2023 NAPRRS NC229 International Conference of Swine Viral Diseases

NORTH AMERICAN PRRS SYMPOSIUM

Intercontinental :
Chicago Magnificent Mile
November 30-December 2

Proceedings

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Welcome to 2023 NAPRRS/NC229 ICSVD

Dear Friends,

We are delighted to gather again in Chicago. The NAPRRS/NC229 International Conference of Swine Viral Diseases (ICSVD) was originated as the North American PRRS Symposium in 2003. Technically, this represents our 20th anniversary. The origin story for the modern PRRSV meeting begins with food. Each year NC229 representatives came together in Chicago in November, meeting for one day before the start of CRWAD. This was a closed meeting for presenting station reports and have discussions on collaboration. In 2003, one of our industry partners made us a proposition: “Let us sit in on the NC229 discussions and we will buy lunch”. It was a huge buffet, typical of animal health company generosity and deep pockets. Other NC groups breaking for lunch saw the big spread and declared “you sold out to industry…can we have some”. Lunch was the event that opened the NC229 meeting to the public and remains a mainstay of the modern PRRS meeting. The 2003 meeting was named, “4TH Annual Meeting of NC-229/International Workshop on PRRSV”. In 2005 the name was changed to the “2005 International PRRS Symposium".

In recent years, the emergence and spread of new swine viruses have expanded the focus to include emerging and transboundary swine diseases. Our 2023 meeting expands into a 2-day international conference, including 5 plenary scientific sessions, a SHIC biosecurity session co-organized with the Swine Health Information Center, two special lunch sessions co-organized with industrial sponsors emphasize modern technologies and translational research in emerging swine diseases. The Saturday afternoon poster session provides a forum for attendees to discuss the latest research and explore collaborative relationships for new research initiatives. In addition, the NC229 business meeting will be held to update the research progress of each station. On Saturday evening, a special banquet with keynote talk and award ceremony will be held to celebrate the 20th anniversary of NAPRRS/NC229 conference.

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 PRRS/NC229 Advisor:

Dr. Raymond (Bob) Rowland
University of Illinois

Executive Director:

Dr. Ying Fang
University of Illinois

Planning Committee:

Dr. Jose Angulo
Zoetis

Dr. Jay Calvert
Zoetis

Dr. John Harding
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Swine Health Information Center

Dr. Tomasz Stadejek
Warsaw University of Life Sciences

Dr. Paul Sundberg
Swine Health Information Center

Dr. Hanchun Yang
China Agriculture University

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Co-Chairs

Dr. Scott Kenney
The Ohio State University

Dr. Roman Pogranichniy
Kansas State University

Committee Members:

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The Ohio State University

Dr. Diego G. Diel
Cornell University

Dr. Mariana Kikuti
University of Minnesota

Dr. Pablo Pineyro
Iowa State University

Dr. Hiep Vu
University of Nebraska

Internal coordinator

Jackie Sturdyvin

A SHIC Director's Journey in Swine Health

- Prepared by Drs. Raymond (Bob) Rowland, Ying Fang, and Megan Niederwerder

Dr. Paul Sundberg attended the University of Nebraska-Lincoln where he earned his bachelor's degree in education in 1976 and obtained his DVM degree from Iowa State University in 1981. He completed his veterinary medicine curriculum and master's degree in clinical science/preventive medicine at Iowa State University in 1992. He also earned a PhD degree in veterinary microbiology with a specialty in preventive medicine from Iowa State University in 1996. He is board certified in the American College of Veterinary Preventive Medicine and is a past president of the College.

From 1981-1990, Dr. Sundberg spent nine years in private practice as owner of a veterinary clinic in Madison, Nebraska. In 1990, he left the practice to pursue further education at Iowa State University College of Veterinary Medicine in Ames, Iowa. In 1994, Dr. Sundberg joined the National Pork Producers Council as the Director of Veterinary Issues, and later served as Vice President of Science and Technology for the National Pork Board. Since July 2015, Dr. Sundberg has served as the Executive Director of Swine Health Information Center.

In his capacities with the NPB and SHIC, Dr. Sundberg was an advocate for developing collaborations with NC229, the PRRS symposium, and other PRRS research programs. Over the years, his leadership provided important support for the PRRS conference and related meetings. Between 2008 and 2013, he served on the advisory board for the PRRS Coordinated Agricultural Project (PRRS CAP). He supported important collaborations, such as the PRRS Host Genomic Consortium (PHGC), which established the groundwork for understanding the connection between host genetics and infectious disease in pigs. He was an important participant in what can be referred to as the golden age of PRRS research collaboration: a result of a confluence of NPB, USDA, and corporate funding. As the director of SHIC, he established a new paradigm by rapidly placing funding in the hands of qualified researchers who could provide key deliverables as a rapid response to new diseases. This approach has left a permanent imprint on how funding agencies engage researchers as problem-solvers in confronting emerging diseases.

Dr. Sundberg is a member of the American Veterinary Medical Association, the American Association of Swine Veterinarians, and the Iowa Veterinary Medical Association. He is a globally recognized expert in swine health. He has represented the U.S. pork industry during the World Organization for Animal Health (WOAH) General Sessions, the World Health Organization (WHO) meetings on antimicrobial use and antimicrobial resistance, the International Meat Secretariat meetings, and other activities for discussions on a variety of swine health topics with the European Union, Sweden, Denmark, Germany, Mexico, China, Japan, and others.

In recognition of his contribution to swine health, Dr. Sundberg received multiple awards, including Howard Dunne Memorial Award, the Meritorious Service Award from AASV, and the Science with Practice award from ISU, etc.

Paul and his wife Debra live in Anderson, South Carolina.

Career Path of the Master of the US Pork Industry

- Prepared by Drs. Ying Fang and David Benfield

Dr. Scott Dee earned a DVM (1987), MS (1985), and PhD (1996) from the University of Minnesota. He is board-certified in veterinary microbiology, past President of the American Association of Swine Veterinarians, past Chair of the American Veterinary Medical Association (AVMA) Council on Biologic and Therapeutic Agents, and currently serves on the AVMA House of Delegates. After 12 years in swine practice (1987-1999) and another 12 years in academia (1999-2011), in 2011, Dr. Dee joined Pipestone Veterinary Services as the Director of Applied Research, currently serving as Emeritus Director of Discovery and Innovation for Pipestone.

Throughout his 36-year career, he has been awarded more than 12.5 M in research funding and published 181 peer reviewed papers, including the initial publications on the proof of concept of PEDV transmission in feed and the transboundary survival of ASFV in feed, along with 37 textbook chapters, 208 abstracts, and 495 proceedings papers. He has guest-edited a Special Issue on Feed Risk in Transboundary and Emerging Diseases. Through his career, he has mentored 20 MS and PhD graduate students.

Dr. Dee's research has been transformative and set a precedence for thinking differently as to how those viral diseases of swine could be transmitted and controlled. His research has had a profound effect on the basic facets of commercial swine production and has resulted in positive economic changes in the industry. He developed the initial knowledge and industry-wide protocols to understand and control Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection at the farm level. The comprehensive application of these protocols has reduced annual PRRS incidence below 10% across the 76 breeding herds in the Pipestone System, the 3rd largest pork production company in the US, over a 2-year period. This is the first report of long-term control of PRRSV in a large-scale commercial swine production system. His later studies also led to the initial discovery that feed and feed ingredients can serve as vehicles for the transport and transmission of Porcine Epidemic Diarrhea Virus (PEDV) and PRRSV. The work has been replicated and expanded by other investigators to include African swine fever virus (ASFV), pseudorabies virus (PRV), foot and mouth disease virus (FMDV), Seneca virus A (SVA), and classical swine fever virus (CSFV). These findings have led to the development and implementation of biosecurity protocols for managing the importation of feed and feed ingredients into North America, Europe, Asia, and Australia from countries of endemically infected with foreign animal diseases.

In recognition of his contribution to the swine industry, Dr. Dee has received multiple awards, including the AASV Practitioner of the Year, the Howard Dunne Memorial, and Leman Science in Practice awards, a Warrior Chip from the FBI, and is a Master of the US Pork Industry, Distinguished Service to the US Pork Industry Award from the National Pork Board, and was also recognized as the Distinguished Research Alumnus by the UMN College of Veterinary Medicine.

Dr. Dee and his wife Lisa live in Alexandria, MN and have two children: Nicholas, a medical student at the University of Minnesota Duluth and Ellen, a CPA at Cargill.

NAPRRS/NC229 ICSVD
David Benfield Award Competition Judges

Oral Presentation Judges:

Scott Kenney, Facilitator

Jose Angula

Daniel Ciobanu

Wenjun Ma

Hans Nauwynck

Roman Pogranichniy

Xiuqing Wang

Poster Judges:

Mariana Kikuti, Facilitator

Jay Calvert

Diego Diel

Shafiqul Chowdhury

Luis Gimenez-Lirola

Paul Sundberg

Yanjin Zhang

NAPRRS/NC229 ICSVD David Benfield Award Winners

Best Oral Presentation

1st Place: Jessica Caroline Gomes Noll, *Characterization of the Biological Function of African Swine Fever Virus p30/p32 Protein*, Cornell University, Ithaca, NY

2nd Place Nakarin Parmornchainavakul, *Predicting PRRSV-2 Variant Emergence: Insights from a Decade of Genomic Analysis*, University of Minnesota, Saint Paul, MN

3rd Place: Julia Baker, *Repeat Offenders: Understanding PRRSV Clinical Rebreaks*, University of Minnesota, Saint Paul, MN

Best Poster Presentation

1st Place: Carolyn Lee, *Evaluation of Multiple Immune Parameters Following Vaccination with an ASFV Multiepitope Protein Nanoparticle-based Subunit vaccine*, The Ohio State University, Wooster, OH

2nd Place: Kaylyn Rudy, *Elevated PRRSV Viral Load in Critical Non-lymphoid Tissues is Associated with Late Gestation Fetal Compromise*, Purdue University, West Lafayette, IN

3rd Place: Michelle Steyn, *Investigating the Role of Mechanism of Host Cell Death in PRRSV Infection*, South Dakota State University, Brookings, SD

2023 Travel Fellowship Recipients

Thank you sponsors!

Each year, a special set of donors provide funding to assist the participation of students to present their research at the conference. This year, we appreciate the support from USDA conference grant and the generous donation from Boehringer Ingelheim.

Amalie Ehlers Bedsted
University of Copenhagen

Ana Fiorella Castillo Espinoza
Iowa State University

Kassandra Durazo Martinez
University of Nebraska Lincoln

Jessica Caroline Gomes Noll
Cornell Univeristy

Jing Huang
University of Minnesota

Danh Lai
University of Nebraska Lincoln

Valeria Lugo
University of Minnesota

Hung Luong
University of Nebraska Lincoln

Marcello Melini
University of Minnesota

The Nguyen
University of Nebraska Lincoln

Jenna Parliament
South Dakota State University

Marie-Jeanne Pesant
Université de Montréal

Sirisha Puducode Parameswaran
Iowa State University

Michelle Steyn
South Dakota State University

Kristen Walker
Morgan State University

Lufan Yang
University of Illinois at Urbana-Champaign

Xiaomei Yue
University of Minnesota

NAPRRS/NC229 ICSVD Keynote Speakers

8:10-8:40AM, December 1st

Ying Fang, University of Illinois

The art of PRRSV infection and replication

10:00-10:30AM, December 1st

Crystal Loving, USDA BARC APDL

Connecting the dots of single-cell analyses for delineating the big picture of porcine immune status

2:00-2:30PM, December 1st

Xiang-Jin (XJ) Meng, Virginia Polytechnic Institute & State University

Novel candidate vaccines against porcine epidemic diarrhea virus

8:00-8:30AM, December 2nd

Jeffrey Zimmerman, Iowa State University

Achieving effective and sustainable regional surveillance

8:30-9:00AM, December 2nd

Kim VanderWaal, University of Minnesota

Exploring the Emergence, Antigenicity, and Immune-Mediated Evolution of PRRSV-2 Variants through Bioinformatics

3:30-4:00PM, December 2nd

Raymond (Bob) R.R. Rowland, University of Illinois

Historical Review of NAPRRS/NC229

4:00-4:30PM, December 2nd

Hans Nauwynck, Ghent University

Evolution of PRRSV in Europe

4:30-5:00PM, December 2nd

Hanchun Yang, China Agricultural University

Current status of PRRS in China: diversified strains of PRRSV and complicated clinical conditions

NAPRRS/NC229 ICSVD
Keynote Speaker

Susan Weiss

Perelman SCHOOL OF MEDICINE
UNIVERSITY of PENNSYLVANIA

Coronaviruses: Old and New
7:00-8:00 PM, December 2

Zoetis Special Lunch Session

Translational Research in the swine industry, the PRRS case

December 1, 11:50AM-2:00PM

Moderators: Jose Angulo, Jay Calvert

11:50AM-12:20PM: Food Service

12:20-12:30:
Jose Angulo, Zoetis
Welcome and Introduction

12:30-1:00PM:
Cesar Corzo, University of Minnesota
Understanding the epidemiology of PRRSV L1C variant in the US

1:00-1:30PM:
Paul Yeske, Swine Vet Center
Control measures applied in the field for controlling and eliminating PRRSV L1C variant

1:30-1:50PM: Panel discussion

1:50-2:00PM: Break

Industry Sponsored Lunch Session

Modern Technologies in Swine Health

December 2, 11:45AM-1:30PM

Moderators: James Lowe, Melissa Rohrer

11:45AM-12:15PM Food Service

12:15-12:45PM
Jianqiang Zhang, Iowa State University
Evaluation of protective efficacy of six commercial PRRSV-2 MLV vaccines against emergent PRRSV 1-4-4 L1C variant strain in weaned pigs

12:45-1:00PM
Roman Pogranichniy, Kansas Veterinary Diagnostic Lab
Field deployable whole genome sequencing of African swine fever virus and classical swine fever virus

1:00-1:15PM
Rolf Rauh, Tetracore
The development, optimization and validation of the Tetracore VetAlert™ MagBead Total Nucleic Acid

1:15-1:30PM
Melanie Dart, Promega Corporation
Lumit Trip: Light up my assay

NAPRRS/NC229 ICSVD
SHIC Special Session
Biosecurity in US Swine Industry

December 2, 10:15-11:45AM

10:15-10:30AM

Megan Niederwerder, Swine Health Information Center

Swine disease monitoring and the need for enhanced wean-to-harvest biosecurity

10:30-10:45AM

Montserrat Torremorell, University of Minnesota

Development and evaluation of an electrostatic precipitator (ESP) prototype to mitigate airborne spread of pathogens under farm conditions

10:45-11:00AM

Gustavo Machado, North Carolina State University

Rerouting between-farm transportation vehicle movements to minimize the dissemination of endemic and emerging diseases in North America

11:00-11:15AM

Dustin Boler, Carthage Veterinary Service, Ltd

Ensuring site and transportation biosecurity using bioluminescence

11:15-11:30AM

Gustavo Silva, Iowa State University

Industry-wide assessment of bioexclusion practices in wean-to-harvest sites, and development and validation of a rapid risk assessment bioexclusion tool

11:30-11:45AM

Scott Dee, Pipestone Veterinary Services

Case study: The impact of Next Generation Biosecurity on preventing PRRSV infections in breeding herds from a large-scale commercial swine production system in the US

2023 NAPRRS/NC229 ICSVD
Celebrating 20 Years of Service!

NAPRRS Director	Affiliation	Years of Service
Mike Murtaugh	University of Minnesota	2002-2003
Jeffrey J. Zimmerman	Iowa State University	2004-2005
Raymond (Bob) R.R. Rowland	Kansas State University	2006-2019
Ying Fang	University of Illinois	2020-Present

NC229 Chair	Affiliation	Years of Service
Dave Benfield	The Ohio State University	2000-2001
Mike Murtaugh	University of Minnesota	2002-2003
Jeffrey J. Zimmerman	Iowa State University	2004-2005
Raymond (Bob) R.R. Rowland	Kansas State University	2006-2007
Joan Lunney	US Department of Agricutlure	2008-2009
Xiang-Jin (XJ) Meng	Virginia Polytechnic Institute & State University	2010-2011
Jane Christopher-Hennings	South Dakota State University	2012-2013
Fernando Osorio	University of Nebraska-Lincoln	2014-2015
Kyoungjin J. Yoon	Iowa State University	2016-2017
Daniel L. Rock	University of Illinois	2018-2019
Sheela Ramamoorthy	North Dakota State University	2020-2021
Roman Pogranichniy	Kansas State University	2022-2023

2023 NAPRRS/NC229 ICSVD Program

Thursday, November 30, 2023

1:00-6:00PM: Conference Check-In/Onsite Registration

4:00-5:00PM NAPRRS/NC229 organizing committee meeting

Friday, December 1, 2023

8:00-8:10AM: Ying Fang, Bob Rowland: Opening Remarks

Session 1: From Entry to Viral Infection

Moderators: Scott Kenney, Laura Miller

8:10AM: Ying Fang; The art of PRRSV infection and replication

8:40AM: Alberto Brandariz Nuez; Specific regions in CD163 required for porcine reproductive and respiratory syndrome virus (PRRSV) infection are not necessary for binding to viral envelope proteins.

8:55AM: Scott Kenney; Susceptibility and Transmission of Emerging Zoonotic Rat Hepatitis E Virus in Swine

9:10-9:30AM Lightning talks

9:10AM: Kasandra Durazo-Martinez; Reassessment of the Susceptibility of Different Porcine Macrophage Populations to Porcine Reproductive and Respiratory Syndrome Virus Infection

9:15AM: Marcello Melini; Evaluation of the infectiousness level of three wild-type porcine reproductive and respiratory syndrome viruses (PRRSV) variants

9:20AM: Ana Fiorella Castillo Espinoza; Organotypic air-liquid interface respiratory cell culture (ALI-REC) system for studying coinfections with Influenza A subtypes H1N1 and H3N2 in pigs

9:25AM: Amalie Ehlers Bedsted; Characterization of porcine respiratory coronavirus (PRCV) strains from Europe and the USA

9:30-10:00AM Break

Session 2: Insights into Viral Pathogenesis and Host Response

Moderators: John Harding, Hiep Vu

10:00AM: Crystal Loving; Connecting the dots of single-cell analyses for delineating the big picture of porcine immune status

10:30AM: Joan Lunney; Characterization of swine reagents for monitoring pig immune responses

10:45AM: Jonathan Pasternak; Elevated PRRSV viral load in critical non-lymphoid tissues is associated with late gestation fetal compromise

11:00AM: Daniel Ciobanu; Deconstructing the role of SYNGR2 in viral disease susceptibility in swine

11:15AM: Weihuan Fang; Classical swine fever virus Npro evades from host cell inate immune responses by destablizing Sp1 and HMGB1

11:30-11:50AM Lightning talks

2023 NAPRRS/NC229 ICSVD Program

Friday, December 1, 2023

11:30AM: Jessica Caroline Gomes Noll; Characterization of the Biological Function of African Swine Fever Virus p30/p32 Protein

11:35AM: Danh Lai; African swine fever virus gene I73R suppresses the type-I IFN production by broadly inhibiting the host transcription process

11:40AM: Kristen Walker; Investigation of fetal liver, heart, and kidney transcriptomes for prediction of reproductive failure

11:45AM: Jing Huang; Differential Antibody Response to PRRSV Glycoprotein 5 by Vaccine and Field Strains

Session 3: Zoetis Lunch Session

Moderators: Jose Angulo, Jay Calvert

11:50AM-2:00PM: Translational Research in the swine industry, the PRRS case

Session 4: Novel Vaccines, Vaccination Strategies, and Therapeutics

Moderators: Diego Diel, Elisa Crisci

2:00PM: Xiang-jin (X.J.) Meng; Novel candidate vaccines against porcine epidemic diarrhea virus

2:30PM: Wenjun Ma; Bat Influenza vectored NS1-truncated live vaccine protects pigs against heterologous virus challenge

2:45PM: Diego Diel; Immunogenicity of chimeric hemagglutinins delivered by an Orf-virus-vector platform against Swine Influenza virus in pigs

3:00PM: Elisa Crisci; Intranasal Ad5 influenza vaccine elicits hemagglutinin-specific antibody response in pregnant and lactating pigs

3:15PM: Changjiang Weng; Development of a New Effective African Swine Fever Virus Vaccine Candidate by Deletion of the H240R and MGF505-7R Genes Results in Protective Immunity against the Eurasia Strain

3:30PM: Lihua Wang; Development of live-attenuated vaccine to protect pigs against the contemporary pandemic African swine fever virus

3:45-4:00 Lightning talks

3:45PM: Hung Luong; Comparative Analysis of Antibody Responses to African Swine Fever Structural Protein Between Attenuated Live and killed vaccine

3:50PM: Lufan Yang; Effects of maternal vaccination on the hippocampal transcriptome and intestinal microbiome of neonatal piglets infected by influenza A virus

3:55PM The Nguyen; A single-dose intramuscular immunization of pigs with lipid-nanoparticle DNA vaccines based on the hemagglutinin antigen confers complete protection against challenge infection with the homologous influenza virus

4:00-4:30PM Break

2023 NAPRRS/NC229 ICSVD Program

Friday, December 1, 2023

4:30-6:00PM NC229 Business Meeting
Moderators: Pablo Pineyro, Roman Pogranichniy

4:30PM: Roman Pogranichniy; NC-229 business meeting opening and introduction

4:35PM: Kathe Bjork; USDA-NIFA update

4:45PM: Pablo Pineyro-Pineiro; NC229 performance highlights in Year 2022

4:50PM: Eric Burrough; Expanding the Toolbox: Viral Disease Diagnosis in Veterinary Diagnostic Laboratories

5:20PM: Juergen Richt; Swine as a model for biomedical research: influenza and beyond

5:50PM: Closing remarks and election of new members

Saturday, December 2, 2023

Session 5: Rapid Sensing of Viral Infection and Disease Counter Measures *Moderators: Mariana Kikuti, Jianqiang Zhang*

8:00AM: Jeff Zimmerman; Achieving effective and sustainable regional surveillance

8:30AM: Kim VanderWaal; Exploring the Emergence, Antigenicity, and Immune-Mediated Evolution of PRRSV-2 Variants through Bioinformatics

9:00AM: Mariana Kikuti; Opening the pools: What is behind tongue tips sampling and other welfare-friendly postmortem samples for accurately detecting PRRSV?

