigenic distance (dissimilarity) using the median antigenic value. In the models, we used amino acid distances in the ectodomains of ORFs 2 to 5 and site-wise amino acid differences between the viruses as potential predictors of antigenic (dis)similarity. The antigenic distance was weakly to moderately associated with ectodomain AA distance for ORFs 2-4 (rho = 0.1) and 5 (rho = 0.3), respectively. Using a gradient boosting model, we estimated the antigenic (dis)similarity (high versus low) between serum-virus pairs with an accuracy of 81% (95% CI 76-85%); sensitivity and specificity were 86% and 75%, respectively. The model negative and positive predictive values were 83% and 80%, respectively. We then identified and used highly ranked AA sites from the variance importance analysis as model covariates in multimodal logistic inference. Some AA differences between serum-virus pairs were significantly ($p < 0.05$) associated with high-vs-low classification: serum-virus pairs that differed at AA sites 127, 79 and 39 in ORFs 2, 3, and 5, respectively, were twice as likely to have high antigenic distance (low antigenic similarity) compared to serum-virus pairs that did not differ at those sites. While experimental studies to determine cross-protection are instrumental, these *in vivo* studies are not always practical or timely for the many co-circulating and emerging PRRSV strains. In this study, we demonstrate the ability to rapidly estimate potential immunologic cross-reaction between different PRRSV strains in silico using sequence data routinely collected by production systems. These models can provide fast turn-around information crucial for improving PRRS management decisions such as: selecting vaccines/live virus inoculation to be used on farms and assessing the risk of outbreaks by emerging strains on farms previously exposed to certain PRRSV strains and vaccine development among others.

**Target-enriched long-read sequencing (TELSVirus): A streamlined method to characterize many viral genomes simultaneously from a single sample**

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Viral co-infections are a frequent health challenge in swine worldwide contributing to aggravated disease outcomes. Whole-genome sequencing (WGS) of viral genomes can improve our understanding of viral co-circulation dynamics and associations with clinical and production outcomes. However, current methods for WGS are time-consuming due to need for pre-sequencing virus isolation; and most WGS workflows rely on short-read technologies, which constrains our ability to accurately reconstruct multiple co-circulating viral genomes. Long-read metagenomic sequencing, i.e., sequencing all of the nucleic acids within a sample using long reads (without prior culture or enrichment), is one way to overcome these limitations. However, the data throughput of long-read sequencers is significantly smaller than short-read sequencers. Because field samples typically contain limited amounts of viral nucleic acid and high amounts of host DNA/RNA, this lower throughput of long-read sequencers limits our ability to recover viruses from metagenomic samples with high sensitivity.

To overcome these limitations, we have developed a workflow called "TELSVirus", or "Target-Enriched Long-read Sequencing of Virus". The workflow combines a "capture and enrichment" method with long-read, real-time sequencing, followed by an ensemble bioinformatics pipeline for data analysis. The pre-sequencing capture and enrichment step allows us to simultaneously target dozens of viral genomes, increasing their relative abundance within the metagenomic sample. We hypothesize that this will allow us to increase the sensitivity of the long-read sequencing data, thus allowing for robust genome reconstruction and variant calling.

To initiate this workflow, we bioinformatically designed a panel of "probes" that selectively target 52 swine viruses. The probes were designed to cover 100% of all complete genomes for the 52 viruses (i.e., >10,672 genomes). To pilot the workflow, five field nasal swabs from weaned pigs with known influenza A virus (IAV) status (n=3 IAV qPCR positive; n=2 IAV qPCR negative) were subjected to the TELSVirus workflow. First, IAV RNA was extracted by QIAamp® Viral RNA Mini Kit, followed by complementary DNA synthesis. Probe hybridization and enrichment were performed using the SureSelect XT HS2 DNA System (Agilent Technologies). Subsequently, library preparation (PCR barcoding kit SQK-PB004) was performed prior to loading the samples in the minION flow cell. Our preliminary data show that the designed probes can increase the percentage of on-target reads for IAV from 0.1% up to 66%. In contrast, there was no increase in the on-target read proportion for IAV qPCR-negative samples or reads that aligned to the host (*Sus scrofa* genome). The average coverage depth for the IAV positive samples was calculated as the total reads obtained for each segment of the target genome (IAV), and values of 6 to 48 were obtained depending on segment. While highly variable, the coverage positively correlates with information content and reliability of the obtained data. An ensemble bioinformatics pipeline was also piloted, which uses RVHaplo to identify variants within the IAV sequence data. We found 5

circulating haplotypes of IAV within a single sample based on the hemagglutinin, and neuraminidase gene segments. One of the haplotypes was dominant at ~80% of the influenza reads. These pilot data demonstrate that the TELSVirus workflow can effectively increase the proportion of sequenced reads for the target virus; generate long-read sequences with moderate coverage; and discriminate among different IAV variants that are simultaneously present in a single sample. The entire workflow takes 24-48 hours. To estimate the limit of detection (LOD) of the TELSVirus workflow and to estimate its correlation with qPCR copy number, we then performed a serial dilution series using an IAV-positive sample. For dilutions that were "undetermined" via qPCR (i.e., $10^{-7}$ to $10^{-9}$), the TELSVirus workflow generated thousands of IAV reads, suggesting that the LOD of TELSVirus is 2-3 orders of magnitude lower than qPCR. There does not appear to be a linear correlation between qPCR copy number and the number of TELSVirus reads produced, suggesting that the probe-based binding and amplification steps of the TELSVirus workflow produce different target copy numbers than the primer-based amplification steps involved in qPCR.

Future work aims to streamline and further validate the TELSVirus workflow by applying it to a greater number of field samples from swine herds, with co-circulating viruses. Prospective results from the TELSVirus workflow will increase understanding of the ecology and epidemiology of important swine viruses, including co-infection dynamics and their importance for swine health and performance.

**Evaluation of infectiousness and virulence of the newly emergent and virulent Minnesota PRRS virus variant**

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The present undergoing study was designed to compare newly emerged PRRS strain L1C 1-4-4 with two other strains (i.e., L1A 1-7-4 and L9 1-4-2) that had caused important national outbreaks in the past and address specific questions such as assessing infectiousness, viremia magnitude, shedding pattern, and histopathological lesions in experimental infected pigs.

For each strain, thirty-six PRRSv negative three-week old barrows were randomly allocated to five different challenge groups and one control group. Each group of pigs was housed in a separate room and within each room pigs were individually housed where no nose-to-nose contact among pigs was possible, but pigs shared the same air space. PRRSv inoculum was titrated, and through 10-fold dilutions five different incula concentrations were obtained ranging from $10^4$ to $10^0$ $TCID_{50}$/ml. Pigs were intranasally (1 ml per nostril) challenged according to the groups selected concentration. The control group was sham-inoculated with minimum-essential media. Blood, nasal, oral, and rectal swabs were collected at day post-challenge (DPC) 0, 1, 2, 4, 7, 11, 16, 21, 26 and 30 to assess infection, viremia and monitor viral shedding though PRRSv RT-PCR. Researchers changed their gloves, mask and gowns between each pig sampling. On DPC 11, two pigs from each group were euthanized and tissues (e.g., brain, lung, lymph nodes) were collected for histopathological assessment. At the time of writing, only the L1A 1-7-4 and L9 1-4-2 groups had been completed and the L1C 1-4-4 challenge was in progress.

At DPC 4 and 7, a total of 23 and 25 pigs were RT-PCR positive for the L1A virus whereas for the L9 a total of 15 and 16 were RT-PCR positive.
PRRSv L1A 1-7-4 had $ID_{50}$ of approximate titer $10^2$ $TCID_{50}$/ml at DPC 4 and for the PRRSv L9 1-4-2 had $ID_{50}$ of $10^2$ $TCID_{50}$/ml. Histopathological results from PRRSv L1A 1-7-4 tissue samples collected at day 11 show no difference of severity between animals inoculated with different concentrations (no lesions to slight or remarkably mild). Histopathological results from the L9 1-4-2 are still pending. PRRSv PCR results on oral, nasal, and rectal swabs are also pending.

**Addressing biocontainment through environmental contamination assessment in farms housing PRRS Lineage 1C 1-4-4 positive pigs**

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An exploratory study was designed to assess whether PRRS virus RNA could be detected in surfaces outside of positive farms as a proxy of dissemination and to understand whether biocontainment interventions needed to be implemented.

A total of 8 conveniently selected farms (3 breeding and 5 growing pig farms) were included in this study. A sampling protocol that included wiping of specific areas and surfaces was developed in conjunction with swine practitioners. A surface sampling protocol previously validated for PRRSv and influenza was used. Briefly, sterile cloths moistened with 20 ml of PBS were used to sample a 1ft-by-1ft area. Once the sample was collected, the cloth was deposited in a sterile plastic bag and fluid was extracted and poured into Corning Falcon™ tubes (Fisher scientific), labeled according to sampled surface and farm. Samples were then refrigerated and submitted for PRRSv RT-PCR testing at the University of Minnesota Veterinary Diagnostic Laboratory.

Out of 169 environmental samples collected at 8 farms housing PRRSv L1C 1-4-4 positive pigs, 19 (11.24%) samples from 6 farms yielded at least one positive RT-PCR result. Eight (42%) of the positive samples originated from exhaust fan housings in 4 farms with a Ct value ranging from 30.29 to 35.98. Main door entry doorknobs in four farms tested positive in 4 occasions with Ct values ranging between 31.68 and 36.35. The remaining positive samples originated from surfaces such as ante-room floors and mortality carts with Ct values ranging from 25.41 to 35.38.

Overall, the detection of viral genetic material on surfaces on the outer areas of a farm housing PRRSv positive pigs is possible using a targeted surface sampling. This exploratory study provides evidence that biocontainment efforts to prevent the spread of PRRSv from infected farms need to be reviewed and further studied.

**Effect of freeze-thaw on PRRSV RNA detection by RT-qPCR**

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Re-testing samples is common both in diagnostic and research settings, but the effect of freeze-thaw cycles on nucleic acid detection by PCR is largely unexplored. The objective of this project was to quantify the effect of multiple freeze-thaw cycles on the detection of PRRSV RNA and a porcine endogenous reference gene (internal sample control) in serum and oral fluids.

Serum samples (n = 10) used in the study were from pigs experimentally inoculated with wild-type PRRSV (n = 5) and corresponding negative control pigs (n = 5). Oral fluid samples (n = 10; 6 PRRSV positive and 4 negative) were from individually housed 14-week-old pigs vaccinated with a PRRSV modified-live vaccine (MLV) (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica, Inc., Duluth, Georgia) and sampled from -7 to 42 days post vaccination (DPV).

The experiment consisted of exposing samples to one of 4 treatments, i.e., 2, 5, 10, or 15 complete freeze-thaw cycles, testing for PRRSV RNA and a porcine internal sample control, and then quantifying the freeze-thaw effect by linear regression on SAS 9.4 (SAS Institute, Cary NC). To conduct the experiment, samples were aliquoted (1 ml) into 2 ml tubes to create 4 complete sample sets (one set per treatment). Samples were submitted to freeze-thaw cycles by freezing at -80°C and then thawing at 4°C overnight. After all freeze-thaw treatments were completed, samples were tested using a commercial RT-qPCR that detects both PRRSV RNA and a porcine internal sample control simultaneously (RealPCR*NA PRRS Types1-2 RNA Mix, IDEXX Laboratories, Inc., Westbrook, Maine, USA).

In serum, regression analysis showed that freeze-thaw cycles had no impact on the detection of PRRSV RNA or the internal sample control. That is, all samples were positive for both targets and the slope of the regression was -0.135 (95% CI: -0.37, 0.34) and 0.018 (95% CI: -0.06, 0.09) for PRRSV and the internal sample control, respectively. In oral fluids, the intended targets were detected in all samples, but a freeze-thaw effect was discernable. That is, the slope of the regression was 0.208 (95% CI: -0.02, 0.43) and 0.193 (95% CI: 0.06, 0.32) for PRRSV RNA and the internal sample control, respectively.