9:15-9:45AM Lightning talks

9:15AM: Sirisha Pudocode Parameswaran; Evaluation of the High-Volume Testing Potential of SmartChip Real-Time PCR System for the detection of Foreign Animal Diseases

9:20AM: Julia Baker; Repeat Offenders: understanding PRRSV clinical rebreaks

9:25AM: Nakarin Pamornchainavakul; Predicting PRRSV-2 Variant Emergence: Insights from a Decade of Genomic Analysis

9:30AM: Xiaomei Yue; An early warning tool for PRRS virus occurrence in the U.S. swine breeding herds

9:35AM: Valeria Lugo Mesa; Survival of five strains of PRRSV in tap water at different temperatures

9:40AM: Igor Paploski; Weathering the storm: extreme weather events and their association with PED and PRRS occurrence

9:45AM-10:15AM Break

2023 NAPRRS/NC229 ICSVD Program

Saturday, December 2, 2023

Session 6: SHIC Special Session

Moderators: Megan Niederwerder, Paul Sundberg

10:15-11:45AM: Biosecurity in US Swine Industry

Session 7: Industry Sponsored Lunch Session

Moderators: James Lowe, Melissa Rohrer

11:45AM-1:30PM: Modern Technologies in Swine Health

1:30-3:30PM Poster Session

Session 8: Global Swine Viral disease Control: Past, Present, and Future

Moderators: Jeff Zimmerman, Joan Lunney

3:30-4:00PM: Raymond (Bob) Rowland; Historical Review of NAPRRS/NC229

4:00-4:30PM: Hans Nauwynck; Evolution of PRRSV in Europe

4:30-5:00PM: Hanchun Yang; Current status of PRRS in China: diversified strains of PRRSV and complicated clinical conditions

5:00-5:30PM: Hualan Chen; Evolution and pandemic potential of the Eurasian avian-like H1N1 swine influenza viruses

Banquet and Award Ceremony

6:00-7:00PM Food Service

7:00-8:00PM: Keynote Presentation - Susan Weiss; Coronaviruses: Old and New

8:00-9:00PM: Award Ceremony

2023 NAPRRS/NC229 ICSVD Posters

Detection, Diagnostics, and Surveillance		
Poster #	Presenter Name	Title
01	Armenta-Leyva, Betsy	What is "normalization" and why should we normalize PCR results?
02	Armenta-Leyva, Betsy	Paper sampling for passive environmental surveillance for swine pathogens
03	Baker, Julia	Repeat offenders: understanding PRRSV clinical rebreaks
04	Bedsted, Amalie Ehlers	Characterization of porcine respiratory coronavirus (PRCV) strains from Europe and the USA
05	Crisci, Elisa	Intranasal Ad5 influenzavaccine elicits hemagglutinin-specific antibody response in pregnant and lactating pigs
06	Crisci, Elisa	The role of pathogens and anti-PRRSV immunity in the porcine respiratory disease complex
07	Crisci, Elisa	Predict and protect against PRRSV (PreProPRRSV): combining PRRSV forecasting technology with vaccine efficacy prediction to prevent PRRSV outbreak
08	Dee, Scott	The impact of next generation biosecurity on preventing porcine reproductive and respiratory syndrome virus infections in breeding herds from a large scale commercial swine production system in the US
09	Kenney, Scott	Susceptibility and transmission of emerging zoonotic rat hepatitis E virus in swine
10	Kikuti, Mariana	Opening the pools: What is behind tongue tips sampling and other welfare-friendly postmortem samples for accurately detecting PRRSV?
11	Kikuti, Mariana	Monitoring of porcine reproductive and respiratory syndrome virus variant emergence
12	Lanka, Saraswathi	Detection of classical swine fever virus RNA using minimal equipment LAMP-PCR
13	Li, Siyan	Digital biosensor assays for rapid detection of ASFV infection
14	Lugo Mesa, Valeria	Survival of five strains of PRRSV in tap water at different temperatures
15	Ma, Wenjun	Pathogenicity of a novel reassortant H1N1 virus from swine responsible for deaths in sows in the US
16	Ma, Wenjun	Bat influenza vectored NS1-truncated live vaccine protects pigs against heterologous virus challenge
17	Melini, Marcello	Evaluation of the infectiousness level of three wild-type porcine reproductive and respiratory syndrome viruses (PRRSV) variants
18	Munguia-Ramirez, Berenice	Effect of storage temperature x time on PRRSV RT-qPCR testing
19	Munguia-Ramirez, Berenice	How should we stabilize PRRSV RNA in oral fluids?
20	Nelli, Rahul	Evaluation of RT-PCR assay for detecting Japanese encephalitis virus in porcine samples
21	Pamornchainavakul, Nakarin	Predicting PRRSV-2 variant emergence: insights from a decade of genomic analysis
22	Paploski, Igor	Weathering the storm: extreme weather events and their association with PED and PRRS occurrence

2023 NAPRRS/NC229 ICSVD Posters

Detection, Diagnostics, and Surveillance		
Poster #	Presenter Name	Title
24	Pogranichniy, Roman	Can producers raise pigs in ASF endemic areas? With high level biosecurity on the farm, it is possible.
25	Rathkjen, PH	Evaluation of PRRSV2 PCR detection using cotton ropes, in oral Fluid, in room-based manure pit and manure collection tank, as nursery PRRSV status monitoring
26	Tarasiuk, Grzegorz	A novel method for evaluating environmental and pig-derived targets in pen-based oral fluids
27	Yang, Hanchun	Current status of PRRS in China: diversified strains of PRRSV and complicated clinical conditions
28	Yue, Xiaomei	An early warning tool for PRRS virus occurrence in the U.S. swine breeding herds
29	Zimmerman, Jeff	Achieving effective and sustainable regional surveillance
Host Response to Infection Including Host Genetics and Innate Adaptive Immunity		
30	Brandariz- Nunez, Alberto	Specific regions in CD163 required for porcine reproductive and respiratory syndrome virus (PRRSV) infection are not necessary for binding to viral envelope proteins
31	Castillo Espinoza, Ana Fiorella	Organotypic air-liquid interface respiratory cell culture (ALIREC) system for studying coinfections with influenza A subtypes H1N1 and H3N2 in pigs
32	Ciobanu, Daniel	Deconstructing the role of SYNGR2 in viral disease susceptibility in swine
33	Ciobanu, Daniel	Understanding host x pathogen associations in swine infectious diseases
34	Dunkelberger, Jenelle	Evaluation of biomarkers measured post PRRS vaccination as potential indicators of resilience to PRRS virus infection
35	Durazo-Martinez, Kassandra	Reassessment of the susceptibility of different porcine macrophage populations to porcine reproductive and respiratory syndrome virus infection
36	Fang, Weihuan	Classical swine fever virus Npro evades from host cell innate immune responses by destabilizing Sp1 and HMGB1
37	Gomes Noll, Jessica Caroline	Characterization of the biological function of African swine fever virus p30/p32 protein
38	Guo, Chunhe	MARCO inhibits porcine reproductive and respiratory syndrome virus infection through intensifying viral GP5- induced apoptosis
39	Han, Jun	Contribution of PRRSV nsp2 genetic variation to viral virulence and persistence
40	Han, Jun	Riding apoptotic bodies for cell- cell transmission by African swine fever virus
41	Huang, Jin	Differential antibody response to PRRSV glycoprotein 5 by vaccine and field strains
42	Jeon, Dayeon	Evaluating the impact of thyroid hormone on the efficiency of in vitro PRRSV-2 infection
43	Lai, Danh	African swine fever virus gene I73R suppresses the type- I IFN production by broadly inhibiting the host transcription process
44	Liu, Pinghuang	Long-term expanding porcine airway organoids reveal age-related divergent IFN responses to respiratory coronavirus

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46	Pinto, Derek	Identification of regions in the viral glycoprotein genes involved in CD163 binding and PRRSV infection
47	Rudy, Kaylyn	Elevated PRRSV viral load in critical non-lymphoid tissues is associated with late gestation fetal compromise
48	Salgado, Briana	Sialoadhesin is not required for infection of pigs with PRRSV-1
49	Walker, Kristen	Investigation of fetal liver, heart, and kidney transcriptomes for prediction of reproductive failure
50	Walker, Lianna	Survey of intraspecific variation within swine ANP32A/B and effects on host permissiveness to avian influenza virus
51	Weng, Changjang	ASFV pH240R affects the virulence of African swine fever virus by inhibiting antiviral innate immune response
52	Weng, Changjang	Porcine reproductive and respiratory syndrome virus infection inhibits NF-kB signaling pathway through cleavage of IKKβ by Nsp 4
53	Weng, Changjang	The “tricks” of African swine fever virus escaping host anti-viral innate immune responses
54	Xu, Jidong	Molecular mechanism of porcine epidemic diarrhea virus evading from host interferon immune responses by inhibiting histone deacetylase 1
55	Yang, Lufan	Effects of maternal vaccination on the hippocampal transcriptome and intestinal microbiome of neonatal piglets infected by influenza A virus
56	Yoo, Dongwan	PRRS virus nsp1 protein downregulates TRIM19 expression and promotes viral replication
57	Yoo, Dongwan	Double mutations in nsp1 and N genes of PRRSV downregulate inflammatory cytokine expressions and confer clinical attenuation in pigs during bacterial co-infection
58	Zhou, Lei	IL-1beta and TNF-alpha released from highly pathogenic porcine reproductive and respiratory syndrome virus-infected pulmonary alveolar macrophages enhance pulmonary vascular permeability by dysregulating claudin-8 and claudin-4
Vaccination Strategies and Therapeutics		
59	Diel, Diego	Immunogenicity of chimeric hemagglutinins delivered by an Orf virus-vector platform against swine influenza virus in pigs
60	Koziol, Marie-Eve	A CSF vaccine based on E2 recombinant glycoprotein and adjuvanted with oil-in-water emulsion induces a full protection in a pig field trial
61	Lee, Carolyn	Evaluation of multiple immune parameters following vaccination with an African swine fever virus multiepitope protein nanoparticle-based subunit vaccine
62	Lunney, Joan	Characterization of swine reagents for monitoring pig immune responses
63	Luong, Hung	Comparative analysis of antibody responses to African swine fever structural protein between attenuated live and killed vaccine
64	Ma, Wenjun	Bat influenza vectored NS1-truncated live vaccine protects pigs against heterologous virus challenge
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67	Nguyen, The	A single-dose intramuscular immunization of pigs with-lnipaindoparticle DNA vaccines based on the hemagglutinin antigen confers complete protectio against challenge infection with the homologous influenza virus strain
68	Paladino, Pesant	Elucidating deoxynivalenols' antiviral effect against porcine reproductive respiratory syndrome virus infections in v–itaromult-iomics approach
69	Sobhy, Nader	In vitro antiviral activity of modified med-icuhmain fatty acids against porcine reproductive and respiratory syndrome virus
70	Wang, Lihua	Development of liv-aettenuated vaccine to protect pigs against the contemporary pandemic African swine fever virus
71	Zhang, Jianqiang	Evaluation of protectiveefficacy of six commercial PRR-2SVMLV vaccines against the emergent PRRS-V4-14 L1C variant strain in weaned pigs
72	Wu, Pei-yin	Efficacy evaluation of a bivalent ready-to-use protein subunit vaccine against PRRSV and Mycoplasma hyorhinis challenge
73	Parliament, Jenan	Abelson (Abl) Tyrosine Kinase Inhibitors Reduce Porcine Reproductive an Respiratory Syndrome Virus (PRRSV) Replication in Vitro
74	Steyn, Micheell	Investigating the role and mechanism of host cell death in PRRSV infection

ABSTRACTS

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

What is "normalization" and why should we normalize PCR results?

B Armenta-Leyva1*, B Munguía-Ramírez1, T-Y Cheng2, F Ye1, A Henao-Díaz3, K Doolittle5, S Zimmerman5, LG Giménez-Lirola1, J Zimmerman1

1Iowa State University, Iowa; 2Ohio State University, Ohio; 3PIC Latin America, Mexico, 4IDEXX Laboratories, Inc., Maine. *Presenting author

Introduction. "Normalization" is re-expressing a test result relative to an agreed-upon reference standard that undergoes the same testing processes as the sample. For example, ELISAs re-express the sample (S) optical density relative to a positive (P) control optical density and report "S/Ps". The purpose of normalization is to account for run-to-run variation, e.g., sample handling, pipetting, technicians, etc. Normalization of PCRs has been routine in basic research for 20+ years, but diagnostic laboratories continue to report non-normalized results (raw Cqs). Unique to PCR, amplification efficiency (E) is also an important source of variation because E directly impacts the final number of PCR copies. PCR results reported as raw Cqs assume that E is 100%, but in real life, nothing is 100% efficient. Cqs can be easily normalized by converting them to "efficiency standardized Cqs" (ECqs):

Eq. 1 ECq = E-DeltaCq

where E = amplification efficiency and DeltaCq = (Sample Cq - Reference Standard Cq). ECqs represent target concentration relative to the reference standard and their interpretation is simple: the larger the ECq value, the more nucleic acid in the sample relative to the reference standard. Because all Cq results can be converted to ECqs, there are no "indeterminate" results. This makes it possible to estimate the diagnostic sensitivity and specificity of the test and set cutoffs.

Methodology. Our objective was to explore routine normalization in PRRSV RT-qPCR testing. Reference standards were created by rehydrating and diluting (1 × 10-4) a PRRSV MLV (Ingelvac) with serum or oral fluid (OF). That is, a serum reference standard was used when testing serum samples and an OF reference standard when testing OF samples. Sample ECqs were calculated using Eq. 1 where the mean E and mean reference standard Cq were calculated from 4 reference standards run on each plate.

Results. Serum (n = 132) and individual OF (n = 130) samples from 12 pigs vaccinated with a PRRSV MLV from -7 to 42 DPV were tested and Cqs converted to ECqs. Testing was done using commercial reagents (IDEXX Laboratories) and the MIC PCR Cycler (Bio Molecular Systems). PRRSV RT-qPCR positives were detected on DPV 3 in serum ($\bar{x}$ ECq 0.90) and OF (mean ECq 1.07). That is, in both serum and oral fluids, the concentration of virus was about the same as in the reference standards. Thereafter, virus concentration peaked on DPV 7 in serum (mean ECq 2.24) and on DPV 9 in OF (mean ECq 2.00). For serum, the diagnostic sensitivity and specificity was estimated as 97.9% and 100% at an ECq cutoff of ≥ 0.20 and, for OF, 82.6% and 100%, respectively, at ECq ≥ 0.45.

Conclusions. Benefits of normalized PCRs are major: 1) results within or between labs using the same reference standard are comparable (Cqs are not comparable); 2) all results have an easily interpreted numeric value - the larger the ECq, the more nucleic acid in the sample relative to the reference standard; 3) diagnostic sensitivity and specificity can be derived for normalized PCRs.

Paper sampling for passive environmental surveillance for swine pathogens

B Armenta-Leyva1*, B Munguía-Ramírez1, LG Giménez-Lirola1, J Zimmerman1

1College of Veterinary Medicine, Iowa State University, Ames, Iowa.
*Presenting author

Introduction

Passive environmental surveillance (PES) detects pathogens circulating in the population of interest by sampling their environment. Unlike point-in-time environmental sampling, PES samplers remain in place over time, thereby increasing the probability of detection. In laboratory animal colonies, PES was shown to be as sensitive as sampling the animals themselves.

Our long-term objective is to adapt PES to swine production. For this application, sampling devices would be placed in production facilities, collected at designated time periods, and tested for the pathogen(s) of interest. The first step in adapting PES to swine production is identifying the best material ("membrane") to use in PES samplers. In this pilot, we used PRRSV as the target.

Methodology

Seven candidate membranes were evaluated: (1) Whatman filter paper; (2) Flinders Technology Associates (FTA) cards; (3) polyester filter paper; (4) SmartSolve cardstock (soluble in water); (5) dry surface polyester; (6) tacky surface polyester; (7) starch foam sheet (soluble in water). In brief, membranes were inoculated with 100 ul of four ten-fold dilutions (10-2 – 10-5) of a PRRSV MLV (Ingelvac) that was rehydrated and diluted with PRRSV-free oral fluids. After overnight storage at 25°C, membranes were eluted with TE buffer and the eluate subjected to extraction and RT-qPCR using commercial reagents (IDEXX Laboratories). Target recovery was expressed in terms of percent recovery from the inoculum.

Results

PRRSV	Candidate membranes (% recovery relative to inoculum)						
dilution	1	2	3	4	5	6	7
1×10^{-1}	21	30	24	5	1	24	10
1×10^{-2}	27	36	20	19	2	15	11
1×10^{-3}	24	28	26	23	3	13	8
1×10^{-4}	15	19	16	11	0	13	7

Conclusion

Preliminary results indicate that PRRSV RNA can be recovered from various types of membranes, albeit more efficiently from some. Future experiments will focus on the optimization of target recovery from the membranes for swine pathogens and stability of pathogens on membranes by time and temperature.

Repeat Offenders: understanding PRRSV clinical rebreaks

J.P. Baker1*, A. Rovira2, K. VanderWaal1.

1Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, 2Veterinary Diagnostic Laboratory, University of Minnesota, Saint Paul, MN.

Immunization-based control efforts on sow farms, such as gilt acclimation with vaccination or live virus inoculation, exist to ensure that herds have been exposed to and developed immunity against the "resident" PRRSV variant. However, repeated clinical outbreaks of PRRSV caused by very closely related viruses do occur, and are incredibly frustrating for the practitioner and producer. The ability of a very closely related virus to induce a second clinical outbreak of disease suggests that the virus has changed, allowing it to reinfect a herd with preexisting immunity. Thus, the objective of this study is to generate and characterize PRRSV whole genomes from viral isolates associated with clinical rebreaks of near identical PRRSV variants on single sow farms, to examine for patterns of change that could be related to viral immune escape.

A participating farm system provided production data (weekly abortion counts) and diagnostic data (weekly PCR and ORF5 sequence diagnostics) for sow farms from 2014 to the present. Weekly abortion counts were evaluated using statistical process control methods (EWMA) to identify spikes in abortion counts above the weighted average baseline. Processing fluid PCR CT values for the concurrent time period of an abortion spike were investigated to confirm that they were PRRSV positive and that CT values were dropping, to indicate that PRRSV was likely involved in abortion spike. These two criteria constitute a "clinical break" of PRRS. In this study, we also focused on breaks where an ORF5 gene sequence could be associated with the abortion spike. A "clinical rebreak" follows the same definition as above with additional criteria: 1) the second clinical break must occur within 3-12 months of a previous break, and 2) the ORF5 gene sequence obtained from the second clinical break must be ≥97% similar, at the nucleotide level, to the first clinical break. Upon fulfilling these criteria, viral isolates from these defined clinical breaks were utilized for whole genome sequencing.

12 sow farms experienced clinical rebreaks, as defined above, between 2017 and 2023. Of these 12 farms, 10 experienced a single rebreak event. The remaining farms (n=2) experienced multiple rebreak events and/or a series of rebreaks – meaning there were a series of 3 or 4 sequential rebreaks that satisfied our definition of rebreaks. The mean percent nucleotide difference (on ORF5) between all clinical rebreak pairs was 1.57% (SD = 0.974%) while the mean number of days between rebreaks was 208 days (SD = 71.7 days). In all, 33 whole genome sequences have been obtained from viral isolates corresponding to clinical breaks.

These results indicate that it is not uncommon for herds to break with PRRSV despite having recently been exposed to a very closely related virus. Though these clinical breaks are potentially milder than those caused by the introduction of a novel (to the farm) virus, they still impact productivity. In this analysis, we characterize genomic differences that frequently accrue *between* rebreak events on a single farm, identifying "hot spot" regions potentially contributing an immune evasion phenotype that may allow the variant to re-infect a previously exposed population.

Characterization of porcine respiratory coronavirus (PRCV) strains from Europe and the USA

A.E. Bedsted1*, T.B. Rasmussen2, P. Normann1, L.D. Martinenghi1, A. Bøtner1, G.J. Belsham1.

1Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870, Frederiksberg, Denmark, 2Department of Virus and Microbiological Special Diagnostics, Statens Serum Institut, Copenhagen, Denmark.

Coronaviruses infect a broad spectrum of avian and mammalian hosts and may cause a wide array of symptoms. As seen with SARS-CoV-2, coronaviruses may also change host- or tissue tropism over time. Porcine respiratory coronavirus is another example of such a virus, having an extensive deletion in the spike protein coding sequence compared to the parental transmissible gastroenteritis virus (TGEV). A prerequisite for a change in coronavirus host- or tissue tropism is the adaptability of the viral structural proteins (spike (S), envelope (E), membrane (M), and nucleocapsid (N)) in order for the virus to enter and replicate in a new environment. In the present study, we aimed to investigate sequence variations in the genes encoding the structural proteins between various TGEV and PRCV sequences, with a focus on variations between strains from Europe and the USA.
RNA was extracted from an Italian PRCV strain (PRCV 15087/12 III NPTV Parma) and the viral genome was sequenced on an Illumina MiSeq sequencing system. Nucleotide sequence alignments were prepared using this sequence along with a sequence of a Belgian strain (PRCV 91V44), as well as seven PRCV strains and about forty TGEV strains from Europe and the USA obtained from GenBank. Phylogenetic trees were built based on these sequence alignments, using the PhyML and MrBayes algorithms.
In the sequence analysis, we found variations among the genes encoding the four structural proteins for the PRCV strains from Europe and from the USA. The deleted region in the PRCV S gene was not the same size for all strains, which could indicate that multiple events have occurred to alter the sequence from TGEV. Phylogenetic analyses showed that PRCV strains from Europe clustered separately from the PRCV strains from the USA.
To conclude, we found differences between the European and US PRCV strains, including variations in the deletion in the S gene of PRCV compared to TGEV.

Specific regions in CD163 required for porcine reproductive and respiratory syndrome virus (PRRSV) infection are not necessary for binding to viral envelope proteins

Ana M.M. Stoian1, Raymond R.R. Rowland2, Alberto Brandariz-Nunez2*

1School of Medicine, Department of Medial Microbiology and Immunology, University of California Davis, Davis, CA
2Department of Pathobiology, College of Veterinary Medicine Administration, University of Illinois at Urbana-Champaign, Champaign, Illinois

CD163, a macrophage-specific membrane scavenger receptor, serves as a cellular entry receptor for porcine reproductive and respiratory syndrome virus (PRRSV). The viral receptor possesses nine scavenger receptor cysteine-rich (SRCR) and two proline-serine threonine (PST) domains.
Previous reports showed that removal of SRCR5 blocks infection. To further identify CD163 regions involved in PRRSV infection, deletion mutants were generated. Constructs were transfected into a PRRSV non-permissive cell line, and then evaluated for infection with PRRSV-2 or PRRSV-1. Infection experiments confirmed that SRCR5 is important for infection. Resistance to infection was observed following deletion of the SRCR4/5 interdomain region. Removal of Exon 13 that encodes a portion of PSTII also conferred resistance. A pentapeptide essential for infection located in SRCR5 and SRCR7 was also identified. By performing immunoprecipitation and colocalization studies, we found that the mutant CD163 proteins that resisted infection retained the ability to interact with the four viral glycoproteins: GP2, GP3, GP4 and GP5; and the nonglycosylated viral membrane protein M. The contribution of multiple domains to infection but not to the binding of viral envelope proteins suggests that the viral proteins may form multiple interactions with CD163, or that receptor regions important for infection have other cellular binding partners required for PRRSV infection.

Organotypic air-liquid interface respiratory cell culture (ALI-REC) system for studying coinfections with Influenza A subtypes H1N1 and H3N2 in pigs

A.F. Castillo-Espinoza1*, R.K. Nelli1, L. Yen1, G. Castillo, J.C. Mora-Diaz1, J. Zimmerman1, L. Gimenez-Lirola1

1Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

Objective: To describe the use of a porcine-derived ALI-REC culture system (ALI-PREC) as model to characterize single vs. coinfection of H1N1 and H3N2, Influenza A virus (IAV) subtypes commonly circulating in pigs, under strictly controlled in vitro conditions.

Methods. Tracheas collected from seven-days-old CD/CD pigs (n=3) were dissected, washed, and enzymatically digested to isolate PRECs, which were seeded into pre-coated transwells and cultured under ALI conditions for 4-5 weeks to allow them to differentiate. Differentiated ALI-PRECs were then inoculated (MOI 0.1 and MOI 1) with H1N1 (A/Swine/Minnesota/37866/1999), H3N2 (A/Swine/Texas/4199-2/1998), or both, along with mock uninfected controls. ALI-PRECs were exposed to viral/mock inoculum for 6 h at 37°C, 5% CO2, after that the inoculum was removed, the wells were washed, and further incubated for 24, 48, 72, and 96 h post inoculation (hpi). The outcome of the infection in ALI-PRECs was assessed microscopically and by immunocytochemistry (ICC), while the subnatants collected from the basolateral compartment were tested for IAV RNA detection by RT-qPCR.

Results. Microscopic evaluation showed active ciliary motility in both virus- and mock-inoculated ALI-PRECs up to 24 hpi. By 48 hpi, ciliary motility was significantly reduced in virus-inoculated ALI-PRECs, and the characteristic cytopathic effects (CPE), including dead/lifting cells, started to appear and became more pronounced by 72 hpi. At 96 hpi, virus-specific CPE was observed in all IAV-inoculated wells, for example, cytoplasmic stranding, vacuolation, rounding of cells, clusters of rounded cells, cell shrinkage, and cell detachment. ICC staining for IAV nucleoprotein further demonstrated that ALI-PRECs were permissive to initial virus entry and replication with detection at 24 hpi in fixed cells. Virus RNA by RT-qPCR was detected in the plate well subnatants of ALI-PRECs co-inoculated and single inoculated with H3N2 (MOI 1) as early as 24 hpi, and at 48 hpi in H1N1-inoculated ALI-PRECs. In summary, microscopic evaluation, ICC, and RT-qPCR data suggest that the ALI-PREC model is a suitable culture system for studying IAV single and coinfections in vitro.

Conclusions. Organotypic ALI-PREC is a suitable culture system for studying IAV single and coinfections of H1N1 and H3N2 subtypes in vitro, contributing to the advancement of research in a more ethical and innovative manner.
Research will continue focusing on the characterization of gene expression profiles associated with the innate immune response during H1N1 and H3N2 coinfection, and the development of an ALI-PREC and macrophages/dendritic cells co-culture system as bridge between the innate and adaptive immunity against IAV

Evolution and pandemic potential of the Eurasian avian-like H1N1 swine influenza viruses

Hualan Chen

Harbin Veterinary Research Institute, CAAS, China

Animal influenza viruses continue to pose a threat to human public health. The Eurasian avian-like H1N1 (EA H1N1) viruses are widespread in pigs throughout Europe and China and have caused human infections in several countries, indicating their pandemic potential. To carefully monitor the evolution of the EA H1N1 viruses in nature, we collected nasal swabs from 103,110 pigs in 22 provinces in China between October 2013 and December 2019, and isolated 855 EA H1N1 viruses. Genomic analysis of 319 representative viruses revealed that these EA H1N1 viruses formed eight different genotypes through reassortment with viruses of other lineages circulating in humans and pigs, and two of these genotypes (G4 and G5) were widely distributed in pigs. Animal studies indicated that some strains have become highly pathogenic in mice and highly transmissible in ferrets via respiratory droplets, and we further revealed that the human-adapted PA gene of influenza virus plays an important role in the pathogenicity and efficient transmission in ferrets of the EAH1N1 reassortants. Moreover, two-thirds of the EA H1N1 viruses reacted poorly with ferret serum antibodies induced by the currently used H1N1 human influenza vaccine, suggesting that existing immunity may not prevent the transmission of the EAH1N1 viruses in humans. Our study reveals the evolution and pandemic potential of EA H1N1 viruses and provides important insights for future pandemic preparedness.

Intranasal Ad5 influenza vaccine elicits hemagglutinin-specific antibody response in pregnant and lactating pigs

John Byrne1, Diego Leal1, Tatiane Watanabe1, Danielle Meritet1, Juliana Ferreira1, Christopher Beverly2, Susan Johnson3, Sean N. Tucker3, Stephanie N. Langel4, Elisa Crisci1*

1Department of Population Health and Pathobiology, North Carolina State University College of Veterinary Medicine, Raleigh, NC.
2Duke Human Vaccine Institute, Duke University School of Medicine, Durham, NC
3Vaxart, South San Francisco, CA
4Center for Global Health and Diseases, Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH

Influenza A virus can cause severe complications for pregnant women and infants. New vaccines and strategies are being implemented to increase global access to vaccination in these vulnerable populations. Additionally, there are no influenza vaccines approved for infants younger than six months. While inactivated intramuscular (IM) vaccines are currently available for pregnant women, IM immunization may not be an ideal route to boost neutralizing specific antibodies in breastmilk. The aim of the study was to evaluate the capacity of a hemagglutinin (HA) (A/California/2009(H1N1)) Ad5 vector vaccine to induce specific passive immunity in pregnant and lactating pigs using different routes of administration. Pigs were used as a translational model to investigate the protective level of passive maternal antibodies in infants, after mucosal immunization. Influenza naïve pregnant pigs were vaccinated via oral or intranasal routes three weeks prepartum and boosted four weeks later (one week postpartum). Serum, colostrum and milk samples, as well as samples from the nasal mucosa and saliva were collected to measure the level of HA-specific antibodies induced by the vaccine over time in different mucosal tissues. Data showed that both HA-specific IgG and IgA antibody responses were induced by vaccination through the intranasal route in serum, colostrum and milk, but not after oral vaccination. Future research will evaluate the neutralization capacity of these HA-specific antibodies, as well as showing if this vaccine induces passive protection in a piglet challenge trial.

The role of pathogens and anti-PRRSV immunity in the Porcine Respiratory Disease Complex

Elisa Crisci1*, Andrew R. Kick1&2, Lizette M. Cortes1, John J. Byrne1, Amanda F. Amaral1, Phillip C. Gauger3, Emily Mahan-Riggs4, Jeremy Pittman4, Tobias Käser1,5

1Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA
2Department of Chemistry & Life Science, United States Military Academy, West Point, NY, USA
3Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA
4 Smithfield Foods, Smithfield, VA, USA
5 Institute of Immunology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria

The Porcine Respiratory Disease Complex (PRDC) is a multifaceted polymicrobial syndrome that results from a combination of environmental stressors, primary infections (e.g., the immunosuppressive PRRSV) and secondary or opportunistic infectious agents (viruses and bacteria). PRDC causes severe lung pathology leading to reduced performance together with increased mortality rates and production costs in the pig industry worldwide. While previous studies have evaluated the role of PRRSV or selected primary/secondary pathogens, our goal was to perform a comprehensive study correlating both the anti-PRRSV immune response as well as 20 secondary infectious agents with PRDC disease severity. To this end, PRRSV-negative weaners were vaccinated with an MLV and put into a farm with PRDC history. Then, the anti-PRRSV cellular and antibody response was followed pre-vaccination, at 4 weeks post vaccination and during PRDC. In addition, NanoString was used to quantify 20 pathogens within the bronchoalveolar lavage (BAL) at day of necropsy – during PRDC outbreak. Nine pathogens out of 20 were detected in at least one pig and PRRSV was present in 53 out of 55 pigs. During PRDC, the median neutralizing antibody titers against the farm-prevalent PRRSV strain in both BAL and serum ranged from 0-64. The anti-PRRSV interferon-gamma response ranged from absent to very strong (>1,000 spots/million PBMC). Surprisingly, single correlation analyses did not reveal significant correlations between the anti-PRRSV responses or pathogens and PRDC disease severity supporting the multifaceted nature of PRDC. Therefore, future studies will use the collected data to perform more in-depth combinatorial correlation analyses to the role of PRRSV and secondary infectious agents in PRDC.

Predict and Protect against PRRSV (PreProPRRSV): Combining PRRSV forecasting technology with vaccine efficacy prediction to prevent PRRSV outbreak

Gustavo Machado1, John Byrne1, Lanre Sulaiman1, Jason Ardila Galvis1, Faith Kennedy1, Juliana Ferreira1, Glen Almond1, Tobias Käser1,2, Elisa Crisci1*

1 Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, USA.
2 Institute of Immunology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria.

The high mutation rate of PRRSV represents a big challenge and raises two important questions for swine producers: Which PRRSV strain will hit my farm next? And which vaccine can best protect my herd against it? Currently no technology can adequately answer those questions. To overcome this issue, we have combined two state-of-the-art technologies – PRRSV forecasting and heterologous vaccine efficacy prediction. These technologies will create the first proactive PRRSV mitigation system: Predict and Protect against PRRSV (PreProPRRSV). Thereby, for the first time, PRRSV outbreak mitigation will become proactive!
The PreProPRRSV system integrates two components:
1. Establishment of PRRSV Forecasting Technology. This forecasting methodology uses computer-based prediction algorithms based on surveillance data relevant to predict PRRSV spread – both intrinsic (e.g., variation of pathogen strains) and extrinsic (e.g., landscape) variables, pig transporting, and farm locations. This technology can precisely predict the spread of PRRSV strains in North Carolina (NC).
2. Establishment of a Vaccine Efficacy Prediction System. This system consists of an immune biobank (cells + serum) from pigs which received different PRRSV vaccinations. This biobank is established at the North Carolina State Veterinary College and will enable to determine which vaccine induces the strongest immune response to an approaching NC PRRSV strain.
This interdisciplinary project combines computer-algorithm-based forecasting with translational immunology to enable precision animal management for PRRSV in North Carolina: It will determine the most effective vaccine before the emerging PRRSV strain arrives at a production site.
The PreProPRRSV system aims to enhance pig hea**lth and production by decreasing the impact of PRRS with a proactive outbreak mitigation approach.**

Case study: The impact of Next Generation Biosecurity on preventing porcine reproductive and respiratory syndrome virus infections in breeding herds from a large-scale commercial swine production system in the US

S. Dee*, J. Nerem, A. Schelkopf, L. Brands, K.A. Havas, B. Kerkaert, G. Spronk

Pipestone Research, Pipestone Veterinary Services, Pipestone, MN.

Objective: Porcine reproductive and respiratory syndrome is the most economically significant disease of pigs and has been estimated to cost the US swine industry $600M/year. The key to controlling PRRS is preventing the introduction of porcine reproductive and respiratory syndrome virus (PRRSV) infection of breeding herds. This case describes how Next Generation Biosecurity successfully reduced wild type PRRSV infections and decreased PRRS incidence over two consecutive disease years across a large-scale commercial swine production company known as the Pipestone System.

Methods: In 2009, PRRS incidence across Pipestone System breeding herds was 50%, with many herds experiencing annual infection with new wild type PRRSV variants. To enhance disease control, Next Generation Biosecurity (NGB) protocols were applied sequentially over time to mitigate the direct routes of PRRSV transmission, (incoming breeding stock and semen), and indirect routes (mechanical/fomite-based, aerosols, and feed). To measure the impact of NGB on preventing the introduction of new wild type PRRSV to system breeding herds, a case study was conducted encompassing two disease years: July 1, 2021 to June 30, 2022 and July 1, 2022 to June 30, 2023. For the study, a new wild type PRRSV introduction was defined as either a new variant entry into a previously naïve herd, or the introduction of a variant having > 2% heterology to historical PRRSV found in the farm. Incidence rates of PRRSV infection were compared between farms that completely incompletely implemented a NGB program. Complete implementation included an audited general biosecurity program, as well as use of mechanical, air filtration and feed mitigation (NGB COMPLETE), while an incomplete program (NGB INCOMPLETE) targeted direct, mechanical, and feed-based routes, but not the aerosol route.

Results: Over the 2-year period, NGB was applied across a total of 81 breeding herds, encompassing 392,592 sows over 10 US states. During this period, 57 of 81 breeding herds employed a NGB COMPLETE program, while 24 herds targeted direct, mechanical, and feed-based routes, but not the aerosol route (NGB INCOMPLETE). From disease year 1 (July 1, 2021, to June 30, 2022), and disease year 2 (July 1, 2022, to June 30, 2023), system wide PRRS incidence was 6.7% and 7.4%, respectively. During this time, 11 new PRRSV introductions were documented, with four new wild type PRRSV variants detected in NGB COMPLETE herds (7.0%) and seven in NGB INCOMPLETE herds (29.2%). In addition, further work will be presented that also considers swine density and other confounding factors.

Conclusions: This case study demonstrates that PRRS can be successfully controlled in breeding herds within a large commercial swine production system for an extended period via a NGB COMPLETE program. These are novel observations which justify the application of NGB protocols across the industry to reduce the impact of PRRS.

Transmission of porcine reproductive and respiratory syndrome virus in domestic pigs via oral ingestion of feed material

Scott Dee, Travis Clement, and Eric Nelson

History: There are many reported routes of transmission of porcine reproductive and respiratory virus (PRRSV), including direct routes (infected pigs and semen) and indirect routes, such as contaminated fomites (transport, personnel boots, coveralls, and supplies) and PRRSV-positive aerosols. While there is experimental evidence of transmission of PRRSV during natural feeding behavior, no information is available regarding the ability of contaminated feed to transport and transmit the virus under field conditions. The purpose of this case study is to describe the transmission of PRRSV under field and experimental conditions via the consumption of PRRSV-positive swine feed.
Clinical Presentation, Progression, and Procedures: A 2500 sow PRRSV-naïve biosecure breeding herd became infected during the autumn months. It experienced a feed outage involving a specific bin on October 23 (Day 0), with the bin refilled on October 24 (Day 1). From October 28 to 30 (Day 5-7), signs of anorexia and hyperemia were observed in 30 gestating sows after consuming feed from this bin. On November 1 (D9), blood samples from 10 affected sows were PRRSV positive by RT-PCR. In contrast, sows in the same room that had consumed feed from other bins were clinically normal and PRRSV negative. To investigate whether the feed delivery introduced PRRSV to the herd, on November 2 (Day 10) four samples of feed material from the interior walls of the index bin were collected and tested by RT-PCR.
Treatment and Outcome: All four samples were positive for PRRSV RNA with Ct values ranging from 26-29. Nucleic acid sequencing indicated that the ORF 5 region of the PRRSV in feed samples was 100% homologous to PRRSV from index cases. To assess viability of the virus, PRRSV-naïve pigs were allowed to consume the index feed bin samples and became infected with PRRSV based on viral RNA in oral fluid samples, clinical signs, and post-mortem lesions.
Clinical Relevance: These results suggest that feed was a likely source of PRRSV introduction to the herd. This is the first report of PRRSV transmission through feed.

Immunogenicity of chimeric hemagglutinins delivered by an Orf-virus-vector platform against Swine Influenza virus in pigs

Gabriela Mansano do Nascimento 1, Pablo Sebastian Britto De Oliveira 1, Salman Latif Butt 1, and Diego G. Diel 1

1Department of Population Medicine and Diagnostic Sciences, Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA.

Orf virus (ORFV) is a large DNA virus that is able to harbor and efficiently deliver viral antigens in swine. Here we used ORFV as a vector platform for chimeric hemagglutinins (HA) of Influenza A virus of swine (IAV-S). Effective vaccination against IAV-S is hindered by the antigenic diversity of the strains circulating in the field. A promising alternative aiming at focusing immune responses on conserved epitopes of the stalk segment of the hemagglutinin (HA2) has recently emerged. Sequential immunization with chimeric HAs comprising the same stalk but distinct exotic head domains can potentially induce cross-reactive immune responses against conserved epitopes of the stalk hemagglutinin (HA2) while breaking the immunodominance of the head (HA1) domain. Here, we generated two recombinant ORFVs expressing chimeric HAs encoding the stalk region of a contemporary H1N1 IAV-S strain and exotic heads derived from either H6 or H8 subtypes (OV-cH6/1 and OV-cH8/1). The resulting recombinant viruses expressed the heterologous protein in vitro. Further, the immunogenicity and cross-protection of these vaccine candidates were assessed in swine after sequential intramuscular immunization with OV-cH6/1 and OV-cH8/1, and subsequent challenge with divergent IAV-S strains. Humoral responses assessed by ELISA showed that animals primed and boosted with OV-cH6/1 and OV-cH8/1 presented increasing IgG responses in sera against a panel of 10 divergent IAV-S. Additionally, cross-reactive IgG responses elicited by immunization were detected by ELISA against different viral clades and a diverse range of contemporary H1N1 IAV-S strains. Importantly, Viral shedding was reduced in nasal swabs from vaccinated piglets after intranasal challenge with either Oh07 (gamma clade) or Ca09 strains (npdm clade) IAV-S strains. These results demonstrated the efficiency of ORFV-based vectors in delivering chimeric IAV-S HA-based vaccine candidates and underline the potential use of chimeric-HAs for prevention and control of influenza in swine.

Evaluation of biomarkers measured post-PRRS vaccination as potential indicators of resilience to PRRS virus infection

Claudia A. Sevillano1, Jenelle. R. Dunkelberger1,2*, Egbert F. Knol1

1Topigs Norsvin Research Center, 's-Hertogenbosch, the Netherlands, 2Topigs Norsvin USA, Burnsville, MN, USA

Selection for enhanced resilience to PRRS requires collection of individual data under challenge, which is time-intensive and costly. Therefore, identifying an indicator trait for resilience to challenge is highly desired. The objective of this study is to evaluate whether PRRS viremia and antibody response, measured following PRRS vaccination, can be used as indicators of resilience, by evaluating their effect on growth rate following experimental challenge. Data were collected as part of an experimental PRRS virus (PRRSV) challenge trial involving 1,786 commercial three-way crossbred pigs. Pigs were vaccinated using a PRRS modified live virus vaccine within one week of placement and subsequently inoculated with 2 x 103.5 TCID50 of PRRSV 1-7-4 via intramuscular injection at six weeks post-vaccination. Individual body weights were recorded at weaning, placement, vaccination, 0 days post-infection (dpi), 21 dpi, and at death. Average daily gain (ADG) was calculated as the ratio of body weight gain from 0 to 21 dpi to phase length in days. Missing body weights for animals that died prior to 21 dpi were imputed using linear regression. Blood samples were collected for 950 pigs at 14 days post-vaccination (dpv) to measure PRRS vaccine viremia and at 42 dpv to measure PRRSV-specific IgG level, reported as sample-to-positive (S/P) ratio. Ear tissue was collected from each pig for genotyping using a custom 25K single nucleotide polymorphism (SNP) panel and imputed to 50K density. Genetic parameters were estimated for vaccine viremia, S/P ratio, and ADG using single-trait animal models and genetic correlations were estimated using bivariate models. An additive, linear genome-wide association study was conducted to identify genomic regions associated with each trait. Heritability of ADG under PRRS challenge was 0.20 (0.04), where litter explained 4% of the phenotypic variance. Heritability estimates for vaccine viremia and S/P ratio were 0.29 (0.07) and 0.63 (0.06), where litter explained 4% and 2% of the phenotypic variance, respectively. Although SEs were large, genetic correlations between ADG and vaccine viremia (-0.13 ± 0.18) were in the expected direction, but not for ADG and S/P ratio (-0.07 ± 0.13). A region on chromosome 4, harboring the GBP5 gene, was associated with both ADG and vaccine viremia. Results from this study validate previous findings, where the favorable “A” allele at the causative mutation within this gene was associated with 42 extra grams of growth per day and a reduction in 0.25 logs of PRRS viremia. A region on chromosome 7, associated with both ADG and S/P ratio, was also detected. This region harbors the swine leukocyte antigen genes, which have known roles in immunity. In conclusion, results from this study show that vaccine viremia and S/P ratio following PRRS vaccination, and ADG following inoculation with a field strain, have a substantial genetic basis. Two main regions were associated with host response to both PRRS vaccination and subsequent experimental challenge. Genetic correlations between PRRS vaccine viremia and growth rate post-challenge suggest that PRRS vaccine viremia may be a promising indicator trait for selecting pigs for enhanced, natural resilience to challenge with a field isolate of PRRSV.