Samples in diagnostic laboratories commonly undergo freeze-thaw cycles in the course of testing and retesting, but the effect of this process on the PCR results is largely unquantified. This study demonstrated that freeze-thaw cycles had little impact on the detection of PRRSV RNA and a porcine internal sample control, albeit more so in oral fluids *vs* serum. It should be noted that these results apply to PRRSV and that further studies are needed to address the effect of freeze-thaw on other pathogens and other matrices.

**Use of a porcine endogenous reference gene (internal sample control) in a PRRSV RT-qPCR**

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Endogenous reference genes (i.e., housekeeping genes inherent to a specimen) have been used extensively as internal sample controls (ISC) in PCRs used in gene expression research and, with increasing frequency, in veterinary diagnostic PCRs. This study evaluated the detection of a porcine endogenous reference control in a commercial PRRSV RT-qPCR (RealPCR*NA PRRS Types1-2 RNA Mix, IDEXX Laboratories, Inc., Westbrook, Maine, USA) using research samples of known PRRSV status.

Two sample sets were evaluated. Set 1 consisted of serum (n = 132), oral fluids (n = 130), and feces (n = 132) collected from 12 14-week-old pigs individually housed under experimental conditions. Pigs were vaccinated with a PRRSV modified-live vaccine (MLV) (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica, Inc., Duluth, Georgia), and samples were collected on days post vaccination (DPV) -7 to 42. Set 2 consisted of serum samples (n = 83) from 7 pigs inoculated with PRRSV, ATCC VR-2332 (American Type Culture Collection, Manassas, VA, USA) from -6 to 182 days post inoculation (DPI).

Samples were tested using a commercial RT-qPCR that detects PRRSV RNA and the ISC simultaneously. ISC results were analyzed in terms of the frequency of detection and distribution of Cq values. Thereafter, the 95th, 97.5th, and 99th percentiles of the ISC Cqs were calculated for each specimen type using R 4.1.0 (R core team, 2020) to establish the upper limits of the expected response.

In Set 1, all serum, oral fluid, and fecal samples (n = 394) were negative for PRRSV RNA at -7 and 0 DPV, with the first positive result at 3 DPV. In Set 2, all pigs (n = 7) were positive for PRRSV at DPI 7.

The ISC was detected in all samples in both sets (n = 477), with mean Cq values of 27.5 in serum, 26.6 in oral fluid, and 27.4 in feces. The ISC Cq values for the 95th, 97.5th, and 99th percentiles were 29.6, 30.0, and 30.2 in serum, 29.5, 30.1, and 30.7 in oral fluids, and 29.3, 29.7, and 30.6 in fecal samples, respectively.

Although not frequently described in veterinary diagnostics, the use of ISCs could be a useful addition to routine PCR testing by providing an assessment of sample quality. In this study, the detection of the ISC in samples collected under the “best-case scenario” (i.e., under experimental conditions and immediately stored) was consistent over the time and unaffected by PRRSV replication. Thus, preliminary data suggest that failure to detect the ISC represents an irregularity either with the sample or the testing process. Work in progress will evaluate the effect of sample "mishandling" on ISC detection.

**Comparative survival of different strains of Porcine Reproductive and Respiratory Syndrome virus at different temperatures**

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**Objective:** Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is a major cause of economic losses to the North American swine industry. An emerging PRRSV-2 classified as 1-4-4 RFLP pattern-lineage 1C is the cause of a regional outbreak in the Midwestern US since 2020. The virus can be transmitted to naïve hosts via direct and indirect routes. For the latter route to be successful, the virus needs to survive in the environment, which depends on temperature, moisture, matrix, and pH. The emergence of new PRRSV variants within the last 20 years and the severity of disease outbreaks raise concerns about their stability in the environment since it can affect virus dissemination. Therefore, the aim of this study was to determine the comparative survival of 10 different strains of PRRSV (one from PRRSV-1 and nine from PRRSV-2) at three different temperatures (4°C, room temperature, and 37°C).

**Methods:** Viruses were propagated and titrated in MARC 145 cells. For each strain, three 24 well plates were labeled appropriately (4°C, ~25°C and 37°C). The virus was applied to the bottom of all wells (100µl of virus/well). The plates were air-dried for 4 hours and placed at their respective temperature. The surviving virus was eluted (from 3 wells each) after 4 hours, day 1, day 3, day 7, day 14, day 21, day 28 and day 35 using 200µl of elution buffer (3% beef extract-0.05 M glycine) per well. Serial 10-fold dilutions of all eluates were prepared and inoculated in monolayers of MARC 145 cells. Then, plates were incubated and examined daily. Virus titers and percent virus inactivation were then calculated.

**Results:** All ten strains of PRRSV survived for at least 35 days at 4°C. However, six strains (1-7-4, Lelystad, 1-8-4, VR 2332, 1-4-2, and 1-4-4 MN) were more resistant (in terms of percent inactivation) than the other four strains. At 25°C, five strains survived for no longer than 1 day. The other five strains (VR2332, Lelystad, 1-4-4 SD, 1-4-4 MN, and 1-8-4) were more resistant surviving for 3 to 7 days. At 37°C, six strains survived for only one day while four of them (Lelystad, 1-4-4 MN, 1-4-4 SD, and 1-8-4) survived for up to 3 days. Two strains of 1-4-4 L1C differed in their survivability at room temperature. The emerging variant 1-4-4 L1C was one of the more resistant strains; it survived for 3 days at both room temperature and at 37°C.

**Conclusions:** These results show differences in the survival of PRRSV strains at different temperatures. It is not surprising that the virus survived longer at cold temperature, as compared to room temperature and 37°C. Biosecurity practices that include a robust disinfection protocol of facilities, equipment, and instruments must be followed since contaminated surfaces at different temperatures could be a potential risk factor for virus transmission.

**Gilt introduction in Danish PRRSV-positive farms**

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An effective gilt introduction program is one of the most important management strategies for controlling PRRSV infections. This study describes the gilt introduction program in 18 Danish sow farms. All 18 farms were identified as porcine reproductive and respiratory syndrome virus (PRRSV) positive; six farms had the PPRS type 1 strain, five farms the PRRS type 2 strain, and seven farms harbored both PRRS stains. The level of clinical symptoms varied. The farm size varied from 900 to 3000 sows.

The procedure for gilt introduction was registered along with a farm visit and an on-site questionnaire. In each herd, 15 gilts were selected for blood sampling the day they entered the sow unit. The serum samples were analyzed at the University of Copenhagen for antibodies against PRRSV using ELISA tests, and real-time RT-PCR analyses for PRRSV.

A quarantine for purchased gilts was standard in 16 farms. Two farms produced own replacement animals and did not acclimatize the gilts in a separate quarantine unit. Eleven of the farms (11/16~69%) had optimal procedures for the quarantine unit. This was defined as all-in/all-out management, separate entrance, and no air contact to other pigs (e.g., a door directly to another section). Duration of the quarantine varied from six weeks to 14 weeks, with an average of 10 weeks.

Gilts were purchased from a single supplier in all 16 farms. On arrival in 14 farms, purchased gilts were vaccinated with one or two modified live PRRS vaccines according to the strain present in the farm, and typically revaccinated three weeks later. The purchased gilts were not vaccinated in two farms. The gilts were blood-sampled just after the quarantine, at the day of entrance in the sow facility. The seroresponse in the gilts was 90-100 % PRRS-positive at farm level in 13 farms. Only 77 % of the gilts were seropositive in one farm. None of the tested gilts were positive in real-time RT-PCR.

In most farms, an immunization of the gilts was achieved after vaccination with modified live PRRS vaccines. None of the tested gilts showed viremia in real-time RT-PCR when entering the sow facility.

The quarantine program could be optimized in some of the farms, by extending the duration in the quarantine facility to a minimum of 12 weeks along with adhering to strictly all-in/all-out management procedures.

**Preventing ASF transmission through intradermal vaccination**

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Shared needles are a possible iatrogenic vector of African Swine Fever virus in farm conditions. The objectives of this study were to evaluate a possible model for transmission of African Swine Fever (ASF) virus through conventional needle and a needle-free device (IDAL® 3G, MSD Animal Health, The Netherlands)

Sixty, 4-week-old, male castrated pigs were procured from a herd free of ASF and PRRSV. Upon arrival, pigs were randomly allocated into two sets. Set 1 (n=12), served as seeders, were randomly allocated into 4 groups (ASF-H, ASF-M, ASF-L and Control). Forty eight, age-matched pigs were divided in 8 groups of 6 pigs each and served as sentinels. Pigs in Set 1 from the ASF-H, ASF-M, ASF-L groups were oronasally challenged with ASFV at 108, 106, and 101 HAD50/ml, respectively. Control served as the negative and no-challenge control. Animals were observed for clinical disease daily. Blood samples were collected on weekly basis, and assayed for the presence of ASFV DNA by Realtime PCR. At 7 days post challenge, corresponding to a peak in viremia, all four groups were intradermally (ID) and intramuscularly (IM) injected with Diluvac Forte using a needle-free device (IDAL® 3G, MSD Animal Health, The Netherlands) and conventional needles, respectively. The same needle or needle-free device was then used to inject the same volume of Diluvac Forte into Set 2 (n = 48) divided into 8 sentinel pigs groups (Sentinel-H ID, Sentinel-H IM, Sentinel-M ID, Sentinel-M IM Sentinel-L ID, Sentinel-L IM , Control ID and Control IM. Sentinel pigs were observed for clinical disease daily and assayed for the presence of ASFV DNA by Realtime PCR.

All ASF seeder groups developed viremia. All animals in ASF-H (CT peak viremia 16.90) and ASF-M (CT peak viremia 17) died by Day 28 of the study. No control animals developed viremia. ASF transmission occurred from viremic pigs to naïve pigs in all the needle groups. Transmission rate by conventional needle correlated to level of viremia. All animals in Sentinel-H IM and Sentinel-M IM died by 28 days of the study. No viremia or clinical signs were observed in the needle-free device groups. No control sentinel animals developed viremia or clinical signs.

Previous studies have demonstrated the possibility of PRRS transmission through conventional needles. To our knowledge this is the first study that confirms this possibility with ASF. Transmission did not occur during the use of the needle-free device but could occur in the case of needles. When transmission occurred through needles, both viremia and mortality could be observed, suggesting that high levels of virus can be transferred on the needle and cause disease in naïve animals. A dose dependent effect was also noted in both the seeder and sentinel groups. Needles may thus pose as a vector of iatrogenic ASF transmission in farms.

**Japanese Encephalitis Virus: an Emerging Transboundary Pathogen**

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Japanese encephalitis virus (JEV) is a zoonotic arthropod-borne pathogen native to Asia and the Pacific Rim, where it is a significant cause of reproductive and neonatal loss in swine and severe encephalitis and death in humans. JEV is transmitted to vertebrate hosts by infected mosquito vectors, and like many other viruses transmitted by arthropods (arboviruses), it poses a current and future threat for invasion into new regions, especially as increasing global transportation of people, animals, plants, and goods provide opportunities for distant introductions of viruses and their vectors. The propensity of JEV to expand into new areas, and the resulting impacts and challenges, were recently highlighted by an unprecedented outbreak in Australia earlier this year. JEV does not currently circulate in the United States; however, the risk of its introduction has been assessed as high. Scientists of the recently established Foreign Arthropod-Borne Animal Diseases Research Unit (FABADRU) at the USDA's National Bio and Agro-Defense Facility address research gaps regarding the vulnerability of the U.S. to JEV through in-house and collaborative research programs. These gaps include identifying and understanding native host and vector species that could transmit JEV following an introduction into the U.S. The knowledge gained from these projects aims to inform risk assessments and predictive models and help identify target points to guide diagnostic development, surveillance programs, and control strategies.