Reassessment of the Susceptibility of Different Porcine Macrophage Populations to Porcine Reproductive and Respiratory Syndrome Virus Infection

K. Durazo-Martinez1*, J. Chaudhari1, S. Kumari2, and H. Vu1

1Dept of Animal Science and Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE, 2School of Vet. Science and Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE

Porcine Reproductive and Respiratory Syndrome (PRRS) has been around the globe for over 40 years. In the U.S., PRRS has remained one of the most economically important swine pathogens generating losses of $1 billion annually. The causative agent, the PRRS virus (PRRSV) is a small enveloped positive sense single-strand RNA virus. PRRSV is known for having a restricted host and cellular tropism, pigs are the only known host, and porcine alveolar macrophages (PAM) are the main cellular target. It has been reported that porcine peritoneal macrophages (PPM) are not susceptible to PRRSV infection. This is intriguing because both PAM and PPM belong to the same family of monocyte/macrophages. The present study aimed to identify host factors responsible for the contrasting susceptibility to PRRSV infection observed between PAM and PPM. Briefly, PPM and PAM were collected from 6- to 8-week-old, PRRSV-negative pigs. The cells were analyzed for the expression of three macrophage makers, including CD163 and CD169, two well know PRRSV receptors, and CD14, a known monocyte/macrophage lineage marker. Preliminary results indicate both PAM and PPM expressed high levels of CD163. However, the levels of CD14 were low on PAM but high on PPM. The two cell types were infected with different PRRSV strains to evaluate their susceptibility to PRRSV infection. As expected, PAMs are highly susceptible to PRRSV infection. Contrary to previous reports, we observed that PPMs were also highly susceptible to both type 1 and type 2 PRRSV strains. To determine if PRRSV infection of PMM was dependent on CD163, an antibody blockage experiment was conducted. Pretreatment of PAMs or PPMs with an anti-CD163 antibody completely prevented the cells from being infected with PRRSV. Overall, our results demonstrate that PPMs express a high level of CD163 and are susceptible to PRRSV infection

Classical swine fever virus Npro evades from host cell innate immune responses by destabilizing Sp1 and HMGB1

X. Han1, R. Chen1, H.K. Xu1, Y.F. Weng1, J.D. Xu1, X.L. Li1, W.H. Fang1,2*
1Zhejiang University Institute of Preventive Veterinary Medicine; 2Zhejiang A&F University College of Veterinary Medicine, Hangzhou, China
* Correspondence: whfang@zju.edu.cn

Classical swine fever virus (CSFV) is known to dampen the host innate immunity by destabilizing IRF3 upon its binding with viral Npro. Viral Npro also interacts with other host proteins, including IB involved in NF-B signaling, antiapoptotic HAX-1, etc. We wonder if Npro interacts with other important host proteins, such as Sp1 (transcriptional regulator) and proinflammatory HMGB1 that we came across while working on virus-mediated host protein acetylation, and what would be the functional activities and mechanisms behind the interactions. IPEC-J2 and porcine alveolar macrophage 3D4/21 cells were used to infect wild-type CSFV or its Npro-deletion mutant virus with or without chemical inhibitors or gene-knockout or siRNA targeting the relevant molecules. The cell samples were subjected to confocal imaging, immunoblotting (IB) or immunoprecipitation plus IB, transcription of target molecules by qPCR and virus titration.
We found that Npro interacts with and downregulate Sp1 via the ubiquitin-proteasomal pathway, leading to reduced expression of class I deacetylase HDAC1, one of its downstream targets. Npro also interacts with HMGB1, and promotes its acetylation and nucleocytoplasmic translocation, resulting in its lysosomal degradation. We also show that both HDAC1 and HMGB1 are antiviral host factors involved in initiating IFN-I/IFN-III responses that are inhibitable by RNAi or chemical inhibitors targeting Sp1, HDAC1 or HMGB1. Using the wild-type CSFV and its Npro-deletion mutant, we are able to show that Npro is involved in evasion from HDAC1- and HMGB1-mediated innate immune responses. Interestingly, CSFV uses its Npro N-terminal domain to interact with Sp1, a region that does not have significant effect on IRF3 stability. Thus, it is clear that CSFV deploys the two domains of its Npro to counteract the innate immune responses, the C-terminal one targeting the IRF3 pathway as previously reported, and the N-terminal one targeting the Sp1-HDAC1-HMGB1 axis. This research provides insights into better understanding of CSFV immune evasion strategies as part of its pathogenetic mechanism.

Acknowledgement: Zhejiang Provincial Department of Science and Technology Key R&D project (2023C02023) and China National Natural Science Foundation (32172819).

The Art of PRRSV Infection and Replication

Ying Fang

Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

PRRSV is an enveloped, positive-sense, single-stranded RNA virus. The viral genome is about 15 kb in length. The 3′ end of the viral genome encodes envelope proteins and also nucleocapsid protein that encapsulates the genomic RNA. The 5′ two-thirds of the viral genome encodes two large replicase polyproteins, pp1a and pp1ab, which are proteolytically processed into at least 14 functional nonstructural proteins. In our previous studies, two novel proteins, nsP2TF and nsp2N, were found to be expressed in the nsp2-coding region through a −2/−1 programmed ribosomal frameshifting (PRF) mechanism. Our recent study revealed that the frameshifting rate at nsp2 PRF site is temporally regulated, in which the increasing of -2 PRF efficiency is likely facilitated by accumulation of the PRF-stimulatory viral protein, nsp1β. Remarkably, we find that PRF efficiency at the canonical ORF1ab frameshift site is also temporally regulated, which challenges the traditional assumption of a fixed efficiency for the numerous other viruses with canonical PRF sites.

PRRSV infection can be divided into at least two distinct stages: acute infection and persistence. Initial acute infection leads to the cytopathic replication of the virus in host cells, resulting in the release of viral particles that subsequently transmit to naive cells through standard receptor-mediated endocytosis. Recently, alternative pathways for intercellular spreading of PRRSV infection have been identified, in which viral infectious materials can be transmitted through intercellular nanotube connections (TNTs) and exosomes. Utilizing these pathways to directly access the cytoplasm of a naive cell present efficient spreading routes for the virus to bypasses many of the otherwise critical assembly, budding, and cell entry steps. Mitochondrion, an important regulator for cell survival/death, appears to be transported through TNTs to regulate the fate of PRRSV-infected cells. PRRSV takes advantage of this pathway and utilizes the mitochondrion as a cargo to transport viral infectious materials for cell-to-cell spreading of the infection. Intercellular transmission through these novel pathways allows the virus to escape the host immune responses, which may contribute to the pathogenesis of viral infection and persistence.

Current advanced technologies have enhanced our capability for in depth studies on the molecular mechanisms of PRRSV persistence. A cellular model of a persistently infected cell line has been established. Using this model system, viral dsRNA was revealed to be associated with the TNT pathway and function as a mediator for PRRSV persistence. Pig models have been used to confirm the findings in the cell culture system. PRRSV dsRNA was detected persisting in lymphoid tissues of infected pigs. Importantly, the germinal center of the lymphoid tissue was identified as a potential reservoir for dsRNA persistence. The RNA array analysis showed that dsRNA in lymphoid tissues of persistently infected pigs had limited ability to stimulate host antiviral responses, suggesting that viral dsRNA persistence in the germinal center allows the virus to escape antiviral immune responses. Studies are underway to identify the potential vial and host factors as indicators for viral persistence.

The ability of PRRSV to invade the host immune system and establish persistent infection significantly impedes our efforts to eliminate PRRS. Novel mechanisms of PRRSV replication and persistence revealed in our studies provide fundamental knowledge and new directions for developing diagnostic assays, therapeutics and other control measures.

MARCO inhibits porcine reproductive and respiratory syndrome virus infection through intensifying viral GP5-induced apoptosis

Xiaoxiao Zhanga, Chunhe Guoa,*

a Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong, PR China

*Correspondence author. E-mail: guochunh@mail.sysu.edu.cn

Abstract

Studying viral glycoprotein-host membrane protein interactions contributes to the discovery of novel cell receptors or entry facilitators for the virus. Glycoprotein 5 (GP5), a major envelope protein of porcine reproductive and respiratory syndrome virus (PRRSV) virions, is a key target for the control of the virus. Here, macrophage receptor with collagenous structure (MARCO), a member of the scavenger receptor family was identified as one of the host interactors of GP5 through a DUALmembrane yeast two-hybrid screening. MARCO was specifically expressed on porcine alveolar macrophages (PAMs) and PRRSV infection down-regulated MARCO expression both in vitro and in vivo. MARCO was not involved in viral adsorption and internalization processes, indicating that MARCO may not be a PRRSV-entry facilitator. Contrarily, MARCO served as a host restriction factor for PRRSV. Knockdown of MARCO in PAMs enhanced PRRSV proliferation while overexpression suppressed viral proliferation. The N-terminal cytoplasmic region of MARCO was responsible for its inhibitory effect on PRRSV. Further, MARCO knockdown weakened virus-induced apoptosis whereas overexpression aggravated apoptosis, and it also could intensify viral GP5-induced apoptosis. These suggest that MARCO is involved in the regulation of apoptosis induced by PRRSV and the interaction between GP5 and MARCO may contribute to the exacerbation effect of MARCO on PRRSV-induced apoptosis. Additionally, inhibition of apoptosis during infection weakened the anti-PRRSV effects of MARCO, indicating that MARCO inhibits PRRSV through apoptosis regulation. Taken together, this study reveals a novel antiviral mechanism of MARCO and suggests a molecular basis for the potential development of therapeutics against PRRSV.

Contribution of PRRSV nsp2 genetic variation to viral virulence and persistence

Can Kong, Dan Li, Yanxin Hu, Peng Gao, Yongning Zhang, Lei Zhou, Xinna Ge, Xin Guo, Hanchun Yang, Jun Han*,

Key Laboratory of Animal Epidemiology of the Ministry of Agriculture and Rural Affairs, College of Veterinary Medicine, China Agricultural University, Beijing 100193, People's Republic of China

Fast evolution in the field of the replicase nsp2 represents a most prominent feature of porcine reproductive and respiratory syndrome virus (PRRSV). Here, we determined its biological significance in viral pathogenesis by constructing inter-lineage chimeric mutants between the Chinese highly pathogenic PRRSV (HP-PRRSV) strain JXwn06 (lineage 8) and the low virulent NADC30-like strain CHsx1401 (lineage 1). Replacement with nsp2 from JXwn06 was surprisingly lethal to the backbone virus CHsx1401, but combined substitution with the structural protein-coding region (SP) gave rise to viable virus CHsx1401-SPnsp2JX. Meanwhile, a derivative carrying only the SP region (CHsx1401-SPJX) served as a control. The gain of function study by animal experiments revealed that the strain CHsx1401 is a sneaky virus in terms of its immune activation ability, and that the HP-PRRSV nsp2, but not the NADC30-like nsp2, is a strong activator of host inflammation and cellular immune responses, and the immune activation status in the individual groups correlated well with the rate of viremia clearance and viral tissue load reduction. In the subsequent vaccination/challenge experiments by using CHsx1401 or its chimeric mutants as immunogens followed by challenge with JXwn06. We found that the SP region is the determinant for inducing homologous immunity, whereas while the HP-PRRSV nsp2 contributed greatly to viral clearance of both vaccine virus and challenge virus in various tissues. Overall, the above results highlight the importance of nsp2 genetic variation in modulating PRRSV virulence and persistence via immune modulation, and provide insight into the mechanisms underlying the protective immunity against PRRSV.

Riding apoptotic bodies for cell-cell transmission by African swine fever virus

Peng Gao1, Lei Zhou1, Jiajun Wu2, Wenlian Weng1, Hua Wang1, Miaomiao Ye1, Yajin Qu1, Yuxin Hao2, Yongning Zhang1, Xinna Ge1, Xin Guo1, Hanchun Yang1, Jun Han*1,

1 National Key Laboratory of Veterinary Public Health and Safety, Key Laboratory of Animal Epidemiology of the Ministry of Agriculture and Rural Affairs, College of Veterinary Medicine, China Agricultural University, Beijing 100193, People's Republic of China.
2 China Animal Disease Control Center, Beijing 100125, People's Republic of China.

African swine fever virus (ASFV), a devastating pathogen to the worldwide swine industry, mainly targets macrophage/monocyte lineage, but how the virus enters host cells have remained unclear. Here we report that ASFV utilizes apoptotic bodies (ApoBDs) for infection and cell-cell transmission. We show that ASFV induces cell apoptosis of primary porcine alveolar macrophages (PAMs) at late stage of infection to productively shed ApoBDs that are subsequently swallowed by neighboring PAMs to initiate a secondary infection as evidenced by electron microscopy and live-cell imaging. Interestingly, the virions loaded within ApoBDs are exclusively single-enveloped particles that are devoid of the layer of viral outer-membrane and represent a predominant form produced during late infection. The in vitro purified ApoBD vesicles are capable of mediating virus infection of naive PAMs, but the transmission can be significantly inhibited by blocking the “eat-me” signal phosphatidyserine (PS) on the surface of ApoBDs via Annexin V or the efferocytosis receptor TIM4 on the recipient PAMs via anti-TIM4 antibody, whereas overexpression of TIM4 enhances virus infection. The same treatment however did not affect the infection by intracellular viruses lacking the shield of outer-envelope or ApoBDs. Importantly, the swine sera to ASFV exert no effect on the ApoBD-mediated transmission, but can partially act on intracellular virions. Thus, ASFV has evolved to hijack a normal cellular pathway for cell-cell spread to evade host responses.

Differential Antibody Response to PRRSV Glycoprotein 5 by Vaccine and Field Strains

J. Huang 1*, V. D. Krishna 1, A. Kennedy 1, I. A. D. Paploski 1, D. N. Makau 1, K. VanderWaal 1, D. C. Schroeder 1, M. C-J. Cheeran 1

1 Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN

The glycoprotein 5 (GP5) ectodomain of porcine reproductive and respiratory syndrome viruses (PRRSV) harbors three major antibody-recognition sites, designated as epitope A, B, and C. Epitope B is conserved across different viral strains and induces neutralizing antibodies, while epitope A is highly variable. Although epitope A has been found only in a minor proportion of virions in cultured VR-2332, potentially due to differential cleavage of the signal peptide on GP5, it can induce a robust antibody response, which is functionally non-neutralizing. It is proposed that antibody response to epitope A may divert the adaptive humoral immune response, consequently delaying the production of neutralizing antibodies against PRRSV. The present study was designed to investigate if antibodies to epitope A are induced differentially by specific PRRSV strains. Understanding the varying antibody responses to the epitope A amongst PRRSVs would benefit optimization of strains in future vaccine development.
Serum samples collected from naïve pigs and pigs inoculated with either of two modified live virus (MLV) vaccines or three field isolates of PRRSV were subjected to indirect ELISA to determine antibody responses to linear GP5 peptides containing the three major epitopes. These peptides were designed to include epitope A fused to a scrambled peptide, while others consist of GP5 ectodomains derived from each of the inoculated viral variants, with or without the epitope A. Antibodies to the recombinant nucleocapsid protein and a non-specific peptide of the same amino acid length served as the positive and negative control, respectively. Finally, protein sequences of GP5 ectodomain (amino acids 1-61) from different stains, including vaccine strains and field isolates, were analyzed for predicted signal peptide cleavage sites using SignalP 4.1.
ELISA results showed that antibody responses to the epitope A were dominant and sequence-specific in MLV-vaccinated animals. However, no significant difference was observed in the antibody response to epitope A between naive and experimentally infected animals. These data suggest differential induction of antibody responses to GP5 epitope A by MLV strains and field isolates, which likely results from alternate signal peptide cleavage in the two MLV strains studied here. Signal peptide cleavage analysis suggested that all selected MLVs retained epitope A on GP5 (cleaved between aa 26 and 27), while the other three field isolates belonging to different viral lineages (lineage 1C, 1A and 1G) were predicted to undergo cleavage between aa 31 and 32 thereby deleting epitope A from the viral GP5. Further serological studies are needed to determine the prevalence of epitope A-specific antibodies in infected or vaccinated animals, which would provide more insights for future PRRSV vaccine design.

ASFV pH240R affects the virulence of African swine fever virus by inhibiting antiviral innate immune response

Li Huang1,* , Changjiang Weng 1

1. Division of Fundamental Immunology, National African Swine Fever Para-reference Laboratory, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150069, China

African swine fever (ASF) is a highly contagious infectious disease caused by African swine fever virus (ASFV). ASFV-infected domestic pigs and wild boars have mortality rate as high as 100%. Upon ASFV infection, pigs initiate antiviral innate immune responses to antagonize viral infection and replication, ASFV has developed multiple strategies to evade host antiviral responses. However, the molecular mechanisms of ASFV-encoded proteins executing immune evasion are not fully understood. In this study, an unbiased screen was performed to screen the ASFV genes involved in the inhibition of the interferon (IFN) signaling, ASFV pH240R was found to inhibit IFN-α-mediated JAK-STAT signaling. ASFV pH240R inhibited both IFN-α-induced activation of the promoters of ISRE, ISGs and the mRNA transcription of Isg54 and Isg56. In addition, ASFV pH240R inhibited the IFN-α-induced phosphorylation and nuclear translocation of STAT1 and STAT2. Mechanistically, pH240R interacted with IFNAR1 and IFNAR2, and overexpressed pH240R significantly disrupted the interaction between IFNAR1 and TYK2 as well as the interaction between IFNAR2 and JAK1. Furthermore, pH240R was found to strongly inhibit IL-1β maturation and secretion. Interestingly, pH240R disrupted the IKK complex to inhibit NF-κB activation by interacting with NEMO and bound to NLRP3 to inhibit NLRP3 inflammasome activation, resulting in reduced IL-1β production. Compared with wild-type ASFV HLJ/18 strain, H240R-deficient ASFV (ASFV-ΔH240R) strain infection induced higher levels of ISGs and inflammatory cytokines expression both in vitro and in vivo. Consistently, H240R deficiency attenuated the virulence of the ASFV HLJ/18 strain in pigs. These findings suggest that pH240R plays a critical role in affecting viral virulence of the ASFV by antagonizing the type I IFN signaling and inflammatory responses, indicating that deletion of H240R gene may serve as a strategy to develop attenuated vaccines against ASFV.

Evaluating the Impact of Thyroid Hormone on the Efficiency of In Vitro PRRSV-2 infection

D. Jeon1* and J.A. Pasternak1

1Department of Animal Sciences, Purdue University, West Lafayette, IN, USA

Objective: Thyroid hormones (TH) are essential for living organisms, as they play fundamental roles in regulating various physiological processes, including metabolism, growth, fetal development, and immune function. This endocrine system is normally under strict homeostatic regulation, however in a previous study, we observed a reduction in circulating levels of Triiodothyronine (T3) and Thyroxine (T4) following Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection. The underlying physiological mechanism and biological purpose of this phenomenon remains unclear. We hypothesize that this suppression is part of the response to protect the host against PRRSV, as low TH will reduce cellular metabolism and thus limit availability of resources necessary for viral replication. Therefore, the overall objective of this preliminary study was to establish an in vitro method for quantitative assessment of cellular infection rate under different concentrations of TH.

Methods: To evaluate the potential in vitro cytotoxicity of TH, MARC 145 cells were first cultured with 3,3',5-Triiodo-L-thyronine (LT3) and L-thyroxine (LT4) at concentrations ranging from 0-1000nM. After 72 hrs of culture, cells were trypsinized, stained with Sytox Red Dead, and the proportion of dead cells evaluated by flow cytometry. Measurements of cell viability were taken from three plates, each with two replicates. To assess the impact of TH treatment on PRRSV infection rate, cells were cultured in media alone or supplemented with either LT3 or LT4 at concentrations of 0, 50, 200, and 1000 nM. After 48 hours of culture, cells were infected with PRRSV strain P129 (TCID50 9.5×106) for 5 hours. At 48 hours post infection, cells were trypsinized, fixed in 3.7% formaldehyde and fluorescently stained with PRRSV specific monoclonal antibody (SDOW-17) for quantitative assessment of infection rate by flow cytometry. Measurements of in vitro infection rate were taken from three plates, with three replicates per hormone concentration and a mock infected control.

Results: Neither T3 nor T4 increased cytotoxicity within the range of concentrations tested. PRRSV infection of MARC 145 cells with 9.5×106 TCID50 for 48 hrs resulted in an average infection rate of 32.75%±9.72 as measured by flow cytometry. Culture of MARC 145 cells for 48 hrs pre- and post infection with TH did not significantly increase the proportion of cells infected at any of the concentrations evaluated.

Conclusions: Exogenous TH does not appear to affect cellular viability of MARC 145 cells, even at high concentrations. In addition, there is no evidence to suggest that exogenous TH supplementation accelerates the infection rate following PRRSV infection in MARC 145 cells. These results do not support our initial hypothesis that the suppression of TH could be a part of a host defense mechanism aimed at reducing PRRSV replication. However, further work in this area is required to evaluate the expression of TH receptors in MARC 145 cells and to repeat the present experiment in more biologically relevant cells, such as primary porcine alveolar macrophages (PAMs).

Porcine reproductive and respiratory syndrome virus infection inhibits NF-κB signaling pathway through cleavage of IKKβ by Nsp4

Shuang Jiao 1, Changjiang Weng 1, *

1National African Swine Fever Para-reference Laboratory, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150069, Heilongjiang, China

Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly contagious porcine pathogen that causes serious economic losses to the world swine industry. The inhibitor kappa B kinase β (IKKβ), a catalytic subunit of the IKK complex, plays multiple roles in regulating the nuclear transcription factor kappa B (NF-κB) activity and a variety of cytokines transcription involved in immune responses. Here, we reported that the nonstructural protein 4 (Nsp4) of PRRSV cleaved IKKβ at the E378 site to inhibit the activation of NF-κB signaling pathway. Additionally, we clearly showed that cleavage of IKKβ by PRRSV Nsp4 depends on the 3 C-like serine protease activity of Nsp4 because the catalytically inactivate mutants of Nsp4 lost the function to cleave IKKβ. Furthermore, we found that hydrophobic patch at the KD-ULD junction of IKKβ could be disrupted by PRRSV Nsp4 via the cleavage of the E378 site, resulting in disruption of NF-κB activity. Of note, the two cleavage fragments of IKKβ lose their function to phosphorylate IκBα and activate NF-κB signaling pathway. Our findings provide a clue to better understand the pathogenic mechanism of PRRSV involved in PRRSV evasion of host antiviral innate immune responses.