**Historical analysis of N-glycosylation patterns in PRRSV-2 in the U.S.**

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One of the main challenges when controlling viral diseases is often related to their high mutation rate, which is sometimes associated to an ability to escape the immune response of the infected host. Glycosylation of proteins is a common post-translational modification in which oligosaccharides are added to specific portions of proteins, which may alter the protein folding and epitope availability for immune recognition, changing the glycosylation profile of a protein and potentially modifying how they are immunologically recognized. Our objective was to describe the N-glycosylation patterns over time and across different phylogenetic lineages of PRRSV-2, with the ultimate goal of better understanding the potential for immune-evasion in field settings and patterns of coexistence and emergence of different lineages. We analyzed data from the University of Minnesota Veterinary Diagnostic Laboratory from 2004 to 2021, comprised of 19,179 PRRSV ORF5 sequences. ORF5 sequences were translated to GP5 proteins and amino acids *sequons* that allow N-glycosylation to occur were identified using a custom-built code. Sequences were summarized according to which residues were potentially N-glycosylated, the year they were identified, and to which phylogenetic (sub-) lineage they belonged. The percentage of sequences with each N-glycosylation pattern per year per (sub-) lineages were used to compare if the emergence of novel strains was associated with changes in N-glycosylation patterns. A time-scaled phylogenetic (ML; GTR+G) tree was constructed and glycosylated residues for each sequence were visualized. We identified nine residues in GP5 where N-glycosylation occurred. The emergence of certain novel combinations of glycosylated sites coincided with past PRRSV-2 epidemics in the U.S. For lineage L1A, sequences glycosylated at sites 32, 33, 44, 51 and 57 first appeared in 2012, and this pattern represented more than 62% of all L1A sequences identified by 2015. This coincides with the emergence of the L1A 1-7-4 strain, which expanded from 8 to 86% of all L1A sequences identified in 2012 to 2015, respectively. The L1C 1-4-4 variant strain that emerged in 2020 also had a relatively distinct N-glycosylation pattern (at residues 32, 33, 44 and 51). In 2020 and 2021, this pattern was responsible for 44 and 47% of the L1C sequences identified in those years, contrasting to <5% in years prior. Our findings support the hypothesis that glycosylation patterns may contribute to the sequential dominance of different PRRSV-2 strains. Glycosylation patterns are almost certainly not solely responsible for new strain emergence; however, in a dynamic landscape of cross-immunity elicited by diverse immunization practices (live virus exposure and vaccination) and from natural infection, the fitness of viruses with specific N-glycosylation patterns may change over time and may contribute to the emergence of new strains. Further exploration of N-glycosylation patterns; how they affect the GP5 folding, and experimental studies could help understand how glycosylation patterns shape the viral ecology of PRRSV-2.

**Tenofovir and pro-drug tenofovir disoproxil fumarate inhibit porcine reproductive and respiratory syndrome virus [PRRSv] *in vitro***

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Infections by PRRSv are commonly followed by other opportunistic pathogen co-infections, forming the porcine respiratory complex [PRC]. *Actinobaccillus pleuropneumoniae* [*App*] is an important bacterium of the PRC and its supernatant is known to inhibit PRRSv replication *in vitro*. Analysis of the *App* supernatant by high resolution mass spectrometry [HRMS] revealed high concentrations of adenosine nucleotides. Many metabolites, such as 2-AMP and 3-AMP, were subsequently tested *in vitro* and were shown to reduce PRRSv replication at 1mM. Interestingly, analogs of these metabolites are one of the biggest family of antiviral drugs on the market and their mechanisms of action are well known.
In this preliminary study, two drugs and their respective prodrugs, four adenosine nucleotide analogs, were tested *in vitro* to evaluate their potential antiviral activity against PRRSv.
St-Jude Porcine Lung [SJPL] epithelial cells were infected with PRRSv and treated with 1uM, 10uM or 100uM of adefovir, adefovir dipivoxil, tenofovir or tenofovir disoproxil fumarate [Tdf]. Viral replication efficiency was determined with a viral antigen immunofluorescence assay [IFA] at 72h post-infection and with a PRRSv RT-qPCR gene specific assay conducted at 24h, 48h and 72h post-infection.

The most promising results were obtained with Tenofovir and Tdf treatments, which significantly inhibited PRRSv infection using both IFA and RT-qPCR assays, especially at concentrations of 10uM. Noteworthy, SJPL cells proliferation was not affected by these two drugs.

This study introduces promising preliminary results, suggesting tenofovir and its pro-drug, a well-established antiviral drug in HIV therapeutics, could inhibit PRRSv. Studies will be repeated in porcine macrophages and drug-repositioning will be evaluated for *in vivo* trials in PRRSv infected pigs.

**Partnership update: Functional genomics approach in livestock to delineate host factors critical for emerging coronavirus replication**

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Coronaviruses (CoVs) are noted for their ability to cross-species barriers and cause significant outbreaks in multiple species. Porcine deltacoronavirus (PDCOV) is primarily a swine pathogen capable of cross-species and zoonotic transmission. High throughput genomics technologies can rapidly identify critical host factors necessary for viral replication in differing species. The objective of these studies is to identify host genes critical for CoV transmission utilizing PDCOV infection in human and porcine cell lines through RNAseq and genomic CRISPR knockout (GeCKO) approaches.

Newborn pig trachea (NPTr), swine testicular (ST), porcine kidney epithelial cells (LLC-PK1), and porcine intestinal epithelial cells (IPEC-J2) were transduced with lentivirus to express Cas9. Cas9 expressing cell lines were assessed for susceptibility to PDCoV. IPEC-J2 and human intestinal epithelial cells (HIEC) cells were utilized for RNAseq experiments. IPEC-J2 and human intestinal epithelial cells (HIEC) were infected with PDCoV. At 24h post infection, total cellular RNA was harvested and analyzed using RNA-sequencing (RNA-seq).

NPTr cells, although susceptible to PDCOV infection, were not efficiently killed by PDCoV limiting their ability to be utilized for negative selection screening of the porcine GeCKO library. Transduction of ST, LLC-PK, and IPEC-J2 with Cas9 yielded multiple clones expressing Cas9. Comparing RNAseq of IPEC-J2 cells and HIEC cells infected with PDCoV identified 1,134 differentially expressed (DEGS) in pig cells and 7,486 DEGS in humans. These DEGs primarily resided in the NF-kappa-B transcription factor family, interferon (IFN) family, protein kinase family, and signaling pathways such as apoptosis, JAK-STAT, inflammation/cytokine, Toll-like receptor, NOD-like receptor, Ras, and cytosolic DNA-sensing pathways. Further DEG comparison of the human PDCoV host response to the host response from the betacoronavirus SARS-CoV-2 shows many similarities for coronavirus infections.

NPTr cells are inefficiently killed by PDCoV, necessitating the creation of new Cas9 expressing porcine cell lines for GeCKO library screening. PDCoV elicits distinct transcriptional profiles in human and pig intestinal cells that have conservation between delta- and betacoronaviruses.
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**Experimental pig study comparing contemporary and historical porcine respiratory coronavirus isolates in pigs with and without subsequent influenza A virus infection**

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*Porcine respiratory coronavirus* (PRCV), first reported in 1984 in Belgium, is a deletion mutant of *transmissible gastroenteritis virus* (TGEV). PRCV has a respiratory tract tropism commonly associated with respiratory disease in young pigs. The primary objective of this study was to compare the infection dynamics of strains isolated in 1991 and 2020. The impact of PRCV on a pig can be two-fold: induction of disease and/or potentiation of other respiratory pathogens. Hence the secondary objective was to investigate the impact of PRCV on subsequent influenza A virus (IAV) infection.

In brief, 35 4-week-old pigs were divided into six groups: PRCV-2020 (n=5), PRCV-1991 (n=5), IAV (n=5), PRCV-2020/IAV (n=5), PRCV-1991/IAV (n=5) and negative control pigs (n=10). On day 0 of the study, pigs were inoculated with 4ml of PRCV-1991 or PRCV-2020 strain intranasally. PRCV-2020, PRCV-1991 and five negative controls were necropsied at D3. Except for the negative controls, all remaining pigs were challenged at D5 with a contemporary IAV H1N1 isolate. Respiratory scores and rectal temperatures were recorded daily. Nasal swabs were taken daily until D10 and were tested by both PRCV and IAV PCR. Serum samples were collected at D3 and D10 and were tested by IAV NP ELISA and TGEV/PRCV differential ELISA. The second necropsy was done at D10, corresponding to 5 days post IAV challenge. Gross lung lesion scores were assessed and tissues were collected for histopathology and immunohistochemistry.

Elevated respiratory scores were observed in the PRCV-infected pigs on D1, D2 and D3. Until D5, PRCV-2020 pigs had significantly higher RNA shedding compared to the PRCV-1991 pigs. Pyrexia was observed at D6 (D1 post-IAV inoculation) in all IAV infected groups, with no significant differences between IAV-infected groups. IAV shedding was uniform across IAV positive control group and both PRCV co-infected groups. Negative controls remained negative over time by both PCRs. Higher ELISA antibody titers were observed in pigs infected with PRCV-2020 compared to PRCV-1991, which correlates with clinical signs and PCR data. Necrosis and inflammation of nasal turbinate were detected in PRCV inoculated pigs at 5 DPI. Necrotizing bronchitis and severe interstitial pneumonia were present in both groups of pigs co-infected with PRCV and IAV.

Compared to the 1991 PRCV, the 2020 PRCV caused slightly more severe clinical respiratory disease and increased amount and length of shedding. Pre-infection of pigs with PRCV did not enhance disease or lesions caused by IAV infection five days later.

**Experimental pig study comparing pathogenicity of PRRSV 1-4-4 L1C variant with other Lineage 1 strains**

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A PRRSV 1-4-4 L1C variant strain emerged in the USA in October 2020 and has spread widely, causing high production losses based on field observations. However, no unequivocal experimental data was available to determine the virulence phenotype of the 1-4-4 L1C variant. In this experimental pig study, the virulence and transmissibility of the 1-4-4 L1C variant strain was characterized in comparison with three other circulating 1-4-4 strains (L1C non-variant, L1A, and L1H) and one highly virulent 1-7-4 L1A strain. Seventy-two 3-week-old PRRSV-naive pigs were divided into 6 groups with 12 pigs per group. Forty-eight pigs (8/group) were for inoculation and 24 pigs (4/group) served as contact pigs. After one-week acclimation, pigs were inoculated with the corresponding virus or negative media intramuscularly and intranasally. At 2 days post inoculation (DPI), contact pigs were added to the pen adjacent to the inoculated pigs in each room. Daily temperature and clinical signs were recorded. Serum and oral fluid samples were collected at 0, 2, 4, 7, 10, 14, 21 and 28 DPI. Pigs were necropsied at 10 & 28 DPI. Fresh and formalin-fixed tissues were collected at necropsy for examinations. For all analyses, SAS was used and a p-value $\leq 0.05$ was considered significant.

The 1-4-4 L1C variant-inoculated pigs became more anorectic and lethargic, had higher mortality, had higher percentage of pigs with fever (>40°C) during 0-10 DPI, and had significantly higher average body temperature than other virus-inoculated groups at several DPIs. The 1-4-4 L1C variant-inoculated group had significantly higher viremia levels compared to all other groups at 2 DPI. Collectively, 4/4, 2/4, 2/4, 0/4, and 2/4 contact pigs in the L1C variant, L1C non-variant, L1A, L1H, and 1-7-4 L1A groups became viremic at 2 DPC. There were more severe gross lung lesions in the 1-4-4 L1C variant-inoculated group compared to others except the 1-7-4 L1A group at 10 DPI. The differences of ADG, microscopic lung lesion score, IHC score, and RNA level in different tissues at 10 DPI were not statistically significant between virus-inoculated groups. Serum antibodies in all virus-inoculated groups were readily detected by the commercial PRRS X3 ELISA and the ISUP virus strain-based IFA antibody assays during 7-28 DPI.

This study provides experimental data in weaned pigs regarding the clinical impact, pathogenicity, transmissibility, and antibody detection of the newly emergent 1-4-4 L1C variant strain, along with comparisons with other PRRSV strains. The findings confirm 1-4-4 L1C variant is highly virulent in weaned pigs. The higher number of contact pigs becoming viremic at 2 days post contact implies the L1C variant strain may have higher transmissibility than other PRRSV strains although it needs to be confirmed with a study involving more pigs.

**Role of PSTII in PRRSV-CD163 interaction**

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Expression of the scavenger receptor, CD163, on porcine macrophages is required for PRRSV infection. The CD163 protein on the surface of macrophages is composed of nine scavenger receptor cysteine-rich (SRCR) domains, two 35-amino-acid proline-serine-threonine (PST)-rich regions, a transmembrane region, and a cytoplasmic tail. To evaluate the role of PSTII in PRRSV infection, HEK cells were transfected with mutated CD163 cDNAs and infected with a Type 2 PRRSV-GFP. The 16 amino acid PSTII peptide sequence of porcine CD163 is encoded by two different exons. The first 12 amino acids are contributed entirely by Exon 13, while the remaining four amino acids, Gly-Arg-Ser-Ser (GRSS), which are located on the cell surface are contributed by Exon14. The first construct consisted of a AASA substitution for the GRSS peptide sequence. Transfected HEK cells supported PRRSV replication. To evaluate the contribution of the Exon 13, the first 10 amino acids of the predicted translation product were deleted. The construct was resistant to PRRSV infection. Flow cytometry using an anti-CD163 antibody showed that all constructs were expressed on the cell surface. Together, these results showed that the deletion of the Exon 13 peptide of CD163 confered resistance to PRRSV.

**Viral load and inflammatory response in non-lymphoid fetal tissues following late gestation PRRSV-2 challenge**

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Objective:
Recent data suggests an impact of PRRSV infection on terminal maturation in a wide array of vital fetal organs; however, the underlying mechanism behind these effects remains a fundamental unknown. While evidence of widespread viral distribution exists, fetal infection status has historically been determined based on viral load in serum and thymus. This study investigates the quantitative distribution of virus among non-lymphoid tissues of fetuses categorized by these classical metrics of infection and investigates the associated local inflammatory response.

Methods:
Fetal tissue samples were obtained from a prior challenge study in which 22 gilts were inoculated with NVSL 97-7895 on gestation day 85 and sample collected 21 days later. A subset of fetuses from PRRSV inoculated gilts were selected based on previously established model of biologically extremes including uninfected (UNIF n=10), high viral load viable (HV-VIA n=10) and high viral load meconium-stained (HV-MEC n=10), and compared with gestational age matched controls (CON n=10) from mock infected gilts. From each selected fetus, total RNA was extracted from six non-lymphoid tissues including brain (BRN), heart (HRT), kidney (KID), liver (LVR), lung (LNG) and skeletal muscle (MUS). PRRSV viral load was determined by absolute quantification relative to a standard curve comprised of plasmid DNA containing the target region in ORF7. Local inflammatory response and recruitment of PRRSV susceptible cells were evaluated in the form of interferon gamma (IFNG) and CD163 expression respectively.

Results:
PRRSV RNA was not detected in any non-lymphoid tissue from CON or UNIF fetuses. In contrast, all six tissues from HV-VIA and HV-MEC fetuses had detectable virus, with the highest viral load observed in HRT, KID, and LNG. No significant differences in tissue viral load were detected between HV-VIA and HVL-MEC groups. Expression of IFNG was significantly elevated in all six tissues from HV-VIA and HV-MEC groups relative to both CON and UNIF. Expression of CD163 was significantly greater in BRN, KID, and LNG of HV-MEC fetuses relative to CON and UNIF, but not HV-VIA.

Conclusions:
The lack of virus detected in fetuses previously categorized as UNIF indicates serum and thymic viral load are sufficient for binary categorization of infection status. The virus shows a surprisingly wide distribution within the highly infected fetus, suggesting infection of tissue specific resident macrophage populations including muscle satellite and glial cells. Viral load in non-lymphoid tissues does not appear to be a factor in fetal resilience, but the resulting local inflammatory response may explain previously described effects on fetal development.

**Codon and conditions optimization for ASFV protein expression in vitro by mRNA expression system: A rapid and adaptable vaccine platform**

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African swine fever virus (ASFV) is a highly contagious disease leading to acute deaths in domestic pigs and wild boars with a 100% mortality rate. ASFV is an icosahedral DNA virus comprising envelope, capsid, inner capsule membrane, core-shell, and inner core. ASFV genome is approximately 170kb-190kb and encodes 150-200 viral proteins, including 68 structural proteins and more than 100 non-structural proteins. Proteins p22 and p72 are encoded by KP177R and B646L genes, respectively. Protein p22 is an ASFV early transcribed structural protein. Although its function is unknown, p22 has been recognized as an interacting partner of several host proteins. Proteins p72 is involved in the virus cycle process, with strong immunogenicity and the ability to induce neutralizing antibody production. We hypothesize that this set of proteins individually or in specific combination could be used as a potential target for an effective non-replicative mRNA-based vaccine against ASFV. Therefore, this study aimed to evaluate the codon optimization, transfection efficacy, and protein production in vitro in different eukaryotic systems of different non-replicative mRNAs for potential ASFV vaccine usage. Briefly, codon-optimized KP177R and B646L genes were ligated into a cloning vector together with the T7 promotor, 5'-untranslated region (UTR) of a human alpha-globin gene, Kozak sequence, and 3'-UTR of a human alpha-globin gene. All recombinant plasmids were linearized by restriction digestion in vitro transcribed before transfection. Different conditions were evaluated, including (1) mRNA doses, (2) transfection reagents, (3) mRNA/transfection reagent ratios, (4) cell lines, and (5) transfection durations. Effective mRNA transfection was confirmed by fluorescence in situ hybridization (FISH) with specific mRNA probes and protein expression by immunofluorescence assay (IFA) and western blot. Different non-replicative mRNAs were effectively transfected into the BHK-21 cells, and p22 protein can be expressed at different mRNA doses. The results suggest that the optimized mRNA conditions with lipoplex can transfect and is a viable method for ASFV p22 protein production in vitro. Future studies include optimization of conditions for successful transfection and expression of p72 protein. In addition, further in vivo studies will be necessary to evaluate the immunogenic efficacy of the non-replicative mRNA-based as a potential vaccine platform against ASFV.

**Senecavirus a (SVA) and foot-and-mouth disease virus (FMDV) viral-like-particle (VLP) based vaccines induced cellular and humoral immune response in pigs**

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Senecavirus A (SVA) and foot-and-mouth disease virus (FMDV) cause vesicular disease characterized by stomatitis, vesicles on the snout, and coronary bands, leading to economic losses in the swine industry worldwide. Clinically, SVA and FMDV infection cannot be distinguished, resulting in increased foreign animal disease investigation in countries free of FMDV and increased economic losses in countries with a concomitant circulation of both viruses. Numerous commercial vaccines are available against FMDV, but no commercial vaccines are available against SVA. This study aims to develop and evaluate the immunogenicity efficacy of SVA and FMDV viral-like-particle (VLP) based vaccines. In this study, SVA and FMDV viral-like-particle (VLP) based vaccines were produced in baculovirus expression system. Forty-five 3-weeks-old pigs were allocated into 9 experimental groups (5 pigs/group). Pigs were vaccinated with SVA VLP with and without AddaVax adjuvant, killed SVA with and without AddaVax adjuvant, AddaVax adjuvant, FMDV VLP with and without AddaVax adjuvant, killed FMDV with and without AddaVax adjuvant, or only AddaVax adjuvant at two weeks intervals. Blood samples were collected at 0, 7, 14, 21, and 28 days post-vaccination (DPV). The humoral and cell-mediated immune response was evaluated. Vaccination with SVA VLP with and without AddaVax induced viral neutralizing (VN) antibodies detectable from 14 DPV until the end of the study. Moreover, the proliferation of the CD4+ T-cell population increased at 28 DPV. Vaccination with FMDV VLP with AddaVax induced VN antibodies at 14 DPV detectable until the end of the study. By 7 DPV, IgG levels against FMDV VLP were elevated and maintained until 28 DPV, while the IgG levels against viral protein 2 (VP2) and VP3 were boosted from 7 to 21 DPV. The IFN-γ–producing CD4+ cells increased at 7 DPV. In addition, the FMDV VLP vaccination group had also stimulated the IFN-γ–producing CD4+CD8+ cells. In conclusion, vaccination with SVA VLPs can induce cellular immune responses either T-cell proliferation or IFN-γ producing T-cell earlier and stronger than killed virus vaccine. Although there is no difference in the percentage of IFN-γ producing T-cell between FMDV VLPs and killed virus vaccinated groups. The vaccination with FMDV VLPs can induce CD8+ and double-positive T-cell proliferation higher than killed virus vaccine. The vaccination with either SVA or FMDV VLPs with AddaVax could effectively increase viruses-specific neutralizing antibodies which are necessary for protection against viral infections. Thus, the developed VLPs vaccines might be used instead of killed virus vaccines since they are safer and can stimulate both humoral and cellular immune responses. However, the viral challenge model should be further clarified.

**Deletion of CD163 domain five protects pigs from infection with porcine reproductive and respiratory syndrome virus (PRRSV) infection**

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Expression of the scavenger receptor, CD163, on porcine macrophages is required for PRRSV infection. CD163 is composed of nine scavenger receptor cysteine-rich (SRCR) domains, two 35-amino-acid proline-serine-threonine (PST)-rich regions, a transmembrane region, and a cytoplasmic tail. CD163 is responsible for scavenging excess hemoglobin from the blood. Previous work by us demonstrated that pigs lacking CD163 are resistant to PRRSV infection. Several studies indicate that SRCR5 (exon-7) is the domain required for infection. In this study, pigs lacking SRCR5 were constructed at the University of Missouri. CRISPR/Cas9 was used to knock out exon-7 within the *CD163* gene. The removal of exon-7 was confirmed by genomic sequencing. In addition to the knockout (KO), the CRISPR technique created a novel mutation that consisted of a 33 bp insertion located at the beginning of exon-7. This construct resulted in 11 amino acids located between the interdomain region and the beginning of the domain 5 polypeptide. Three wild type (WT), five SRCR5 KO, and two 33bp pigs were infected with a PRRSV-2 isolate and followed for 14 days. Virus was detected using RT-PCR for amplifying virus nucleic acid in serum. The results showed that all WT and the 33bp pigs were viremic, whereas the SRCR5KO pigs remained PCR-negative. The results demonstrate that the SRCR5 KO pigs are resistant to PRRSV, which creates the opportunity to construct pigs that are resistant to PRRSV but retain normal CD163 functions.

**A participatory approach to enhancing the passive surveillance of African and Classical swine fevers**

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African swine fever (ASF) and classical swine fever (CSF) are hemorrhagic, viral diseases of swine causing widespread and severe impact to the global swine industry. Despite the efforts that animal health officials have dedicated to controlling these diseases, new introductions are occurring each year. These viruses cause significant economic impact and animal health burden to affected countries, and an introduction in the US would be disastrous. For example, it has been estimated that an ASF incursion in the US would cost over $50 billion USD in 10-year scenario. In many cases, the first detection of these diseases in free areas has been delayed for weeks to months despite ongoing active surveillance and resulting in widespread virus circulation. Passive surveillance activities performed by producers and private veterinarians may be the best opportunity for the early detection of ASF and CSF introductions in the US and other free areas. To enable wide-scale and consistent implementation of passive surveillance, primary reporters need tools to standardize the collection and interpretation of surveillance data. Here, we developed and piloted a participatory, enhanced passive surveillance (EPS) protocol based on regularly collecting data and applying an objective and adaptable scoring system to aid the early detection of ASF or CSF incursions at the farm level. The EPS protocol contains three components: the biosecurity of the farm, syndromic surveillance of clinical findings, and necropsy findings. To evaluate its use and application for disease detection, the protocol was piloted for 21 weeks in two commercial pig farms in the Dominican Republic, a CSF- and ASF-infected country. After an initial biosecurity assessment, swine producers or their veterinarian regularly recorded clinical and necropsy findings, and the assigned scores were monitored using temporal detection in SatScan. The EPS protocol detected substantial variations in the risk score and triggered testing in one of the farms based on that variation, although the test results were negative. Findings from this pilot have led to proposed changes to improve its feasibility to implement in the context of US swine farms. Additionally, lessons learned here about identifying suspect pigs for ASF and CSF testing may inform future efforts to adapt the protocol for detection of any health event, such as endemic diseases in the US like porcine reproductive and respiratory virus. Overall, results suggest that standardized EPS protocols may contribute to the early detection of disease introductions by triggering sampling activities to confirm or rule out suspects, with the ultimate goal of promoting public-private partnership to mitigate the impact of foreign and endemic animal disease incursions into free regions and farms.