Susceptibility and Transmission of Emerging Zoonotic Rat Hepatitis E Virus in Swine

K.K. Yadav1,2, P.A. Boley1, C.L. Lee1,2, S. Khatiwada1, T. Laocharoensuk1,2, Jake Hofstetter1 K. Jung1, R. Wood1, J. Hanson1,2, S P Kenney1,2*

1Center for Food Animal Health, Department of Animal Sciences, The Ohio State University, Wooster, Ohio
2Department of Veterinary Preventive Medicine, The Ohio State University College of Veterinary Medicine, Columbus Ohio

Rocahepevirus ratti is an emerging hepatitis E virus (HEV). Known more commonly as "rat HEV" for its primary reservoir host, Norway rats, emerging strains have recently been found to be infectious to immunocompromised and immunocompetent humans. While zoonotic Paslahepevirus HEV strains primarily infect humans through consumption of tainted meat, how rat HEV transmits to humans is currently unknown. The objective of this study was to construct and characterize an infectious cDNA clone of zoonotic rat HEV and test whether swine would be susceptible to infection. To accomplish this objective, the complete genome of rat HEV strain LCK-3110 was inserted into a plasmid downstream of an SP6 promoter and capped genomic RNA was generated by in vitro transcription. Capped viral RNA infectivity was assessed by in vitro transfection of human hepatoma (Huh7), mouse subcutaneous tissue (LMTK), human carcinoma lung tissue (A549), and swine testicular (ST) cells. Direct intrahepatic inoculation of gnotobiotic pigs with capped RNA transcripts was performed to study the replication competence of rat HEV in vivo. Fecal suspension of intestinal contents derived from the rat HEV positive gnotobiotic pigs was intravenously inoculated via ear vein into conventional pigs. Sentinel pigs were comingled with the rat HEV inoculated pigs after 7 days post inoculation (DPI). Pigs were also inoculated with Paslahepevirus balyani human HEV (US-2) strain and PBS, as positive and negative controls, respectively. The results demonstrated that capped RNA transcripts of rat HEV were replication competent when transfected into A549, LMTK, and ST cells as shown by detection of HEV ORF2 positive cells using immunofluorescence and flow cytometry. Capped RNA transcripts of rat HEV produced active infection as evidenced by viremia and fecal virus shedding in gnotobiotic pigs. The infectivity was further confirmed by the successful infection of conventional pigs and transmission to sentinel pigs as shown by seroconversion, viremia, fecal virus shedding, and immunohistochemistry. These results suggest that swine have the potential to serve as a transmission vector for rat HEV to humans. The availability of this infectious clone of rat HEV and pig model now affords an opportunity to genetically study the mechanisms of HEV cross-species infection and tissue tropism.

Opening the pools: What is behind tongue tips sampling and other welfare-friendly postmortem samples for accurately detecting PRRSV?

M. Kikuti1*, C.M. Melini1, X. Yue1, M. Culhane1, C.A. Corzo1

1Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA

Objective
Specimens collected from dead pigs are a welfare friendly and possibly cost-effective active surveillance method with comparable sensitivity to antemortem specimens for PRRSV detection. We aimed to determine whether postmortem sampling of suckling piglets allows for the detection of PRRSV in farms undergoing elimination and to determine the sensitivity of each postmortem specimen studied.

Methods
Two farrow-to-wean farms undergoing a wild-type PRRS outbreak and that had decided to pursue elimination were conveniently selected. Each farm was sampled at approximately 8 and 20 weeks after the outbreak detection. Postmortem sampling of piglets found dead or euthanized according to farms' regular animal care protocols was conducted. Postmortem specimens consisted of individual sterile swabs of the nasal, oral, and rectal cavities, tongue tips, and serum collected from intracardiac blood. All specimens were stored and tested individually for PRRSV by rRT-PCR Additionally, we collected serum from 30 and 60 live piglets during the first and second sampling points, respectively, via jugular venipuncture. Sera from live piglets were tested for PRRSV by rRT-PCR in pools of five; any blood from positive pools were then tested individually. Sera from live piglets were used to estimate the within-farm PRRS prevalence using the current industry monitoring sampling approach. The sensitivity and agreement of tongue tips and oral, nasal, and rectal swabs were calculated using the postmortem intracardiac sera as the assumed gold standard.

Results
PRRSV was detected by rRT-PCR in 79% of live piglets' sera on farm 1 visit 1 (F1V1), 10% on farm 1 visit 2 (F1V2), 63% on farm 2 visit 1 (F2V1), and 0% on farm 2 visit 2 (F2V2). Overall, oral swab sensitivity when compared to the same dead animal's serum was 90.9%, 84.8% for nasal swabs, 75.8% for rectal swabs, and 97.0% for tongue tips. Compared to sera, agreement ranged from 70.2% for tongue tips (kappa 0.45, $p<0.001$) to 89.6% for oral and nasal swabs (kappa 0.77, $p<0.001$).

Conclusion
Postmortem sampling of piglets to detect PRRSV is a welfare-friendly alternative. Any of the assessed specimen types were capable of identifying PRRSV on F1V1, F1V2, and F2V1. PRRSV was identified in F2V2 through serum and tongue tips, possibly by due to environmental contamination. Although this might be an issue when trying to assess within-herd prevalence, having highly sensitive detection methods, even those that detect environmental contamination, might represent an improvement in detecting presence of the virus on a farm.

Monitoring of porcine reproductive and respiratory syndrome virus variant emergence

M. Kikuti1*, C.M. Melini1, X. Yue1, I.A.D. Paploski1, N. Pamornchainavakul1, J. P. Baker1, D. Makau1, K. VanderWaal1, C.A. Corzo1

1Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA

Objective
To describe the emergence of a new PRRSV variant within sub-lineage 1C (RFLP 1-2-4), including its space-time distribution and impact on affected herds, and discuss considerations on how to monitor emerging PRRSV variants.

Methods
Monitoring of this newly emerging clade started in June 2022, triggered by one pig production system that required sequence comparison between a sequence originating from a sow herd undergoing a mild PRRS outbreak and the Morrison Swine Health Monitoring Project (MSHMP) PRRSV sequence database. The obtained sequence was used as a seed and a case was defined by nucleotide identity of ≥98% between samples with any additional case identified used as seeds for subsequent rounds. Epidemiological curves with number of sequences detected over time and number of sites in which these sequences were detected were constructed. Space-time permutation models were used to identify space-time clusters up to November 2022. Time to stability was calculated for breeding herds.

Results
A total of 276 case sequences were identified by November 2022. Twelve production systems were involved. Most (60.5%, 167) sequences originated from breeding sites while 31.2% (86) from grow-finishing sites. Notably, these 276 sequences originated from 91 individual sites (55 grow-finishing sites, 33 breeding sites, and 3 had no farm type information). No space-time clusters were found when accounting for system. However, two clusters were detected when system was not accounted for, occurring in Western Illinois/Northeast Missouri and Southern Iowa/Northern Missouri. Breeding herd stability, indicated by the weaning of PRRSV-free piglets, was reached in 62.07% (18/29) of the breeding sites, within a median of 88 weeks (median of 57 weeks for outbreaks in which only the studied variant was detected during the unstable period and 88 weeks for outbreaks with multiple PRRSV variants).

Conclusion
We highlight the need to use a genetic-based approach when identifying PRRSV variants. Epidemiological curves based on sequence counts might be inflated since it is not uncommon for multiple sequences to be generated by the same site over time. Case counts per region or state are informative but these need to account for the amount of data being generated in each unit of analysis as surveillance is not equal throughout space. Case descriptions, although informative, might generate a bias in perceived virulence since comparisons with more typical cases are lacking. An in-depth rigorous investigation is crucial before raising concerns of a new emerging variant.

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

Marie-Eve Koziol[1], Sarah Kharief[2], Juliette Ben Arous[2], Nicolas Versillé[3]
[1] Seppic - USA - 30 Two Bridges Road, Ste 210 - Fairfield, NJ 07004
[2] Seppic - Paris La Défense - 50 Boulevard National - 92257 La Garenne Colombes, France
[3] Seppic - 127 chemin de la Poudrerie - BP 90228 - 81105 Castres Cedex, France

Abstract
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.

African swine fever virus gene I73R suppresses the type-I IFN production by broadly inhibiting the host transcription process

Danh Cong Lai1*, Jayeshbhai Chaudhari2, and Hiep L. X. Vu1,2

1 Nebraska Center for Virology, University of Nebraska-Lincoln, 2 Department of Animal Science, University of Nebraska-Lincoln

African swine fever virus (ASFV) poses a significant threat to the global swine industry due to its high mortality rate in domestic pigs and the lack of approved vaccines or antiviral treatments. The ASFV genome contains over 150 genes, of which the immediate early and early genes are particularly important for viral replication and evasion of the host immune response. This study aimed to investigate the ability of the ASFV immediate early and early genes to suppress the type I IFNs induced by the cGAS-STING pathway.

Among the 21 immediate early and early genes analyzed, I73R showed significant inhibition of the cGAS-STING-induced IFN-β promoter activity by targeting both the NFκB response element and the IRF3 response element within the IFN-β promoter region. As a result, I73R inhibited IRF3 nuclear translocation following the treatment of cells with poly(dA:dT), a strong inducer of the cGAS-STING signaling pathway. We consistently observed that both endogenous and exogenous protein levels of major signaling molecules, including cGAS, STING, TBK1, and IRF3, were significantly lower in cells expressing I73R than those transfected with an empty plasmid, suggesting that I73R can suppress host gene expression. Further analysis revealed that I73R interfered with the host gene transcriptional, not the translational process. The I73R protein shares structural homology with the Z-DNA binding domains (Zα) of the vaccinia virus E3L protein, a known suppressor of type I IFNs signaling. However, I73R's ability to suppress cGAS-STING induction of IFN- β was independent of its Z-DNA binding activity. Instead, the α1, β1, α2, and α3 domains of I73R played a significant role in suppressing cGAS-STING induction of IFN- β.

Overall, our findings suggest that I73R hinders the host's innate immune response by broadly inhibiting the transcription of host genes, including those involved in the cGAS-STING pathway. This knowledge provides insights into the viral pathogenesis and may contribute to the rational design of a live-attenuated ASFV vaccine.

Evaluation of Multiple Immune Parameters following Vaccination with an African Swine Fever Virus Multiepitope Protein Nanoparticle-based Subunit Vaccine

C.M. Lee1*, P. Boley1, R. Suresh2, O. Shekoni2, J. Schrock1, S. Dolatyabi2, M. Singh2, S. Khatiwada2, K. Yadav1, D. Bugybayeva2, R. Gourapura2, S.P. Kenney1,2.

1Center for Food Animal Health, Department of Veterinary Preventative Medicine, The Ohio State University, Wooster, OH.
2Center for Food Animal Health, Department of Animal Sciences, The Ohio State University, Wooster, OH.

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, and recently the Dominican Republic and Haiti, bringing the virus closer to the United States (U.S.) border. If ASF were to jump to the 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, and if, the disease makes its way to 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 ASFV sites. The aim being to induce robust, cross-protective immune responses utilizing a single protein vaccine antigen when coupled with existing nanoparticle vaccine technology. The multiepitope protein was subsequently expressed using a bacterial expression system, purified, and entrapped into nanoparticles for vaccine formulation. The candidate vaccine, along with nanoparticle entrapped adjuvant, was delivered intramuscularly to pigs, and T- and B-cell responses were evaluated following initial (DPV 22) and booster (DPV 42) vaccine dosages. Nanoparticle entrapped multiepitope protein vaccinates showed a positive multiepitope-specific IgG antibody response earlier, at DPV22, than unentrapped protein vaccinates. In both vaccinated groups, we detected a significantly higher multiepitope-specific IgG antibody response at DPV42 compared to mock (PBS vaccinates) and DPV22 titers. The nanoparticle entrapped multiepitope vaccine also induced a greater number of cytotoxic and T-helper cells in vaccinates, compared to mock and unentrapped vaccinates. Overall, our nanoparticle entrapped multiepitope vaccine induced antigen specific T- and B-cell responses, both of which have been identified as important correlates of protection against ASFV. This promising preliminary data suggests that our nanoparticle entrapped multiepitope protein vaccine could provide protection against ASFV challenge, which will be evaluated in future studies.

Detection of Classical Swine Fever Virus RNA using minimal equipment LAMP-PCR

Jose Alejandro Bohorquez [1], Adriana Muñoz [2,3], Saraswathi Lanka [1], Liani Coronado [3,4]; Rosa Rosell [3,5], Mònica Alberch [3,4], Patrick Roady [1,6], Llilianne Ganges [3,4†] and Carol W Maddox [1,7†]

[1] Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, United States; [2] Instituto Colombiano Agropecuario (ICA), Bogotá DC, Colombia; [3] WOAH Reference Laboratory for classical swine fever, IRTA-CReSA, Barcelona, Spain; [4] Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain; [5] Departament d'Acció Climàtica, Alimentació i Agenda Rural, Generalitat de Catalunya, Barcelona, Spain. [6] Department of Clinical Veterinary Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, United States; [7] Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, United States
Corresponding authors: maddox@illinois.edu and Llilianne.ganges@irta.cat

Classical swine fever (CSF) remains one of the most important challenges to swine health worldwide. Due to its severe economic and sanitary impact, CSF is notifiable to the world organization for animal health (WOAH). The disease is caused by the CSF virus (CSFV), a member of the *Pestivirus* genus with a single-stranded RNA genome. Even though vaccination and serological surveillance programs are implemented worldwide, eradication of CSFV remains elusive. Molecular diagnosis is a cornerstone for CSF control, which requires specialized equipment and facilities as well as specific personnel training. This study led to development of a new LAMP-PCR assay for detection of CSFV RNA, a technique that can be implemented using minimal equipment, largely accessible in in laboratories that do not have the appropriate infrastructure for qPCR or even farm conditions. Over 400 CSFV RNA sequences, downloaded from NCBI, were analyzed, and two primer sets were selected within the most conserved region of the CSFV genome. These primer sets were initially tested, through fluorometric and colorimetric LAMP methods, using a plasmid that included the consensus sequence for the target region.

One of the primer sets, CSFV-LAMP-JB3, was able to detect as few as 10 genome copies/µL, using both detection methods. This primer set was selected for validation using RNA extracted from field viral isolates. CSFV-LAMP-JB3 proved able to efficiently detect RNA from all the CSFV strains tested, including viral isolates from all three CSFV genotypes, using colorimetric and fluorometric detection. The sensitivity of the CSFV-LAMP assay was compared with the gold standard RT-qPCR techniques from USDA and WOAH. The CSFV-LAMP-JB3 showed remarkable detection of CSFV RNA, comparable with Ct values >37, near the limit of detection for the RT-qPCR reactions. The LAMP assay also proved successful in detecting viral RNA extracted through sample heating, to avoid the use of extraction kits not readily available in farm conditions.

The specificity of the primer set was also evaluated, using DNA and RNA from multiple pathogens infecting swine, as well as isolates from the other viruses in the *Pestivirus* genus. The technique did not amplify nucleic acid from any of the other pathogens tested, showing specificity of 100%.

The CSFV-LAMP-JB3 assay provides the basis for a pen-side, feasible alternative for CSFV diagnosis. This tool can be highly valuable for control and eradication of CSFV, reducing the equipment needs, and the time to detection, both of which will result in faster control measures, limiting the spread and impact of the disease.

[†] Authors share senior authorship

Development of a New Effective African Swine Fever Virus Vaccine Candidate by Deletion of the H240R and MGF505-7R Genes Results in Protective Immunity against the Eurasia Strain

Jiangnan Li 1,*, Changjiang Weng 1

1 National African Swine Fever Para-reference Laboratory, State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150069, Heilongjiang, China

African swine fever is a contagious and lethal disease of domestic pigs and wild boars that has caused significant economic loss for the swine industry. African swine fever virus (ASFV) is the etiological agent of ASF. Until now, no effective commercial vaccine and antiviral drugs are available for ASF control. Our previous studies demonstrated that pMGF505-7R interacted with the IKK complex and NLRP3 to decrease interleukin-1β (IL-1β) production, and a recombinant ASFV-Δ7R generated by deleting MGF505-7R gene displayed an attenuated phenotype in pigs. Our previous studies also showed that H240R gene is a new virulence-related gene, but a recombinant ASFV with deletion H240R gene still have residual virulence. Here, we report a new designed live attenuated mutant (ASFV-ΔH240R-Δ7R) by deleting H240R and MGF505-7R genes based on the highly pathogenic ASFV HLJ/18 backbone. The viral titer of ASFV-ΔH240R-Δ7R was decreased approximately 1.0 log in PAMs compared with its parental ASFV HLJ/18. It is highly stability following 30 serial passages in vitro. Piglets immunized by intramuscular inoculation with 103 or 105 HAD50 of ASFV-ΔH240R-Δ7R displayed safety without any ASF-related signs and could not be transmitted by direct contact. In addition, the immunized pigs produced specific antibodies against p30, but not those cohabitation piglets. Challenged with a virulent ASFV isolate HLJ/18, the piglets in the immunized group with 103 HAD50 of ASFV-ΔH240R-Δ7R obtained clinically significant 100% protection with mild clinical symptoms, whereas the piglets in the immunized group with 105 HAD50 of ASFV-ΔH240R-Δ7R obtained 100% protection without clinical symptoms. Moreover, the piglets in the immunized group with 105 HAD50 of ASFV-ΔH240R-Δ7R exhibited low levels of virus replication and with no observed pathological changes by postmortem and histological analysis. Overall, our results provided a new designed live vaccine candidate and rationalized the understanding of ASFV gene function, virus attenuation, and protection against ASFV infection.

Digital biosensor assays for rapid detection of ASFV infection

Siyan Li1, Weijing Wang2, Fangfeng Yuan1, Brian Cunningham2,3, Ying Fang1,3

1Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign; 2Department of Electrical and Computer Engineering, The Grainger College of engineering, University of Illinois at Urbana-Champaign; 3Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign

The emergence of the African Swine Fever Virus (ASFV) in Eurasia poses a significant threat to the global swine industry. There is an immediate and critical need for highly sensitive and specific diagnostic assays to enable swift detection during outbreaks, facilitate post-outbreak investigations, and support ongoing disease surveillance efforts. In this study, we present a blocking activate capture + digital counting (bAC+DC) approach derived from a monoclonal-antibody (mAb)-based blocking Enzyme-Linked Immunosorbent Assay (bELISA), which are using a recombinant p30 protein as the antigen combined with mAb against p30 as the detection antibody. This approach employs a photonic crystal (PC) biosensor and gold-nanoparticles (AuNP) to develop a blocking assay for detecting antibody responses in animals infected with ASFV. This test platform facilitates rapid field surveillance across multiple species, providing valuable support in the efforts to control and prevent the spread of ASFV.

Long-term Expanding Porcine Airway Organoids Reveal Age-related Divergent IFN Responses to Respiratory Coronavirus

Chengfan Jiang2, Liang Li2,Shihao Ding1 , Xiang Liu1, Li Feng2, Pinghuang Liu1,*

1 College of Veterinary Medicine, China Agricultural University, Beijing 100193, China;
2 State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China, 150069.

Respiratory coronaviruses cause serious health threats to humans and animals. Porcine respiratory coronavirus (PRCoV) is a natural mutant of transmissible gastroenteritis virus (TGEV) that causes mild respiratory illness in pigs. As an animal model for human respiratory coronaviruses, PRCoV presents an opportunity to better understand coronavirus pathogenesis. However, research has been hindered by a lack of robust models of the porcine airway epithelium. Here, we generated long-term expanding 3D and 2D porcine airway organoids (AOs) containing the major airway epithelial cell types. In contrast to TGEV, which readily infects both intestinal organoids and AOs, PRCoV amplification occurred primarily in the AOs. Moreover, AOs derived from 28-day old piglets supported significantly higher PRCoV infection compared to those from 2-day old piglets, whereas TGEV displayed the opposite pattern. Mechanistically, increased expression of the viral receptor Aminopeptidase N and decreased type I and III interferon responses in the 28-day AOs accounted for their divergent PRCoV susceptibility. Overall, our long-term porcine AOs morphologically and functionally resemble the porcine airway epithelium. As PRCoV replication differs between neonatal and 28-day AOs, mimicking the robust innate immunity of children versus adults to SARS-CoV-2, these AOs provide a promising model for studying respiratory coronavirus pathogenesis.

Keywords: Coronavirus, PRCoV, TGEV, Airway organoids, Interferons, ISG

Connecting the dots of single-cell analyses for delineating the big picture of porcine immune status

Crystal L. Loving

[1]National Animal Disease Center, ARS, USDA, Ames, Iowa, USA

Traditional 'bulk' assessment of gene expression via RNA sequencing captures overall patterns of transcriptional activity but fails to define cellular or spatial dynamics that influence functional outcomes related to immune status. High-resolution transcriptomics have revolutionized understanding of livestock immune status by resolving gene expression to the level of individual cells via single-cell RNA sequencing (scRNA-seq) and small-area tissue space via spatial transcriptomics (STomics). Blood is routinely obtained via minimally invasive procedures and can be ideal for assessing livestock immune status. While scRNA-seq reveals immune status in peripheral immune cells, delineating how immune cells function within an organized tissue landscape is complicated because spatial context of cells is lost via scRNA-seq. Spatial organization of cells recovered from immunologically complex tissues, such as pig intestine, was achieved through integration of scRNA-seq and Stomics datasets. Dataset integration allowed for prediction of cellular locations within transcriptionally and morphologically distinct intestinal regions, revealing biologically-relevant routes of immune cell activation and differentiation that were highly organized according to spatial locations. Assessment of immune status at the single-cell level allows for more detailed understanding of complex responses to infection, enhancing our ability to intervene in disease processes. As scRNA-seq and STomics become common practice in research it is critical to be cognizant of and overcome obstacles related to both technology and data analysis limitations, including tgenome annotation, sampling capacities, data interpretation, and validation. Overall, scRNA-seq and Stomics are useful for gaining high-resolution cellular insights of immune status in food animals and ideal for delineating cellular functions contributing to overall health.

Survival of five strains of PRRSV in tap water at different temperatures

V. Lugo1*, N.M. Sobhy1, M.M. Luqman1, C.A. Corzo1 and S.M. Goyal1.

1Department of Veterinary Population Medicine, University of Minnesota, St. Paul, Minnesota, USA.

PRRS is still considered one of the most economically important diseases of swine, with losses mainly related to poor reproductive performance and mortality. Currently, management and biosecurity measures are important strategies used to control PRRS; however, outbreaks are still common. Little information is available regarding viral stability in water and the possibility of this milieu to help disseminate the virus. In this study, we tested the survival dynamics of PRRSV in sterile and unsterile tap water at three different temperatures (4°C, ~25°C, and 37°C). Tap water obtained from laboratory faucet was collected in glass bottles, which were then labelled for the following conditions: autoclaved and non-autoclaved chlorinated tap water and autoclaved and non-autoclaved dechlorinated tap water. Sodium thiosulfate at 30 mg/L was used for dechlorination. Four strains of PRRSV-2 (L1A 1-7-4, L1C 1-4-4 SD, L9 MN-30100, VR-2332) and one PRRSV-1 (Lelystad) were grown in the MARC-145 cell line to titres of 104.5 to 105.5 TCID50/0.1 ml. Then, 0.75 ml of each virus suspension was added to each 3.75 ml-aliquot of the four different tap water samples, for a final 1:6 dilution. The mixed aliquots were tested for an initial titre by TCID50/ml microtitration assay in the MARC-145 cell line and then placed at their respective temperatures for further sampling over time. At each time point, 0.5 ml of sample were collected, homogenized, and immediately tested by the microtitration assay in triplicates. Results show prolonged survival (>35 days) of all strains at 4°C with lower survival time at 25°C (3 to 7 days), and lowest survival at 37°C (< 1 day). These results indicate a potential risk of water as a PRRSV carrier, especially at cold temperatures.

Characterization of swine reagents for monitoring pig immune responses

JK Lunney1, C Loving2, S Kenney3, J LaBresh4, V Patil3, R Suresh3, KE Walker1, J Aquino1, GJ Renukaradhya3

1 Animal Parasitic Diseases Laboratory, BARC, ARS, USDA, Beltsville, MD USA
2 Food Safety & Enteric Pathogens, USDA-ARS-NADC, Ames, IA 50010, USA
3 Center for Food Animal Health, CFAES, The Ohio State University, Wooster, OH 44691, USA
4 Kingfisher Biotech, Inc., St. Paul, MN, USA

The USDA-NIFA Swine Immune Toolkit Initiative has been involved in generating priority immune reagents for understanding swine immunity and vaccine responses and pipelining the tools for marketing. We expressed each target immune molecule as a soluble protein using yeast expression system and then with the help of commercial partners, produced panels of monoclonal antibodies (mAbs) against each target. We have successfully generated panels of mAbs reactive with porcine IL-6, IL-13, IL-28B, CXCL10, and BAFF and are screening their reactivity in multiple immune assays. We determined mAb reactivity with orthologous proteins for most panels of mAbs.

A sensitive quantitative sandwich ELISA is now available for IL-13 and CXCL10, but not for IL-6. Other targets are being screened for best mAb pairs. Reactivity tests for intracellular staining of porcine immune cells labeled α-CXCL10 using intracellular staining and flow cytometry was successful, showing that of the 9 mAbs only 2 detected intracellular CXCL10 expression in PMA/ionomycin or rPoIFNg-stimulated porcine cells. Further, cell characterization assays verified CXCL10+ cells as CD3-CD4-CD172+, with occasional CD4+ subsets. Immunohistochemical staining has also identified positive mAbs on formalin fixed pig lymph nodes and spleen tissues.

For α-IL-6 and α-IL-13 mAbs intracellular staining is underway using different cell stimulation conditions. For each target, our goal is to provide the veterinary community with new commercial reagents and standardized assay techniques for their research efforts. Tools and reagents generated by this project will undoubtedly advance our understanding of swine immune responses to disease and vaccine and use for biomedical research efforts. Supported by USDA-NIFA AFRI grant # 2019-67015-29815 and USDA ARS project 8042-32000-117.

Comparative Analysis of Antibody Responses to African Swine Fever Structural Protein Between Attenuated Live and killed vaccine

Hung Luong1,2, The Nguyen1,2, Hanh Vu3, Huong Lai3, Hoa Nguyen3, Lam Truong3, Hiep Vu1,2

1Nebraska Center for Virology, University of Nebraska-Lincoln, 2School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, 68583-0900, USA. 3Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi 12406, Vietnam

African swine fever virus (ASFV) affects many swine-producing countries, causing significant economic losses. It is observed that pigs vaccinated with a live-attenuated virus (LAV) but not a killed virus (KV) vaccine strain develop solid homologous protective immunity. Therefore, we hypothesize that pigs vaccinated with a LAV vaccine develop a different antibody profile than those vaccinated with a KV vaccine. The main objective of this study was to comparatively analyze antibody profiles between pigs vaccinated with a LAV vaccine and those vaccinated with a KV vaccine.

Thirty ASFV seronegative pigs were divided into three groups. Group 1 received a single intramuscular vaccination with an experimental LAV vaccine. Group 2 received two doses of a killed virus vaccine at three-week intervals. Group 3 was injected with PBS to serve as a non-vaccinated control. At day 42 post-vaccination, all pigs were challenged by an intramuscular inoculation with a highly virulent ASFV strain and observed until day 21 post-challenge. All pigs vaccinated with the LAV vaccine survived the challenge infection. In contrast, 8 out of 10 pigs from the KV group and 7 out of 10 pigs from the PBS group died within 14 days post-challenge.

Serum samples collected on day 41 post-vaccination and 7 post-challenge were analyzed for their reactivity against a panel of 29 viral structural proteins. On day 41 post-vaccination, sera of pigs from the LAV group exhibited a strong antibody reactivity against various viral structural proteins, including the capsid (p14.5), inner envelope (p32, p54, p17), core-shell (pp62, p15, p34), nucleoid and nucleus (pE165R, pD339L, pI73R), C-type lectin, and pCP123L. Conversely, the sera of pigs in the KV group only displayed weak antibody reactivity against the inner envelope (p32, p54, p17, p12) and core-shell (pp62, p34). After the challenge, there was an overall increase in antibody reactivity against viral proteins that were recognized by sera collected on day 41 post-vaccination.

These findings highlight the distinct antibody profiles induced by LAV and KV vaccines, as well as their ability to mediate protection against a lethal challenge with virulent ASFV. The viral proteins that are exclusively recognized by sera from the LAV group hold promise as potential candidate antigens for developing subunit vaccines.

Pathogenicity of a novel reassortant H1N1 virus from swine responsible for deaths in sows in the US

W. Ma1,2, R. Palinski2, J. Lee2, J. Crumpton3, Y. Li2, L. Wang1,2, J.A. Richt2, R. Webby3.

1Department of Veterinary Pathology and Department of Molecular Microbiology & Immunology, University of Missouri, Columbia, MO; 2 Department of Diagnostic Medicine & Pathobiology, Kansas State University, Manhattan KS; 3 St. Jude Children's Research Hospital, Memphis TN

An H1N1 A/swine/Kansas/17211/2017 (KS17) was isolated from a commercial sow farm in which 1000 sows showed clinical signs of respiratory disease and 5 sows died. The farm had previously tested negative for porcine reproductive and respiratory syndrome virus and mycoplasma. Sequence and phylogenic analysis revealed that the KS17 H1N1 virus was a reassortant virus containing the HA gene from the 2009 H1N1 pandemic (H1N1pdm09) virus and the remaining genes from North American triple reassortant swine influenza viruses. We investigated the pathogenicity and transmissibility of this virus in pigs and ferrets using the H1N1pdm09 A/California/04/2009 (CA09) virus as the control. The KS17 H1N1 virus was more pathogenic than the CA09 H1N1 virus in pigs in terms of macroscopic and microscopic lung lesions and virus nasal shedding, although there was no difference between the 2 viruses in virus titers present in the lungs of the principal infected and contact pigs. The KS17 H1N1 virus displayed similar viral replication kinetics and transmission capacity as the CA09 H1N1 virus in ferrets. Taken together, the reassortant KS17 H1N1 swine influenza virus is pathogenic and transmissible in both pigs and ferrets and may be a potential threat to public health if it spills over to humans.

Bat influenza vectored NS1-truncated live vaccine protects pigs against heterologous virus challenge

J. Lee1, Y. Li1, Y. Li1, A. G. Cino-Ozuna1, M. Duff1, Y. Lang1, J. Ma1, S. Sunwoo1, J.A. Richt1, W. Ma1,2,3

1Department of Diagnostic Medicine & Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; 2Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO; 3Department of Molecular Microbiology & Immunology, School of Medicine, University of Missouri, Columbia, MO

Swine influenza is an important disease for the swine industry. Currently used whole inactivated virus (WIV) vaccines can induce vaccine-associated enhanced respiratory disease (VAERD) in pigs when the vaccine strains mismatch with the infected viruses. Live attenuated influenza virus vaccine (LAIV) is effective to protect pigs against homologous and heterologous swine influenza virus infections without inducing VAERD but has safety concerns due to potential reassortment with circulating viruses. Herein, we used a chimeric bat influenza Bat09:mH3mN2 virus, which contains both surface HA and NA gene open reading frames of the A/swine/Texas/4199-2/1998 (H3N2) and six internal genes from the novel bat H17N10 virus, to develop modified live-attenuated viruses (MLVs) as vaccine candidates which cannot reassort with canonical influenza A viruses by co-infection. Two attenuated MLV vaccine candidates including the virus that expresses a truncated NS1 (Bat09:mH3mN2-NS1-128, MLV1) or expresses both a truncated NS1 and the swine IL-18 (Bat09:mH3mN2-NS1-128-IL-18, MLV2) were generated and evaluated in pigs against a heterologous H3N2 virus using the WIV vaccine as a control. Compared to the WIV vaccine, both MLV vaccines were able to reduce lesions and virus replication in lungs and limit nasal virus shedding without VAERD, also induced significantly higher levels of mucosal IgA response in lungs and significantly increased numbers of antigen-specific IFN-gamma secreting cells against the challenge virus. However, no significant difference was observed in efficacy between the MLV1 and MLV2. These results indicate that bat influenza vectored MLV vaccines can be used as a safe live vaccine to prevent swine influenza.

Evaluation of the infectiousness level of three wild-type porcine reproductive and respiratory syndrome viruses (PRRSV) variants

C.M. Melini1*, M. Kikuti1, M. Torremorell1, K. VanderWaal1, S. Rossow2, C. A. Corzo1

1Department of Veterinary Population Medicine, University of Minnesota, 2Veterinary Diagnostic Laboratory, University of Minnesota,

The present study was designed to compare the recently emerged PRRS variant L1C 1-4-4 with two other variants (i.e., L1A 1-7-4 and L9 1-4-2) that had caused important national outbreaks in the past to address specific questions such as whether these variants have the same level of infectiousness, shedding patterns and generate similar pathologic changes in infected pigs.

For each variant, thirty-six PRRSV ELISA and RT-PCR negative three-week-old barrows were randomly allocated to five different challenge groups and one control group. Pigs were individually housed in separate rooms according to treatment groups. Pigs had no nose-to-nose contact but did share the same air space. All three viruses were grown in MARC-145 cells to a 105 TCID50/ml and through 10-fold dilutions five different inocula concentrations were obtained ranging from 104 to 100 TCID50/ml. Pigs were intranasally (1 ml per nostril) challenged according to the group's selected virus concentration starting with the sham inoculated control group followed by groups challenged with the virus in an ascendent concentration manner. Blood, nasal and rectal swabs were collected at 0, 1, 2, 4, 7, 11, 16, 21, 26, and 30 days post-challenge (DPC) to assess infection and viral shedding through PRRSV RT-PCR. Researchers changed their gloves, mask, and gowns between sampling each pig. On DPC 11, two pigs from each group were euthanized and tissues (e.g., brain, lung, lymph nodes) were collected for histopathological assessment. For this study, we analyzed experimental outcomes at DPC 4, as this was the time point that showed the greatest variability in the number of pigs infected across treatments. On 4 DPC, the estimated Minimum Infectious Dose (MID) for the L1C 1-4-4 virus was 101.5 TCID50/ml with 4/6 infected pigs, for the L1A 1-7-4 virus the MID was 101.5 TCID50/ml with 2 out of 6 infected pigs, and for the L9 1-4-2 virus was 101.8 TCID50/ml with 1 out of 6 infected pigs, titer values were obtained using the Karber method to a sample from each inoculum concentration. Using a probit model, the estimated Median Infectious Dose (ID50) (infective dose needed to infect 50% of exposed individuals) was 101.3 TCID50/ml (95% CI 100.4, 102.2) for the L1C 1-4-4 variant, 102.3 TCID50/ml (95% CI 101.6, 103.0) for the L1A 1-7-4 variant, and 102.6 TCID50/ml (95% CI 102.0, 103.2) for the L9 1-4-2 variant. Detection of the virus in nasal swabs began at 1 DPC and cycle threshold values decreased until DPC 7. Fecal shedding started at DPC 4 and peaked at DPC 7; intermittent nasal and rectal shedding patterns were similar for all viruses with slight differences between inoculum groups during the study period. The histopathology assessment concluded that lung samples were found to have lesions of different severity degrees in all challenged groups regardless of concentration, but the ones infected with the L1C 1-4-4 were consistently more severe and present for longer time periods compared to the other two viruses. At least 1 pig per concentration group in the L1C 1-4-4 challenged group had brain lesions, in the other viral groups, they were only present in 1 pig of each. Heart lesions were mostly present in the L1C 1-4-4 variant group. Results from this study suggest that compared to L9 1-4-2 and L1A 1-7-4 variants, the 4L1C 1-4-4 virus requires fewer viral particles to successfully infect half of the pigs by 4 DPC. In addition, our data show that the L1C-1--4 virus leads to more pathological changes with tissue tropism towards the lung, heart, and brain as compared to the other evaluated variants.

Novel Candidate Vaccines against Porcine Epidemic Diarrhea Virus

X.J. Meng

Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Porcine Epidemic Diarrhea Virus (PEDV) is an economically-important pathogen in the global swine industry, and its control remains challenging as the available vaccines do not confer adequate protection against PEDV infection. In collaborations with Steven L. Zeichner of the University of Virginia School of Medicine, and Chenming (Mike) Zhang of Virginia Tech Department of Biological System Engineering, we recently developed novel candidate PEDV vaccines with the goals of enhancing both cell-mediated and humoral immune responses. By utilizing dendritic cell (DC) targeting vaccine approach, we delivered PEDV S antigen directly to the porcine DCs through a single-chain antibody specific to porcine Langerin, a C-type lectin receptor expressed on DCs. The candidate DC-targeting PEDV vaccine greatly improved PEDV S antigen-specific T cell IFN-γ responses in the $CD4^{pos}CD8^{pos}$ T cell compartment and induced higher serum IgG and IgA responses in pigs. By utilizing the hepatitis B virus core capsid antigen (HBcAg) that can self-assemble into virus-like particle (VLP), we expressed a PEDV S-specific B-cell epitope in the backbone of the HBcAg that subsequently assembles into VLPs for use as a candidate vaccine. The candidate PEDV-HBcAg VLP vaccine induced higher virus neutralization response in gilt milk, and provided alleviation of clinical signs for piglets experimentally infected with PEDV. By employing a synthetic biology-based novel vaccine platform that expresses the conserved PEDV fusion peptide (FP) on the surface of genome-reduced bacteria, we evaluated the use of killed whole-bacterial cells expressing PEDV FP as candidate vaccine to enhance interaction of vaccine antigen with the immune system, and showed that the candidate genome-reduced bacteria vaccine expressing PEDV FP induced potent anamnestic responses upon virus challenge, potentiated IFN-γ responses, and provided significant protection against clinical disease.

Efficacy of a Modified Live Virus (MLV) prototype of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) vaccine with replication-competent expression of porcine interferons (IFNs)

Buckley, A.1, Sarlo Davila, K.2, Fleming, D.3, Anderson, S.1, Li, J.4, Van Geelen A.5, Lager, K.1, Sang, Y.4, Miller, L.6*

1VPRU, USDA-ARS-NADC, 2IBDRU, USDA-ARS-NADC, 3APDL, USDA-ARS-BARC
4Department of Agricultural and Environmental Sciences, College of Agriculture, Human and Natural Sciences, Tennessee State University, 5USDA-APHIS-NACH, 6Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

Porcine reproductive and respiratory syndrome (PRRS) is a complex and costly disease in the swine industry, due in part to the high degree of genetic variation among PRRS virus (PRRSV) field isolates. Although there are PRRSV vaccines currently available, they can have varying degrees of cross-protection depending on genetic similarity.

We have identified several host interferons that have superior antiviral properties potentiating immune responses in pigs. Preliminary evaluation of selected interferons (IFNs) was demonstrated in vitro through reverse genetic incorporation into the Type 2 PRRSV p129 vaccine backbone (Zoetis). We performed in vivo studies in pigs to compare novel vaccine candidates to commercially available MLV vaccines using a contemporary challenge virus (NADC-34). Here, we present the clinical data.

The animal study was conducted in commercial pigs (n = 10/group): sham vaccine + sham challenge, sham vaccine + challenge, MLV-commercial + challenge, MLV-PRRSVp129-IFN-omega + challenge, MLV-PRRSVp129-IFNmix + challenge. Pigs in all treatment groups were monitored for clinical signs, weighed, and temperature recorded throughout the study. Serum was collected to evaluate viral load with real-time-RT-PCR and the immune response with a commercial PRRSV ELISA, and whole blood was used to evaluate gene expression.

The pilot study demonstrated that antiviral IFN vaccine prototype efficacy was comparable to commercially available PRRSV MLV vaccine. In this study, pigs administered the novel vaccines had similar ELISA titers prior to challenge and reduction in viral load in the serum after challenge to those given the commercial MLV. In addition, the MLV-PRRSVp129-IFNmix numerically reduced temperature and viral load greater than MLV-PRRSVp129-IFN-omega.

A DNA-launched reverse genetics system for PRRSV and co-expression of immunomodulatory peptides designed to directly reverse PRRSV suppression on the pig's IFN signaling and associated immune response has the potential to enhance vaccine efficacy against heterologous PRRSV strains compared to currently available vaccines.

Effect of storage temperature time on PRRSV RT-qPCR testing

B. Munguía-Ramírez1*, B. Armenta-Leyva1, A. Henao-Díaz3, F. Ye2, K. Doolittle4, S. Zimmerman4, L. Giménez-Lirola1, J. Zimmerman1

1Veterinary Diagnostic and Production Animal Medicine, 2Department of Statistics, Iowa State University, Ames, Iowa. 3PIC Latinoamérica, México. 4IDEXX Laboratories, Inc., USA.

In the course of sample collection, storage, transport, and processing before PCR testing, viral nucleic acid targets in diagnostic samples are subjected to a variety of adverse conditions, i.e., temperature time, that could affect PCR results. Although widely recognized, the effect of these conditions on specimens containing viral RNA is largely unexplored and rarely quantified. Herein, we quantified the effect of storage temperature time on the detection of PRRSV RNA and a pig-specific internal sample control (ISC) in serum, oral fluids, and fecal samples by RT-qPCR. Serum samples (n = 5) used in the study were from pigs experimentally inoculated with wild-type PRRSV. Oral fluids (n = 5), and fecal samples (n = 5) were from individually housed pigs vaccinated with a PRRSV MLV (Ingelvac® PRRS MLV).

Each sample was divided into 28 aliquots (500 uL) and each aliquot was subjected to one combination of (temperature time) treatment, i.e., 4°, 10°, 20°, or 30°C for 24, 48, 72, 96, 120, 144, or 168 hr. After completing all treatments, samples were tested by RT-qPCR (IDEXX Laboratories, Inc.) for the simultaneous detection of both PRRSV and the ISC. The resulting RT-qPCR quantification cycle (Cq) values were re-expressed as "efficiency standardized Cqs (ECqs)" and cube root transformed for analysis. The effect of (temperature time) was analyzed by a mixed-effects regression model using R v.4.2.1 (Table 1).

Table 1. Decline of PRRSV RNA every 24 hr as function of storage temperature in swine specimens tested by RT-qPCR.

Target	Specimen	PRRSV ECq decline by temperature (% decline)			
		4°C	10°C	20°C	30°C
PRRSV	Serum	0.00[1]	0.00[1]	0.00[1]	0.11 (5.3%)
	Oral fluid	0.03 (6.3%)	0.05 (9.4%)	0.07 (13.6%)	0.08 (16.6%)
	Feces	0.05 (6.0%)	0.06 (7.9%)	0.05 (14.3%)	0.05 (15.3%)
ISC	Serum	0.00[1]	0.00[1]	0.07 (3.6%)	0.10 (5.7%)
	Oral fluid	0.03 (4.2%)	0.05 (8.3%)	0.07 (12.5%)	0.08 (14.2%)
	Feces	0.06 (3.6%)	0.05 (4.2%)	0.08 (7.1%)	0.10 (7.8%)

[1] No significant ECq decline ($p > 0.05$; mixed-effects regression model).

Overall, our results showed that storage of serum at 4°, 10°, and 20°C had no impact on the detection of PRRSV for up to 168 hr, but storage at 30°C resulted in loss of detectable PRRSV RNA. The ISC in serum was stable at 4° and 10°C, but a discernible effect was observed at 20° and 30°C. In oral fluids and feces, a marked loss of PRRSV and ISC RNA was observed when stored at 4°, 10°, 20°, or 30°C. Based on these data, we recommend storing serum for PRRSV RT-qPCR testing at ≤ 20°C. In contrast, oral fluid and fecal samples should be stored frozen (-80°C) until tested to avoid losing detectable PRRSV RNA, i.e., RT-qPCR false negatives.

How should we stabilize PRRSV RNA in oral fluids? – Preliminary results

B. Munguía-Ramírez1*, B. Armenta-Leyva1, L. Giménez-Lirola1, J. Zimmerman1

1Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa.