**Contrasting PRRSV temporal lineage patterns at the production system, state, and regional levels**

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Porcine reproductive and respiratory virus (PRRSV), one of the most significant diseases in the swine industry, has been challenging to control due to its high mutation rate and complexity. The objective of this retrospective study was to describe and compare the dominance of PRRSV lineages obtained at the system, state, and multi-state levels.

PRRSV sequences obtained from Ohio swine farms during 2017 – 2021 were obtained from one regional swine production system (n = 148) and from the regional state laboratory (Ohio Department of Agriculture, Animal Disease Diagnostic Center (ODA-ADDL, n = 426). The MUSCLE algorithm on Geneious Prime® was used to align the ORF5 region of PRRSV sequences along with PRRSV vaccine strains (n = 15) and lineage anchors (n = 169). Isolated PRRSV sequences were assigned to the most identical lineage anchor sequences. Results were compared to PRRSV lineages from Midwest states using the Swine Disease Reporting System (SDRS) publicly available database.

At the production system level, lineage L1A was dominant over the 5 years (> 50% of sequences), except in 2018 where nearly half of the sequences were classified as L1H. In contrast, among sequences from ODA-ADDL, L5 was dominant in 2017 (61%) and 2019 (54%), while L1A was dominant in 2018 (63%), 2020 (65%), and 2021 (72%). A small proportion (< 4%) of PRRSV sequences in L1C, L1F, L1H, and L8 were identified at both levels. At the multi-state level, lineages L1A (24.4% – 38.6%) and L5 (16.7% - 23.7%) were the top two identified lineages in the SDRS database in 2017 – 2021.

**Porcine reproductive and respiratory syndrome virus induces degradation of the promyelocytic leukemia protein and promotes viral replication**

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The promyelocytic leukemia (PML) protein, also known as TRIM19, is one of the interferon (IFN) stimulated genes and plays a critical role in antiviral activities. Seven different PML isoforms share the identical sequence in the N-terminal region, but the C-terminal regions are variable. Porcine reproductive and respiratory syndrome virus (PRRSV) inhibits the host IFN response, and the nonstructural protein (nsp) 1beta of PRRSV has been determined as the main IFN antagonist. In the present study, we investigated the interaction of PRRSV and PML and showed that overexpressing all six isoforms of PML in MARC-145 cells restricted the PRRSV replication. Among the seven isoforms. PML-II or PML-IV showed the most significant suppression of PRRSV growth. PRRSV replication was increased in PML gene-knockdown cells. Furthermore, PRRSV infection downregulated the PML protein expressions by 24 hours postinfection. The nsp1beta protein of PRRSV was the viral protein that mediates PML degradation. The nsp1beta-mediated reduction of the PML expression was not the post-transcriptional event but rather post-translational regulation. PML was found to directly bind to nsp1beta, as demonstrated by GST pull-down, colocalization, and co-IP assays. The mutations at two of the four potential SUMO interacting motifs (SIMs) in nsp1beta resulted in the reduced ability to bind to PML proteins. Subsequently, double mutations in the SIM motif of nsp1beta did not bind to PML, and the PML expression was restored compared to that in wild-type nsp1beta-expressing cells. Our findings demonstrate that SIMs of nsp1beta play a critical role in the binding to PML and PML degradation. This study reveals a novel strategy for PRRSV to promote viral replication during infection.

**Effect of pen size and number of ropes on behaviors associated with oral fluid sampling**

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Introduction. Oral fluids are the sample of choice for routine surveillance on commercial farms. The method is well established in the swine industry because of the advantages it offers over individual samples, i.e., oral fluids are easily collected with no stress to workers or pigs and, overall, oral fluids are more likely to detect the pathogen of interest and at lower cost than individual pig samples. There are many refereed publications reporting the use of oral fluids for the detection of specific pathogens, but there are few studies elucidating the pig behaviors associated with oral fluid collection. In pens of ~25 pigs, White et al. (2014) reported that > 70% of pigs participated in OF collection in 30 minutes. In pens of 16 - 30 pigs, Graage et al. (2019) reported that 76% -82% of pigs contributed to the sample. However, given the wide range of pen sizes used in the US industry (20 - 1000 pigs), more extensive information about pig participation in oral fluid sampling is required for surveillance design.

Material and Methods. Our long-term objective is to develop industry guidelines for OF collection across pen sizes. The aim of this study was to measure the effect of pen size and number of ropes on pig-rope interactions. The study was performed in 10-14 week-old pigs housed in pens of ~25, ~65, ~100, and ~130 pigs (32 pens for each size category) on commercial wean-to-finish production sites. In each pen, ~12% of pigs were individually marked for observation. Treatments consisted of placing 1, 2, 3, or 4 ropes in a pen (0.5-inch, 3-strand, twisted 100% cotton) and video recording pig interactions with the rope for 30 minutes. Each treatment (number of ropes) was randomly assigned to pens and repeated 8 times, i.e., once in each of 8 different pens. Video recordings were reviewed and rope contacts were counted for each marked pig by minute of the observation period, with "contact" defined as a video image of the pig with the rope in its mouth.

Results. Pig behavior related to pig-rope interaction was affected by pen size and the number of ropes provided. For example, when one rope was placed in the 8 pens of 25 pigs, 71% (17 of 24) of the 3 marked pigs interacted with the rope; when 4 ropes were provided, 92% (22 of 24) of the marked pigs interacted with the rope(s). In contrast, when one rope was placed in 8 pens of ~130 pigs, 23% (28 of 120) of the marked pigs interacted with the rope; 48% (58 of 120) when 4 ropes were provided.

Discussion. This study documented changes in oral fluid sampling behaviors as a function of pen size and the number of ropes provided in the pen. Importantly, this work is strictly focused on pig behavior. Future work will expand to include the probability of detection as a function of pen size, number of ropes, and pig behavior.

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**Characterization of Carbapenem Resistant *Enterobacteriaceae* from Nairobi River, Wastewater Treatment Plant and Slaughterhouse in Nairobi**

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Introduction: Carbapenemase-producing *Enterobacteriaceae* (CPE) are rare, highly multidrug resistant bacteria often associated with hospitalized patients. CPE can be disseminated from healthcare settings through wastewater flows to wastewater treatment plants (WWTP). WWTPs are not designed to sterilize wastewater and may serve as reservoirs for the dissemination of bacteria of clinical concern, such as CPE, into receiving surface waters. Currently, there is little information regarding the frequency and diversity of CPE entering WWTPs and the potential impact of CPE on the downstream ecosystem as well as animal agriculture facilities. Therefore, this study sought to determine frequency and characterization of Carbapenem Resistant *Enterobacteriaceae* from Nairobi River, Wastewater Treatment Plant and Slaughterhouse in Nairobi, Kenya.

Methodology: Wastewater samples were collected in 250ml bottles from Dandora WWTP influent, effluent, upstream, downstream and way downstream of Nairobi River and Swiffer samples from Dagoretti slaughterhouse in Nairobi. Water samples were filtered to capture bacteria. The resulting filters plus Swiffer samples were incubated overnight in MacConkey (MAC) broth modified with 0.5 µg/ml meropenem and 70 µg/ml of ZnSO4 at 37°C. Selective MAC agar supplemented with 0.5 µg/ml of meropenem and 70 µg/ml of ZnSO4, were inoculated and incubated overnight. Based on morphological characteristics, up to three morphologically distinct colonies were tested for carbapenemase production using the Carba NP test.
Results: In total 594 isolates have been recovered, and (688, 67.19%) were phenotypically positive for CPE using carba-NP test from 256 samples collected. Of the samples collected, 108 (42.19%) were from WWTP, 62 (24.22%) from Nairobi River and 74 (28.91%) from slaughterhouse. CPE resistance genotypes were $bla_{\text{NDM}}$ (415, 69.98%), $bla_{\text{KPC}}$ (26, 4.38%) and $bla_{\text{VIM}}$ (21, 4.26%) genes respectively. Organisms identified were *E. coli, Klebsiella pneumonia, Enterobacter, Acinetobacter* and *Citrobacter species*.

Conclusion: Wastewater acts as a reservoir for the dissemination of hospital associated resistance bacteria of critical public health concern.

**Diagnostic performance of two pen-side tests for detection of African swine fever virus in Vietnam**

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African swine fever (ASF) is one of the most devastating livestock diseases, with a mortality rate that can approach 100% of domestic pigs. Early identification of ASF is critical for disease control. The main objective of this research was to evaluate diagnostic performance of two pen-side tests for ASFV detection: PCR test for the detection of viral genomic DNA and lateral flow test for the detection of viral antigens.

To determine the time from infection to the earliest detection, 10 ASFV- seronegative pigs were housed in purchased from a high heath farm and accommodated at the animal facility at Vietnam National University of Agriculture (VNUA). Whole blood samples were collected from all pigs at 3 time-points prior to inoculation to serve as negative controls. After that the pigs were inoculated intramuscularly with $10^{4.0}$ hemadsorption dose 50 (HAD50) of a virulent strain of ASFV currently circulating in Vietnam. Whole blood samples were alternatively collected from each group of pigs every other day until all pig died from the infection (by 10 days). The samples were tested by the two pen-side tests immediately after collection. A portion of samples were then transported to the veterinary diagnostic lab to test with a reference real-time PCR test to determine the true status of the samples. A total of 87 samples were collected including 30 samples collected before infection and 57 samples collected after infection. The pen-side PCR test detected infected pigs starting from 2 dpi and consistently detected infection until the end of the study (10dpi). The antigen test detected infected pigs starting from 3 dpi and no longer detected infection at 10 dpi. Both pen-side PCR and antigen tests accurately detected the 41 ASFV-negative samples with the specificity of 100%. Of 46 samples tested positive by the reference real-time PCR test, the pen-side PCR test identified 45 samples positive (97.8% sensitivity) while the pen-side antigen test detected 22 samples positive (47.8% sensitivity). The low sensitivity of the antigen test was mainly because it did not to detect infection from samples collected early or late after infection.

To evaluate the diagnostic performance of the tests with samples collected from commercial farms, 105 whole blood samples were collected from 14 farms with typical clinical signs of ASF. Additionally, one hundred whole blood samples were collected from 10 farms without signs of ASFV to serve as negative controls. In total 205 samples were collected 34 tested positive and 171 samples tested negative by the reference PCR test. Both pen-side tests had 100% specificity. The sensitivity of the pen-side PCR test was 88.2%, while the sensitivity of the antigen test was 50% when tested with whole blood.

In summary, under the conditions of this study, the pen-side PCR test exhibited greater sensitivity and detected infected pigs earlier and for a longer duration after infection than the antigen test.

**Investigation of fetal gene expression patterns in the liver, heart, and kidney for prediction of reproductive failure**

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Porcine reproductive and respiratory syndrome (PRRS) is one of the costliest diseases to pork producers worldwide. Since its discovery there have been multi-pronged initiatives aimed at addressing this viral disease. The pregnant gilt PRRS infection model has emerged as an essential design for facilitating research into vertical transmission and the fetal response to infection. The goal of this work is to identify critical tissues and genes that forecast fetal resilience or susceptibility following PRRS virus (PRRSV) infection. In this study, pregnant gilts ($n$ = 30) were infected with PRRSV at day 85 of gestation. At 21 days post maternal infection, gilts and fetuses were euthanized, and fetal tissues collected for further investigation.

The fetuses in this study were selected based on viral load in fetal serum and thymus which was determined by quantitative PCR and used in combination with meconium staining status to select three distinct subsets including uninfected (UNIF), resilient (> 5 log viral load but viable) and susceptible (> 5 log viral load and meconium stained). Total RNA was extracted from the liver, heart, and kidney of $n$ =10 fetuses per group. RNA samples were assessed using Qubit for RNA concentration and overall quality. Gene expression was determined using a 58 gene NanoString array. Genes were selected to target five pathways hypothesized to be involved in fetal resilience or susceptibility. Analysis of liver samples revealed several ($n$ = X) differentially expressed genes associated with immune response, transporters, and growth modulating pathways. Further analysis of the fetal kidney and heart should provide insight into the mechanisms of fetal compromise in PRRSV infected gilts, and inform clinical treatments, and virus management approaches in herds.
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**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 candidate gene (*SYNGR2*) and a missense DNA polymorphism (*SYNGR p.Arg63Cys*) associated with susceptibility to PCV2b, a prevalent swine DNA virus. The objective of this study was to validate, characterize and provide direct evidence of the role of *SYNGR2* and its alleles in susceptibility to different PCV2 isolates. The porcine kidney 15 cell line (PK15) is of epithelial origin and a well-established model for PCV2 infection and cellular pathogenesis. PK15 cells are naturally homozygous for the *SYNGR2 p.63Arg* allele, associated with increased susceptibility to PCV2b during *in vivo* experimental infection, while the alternate *SYNGR2 p.63Cys* allele was associated with PCV2b resilience. Using CRISPR-Cas9 mediated gene editing of the PK15 cell line (*wtSYNGR2+p.63Arg*), we generated a predicted *SYNGR2* knock-out clone (*emSYNGR2-del*) and a clone homozygous for the alternate *SYNGR2 p.63Cys* allele (*emSYNGR2+p.63Cys*). The knock-out clone is homozygous for a partial deletion (106 bp) of the second exon that includes the *SYNGR2 p.Arg63Cys* polymorphism and a key motif predicted to influence *SYNGR2* function. Wild type and edited PK15 cells were cultured in 12 well plates and after 24 hours were infected with inoculates representing three PCV2 subtypes (PCV2b, PCV2a and PCV2d). Cell and supernatant samples were collected at multiple time points post infection and viral copy number was obtained by qPCR specific for the PCV2 Capsid gene. PCV2b infection of both edited PK15 clones, *emSYNGR2-del* and *emSYNGR2+p.63Cys*, resulted in decreased viral copy number compared to wildtype PK15, *wtSYNGR2+p.63Arg* ($P<0.05$). This effect was consistent across the other genetically distinct PCV2a and PCV2d isolates. A reduction in total viral genome copies within edited clones at 24 hours compared to wildtype PK15, indicates a potential function of SYNGR2 and the *SYNGR2 p.Arg63Cys* alleles in the early stages of PCV2 infection, prior to viral replication. Specifically, we hypothesize that *SYNGR2* has a role in the endocytosis and/or nuclear translocation of virions. To our knowledge, this is the first study to validate the causality of a DNA polymorphism in viral disease susceptibility in swine. This research will generate knowledge that could be applied to improve animal health and welfare as well as reduce economic losses to the swine industry.

**Differentiation of PRRS Vaccine Strains Using Luminex Bead-Based Technology**

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Since its emergence in North America in the late 1980s, PRRSV has become one of the most problematic pathogens in swine production systems, and has caused significant economic losses in the US. The viral genome has undergone constant changes and new variants have evolved over time, which makes molecular diagnosis of PRRSV very challenging especially in differentiation of field strains from vaccine strains. Traditionally, there are four PRRS vaccines, Ingelvac MLV, Ingelvac ATP, Fostera and Prime Pac, have been used in the US. Differentiating vaccine strains from the field strains is an important aspect to guide and improve vaccine applications. The vaccine strains are very similar to some of the field strains and are primarily differentiated by ORF5 sequencing, which is expensive and time consuming. The Luminex xTAG assay is a bead-based nucleic acid detection that hypothetically can analyze more than 100 different targets in a single reaction. In this study, a Luminex multiplex assay was developed to detect the vast majority of PRRSV-2 field strains, and to differentiate the four US vaccine strains. A collection of 694 full or near-full genome sequences of PRRSV-2 strains was analyzed. A pair of primers each targeting the M and N genes were designed for general detection of viral strains. The coverage for each set is 85.4% and 91.2%, respectively, with a combined coverage of 98.1%. Four pairs of primers targeting the nsp2 gene of vaccine strains were designed for vaccine differentiations. Testing on field samples and the four vaccine strains indicated that the assay detected all PRRSV-2 strains and identified each of the four vaccine strains accurately. To evaluate the limit of detections (LODs), a real-time RT-PCR was used for comparison. The LODs of the Luminex assay were equivalent to Ct 35.8 for MLV, Ct 33.2 for ATP, Ct 31.2 for Fostera, and Ct 36.1 for Prime Pac. The assay also included PCV2 and PCV3 detection with diagnostic coverage of 98.9% for PCV2 and 100% for PCV3 strains. LODs were equivalent Ct 36.4 by PCR for PCV2 and Ct 35.6 for PCV3. Work on adding the new PRRS vaccines, Prevacent PRRS and PRRSGard, into the assay is in progress.

**National PRRS reduction strategy launched in Denmark**

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PRRS is one of the most significant pig diseases in Denmark. The most common transmission route of PRRSV is by trade with infected pigs and by local spread which in recent years has resulted in an increase of PRRSV seropositive pig herds in Denmark.
Eradicating PRRSV at herd level by using well-established protocols are common in Denmark but the risk of reinfection by local spread of the virus from neighboring pig herds can be high in pig dense areas. A joint strategy to reduce PRRSV in Denmark has therefore been developed in a collaboration between veterinary authorities, the pig industry, slaughter companies, veterinarians, and producers.

The goal of the national strategy is that 75% of all finishers will be PRRSV seronegative at slaughter and 85% of all sow herds will be PRRSV seronegative. This will be achieved through targeted management of PRRSV, which will provide an incentive for increasing the numbers of PRRSV negative finisher herds. This, in turn, will increase demand for PRRSV seronegative piglets and provide an incentive for eradicating PRRSV from sow herds.
Danish pig production has good preconditions for combating PRRSV at national level; there is no import of live pigs, nucleus and multiplier herds are PRRSV negative, and there is access to high-quality databases for geographic location of herds, health status and trading/movement of pigs. Furthermore, Denmark is surrounded by sea, which limits the risk of infection from neighboring countries.

A cornerstone of the reduction strategy in Denmark is mandatory declaration of serological PRRS status, based on annually blood analyses, of all pig herds. The serological PRRSV status from each herd is shared real-time via a public database. This means that producers and veterinarians get access to information on regional and national prevalence of PRRS and thereby enable initiatives for regional eradication of PRRS to be taken on a professionally informed basis. The mandatory herd declaration applies to all herds in Denmark with more than 10 sows or more than 100 animals in total. The PRRSV surveillance will be based on the Danish SPF-system principle, that health status is declared for the connected herds. Health status of pigs is considered in case of trading and transport between herds.

Danish Crown, which slaughters about 70% of all Danish pigs, have decided to make a deduction of DKK 0.20 per kg (= 0.027 euros/Kg carcass) the first 17 weeks that a herd is PRRS virus-positive, and the deduction will subsequently increase gradually. This will, in line with the rest of the strategy, give motivation for finishing pig producers to eradicate PRRSV from their herd. Afterwards the other Danish slaughter companies also decided a deduction for PRRS virus-positive herds.

Experience from previous voluntary initiatives has shown that regional eradication programs for PRRSV can be difficult and require thorough coordination and communication between veterinarians and pig producers. The Danish Agriculture & Food Council will therefore organize regional initiatives to ensure that a systematic approach is applied to eradication in specific regional areas with high density of PRRS seropositive herds. The goal is to bring veterinarians and pig producers in the area together to develop a common strategy for reducing PRRS in the geographical area.

**Incidence of porcine parainfluenza virus 1 (PPIV1) co-infections with influenza A virus (IAV) and porcine reproductive and respiratory syndrome virus (PRRSV) in herds with respiratory disease**

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Porcine parainfluenza virus 1 (PPIV1) is also known as *porcine respirovirus* 1 (PRV1). The virus has been detected in Asia, the Americas and Europe, but knowledge on its epidemiology and genetic diversity remains very limited. The virus was detected in clinically healthy animals, but also in pigs with respiratory clinical signs, including sneezing, coughing, and nasal discharge. Since PPIV1 has been detected in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV), the virus was proposed to contribute to the porcine respiratory disease complex (PRDC). The aim of our study was to assess the prevalence of PPIV1 in Poland, with special attention on herds where respiratory disease was observed and the clinical signs suggested the involvement of viral respiratory pathogens.
The study was performed on 30 commercial Polish pig farms. Nasal swabs and oral fluids were collected from pigs at the age of 5-20-week-old. Before nucleic acids extraction nasal swabs were pooled by 5. Extracted RNA was examined with *in house* RT real-time PCR. IAV and PRRSV infection statuses of PPIV1-positive samples were also tested.
The results showed that 76.7% farms were PPIV1-positive. Different patterns of PPIV1 circulation in herds with mild–moderate respiratory disease were observed. Co-infections with IAV and PRRSV were infrequent and detected in 8 (23.5%) and 4 (11.8%) out of 34 PPIV1-positive nasal swab pools from pens of diseased pigs, respectively. In one pen PPIV1, IAV, and PRRSV were detected at the same time. Interestingly, PPIV1 mean Ct value in samples with a single PPIV1 infection was significantly higher (32.5±3.6) than in samples with co-infections (29.8±3.1) ($p < 0.05$).
The virus detection in pig populations exhibiting respiratory clinical signs, negative for PRRSV and IAV, suggests that PPIV1 should be included in differential diagnosis of respiratory problems. More studies are necessary to understand the virus' emergence, epidemiology, and it's role for pigs health.

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**Characterization of the subclinical infection of porcine deltacoronavirus in grower pigs under experimental conditions**

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Porcine deltacoronavirus (PDCoV) is a transboundary and emerging pathogen that causes enteric disease in suckling pigs. There are no previous reports of PDCoV outbreaks in grower or adult pigs. This study characterized the susceptibility and dynamic of PDCoV infection in grower pigs under experimental conditions using a combination of syndromic and laboratory assessment. Seven-week-old conventional pigs (n=24) were randomly distributed into PDCoV- (n=12) and mock-inoculated (n=12) groups. Serum was collected at day post inoculation (DPI) -7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42 to evaluate viremia via RT-qPCR, antibody response using a S1-based indirect ELISA, and cytokine/chemokine response with a 9-plex immunoassay. Viral shedding (RT-qPCR) and potential infectivity (cytopathic effect and viral N protein-based immunofluorescence staining in swine testicular cells) were determined using pen-based oral fluids and feces collected every other day between DPI 0 and 42. Pigs showed no clinical signs or viremia throughout the study. Active virus shedding was detected in feces (6-22 DPI) and oral fluids (2-30 DPI), peaking at DPI 10. Further analysis comparing shedding levels across specimens showed significantly higher ($p < 0.05$) levels in oral fluid compared to feces at DPI 8. No viable virus was isolated or detected *in vitro* by IFA on ST cell cultures inoculated with fecal and oral fluid samples collected from PDCoV infected pigs. An IgG response was first detected at DPI 10, being statistically significant ($p < 0.05$) after DPI 14 and increasing thereafter, coinciding with the progressive resolution of the infection. Likewise, a significant increase in proinflammatory IL-12 was detected between DPI 10 and 21 in PDCoV-inoculated pigs, which could enhance innate resistance to PDCoV infection. It is important to be reminded that the absence of clinical disease is not the same as absence of pathogen shedding and the ability to trigger a specific immune response. Under the specific conditions of this study, it was demonstrated that PDCoV inoculation of grower pigs can result in subclinical infection. Asymptomatic carriers can provide a source of a pathogen for other susceptible populations of pigs if fomites are shared among different populations. This study demonstrated that active surveillance based on systematic sampling and laboratory testing combining molecular and serological tools is critical for accurate detection of subclinical circulation of PDCoV in pigs after weaning.