Oral fluid samples are widely used in swine surveillance for essentially all pathogens of economic importance in the swine industry. Measures taken for surveillance programs require collecting specimens for PCR, with testing results forming the basis of herd health decisions for disease prevention and control.
Previous data revealed that storage temperature × time had a detrimental effect on PRRSV detection in oral fluids. Although sample handling recommendations include prompt chilling or freezing samples after collection to preserve viral nucleic acid, it is not always logistically possible to meet this requirement. It follows that protecting viral RNA from degradation in oral fluids prior to PCR testing is crucial. While a variety of commercial products are sold to preserve nucleic acids in specimens, e.g., RNAlater®, these products are expensive, virucidal, and there is little data documenting their efficacy in RNA stabilization. Herein, we tested specific chemical agents for their capacity to stabilize PRRSV RNA in oral fluid samples stored at high temperature (30°C).
PRRSV-negative oral fluids collected from the ISU-VDL were pooled and centrifuged (3,300× g for 3 hr) to obtain a 30 ml stock solution. The stock solution was inoculated with a PRRSV MLV (Ingelvac®) to a 10-3 dilution, aliquoted in triplicates (1 ml), and assigned to one of 9 treatments: 3 proteins at 5% w/v, 4 carbohydrates at 20% w/v, no stabilizer, and control (-80°C). With the exception of the control, all samples were stored at 30°C for 24 hr and tested for PRRSV RNA and a porcine-specific internal sample control (ISC) by RT-qPCR (IDEXX Laboratories). Quantification cycle (Cq) values were re-expressed as function of the PCR efficiency as "efficiency standardized Cqs (ECqs)" and PRRSV RNA stabilization was evaluated for each treatment in terms of percent (%) PRRSV RNA recovery relative to the control held at -80°C.

Table 1. % PRRSV RNA recovery by RT-qPCR in oral fluids stored at 30°C for 24 h.

Chemical agent	% PRRSV RNA recovery
No stabilizer	68.5%
Protein 1	83.7%
Protein 2	31.4%
Protein 3	48.5%
Carbohydrate 1	92.5%
Carbohydrate 2	91.1%
Carbohydrate 3	91.6%
Carbohydrate 4	90.0%

These data provide evidence that PRRSV RNA can be stabilized in oral fluids using low-cost, readily available chemical agents. Future work will focus on identifying the combination of agents and concentrations that optimize RNA stability. The selected formulation will be then evaluated for its capacity to stabilize other swine pathogens of economic importance in oral fluids.

Development of a broadly protective PRRS vaccine candidate: Application of non-toxic enterotoxin and E. coli as the adjuvant-delivery system

A. Naru1*, I. Upadhyay1, R. R. R. Rowland1, B. Bawa2, R. Gourapura3, W. Zhang1, Y. Fang1

1Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL. 2AbbVie, Inc., Chicago, IL. 3Center for Food Animal Health, College of Food Agricultural and Environmental Sciences, The Ohio State University, Wooster, OH

Porcine reproductive and respiratory syndrome or PRRS, still ranks among the most financially devastating diseases affecting the swine population in the United States of America. Current commercially available vaccines are not always efficacious in protection against infection from a wide array of heterologous PRRS virus (PRRSV) isolates in the field. A novel approach of vaccine development using conserved epitopes has proven to be effective for multiple diseases in preclinical trials. The objective of this study was to construct and evaluate the immunogenicity of an epitope-based candidate vaccine for its potential application in future PRRS vaccine development. Initially, four multi-epitope antigens (chimera A-D) were constructed, which contain a set of consensus T- cell epitopes derived from PRRSV proteins of nsp9, nsp10, GP4, GP5, and N. To enhance immune responses of PRRSV T-cell epitopes, these multi-epitope antigens were genetically linked to a detoxified bacterial heat-labile enterotoxin (LT192) as an adjuvant. These four epitope-toxin chimeras were transformed into a swine non-pathogenic E. coli strain to use as a potential live attenuated vaccine (designated as E. coli /epitope-toxin mix). The in vitro expression of each multi-epitope antigen was detected in the supernatant of transformed E. coli culture by verifying LT192 binding to GM1 receptors. Immunogenicity of this candidate vaccine was evaluated in a PRRSV challenge pig model. The result demonstrated that specific T-cell immune responses were stimulated after immunization. At 28 days post vaccination, we observed the increased frequency of IFN- gamma+CD4+CD8+ cells and IFN- gamma + gamma- delta T-cell populations in PBMCs stimulated by pooled synthetic peptides of T-cell epitopes. After the challenge, a strong IFN- gamma + gamma- delta T cell response was observed. Peptide D2 (VRHHFTPSE) from N protein was identified as the epitope of gamma- delta T cell lymphocytes; peptides A3 (CPGKNSFLDEAAYCNHL) and C3 (VRILAGGWCPGKNSFLD) from nsp10 were identified as epitopes of CD4+T lymphocytes; peptides B3 (VRGNPERVKGVLQNTRF) from nsp2 and C2 (KGRLYRWRSPVIIEK) from GP5 were identified as epitopes of CD8+T lymphocytes. In comparison to the non-immunized pigs, pigs immunized with the E. coli /epitope-toxin mix showed improved protection against virulent PRRSV challenge, with about 50% decrease of pneumonic lung lesions and 10-fold reduction of the viral load in serum and lung tissues at 14 days post challenge. This study establishes a platform for future construction of epitope-based vaccines against PRRSV infection.

Evolution of PRRSV in Europe

Hans Nauwynck

Laboratory of Virology, Department of Translational Physiology, Infectiology and Public Health, Faculty of Veterinary Medicine, Ghent University

Lelystad virus was first isolated in Europe in 1991 as etiology of a new mystery disease, mainly characterized by reproductive problems. Related viruses spread all over Western Europe. This virus (PRRSV-1) was genetically and phenotypically strongly different from the strains that were circulating in the US (PRRSV-2). Lelystad-like viruses were slow-spreading viruses; infections started in the nursery and it took a whole fattening period before all animals became infected. This was correlated with a restricted replication level in the nasal mucosa; tonsils and lungs were main primary targets. The viremia, which is the mirror of the PRRSV replication remained at a low level (10^2-10^3 TCID50/ml). Single infections did not cause general and respiratory problems. Reproductive failure was the main problem, characterized by late abortion/early farrowing and weakborn piglets. From the moment that PRRSV vaccines became available, PRRS was effectively controlled. This situation changed with the appearance of more heterologous strains which primarily emerged in Italy. Later on, heterologous strains appeared all over Europe. Within a period of ten years, PRRSV-1 found its way to escape from immunity upon vaccination and infection. In addition, between 2005 and 2015, the virus succeeded in replicating in additional subsets of macrophages, such as those residing in the superficial layers of the nasal mucosa, giving more power to the virus to spread between pigs and resulting in a one log increase of virus titers in the blood (10^3-10^4 $TCID_{50}$/ml). However, single infections did still not cause overt clinical signs in young pigs. In 2006, highly virulent PRRSV-1 strains were circulating in Eastern Europe, at the same moment as the Chinese high fever PRRSV-2 strains in Asia. They (subtypes 2 and 3) were genetically quite different from the strains present in Western Europe (subtype 1). Lena (prototype PRRSV-1 subtype 3) has been extensively studied. Single infections with this virus resulted in an extremely high replication in all organs, a top viremia (10^4-10^6 $TCID_{50}$/ml), extreme exudate formation in body cavities, high fever for weeks, listlessness, anorexia and respiratory problems in young pigs. Co-infections even killed some of the affected pigs. This type of viruses luckily never spread to Western Europe. More recently, more virulent and pathogenic PRRSV-1 subtype 1 strains were popping up in Italy (PR40/2014; 2014) and Spain (Rosalia (2020)). This evolution stimulated all Western European countries to activate surveillance by whole genome sequencing.

Evaluation of RT-rtPCR assay for detecting Japanese Encephalitis Virus in porcine samples.

R.K. Nelli*, L. K. Bradner, P. C. Gauger, A. De Jong

Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa
Corresponding author: rknelli@iastate.edu

Japanese Encephalitis Virus (JEV) is a mosquito-borne virus that causes viral encephalitis. Pigs become infected through a bite from infected mosquitoes, primarily Culex species. In general, transmission is through the movement of infected mosquitoes, often over long distances, due to wind dispersal and the movement of migratory water birds. Water birds can carry the virus but do not show illness. JEV is endemic in many parts of Asia, but with the re-emergence of JEV in February 2022 in Southern Australia, a potential outbreak in the pig population in the USA can have detrimental economic and zoonotic implications globally. Therefore, it is crucial to be vigilant and have a screening method for early detection of this virus in the pig population when clinical signs suggest JEV as a differential.

The current study aims to establish an RT-rtPCR for JEV (genus level) and its five genotypes (G-1, -2, -3, -4, -5). The TaqMan-based primer/probe (p/p) sequences (FAM/VIC/CY5) used in this study were either designed or previously published that target viral genes NS1, NS2, and M. All reactions included controls with thermal cycling conditions: 50 °C for 5 min holding for reverse transcription, 40 cycles of 95°C for 10 min denaturation, 95°C for 3 s, and 60°C for 30 s for amplification. Standard curves generated with known quantities of synthetic RNA oligos were used to determine the limit of detection (LOD) of RT-rtPCR assays.

Our study developed a genus level (screening) RT-rtPCR assay for JEV and compared its specificity to the previously published assay. The screening RT-rtPCR assays were negative for St. Louis encephalitis, Zika, Dengue, Yellow fever, and other arthropod-borne viruses (Tahyna orthobunyavirus, Getah Virus, and Sindbis Virus). In addition, RT-rtPCR assay did not cross react with PRRSV, PRV, PHEV, PPV1, PTV, PEDV, PDCoV, TGEV, PSV, porcine Astro3/Astro4, and PPV2. However, p/p from the published assay cross-reacted with West-Nile virus RNA. In the case of genotype-specific RT-rtPCR assays, p/p of G-1 and G-3 were specific for different regions of viral gene NS2 (non-structural protein 2) and can detect as low as 125 copies/µL of synthetic RNA. The genotype-specific RT-rtPCR amplification efficiency for JEV G-1 and -3 was 100.1% and 106.1%, respectively. Five virus strains belonging to G-1 and G-3 were tested and had a Ct < 20 with both screening and genotype-specific RT-rtPCR assays.

Our study developed a specific genus level RT-rtPCR for JEV and evaluated the analytical sensitivity and specificity of JEV RT-rtPCR for G-1 and G-3. Currently, more studies are in progress to validate the RT-rtPCR assays for G-2, G-4, and G-5, followed by an evaluation of diagnostic sensitivity and specificity.

A single-dose intramuscular immunization of pigs with lipid-nanoparticle DNA vaccines based on the hemagglutinin antigen confers complete protection against challenge infection with the homologous influenza virus strain

The Nhu Nguyen1*, Sushmita Kumari1,2, Sarah Sillman1,3, Jayeshbhai Chaudhari1,4, Danh Cong Lai1, and Hiep L.X. Vu1,4#

1Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln NE, 2School of Veterinary Medicine and Biomedical Sciences and Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln NE, 3Nebraska Veterinary Diagnostic Center, University of Nebraska-Lincoln, Lincoln NE, 4Department of Animal Science, University of Nebraska-Lincoln, Lincoln NE, #Corresponding author.

Abstract

Influenza A virus of swine (IAV-S) is highly prevalent and causes significant economic losses to swine producers. Due to the highly variable and rapidly evolving nature of the virus, it is critical to develop a safe and versatile vaccine platform that allows frequent updates of the vaccine immunogens to cope with the emergence of new viral strains. The main objective of this study was to assess the feasibility of using lipid nanoparticles (LNPs) as a nanocarrier to deliver DNA plasmid encoding the viral hemagglutinin (HA) gene in pigs. Intramuscular administration of a single dose of the LNP-DNA vaccines resulted in robust systemic and mucosal in pigs. Importantly, the vaccinated pigs were fully protected against challenge infection with the homologous IAV-S strain, with only one out of 12 vaccinated pigs shedding a low amount of viral genomic RNA in its nasal cavity. No gross or microscopic lesions were observed in the lungs of the vaccinated pigs at necropsy. Thus, the LNP-DNA vaccines are highly effective in protecting pigs against the homologous IAV-S strain and can serve as a promising platform for the rapid development of IAV-S vaccines.

T Cell mediated clearance of PRRSV in the naïve porcine lung in the absence of neutralizing antibodies

Andrew Noel1*, Jianqiang Zhang1, Baoqing Guo1, Panchan Sitthicharoenchai1,2, Reid Phillips3, Marius Kunze3, Oliver Gomez-Duran3, Jennifer Groeltz-Thrush1, Michael Rahe1,2

1 Iowa State University, Ames, IA; 2 North Carolina State University, Raleigh, NC; 3 Boehringer Ingelheim Vetmedica Inc.

The T cell response to PRRSV infection plays an important role in conferring heterologous protection due to its ability to recognize conserved non-structural proteins. Typically, this response is studied in peripheral blood mononuclear cells. However, quantitative characterization of effector T cells in the lung, the primary tissue of interest in PRRSV infection, is possible with immunohistochemistry (IHC) staining for CD3 paired with RNA in situ hybridization (ISH) using RNAScope® for interferon gamma (IFN-gamma) and granzyme B and evaluation with the HALO® image analysis software. This assay and analysis do not require immediate fresh tissue processing and can be performed at the convenience of the researcher. In this study, PRRSV MLV vaccinated and non-vaccinated nursery age pigs were challenged with a wildtype lineage 1, RFLP 1-7-4 PRRSV-2 isolate at 28 days post-vaccination (DPV) with tissue samples collected at 14 and 21 days post-challenge (DPC). Utilizing paired IHC/ISH, we found a positive correlation across treatment groups between PRRSV nucleic acid and IFN-gamma expression in the lung, as well as a positive correlation between IFN-gamma expression and granzyme B expression on DPC 14. Vaccinated pigs had significantly lower PRRSV hybridization in the lung than non-vaccinated pigs at DPC 14. From DPC 14 to 21, non-vaccinated pigs showed significant reductions in PRRSV hybridization as well as IFN-gamma and granzyme B expression in the lung. Reductions were drastic enough that, at 21 DPC, vaccinated and non-vaccinated pigs had similar levels of interferon gamma and granzyme B expression and PRRSV hybridization. Furthermore, neutralizing antibodies specific to the challenge virus were not detected in non-vaccinated pigs at DPC 14 or 21. These results demonstrate the effectiveness of quantitative direct detection techniques for the study of the immune response while also suggesting that the T cell is the most important immune cell driving the clearance of PRRSV from the lung.

Characterization of the Biological Function of African Swine Fever Virus p30/p32 Protein

J. C. Gomes Noll1*, M. Martins1,2, D. G. Diel1

1Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY

2Texas A&M Veterinary Medical Diagnostic Laboratory

African Swine Fever Virus (ASFV) is a complex virus that threatens the swine industry worldwide. African swine fever (ASF), caused by ASFV, is a hemorrhagic transboundary disease of high social and economic impact with mortality rates reaching up to 100% in domestic pigs. Due to the high complexity of the virus, there are no vaccines or therapeutics available for ASF. The ASFV phosphoprotein p30/p32, encoded by gene CP204L, is a structural protein of ASFV. Our goal is to characterize p30/p32 focusing on the functional characterization of antibody and T cell responses elicited against the protein. For this, we generated a recombinant poxvirus encoding the gene CP204L of ASFV (ORFV-ASFV-CP204L). Characterization of the resultant recombinant virus revealed similar replication properties of titers as the WT virus in both ovine and swine cell lines. Additionally, like in ASFV infection, p30/p32 is expressed on the membrane of cells infected with ORFV-ASFV-CP204L. Next, we immunized 4-week-old piglets with ORFV-ASFV-CP204L. ELISA tests showed high titers of antibodies against p30/p32. Using peptide ELISAs we identified a 12aa epitope that appears to be the most immunogenic linear epitope of p30/p32. Antibody dependent cellular cytotoxicity (ADCC) assays in cells stably expressing p30/p32 revealed a trend of higher cell death in cells incubated with serum from pigs immunized with p30/p32 when compared to control serum, suggesting that p30/p32 antibodies might play a role in driving antibody dependent cellular cytotoxicity (ADCC). Flow cytometry analysis of PBMC collected from the immunized animals showed that T CD3+ cells stained with cFSE proliferated upon stimulation with different concentrations of p30/p32. These results reveal important aspects of ASFV p30/p32 protein on the host immune responses to the virus. Ongoing experiments include the characterization of neutralizing antibodies elicited by p30/p32, and to determine the nature and magnitude of the T cell responses against p30/p32

Effect of live virus exposure on pregnant sow performance in a PRRS elimination protocol

E.S. PALADINO1*, C. CORZO1, ALYSSA BETLACH2, AARON RENDAHL1, MARIA PIETERS1, RANDALL SINGER1

1. College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA
2. Swine Vet Center P.A., St. Peter, MN, USA

Introduction

Pig production systems commonly implement different measures to control or eliminate PRRS through herd closure and whole herd exposure, utilizing the resident virus to expose all pregnant sows, aiming to wean virus-free piglets. However, concerns regarding the production impact of this practice remain. The objective of this study was to quantify the number of weaned piglets per sow after the implementation of a live-virus inoculation (LVI) protocol in pregnant sows at different times of gestation.

Methods

Production performance records from twelve previously naïve sow farms, belonging to one system that experienced PRRS outbreaks, were used for this study. Upon PRRS detection, a load-close-expose approach was implemented. Only pregnant sows were included in this analysis. Production impact was characterized according to each sow time of gestation, and the interval between outbreak and LVI implementation. Gestation was categorized into three periods, and the outcome variables of interest were number of liveborn and weaned piglets.

Results

Records from a total of 28,331 pregnant sows were analyzed. On average, the farrowing rate was 75.6%, with 14.5% of those sows not weaning live piglets. The interval between outbreak and LVI ranged between 38 and 91 days, and were categorized as: short (38-55 days; n=6 farms) and long (56-91 days; n=6 farms). Results are summarized in table 1.

Table 1. Number of liveborn and weaned piglets (median and interquartile ranges).

Gestation Periods	Short interval	long interval
Liveborn piglets		
Early – 0-5 weeks	15 [12 - 17]	15 [13 - 17]
Mid – 6-10 weeks	11 [6 – 15]	14 [10 – 16]
Late – 11-16 weeks	9 [4 – 14]	10 [5 – 14]
Weaned piglets		
Early – 0-5 weeks	13 [8 - 14]	13 [11 - 15]
Mid – 6-10 weeks	9 [0 - 12]	11 [0 – 13]
Late – 11-16 weeks	3 [0 - 11]	7 [0 – 10]

Discussion and conclusions

The most severe losses occurred in the latest gestation group. Exposure to LVI at the end of gestation precipitates the development of congenital PRRS. Furthermore, sows in the "long interval" group had numerically fewer losses, suggesting that herd immunity might play a role in mitigating the potential impact of LVI and waiting to implement the protocol might be beneficial. As a next step, current analysis will be used in a forecasting model to predict the number of weaned piglets after implementing LVI protocols in pregnant sows.

Predicting PRRSV-2 Variant Emergence: Insights from a Decade of Genomic Analysis

N. Pamornchainavakul1*, M. Kikuti1, I.A.D. Paploski1, C.A. Corzo1, and K.VanderWaal1.

1Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN 55108, USA.

Porcine reproductive and respiratory syndrome (PRRS) presents a formidable challenge, causing significant annual economic losses exceeding half a billion dollars in the United States pork industry. The co-circulation and prevalence of distinct PRRSV-2 viral strains impede effective control measures, including the development of vaccines. Inspired by predictive tools used to combat viral infections like seasonal flu and COVID-19, early identification of emerging viral variants holds promise for proactive disease mitigation. Despite the wealth of available data, such predictive models have yet to be established for PRRSV-2. To address this knowledge gap, we conducted an in-depth analysis of a decade's worth of virus ORF5 sequences (n = 20,700) and associated metadata. Our primary objective was to identify early indicators of variant emergence for both short-term (12 months) and longer-term (24 months) timeframes, based on six phylogenetic parameters. We examined 12 measures of viral variant "success" that represented population expansion, spatial distribution, and genetic diversity of each variant across time. Utilizing a matched case-control study and conditional logistic regression, we determined the optimal predictive models and significant associations between early indicators and variant success. Our comprehensive investigation unveiled that "successful" variants characterized by population expansion also tends to exhibit widespread geographic spread, while maintaining limited genetic diversification at the timescales examined here. Regression analysis identified the local branching index (LBI) as the sole informative indicator for predicting population expansion, while ancestral mutation length displayed a strong association with short-term genetic diversity. Although multiple predictors were employed to estimate spatial dispersion, the intricate causal relationships remain elusive due to external factors influencing disease spread geographically. While our predictive models successfully captured the majority of emerging variants, they demonstrated relatively low precision due to a high occurrence of false positives (i.e., variants predicted to have emergence potential, but did not become particularly successful). However, this preliminary endeavor in PRRSV-2 prediction, combined with forthcoming research and advancements in PRRSV-2 immunology, establishes the foundation for more precise prevention strategies against PRRS in the future.

Weathering the storm: extreme weather events and their association with PED and PRRS occurrence

Igor A. D. Paploski1*, Gabriela Kiesel1, Dennis N. Makau1, Nakarin Pamornchainavakul1, Julia P. Baker1, Mariana Kikuti1, Cesar A. Corzo1, Kimberly VanderWaal1
1 – Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, US, 55108.
* – Presenter

Despite the wealth of knowledge regarding risk factors for Porcine Epidemic Diarrhea (PED) and Porcine Reproductive and Respiratory Syndrome (PRRS), weather risk factors historically received little attention to their association with the occurrence of both diseases. Our objective was to investigate the relationship between exposure to extreme weather events and PED or PRRS outbreaks.

We conducted a case-control study to investigate if the frequency of exposure to extreme weather events was different between cases (farms experiencing new outbreaks of PRRS or PED) and controls (farms not experiencing a new outbreak of PRRS or PED). Outbreaks of both PED and PRRS were captured by the Morrison Swine Health Monitoring Project (MSHMP). County-level extreme weather event data were obtained from the National Oceanic and Atmospheric Administration storm events database for the states in the SE US. We modelled the association between weather events and PED/PRRS occurrence using a 1:4 unmatched logistic regression. For each case (a farm in a given week in which an outbreak occurred), four different controls (farm in weeks in which an outbreak had not occurred) were randomly selected. For each of the analyzed weather event (flood, heavy rain, high wind and tornadoes), we ran ten different models, with each model including a different lag of time between exposure and outbreak occurrence, ranging from lag 1 (if the weather event occurred during the outbreak week) to lag 10 (if the weather event occurred nine weeks before the outbreak). Analyses were run on three periods of time based on trends on PEDV incidence: epidemic (2014-15); endemic (2016-19) and overall (2014-19).