**Establish a pregnant sow-neonatal piglet model system to study microbiome-influenza interactions**

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The interplay between the microbiome and pathogenic viral infection has gained more attention in recent years with the advancement of next-generation sequencing technologies. Microbiome-based integrated health approaches have been explored to treat various viral diseases. Pregnancy-incurred immune adaptation makes pregnant women a vulnerable population for influenza infection. Given the importance of the microbiome in health and a lack of pregnant animal models, the purpose of this study is to establish a pregnant sow-neonatal piglet model system to aid the study of microbiome-influenza interactions. In the pregnant sow model, the placental microbiome diversity in influenza virus-infected sows were assessed using 16S rRNA gene sequencing. Infection of pregnant sows resulted in elevated inflammatory cytokine response accompanied by increased lung lesions and decreased fetal body weight. Microbial community analysis demonstrated that the clinical presentation of influenza virus infection is strongly associated with altered placental microbiome diversity. In the neonatal piglet model, piglets were challenged with recombinant viruses expressing hemagglutinins (HAs) derived from different H1N1 subtypes with zoonotic transmission in history. Viruses expressing different parental HAs showed altered pathogenicity in neonatal piglets, demonstrating the virulence determinant role of HA. Microbial community analysis revealed a distinct pattern of nasal microbiome diversity between different groups after influenza virus challenge and these microbial signatures were predictive of infection status. Overall, findings in this study will facilitate the development of microbiome-based intervention strategies to combat the influenza disease in susceptible populations.

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

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The ability of maternally derived immunity to provide fetal/neonatal protection has increased attention on maternal immunization. In our previous studies, we constructed a candidate influenza vaccine, in which a chimeric HA construct, HA-129, was expressed in the context of a whole virus backbone of A/swine/Texas/4199-2/98-H3N2 as a recombinant virus (TX98-129). When evaluated in a nursery pig model, the TX98-129 had ability to induce broadly protective immune response against genetically diversified influenza viruses [McCormick et al., 2015; PLoS One, 10(6):e0127649]. In the current study, we established pregnant sow-fetus models to evaluate the safety and efficacy of this candidate vaccine. In pregnant sows, the results consistently showed that TX98-129 induced an immune response against the TX98-129 virus and the parental viruses used to construct the chimeric HA antigen. After challenge with a virulent IAV, a significant increase in antibody titers was observed in vaccinated sows (n=4) at 5 and 22 days post challenge (dpc), and challenge virus was detected in nasal swab of only one vaccinated sow with low titer at 5 dpc. Challenge virus was not detected in the fetuses. A panel of immune cytokine genes was analyzed in blood and tissue (lung) samples collected from sows. The results showed that expression levels of IFN-α and IL-1β were higher in the lung of vaccinated sows than those of un-vaccinated pigs at 5 dpc. T cell subpopulation analysis conducted in peripheral blood mononuclear cells (PBMC) showed a relatively higher ratio of $CD4^+CD8^+$ and $CD8^+$ cells in vaccinated sows at 22 dpc after stimulation of PBMCs with either challenge virus or vaccine. Next, we evaluated vaccine-induced maternal passive immunity. Neonatal piglets from both immunized and unimmunized sows were challenged at 6 days post-farrowing. Results showed that an increased antibody titer and deceased viral shedding were observed from neonates born from immunized sows. These results indicate that the challenge virus was cleared due to the induction of innate and adaptive immune responses in vaccinated sows; and vaccine-induced maternal passive immunity was able to protect neonatal pigs from influenza virus infection. Taken together, this study provides a comparative swine model system to study the effect of influenza vaccine in host (maternal) immunity and fetal development.

**Evaluation of the stability and immunogenicity of a novel PPIV1 vector for expression of ASFV p30/p54 antigens**

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Several viruses of human or other animal species in the *paramyxoviridae* family have been well-characterized for use as viral vectors to deliver foreign antigens for vaccine development. In our previous study, we isolated an emerging porcine parainfluenza virus 1 (PPIV1, KS17-258) from a US swine farm and established the reverse genetic system (*Li et al., Virology, 2022, 570:107-116*). To explore the feasibility for use as a viral backbone to express foreign antigens, recombinant viruses (vPPIV1-p30 and vPPIV1-p54) were generated to express p30 and p54 proteins of African swine fever virus (ASFV) as the model antigens. Growth kinetics analysis showed that the vPPIV1-p30 and vPPIV1-p54 maintained similar growth ability to those of the cloned and parental viruses. Genetic stability of the foreign genes was initially assessed by serially passage the recombinant viruses in cell culture. The p30 and p54 genes were stable in the genome of the recombinant viruses upon 10 passages. Subsequently, genetic stability of the PPIV1-p30 virus and PPIV1-p54 viruses was evaluated in nursery pigs. Viral RNA was detected in multiple tissues, including nasal turbinate, trachea, and lung at 4 days post inoculation, while viral RNA in nasal swab samples can be detected throughout the 28 days post infection. Sequence analysis showed that p30 and p54 genes were stably expressed in the genome of the PRV1 recombinant viruses from infected pigs. More importantly, infected pigs generated significant antibody response against p30 and p54 proteins, which further confirmed that these ASFV antigens were expressed by the PPIV1 vector in infected pigs. This study provides a potential platform for future development of vectored vaccines against swine diseases.

**Refining PRRSV-2 genetic classification based on global ORF5 sequences and investigation of geographic distributions and temporal changes**

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an important swine pathogen affecting the global swine industry. The first ORF5-based genetic lineage classification system describing global PRRSV-2 genetic diversity was introduced a decade ago. A recent study proposed a refinement by dividing the lineage 1 into 9 sublineages based on U.S. PRRSV-2 ORF5 sequences. However, PRRSV-2 genetic diversity at international levels has not been thoroughly explored after 2010 and the sublineages within other lineages have not been evaluated and updated. Thus, the classification system needed to be refined using more contemporary global sequences.

In this study, based on analysis of 82,237 global ORF5 sequences reported during 1989-2021, we classified PRRSV-2 into ten genetic lineages (L1-L10) and 21 sublineages (L1A-L1F, L1H-L1J, L5A-L5B, L8A-L8E, and L9A-L9E). The proposed classification system is flexible for growth if additional lineages, sublineages, or more granular classifications are needed in the future. As an example, to meet epidemiological investigation needs, the sublineage L1C was further divided into five groups (L1C.1-L1C.5) with L1C.5 corresponding to recently emerged L1C variant strains. Subsequently, comparison between RFLP typing and phylogenetic classification revealed that some RFLP patterns (e.g. 2-5-2 and 1-7-4) were mostly detected in a particular lineage or sublineage while others (e.g. 1-4-4, 1-8-4, 1-4-2, and 1-3-2) were distributed across multiple genetic lineages, reflecting the inaccuracy of using RFLP typing to determine PRRSV-2 genetic relatedness in most scenarios. Genetic homology of five commercial PRRSV-2 modified live virus vaccines to each of the proposed lineage/sublineage and detection frequency of vaccine-like viruses were determined. Geographic distribution analyses revealed that L1 (L1A-1C, L1D-1F, and L1H), L5 (L5A), L8 (L8A-L8C), and L9 (L9A and L9C) were the major lineages and sublineages in the U.S., which differed from the situation in Canada (mainly L1H, L1C, L5A and L8A), Mexico (mainly L1B, L1A, L5A and L8D), China (mainly L1C, L3, L5A, L8E [HP-PRRSV]), South Korea (mainly L1J, L2, L5A and L5B), Japan (mainly L4), Thailand (mainly L1I, L5A, L8E and L10), Europe (mainly L5A), and South America (mainly L1A and L1B). Temporal dynamic changes of PRRSV-2 in the U.S. were investigated by analyzing 73,092 PRRSV-2 ORF5 sequences collected during 1989-2021 and the data will be discussed (not included here due to the length limit).

In summary, this study refined PRRSV-2 ORF5-based phylogenetic classification globally after comprehensive sequence analysis and investigated the geographic distribution and dynamic changes of PRRSV-2 at the lineage/sublineage levels, including vaccine-like viruses. The refined classification system and molecular epidemiology data in this study will be invaluable for future characterization of PRRSV-2. In addition, reference sequences based on the new genetic classification are available for future epidemiological and diagnostic applications.

**Benefits and Barriers to Implementation of an Enhanced Passive Surveillance System for African Swine Fever in U.S. Swine Farms**

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With the threat of an introduction of African swine fever (ASF) to the United States increasing as the disease spreads globally, animal health officials, producers, and veterinarians need to implement additional measures for early detection and surveillance of disease. While the United States Department of Agriculture (USDA) routinely performs active surveillance, additional tools are needed to aid early detection and surveillance. Current passive surveillance measures performed by swine producers and veterinarians remain critical for identifying disease outbreaks and enhancing these activities may be the best opportunity for early ASF detection. However, these methods are often limited to diagnostic testing after increased mortality rates have already been identified. To aid both producers and veterinarians with decreasing the time to first detection of a potential ASF introduction, an enhanced passive surveillance (EPS) tool is needed. One potential opportunity may be by applying easily implemented EPS protocols to production and diagnostic data already being collected. To better understand what data is regularly collected and how it could be used in an EPS protocol, a questionnaire was distributed in the summer of 2022 across multiple channels of Minnesota swine producers. Thirty responses to the questionnaire indicated most farms regularly collect various types of disease and production information and perform various levels of necropsy and diagnostic submissions. Following the distribution and collection of results from the questionnaire, a focus group was held at the 2022 Leman Swine Conference to further discuss EPS implementation on US swine farms. The focus group included both public and private stakeholders in the swine industry. Participants discussed the potential value, challenges, opportunities, and data required to implement a potential EPS system. The reported value and opportunities for EPS protocols varied by stakeholder group, but generally they felt it would be beneficial for producers and packers in identifying an initial introduction of ASF. Opportunities for collaboration with industry initiatives and swine management software were identified through the discussion. Challenges presented by stakeholders included motivation for participation in an ASF-free area, labor, potential data sharing issues, and the cost of diagnostic testing. These concerns highlight important areas to address before the implementation of an EPS system. Stakeholders felt that the value of such a system included the reduction of secondarily infected farms, aiding the allocation of USDA and veterinary diagnostic laboratory resources in the face of an outbreak, and potentially using EPS to identify endemic disease outbreaks in addition to foreign animal diseases such as ASF. In conclusion, an EPS system can provide value to the United States swine industry in the face of an ASF or other foreign animal diseases if the identified or perceived barriers can be addressed. Continued collaboration with both private and public stakeholders can aid in reducing the barriers to the development of practical, fit-for-purpose, and valuable EPS protocols for ASF detection in the swine industry.

**IFN suppression-negative and NF-κB activation-negative PRRSV in pigs during coinfection with Streptococcus suis**

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The immunological hallmarks of PRRSV infection are represented by the negligible response of type I interferons (IFNs) and the upregulation of proinflammatory cytokines. Such unusual host responses lead to inappropriate innate and adaptive immune responses and enhanced clinical disease during co-infection of pigs with secondary bacterial pathogens, causing porcine respiratory disease complex (PRDC). We have shown that PRRSV non-structure protein 1-beta (nsp1) is the potent IFN antagonist, and the nucleocapsid (N) protein is the NF-kB activator. Subsequently, we have identified the leucine at position 126 (L126) of nsp1 as the catalytic residue for IFN suppression and the nuclear localization signal (NLS) of N as the NF-kB activation motif. In the present study, we generated a series of PRRSV mutants by reverse genetics to eliminate the IFN suppression function and the NF-kB activation function by mutating L126 and NLS, respectively.

The L126A mutant virus did not suppress IFN response in PAMs compared to wild-type PRRSV, and the NLS mutant induced only a lower level of NF-kB activation in PAMs. In addition, the NLS mutant produced reduced levels of proinflammatory cytokines, including IL-1, IL-6, IL-8, and TNF-alpha, compared to the wild-type virus. Based on these findings, a double-mutant PRRSV was generated, and the phenotype of the double-mutant was IFN suppression-negative and NF-κB activation-negative. Since the NF-kB function was removed from the virus, this mutant was expected to reduce the clinical severity in pigs during coinfection with Streptococcus suis (Strep. suis). To evaluate the biological consequence of the mutant PRRSV in pigs, 40 piglets were randomly allotted to 6 groups (7 animals per test group and 5 animals for control) and infected with the wild-type PRRSV, double-mutant PRRSV, or Strep. suis. At 7 days post-infection, the pigs were superinfected with Strep. suis, and at 7 days of coinfection, all animals were sacrificed.

While pigs coinfected with wild-type PRRSV and Strep. suis exhibited enhanced clinical severity represented by heavy breathing, coughing, and depression, coinfection with the double-mutant PRRSV and Strep. suis resulted in reduced clinical symptoms, indicating attenuation of the double-mutant PRRSV. PAMs prepared from bronchoalveolar lavages also showed reduced expressions of proinflammatory cytokines and chemokines, including IL-6, MCP-1, and MCP-2 which are the markers for cytokine storms in COVID-19 patients. Taken together, our data demonstrate that the double-mutant PRRSV enhanced immunity, possibly due to the IFN suppression-negative phenotype, and is clinically attenuated during coinfection with a bacterial pathogen, likely due to the NF-kB activation-negative and thus, reduced expression of proinflammatory cytokines. Our study paves the way for developing a novel vaccine platform for PRRS.

## Low-cost biosensors for rapid, on-chip, on-site detection of swine respiratory viruses

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Respiratory disease is one of the most important causes of economic loss in swine production. In the USA, porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) are currently the top two primary viruses causing swine respiratory diseases. The commonly used PCR-based virus detection methods require virus extraction, nucleic acid purification, and detection, which are relatively time consuming and expensive. We report two low-cost portable biosensors for rapid detection of swine respiratory viruses. The first biosensor is built upon an innovative integrated magneto-opto-fluidic (iMOF) platform, where antibody functionalized magnetic nanoparticles (MNPs) can enable efficient enrichment of multiple swine respiratory viruses and a photonic crystal (PC) biosensor can transduce the amount of captured MNP–virus nanoparticles to the change of their reflection signatures. Owing to the high refractive index of $Fe_2O_3$ MNPs, the use of MNPs can significantly enhance the PC sensor output. The validation utilizes antibody-functionalized MNPs to recognize IAV and PRRSV and transfers the formed MNP–virus conjugates onto the surface of the PC biosensors to quantify these viruses. The iMOF biosensor offers a high sensitivity of 3.5 TCID50 $mL^{-1}$ and 5.9 TCID50 $mL^{-1}$ for detecting IAV and PRRSV, respectively, and a rapid turnaround within one hour, including the MNP–virus conjugation, enrichment, and detection. The second biosensor is a microfluidic device for detecting H1N1 swine influenza A virus (IAV). The device integrates six sensing elements with measurement chambers and passive mixers for parallel processing and analysis of multiple samples. The virus sensing element uses a conducting virus-imprinted polymer (VIP) on the working electrode to recognize and qualify the target virus. The VIP contains nanocavities complementary to the target virus in shape, size, and functional groups. The VIP exhibits high conductance and charge transfer characteristics, making it suitable for an electrochemical sensing interface. The integration of the sensing elements into microfluidic channels allows easy handling of samples and reduced consumption of materials. With electrochemical impedance spectrometry, the sensor demonstrates a linear response to the logarithmic concentration of H1N1 virus in a concentration range from 50 to $5 \times 10^6$ TCID50/mL with a detection limit of 9 TCID50$mL^{-1}$. The sensor also exhibits considerable repeatability, high specificity, and exclusivity in the presence of a wide range of non-specific pathogens. The sensor is validated in detecting IAV-H1N1 in samples of nasal swab, oral fluid, and lung homogenate, demonstrating a good agreement with results obtained from the gold standard polymerase chain reaction method. The presented two virus detection tools have a great potential for in-field surveillance of viral infections.

**PRRSView: An analytical platform for the assessment of PRRSV ORF5 genetic sequences**

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important swine pathogens in the world. Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) performs diagnostic tests to monitor the presence of PRRSV in swine population including sequencing the open reading frame 5 (ORF5) gene to track the epidemiology of the virus and lateral introductions into a farm. The objective was to develop an online platform to assist veterinarians and producers with interactive tools to analyze their ORF5 sequence data by comparison to wild-type and vaccine-like reference strains in the ISU VDL database. This platform works in conjunction with the broader Swine Disease Reporting System (SDRS) project to contextualize the ever-changing patterns of PRRSV diversity.

PRRSView is a web portal created at the ISU VDL to host analytical and phylogenetic tools related to PRRSV ORF5 sequences. PRRSView uses ISU VDL's internal API to update the platform database built on MariaDB each night with new ORF5 sequence and associated metadata. A logistic regression classifier built using Python assigns genetic lineages to these sequences and appends them to a BLAST database. Lineages and RFLP patterns are stored in the database. Additional tools for comparison to vaccine strain and neighbor-joining phylogenetics were built using PHP and JavaScript, and are designed to run through the user's web browser.

The PRRSView (https://prrsv.vdl.iastate.edu) homepage provides a phylogenetic overview of the sequences processed by the ISU VDL within the previous month, indicating the current strains detected in circulation. At present, three analytical tools are available for ORF5 gene sequences on PRRSView: a genetic sequence BLAST tool, a vaccine identity tool, and an RFLP tool. The ORF5 BLAST tool returns up to 10 closely related sequences from the ISU VDL database along with the state, genetic lineage, RFLP, and identity to the query sequence. The vaccine identity tool quickly calculates the percent homology to five different PRRSV vaccines: Inglevac PRRS ATP, Inglevac PRRS MLV, Prime Pac PRRS, Fostera PRRS, and Prevacent PRRS, as well as the distance to the Lelystad strain. This tool also renders a neighbor-joining tree built with a set of curated strains to estimate the genetic lineage of the sequence. Additionally, this tool will calculate the RFLP of the sequence along with cut sites when hovering over the RFLP value. The last analytical tool provided is the ORF5 RFLP tool, which quickly calculates the RFLP pattern of the input sequences.

These analytical tools are designed to allow veterinarians, researchers and animal health industry to easily analyze their PRRSV ORF5 sequences against the expansive ISU VDL database to gain valuable epidemiologic information and comparative data regarding the genetic lineages and related metadata of the PRRSV circulating in a production system, while lowering the barrier of entry for use.

**Development of a broadly protective vaccine against swine influenza virus based on the M2 envelope protein**

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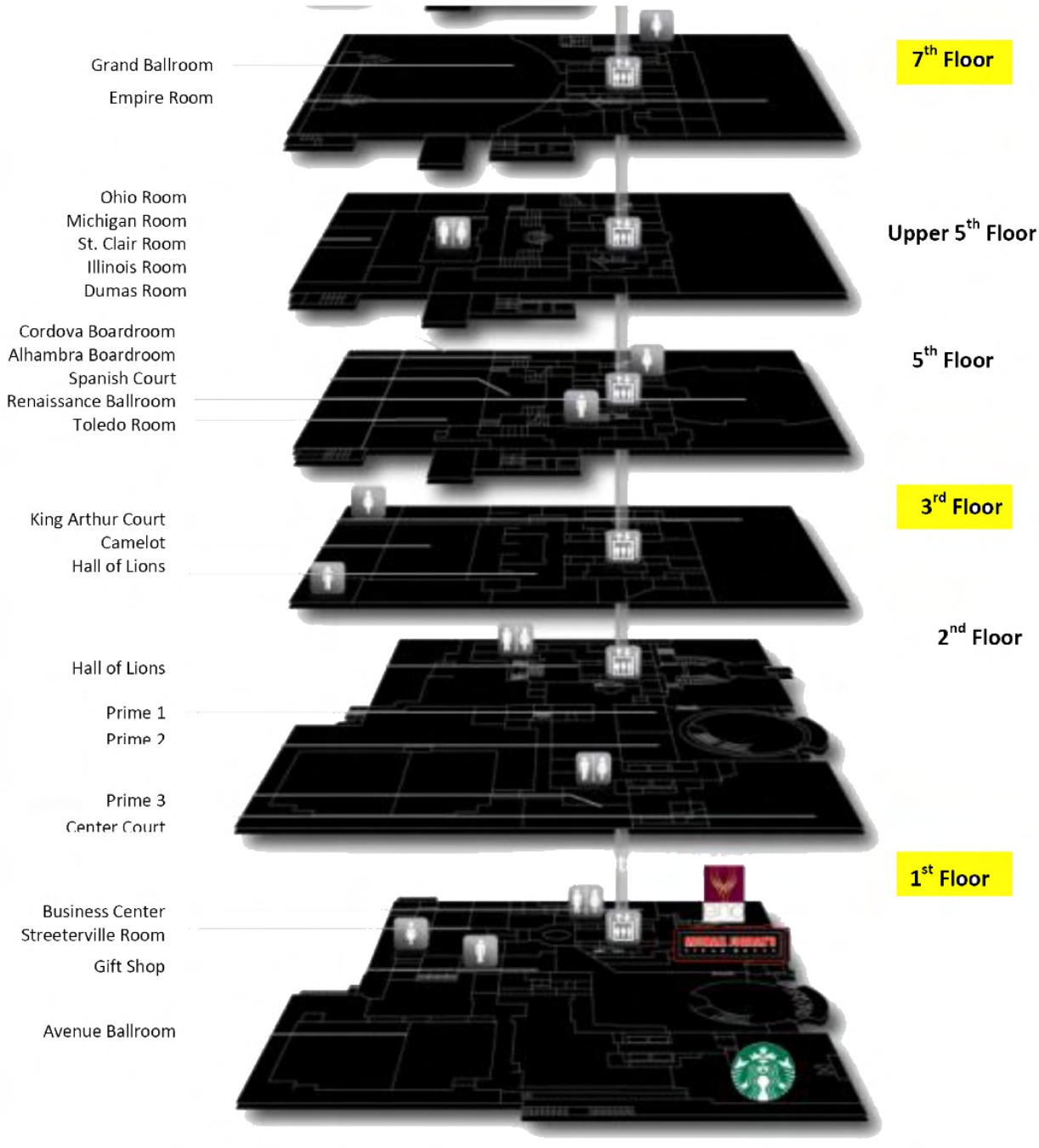
The syndrome resulting from the infection of swine with influenza A virus (IAV) presents a major economic burden to the pork industry and is one of the top three diseases affecting pigs in all phases of pork production. Effective vaccines are the cornerstone of defenses against acute influenza virus 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 swine (swIAV) is limited, which is due in part to the heterologous antigenic nature of the constantly increasing diversity of the hemagglutinin (HA) of swIAV. 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 IAV strains circulating in U.S. swine herds share the same M2 isoform. M2 is a 97 amino acid residues long tetrameric type III membrane protein that 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 antigen of a potential universal influenza vaccine for humans, mostly due to the difficulty of assembling the entire M2 protein in its natural transmembrane configuration. In this study 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 (M2:NDs). We determined that M2:NDs elicit the production of antibodies capable of recognizing IAV virions as well the generation of virus-specific interferon-gamma-producing cells. Further, intramuscular immunization of swine with M2:NDs elicited the development of protective immunity from SIV challenge as determined by a reduction of viral load in the lungs of swine challenged intratracheally with H3N2 swIAV. These studies demonstrate that the M2 protein incorporated into the NDs are immunogenic and can provide protective immunity against SIV. Future work will aim to improve the formulation of the M2:ND vaccine to increase its immunogenicity and the level protective efficacy afforded by this novel vaccine.

# Hotel Map


Grand Ballroom
Empire Room
7th Floor
Ohio Room
Michigan Room
St. Clair Room
Illinois Room
Dumas Room
Upper 5th Floor
Cordova Boardroom
Alhambra Boardroom
Spanish Court
Renaissance Ballroom
Toledo Room
5th Floor
King Arthur Court
Camelot
Hall of Lions
3rd Floor
2nd Floor
Hall of Lions
Prime 1
Prime 2
Prime 3
Center Court
1st Floor
Business Center
Streeterville Room
Gift Shop
Avenue Ballroom