We found that certain extreme weather events are consistently associated with the occurrence of PED. During the endemic period, farms located in counties exposed to floods had three to four times higher odds of having a PED outbreak in the four to nine weeks prior to the flood compared to controls. We also saw increased odds of PED occurrence after heavy rain (endemic period) and high wind (epidemic period). Associations of weather events and PRRS were less clear. We found that PRRS cases only had increased odds of being exposed to high wind in 2014-19 and 2016-19. These result held, with minor changes in the point estimates and width of the confidence intervals, when alternative statistical analyses were performed.

Overall, associations tended to be weak and with wide confidence intervals. The exact mechanism by which each weather event influences the risk of disease occurrence was not possible to investigate, but weather events may potentially influence outbreak occurrence by disrupting aspects of farm management, for example, by increasing the likelihood of biosecurity breaches or changing truck routes and delivery schedules. We suggest that swine-producing companies in the U.S. develop biosecurity protocols to account for and mitigate the impact of extreme weather events. These could potentially include strengthening protocols for visits of maintenance teams assessing physical damages to farms, and more stringent biosecurity practices in the immediate weeks following an extreme weather event.

Evaluation of the High-Volume Testing Potential of SmartChip Real-Time PCR System for the detection of Foreign Animal Diseases

S.Parameswaran, L.G. Giménez-Lirola, P.C. Gauger, R.K. Nelli

Veterinary Diagnostic Laboratory, College of Veterinary Medicine,
Iowa State University, Ames, Iowa
Corresponding author: rknelli@iastate.edu

During outbreaks of foreign animal diseases (FAD) like African swine fever (ASF), classical swine fever (CSF), and foot and mouth disease (FMD), the priority is to diagnose infections early to prevent their rapid spread. While current 96-well-based real-time polymerase chain reactions (rtPCR) are effective at identifying these pathogens, there is a need for a high-throughput rtPCR system that can handle the massive volumes of testing required during a FAD outbreak. The SmartChip real-time PCR system (SCRPS) offers a solution with its advanced microfluidic rtPCR assays in a 5,184 Nano-well chip that can accurately detect pathogens in nanoliter volumes.

This study aims to validate the relative specificity and sensitivity of standard NAHLN-approved 96-well ASF, CSF, and FMD reverse transcription-rtPCR (RT-rtPCR) assays to SCRPS-based RT-rtPCR assays. A total of 341 samples (276 negative cohort and 57 proficiency panel samples) were tested. Negative cohort samples were part of another NAHLN-funded study in which oral fluid (n=92) and processing fluid (n=184) samples were collected from different farm sites across the United States between April- May 2022 and previously confirmed negative at Iowa State University-Veterinary Diagnostic Laboratory (ISU-VDL). Non-infectious proficiency panels (PT, Reference, or training panels) dated between 2014-2020 were provided by National Veterinary Services Laboratory - Foreign Animal Disease Diagnostic Laboratory (NVSL-FADDL). Magnetic bead-based nucleic acid extractions were performed using a MagMax kit and Kingfisher Apex (ThermoFisher). Samples were handled or dispensed using Bravo Liquid handler (Agilent) and Multi-Sample Nano Dispenser (MSND; Takara) instruments. All RT-rtPCR assays were TaqMan-based single plex FAM-labelled reactions specific for ASF, CSF, and FMD, with 4X master mix (ThermoFisher) and similar primer/probe concentrations and modified thermocycling conditions (50° for 5 min, 95°C for 20 sec, 95°C for 10 sec and 60°C for 45 sec for 45 cycles) as described in NVSL protocols. Each triplicate run included samples with XENO as an exogenous internal control.

The SCRPS RT-rtPCR assays provided consistent and reproducible triplicate results, with Ct values ranging from 20.0 to 36.0. As expected, all 276 negative cohort samples tested negative on SCRPS chips, which aligns with the 96-well RT-rtPCR assays. The Ct values of 57 non-infectious proficiency panels fell within the expected ranges provided by FADDL. Our findings indicate that the relative sensitivity and specificity are 100% and 99.9%, respectively, between SCRPS and 96-well RT-rtPCR assays.

Our research has successfully demonstrated the implementation of the FAD RT-rtPCR assay on microfluidic platforms. This first proof-of-concept study will pave the way for innovative opportunities in veterinary molecular diagnostics, including the ability to manage high-volume testing (>30,000 reactions/24 hours) and lower laboratory expenses for VDLs.

Abelson (Abl) Tyrosine Kinase Inhibitors Reduce Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Replication *in Vitro*

J. Parliament[1*], X. Wang[1]

[1]Department of Biology and Microbiology, South Dakota State University, Brookings, SD-57007, USA.

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive-sense single-stranded RNA virus from the *Arteriviridae* family. PRRSV causes respiratory illness and reproductive failures in infected pigs. Outbreaks occurring in the United States and worldwide cause significant economic losses annually. Abelson (Abl) tyrosine kinase is a non-receptor tyrosine kinase, which plays a central role in regulating many cellular processes such as actin reorganization, cell proliferation and differentiation by transducing extracellular and intracellular signals. Abl inhibitors have been shown to restrict the entry of membrane fusion of enveloped viruses, such as severe acute respiratory syndrome coronavirus, Middle Eastern respiratory syndrome virus, infectious bronchitis virus and others, to reduce virus replication. The objective of this study is to investigate the role of Ablson inhibitors against PRRSV replication.

To achieve this, we pre-treated Marc-145 cells with varying concentrations of two Abl kinase inhibitors, Imatinib and GNF-2, followed by a second treatment and an infection with PRRSV. To determine infection rates, the cells were stained with a FITC-labeled monoclonal antibody against PRRSV nucleocapsid protein (FITC-SDOW17) and quantified with flow cytometry. Further confirmation was performed with immunofluorescence staining, microscopy, and $TCID_{50}$ assays.

Cells treated with Imatinib show a reduction in virus positive cells as compared to the untreated controls groups by 3-fold while GNF-2 treated cells showed no significant difference. These differences were further observed with both microscopy and TCID50 assays, with a dose-dependent reduction in viral titers and number of virus positive cells as the concentration of the Imatinib increased. This indicates that the Abl kinases that Imatinib block in Marc-145 cells participate in viral replication.

In summary, our preliminary data suggests that Abl kinase mediated signaling pathways play a role in PRRS replication and by blocking them viral infection can be reduced. Future studies will investigate the mechanism by which Abl kinase inhibitors reduce PRRSV replication *in vitro*.

Elucidating deoxynivalenols' antiviral effect against porcine reproductive and respiratory syndrome virus infections in vitro – a multi-omics approach

MJ. Pesant1,3*, C. Provost2, Y. Chorfi1,3, F. Beaudry1,3, C.A. Gagnon1,2,3

1Swine and Poultry Infectious Diseases Research Center [CRIPA-FRQ], Faculté de médecine vétérinaire, Université de Montréal, Québec, Canada, 2Molecular diagnostic laboratory, Centre de diagnostic vétérinaire de l'Université de Montréal, Québec, Canada, 3Research Group on Infectious Diseases in Animal Production [GREMIP], Faculté de médecine vétérinaire, Université de Montréal, Québec, Canada

Deoxynivalenol (DON) is a mycotoxin produced by Fusarium spp. and often found in animal feed. Swine are notably sensible to its harmful effects, such as weigh loss and vomiting, and studying their interaction has been of importance in the past 15 years. Our research team has highlighted a significant antiviral effect against porcine reproductive and respiratory syndrome virus (PRRSv) infection in vitro but have not explored a cellular and mechanistic explanation for this effect.
This study aims to elucidate in vitro DONs' antiviral mechanisms against PRRSv using transcriptome and proteome approaches.

Briefly, MARC-145 cells were infected at an MOI of 0.5 with a Québec reference strain, IAF Klop, treated post-infection (p.i.) with DON [280ng/mL] supplemented medium. Finally, total RNA and proteins were extracted 72h p.i. and with relevant controls, a total of 16 samples (N=4) of 3' mRNA librairies were sequenced with Illumina MiSeq platform and 12 samples (N=3) of total protein were run through an UHPLC coupled with a Q-Orbitrap Mass Spectrometer (MS/MS) (Thermo Scientifics). Curation of reads and differential expression analysis were performed with CLC Genomics WorkBench (RNAseq) and Proteome Discoverer (Untargeted proteomics). RNAseq results revealed, uniquely in treated and infected cells, 154 differentially expressed genes (DEGs) [p-value<0,05; -1 > Log2FC > 1] whereas proteomics results revealed 23 DEGs [p-value<0,05; -1 > Log2FC > 1]. A ranked gene-list from these combined DEGs was generated and enriched with the computational method GSEA and visualised with Cytoscape. Early analysis suggests a strong link with downregulation of some genes such as APOE, PDCD6IP and LAMP1, which play crucial roles in viral life cycles. Identical results from porcine macrophages are currently being curated to cross-analyse DEGs through this bioinformatic pipeline and more comprehensively select genes from significant enriched pathways. Subsequently, it is planned to confirm transcriptomic and proteomic results using alternative methods such as RT-qPCR, Western Blot and siRNA technology.

This study is based on a trending bioinformatic method using multi-omics to reveal potential cellular mechanisms and genes crucial to an antiviral activity against PRRSv. DON, which has no realistic direct application against PRRS, could hence point towards novel targets for future antiviral strategies against this costly swine virus.

Identification of regions in the viral glycoprotein genes involved in CD163 binding and PRRSV infection.

Derek Pinto*, Raymond R.R. Rowland, Alberto Brandariz-Nuñez

Department of Pathobiology, University of Illinois Urbana-Champaign

Porcine reproductive and respiratory syndrome (PRRS) is responsible for major losses to the global supply of quality protein. Clinical signs following infection range from respiratory distress, reproductive failure, high piglet mortality, and slow growth. Belonging to the genus Betaarterivirus, the virus consists of ~15 kb genome with 10 open-reading-frames; genes for four virus surface glycoproteins GP2, GP3, GP4 and GP5 (which is covalently linked to non-glycosylated matrix (M) protein).

Infection of macrophages begins with the interaction of surface viral glycoproteins with the CD163 receptor on macrophages. The purpose of this research is to identify the regions within the glycoproteins that interact with CD163. The experimental approach is to identify regions in the glycoproteins and M protein that interact with CD163 by creating truncation deletion mutants. Our approach involved creating mutations by truncating the genes in multiple segments. The mutant glycoprotein genes will be cloned individually into vectors and co-expressed with CD163 in culture cells and be assessed by co-localization and co-immunoprecipitation experiments.

So far, we have identified the truncated region within the glycoprotein that interacts with CD163 by co-localization using microscopy and co-immunoprecipitation. Our goal is to determine the specific regions or residues in the glycoproteins that are involved in the interaction with CD163 and to further generate infectious clones that contains these deletions and evaluate the effect of CD163 interaction and its role in viral infection.

Evaluation of PRRSV2 PCR detection using cotton ropes, in Oral Fluid, in room-based manure pit and manure collection tank, as nursery PRRSV status monitoring

PH Rathkjen*
Boehringer Ingelheim AH, Weidekampsgade 14, DK-2300 Copenhagen S,

Study objective
The objective of this study was to compare the detection of PRRSV in pooled OF and manure samples using cotton ropes in order to establish a cost effective, non-invasive monitoring of PRRSV status.

Methods
A Danish nursery site with 4 barns with 23 rooms was selected for sampling. The source of the pigs was one PRRSV negative breeding herd. The manure was collected per room (RM) and cooled at 10 - 15 ºC. Manure pits were emptied twice a week and manure transferred to an outside tank (CMT) which is also emptied with the same frequency. Two consecutive weaning batches, divided into two rooms, were monitored weekly for the entire nursery period. Four ropes per room for 30 minutes were placed. Each rope was placed between two pens. OF were pooled by room and sampling day (240 pigs). Pit samples in the same room and the CMT were collected by dipping a cotton rope in the manure. Furthermore, all rooms containing pigs were sampled using OF at the beginning and the end of each batch. Oral fluid and manure samples were tested by PCR.

Results
In the first batch, 57% (8/14) OF, 64% (9/14) RM and 100% (7/7) CMT samples were positive (see table) from CT values ranging from 24.5 to 29.5 in OF, from 29.3 to 37.5 in RM and from 30.7 to 32.5 in CMT samples. In the second batch, 0% (0/14) OF, 7% (1/14) RM and 29% (2/7) CMT samples were positive. The proportion of positive OF rooms at the beginning and end of each batch were 19% (3/16) and 16% (3/18) for batch 1 and 0% (0/13) and 0% (0/17) for second batch.

Batch	Room	Sample origin	(1w)	(2w)	(3w)	(4w)	(5w)	(6w)	(7w)
1	1	RM Ct	32,3	30,1	32,2	31,3	neg	-	30,6
		OF Ct	neg	neg	neg	29,2	25,3	27,7	24,5
	2	RM Ct	31,9	30,4	37,5	-	neg	-	29,3
		OF Ct	neg	neg	neg	29,5	27,1	25,5	24,8
	All 23	CMT Ct	32,5	30,7	31,6	32,1	30,8	32,1	30,9

Conclusions
The collection tank is a cheap (one sample per week), non-invasive and accessible (from the outside of the farm) option for routine monitoring of the PRRSV status on a nursery site. The CMT samples were positive at all sampling points during the first batch reflecting of the status of RM and/or OF sampling. Manure cooling possibly helped to the conservation of the PRRSV RNA in RM and CMT. During the first sampling weeks of batch 2 the CMT samples were positive, without positivity in any tested rooms. Partial removal of manure from the CMT is allowing PRRSV positive manure from previous batches to be mixed for some time, this could lead to a false positive result regarding the status of the pigs as indicated by the negative OF from all batches during batch 2 and also a risk for reinfection if manure is not handled well. CMT testing offers cost effective monitoring of site PRRSV status.

Historical Review of NAPRRS/NC229

Raymond (Bob) Rowland
Department of Pathobiology, University of Illinois, Urbana-Champaign

The history of NAPRRS/NC229 begins in 1999 (see Benfield et al. 2020, The NC229 multi-station research consortium on emerging viral diseases of swine, published in *Virus Research*). The preparation and writing of the first NC229 proposal were organized by David Benfield at Southy Dakota State university. He went on to become the first NC229 advisor. The writing team and input came from researchers, South Dakota State, Iowa State, USDA, University of Nebraska, North Carolina State, University of Minnesota, University of Missouri, University of Illinois, and others. In addition to research, NC229 paved the way for the inclusion of partners from other countries and the exploration of PRRS regional elimination and control strategies. The NC229 collaborative philosophy laid the groundwork for the successful funding of PRRS CAP I (2004-2008, M. Murtaugh director), PRRS CAP II (2009-2013, R. Rowland director), the PRRS Host Genetic Consortium (2007-present, J. Lunney director) and the PRRS Symposium. The symposium originated out of the yearly NC229 meeting and was called the International Workshop on PRRS, which was held from 10:00 to 5:00 pm, November 8, 2003, in the Windsor Room, Congress Hotel, Chicago. In 2005, the meeting name was changed to the International PRRS Symposium (IPRRS), which remained a one-day meeting. A total of 77 abstracts were submitted. By 2007 the conference became a standalone meeting and was expanded to one and a half days, including the addition of a reception and concurrent poster session. Another notable development was the establishment of travel fellowships in 2009; the result of an initial donation by David Benfield. Hanchun Yang from China hosted the conference in 2013 in Beijing. There were nearly 1,000 attendees from all over the world. To distinguish the periodic international conferences from the annual national meeting, the name was changed to NAPRRSS (North American PRRS Symposium). In 2015, Hans Nauwynck hosted the International Porcine Reproductive and Respiratory Congress, in Gent, Belgium. Over the last 25 years, several cities have hosted the PRRS conference, including Chicago, St Louis, Kansas City, Beijing, and Ghent. The efforts to control PRRS are dedicated to those who helped pave the way and are no longer with us: Michael Murtaugh, Robert Morrison, Monte McCall, and others.

Elevated PRRSV Viral Load in Critical Non-lymphoid Tissues is Associated with Late Gestation Fetal Compromise

K. Rudy1*, D.Y. Jeon1, A. Smith1, J.C.S. Harding2, J.A. Pasternak1
1Department of Animal Sciences, Purdue University, West Lafayette, IN.
2 Western College of Veterinary Medicine, Saskatoon, SK.

Objective:
The impact of late gestation PRRSV infection is highly variable within a litter. While some fetuses remain viable despite significant viral load, others display varying degrees of compromise, with the initial stages identified by the presence of in-utero meconium staining. The fetal thymus is generally considered to be the primary site of PRRSV replication; however, in-depth molecular investigation of this tissue has failed to identify a clear mechanism underpinning fetal resilience or susceptibility. The goal of this project is to examine the distribution of virus within non-lymphoid tissues of highly infected fetuses and evaluate the local response and association with fetal viability.
Methods:
Samples of brain, heart, kidney, liver, lung, and skeletal muscle were obtained from fetuses of pregnant gilts at gestation day 85, and the presence and distribution of CD163+ cells within each tissue evaluated using immunohistofluorescence with monoclonal antibody LND68A. PRRSV infected fetal tissue samples were obtained from a prior challenge study in which gilts were inoculated with NVSL 97-7895 on gestation day 85 and fetal tissues collected 21 days later. A subset of fetuses were selected based on a previously established model of biological extremes including, high viral load viable (HV-VIA) and high viral load meconium-stained (HV-MEC), along with gestational age matched controls (CON n=10) from mock infected gilts. Viral load and local inflammation, in the form of interferon gamma, were initially assessed across the six tissues using quantitative PCR. To verify the presence of infectious viral particles, individual tissue lysates were used to inoculate MARC-145 cells, and infection evaluated three days later using PRRSV specific monoclonal antibody (SDOW-17).
Results:
An objectively substantial population of CD163+ cells were observed in all six non-lymphoid tissues from healthy day 85 fetuses, though relative density and the morphology of positive cells varied between tissue. The most robust and widely distributed population of CD163+ cells was observed in the fetal liver, whereas the population in the brain was limited and found to be restricted to the pia mater. Viral RNA was detected in all six tissues derived from fetuses previously classified as highly infected. Significantly more viral RNA was detected in the heart, brain, lung, and muscle of meconium-stained fetuses relative to their HV-VIA counterparts. The presence of viral RNA in each tissue coincided with a significant upregulation in the expression of interferon gamma. Virus was successfully recovered from 8/8 samples of fetal heart from meconium-stained fetuses.
Conclusions:
Late gestation non-lymphoid fetal tissues contain a presumptively susceptible population of CD163+ cells. Following vertical transmission, PRRSV is widely distributed across these non-lymphoid tissues resulting in a local inflammatory response. Most critically, the viral load in key tissues such as the fetal heart appears to be associated with meconium staining, an established marker of reduced fetal viability.

Sialoadhesin is not required for infection of pigs with PRRSV-1

Brianna C. Salgado1*, Kelly Lechtenberg2, Ying Fang1, Randal Prather3, and Raymond R.R. Rowland1

1Department of Pathobiology, University of Illinois Urbana Champaign
2Midwest Veterinary Services, Inc.
3Department of Animal Science, University of Missouri, Columbia

Sialoadhesin (Sn/SIGLEC1/CD169) is a macrophage surface protein belonging to a family of sialic acid binding immunoglobulin-like lectins. Classic models of PRRSV infection show that the first step in the infection of a macrophage is the binding of CD169 with the virion surface protein GP5. Previous research by our lab shows that pigs possessing a knockout (KO) of SIGLEC1 do not express CD169 but are able to support productive infection with a PRRSV-2 isolate. Since PRRSV-1 and PRRSV-2 share only about 70% nucleotide identity and possess different requirements for infection of macrophages, we conducted a second study to determine if CD169 KO pigs could be infected with a PRRSV-1 isolate. KO pigs (n=8) and wild type pigs (WT, n=9) were infected with EU PRRSV-1 strain, SD01-08, and maintained for 21 days. Both genotypes were maintained in the same room and personnel were blind to the genotype of individual pigs. Serum was collected and evaluated for infection using a Tetracore commercial RT-qPCR kit with standards. Antibody was measured using a commercial ELISA kit from IDEXX. Results from both assays showed that all pigs in the WT and KO groups supported PRRSV infection. Statistical analysis between groups was performed using a Wilcoxon rank sum test with continuity correction ($p < 0.05$). Significant differences between WT and KO pigs were observed at 21 ($p=0.002$) and 28 ($p=0.01$) DPI. Unexpectedly, the KO pigs supported higher levels of viral replication on both days. Template copies per pig were plotted and the area under the curve (AUC) was calculated to determine virus load. Higher viral loads were observed for the KO pigs ($p<0.05$). These results confirm that the presence of CD169 is not biologically relevant for PRRSV infection. Current work is directed at measuring virus nucleic acid in tissues.

In vitro antiviral activity of modified medium-chain fatty acids against porcine reproductive and respiratory syndrome virus

N.M. Sobhy1*, A.Q. Munoz1, Y.-T. Hung2, G.D. Reznik2, S.M. Goyal1
1Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN 55108, 2Devenish Nutrition LLC, 2320 Lake Ave., Fairmont, MN 56031.

Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), is a disease with disastrous impact on the pig industry worldwide. Rapid mutation of the virus, prolonged stability of some strains in the environment, and easy transmission to susceptible hosts are crucial factors in maintaining the disease in pig populations. Despite widespread use of vaccination, limited control of PRRS has been achieved and the prevalence of PRRSV infection among swine herds is still high. Although non-modified medium-chain fatty acids (MCFAs) are known for their antibacterial and antiviral properties, their in vivo efficacy is lost or limited due to chemical and enzymatic hydrolysis in the body. Modified MCFAs have been recently developed and are more structurally stable compared to non-modified MCFAs, which is critical for the antimicrobial properties of MCFAs. Hence, modified MCFAs could be a novel approach to support animals during pathogenic challenges, such as Gram positive bacteria and enveloped viruses. A proof-of-the-concept study was conducted to determine if proprietary blend of modified MCFAs (D-Strike product, Devenish Nutrition) can kill PRRSV in cell culture as a novel anti-PRRSV formula. Undiluted modified MCFA was very potent; it killed 4.5 log10 of PRRSV almost immediately. At 1:1000 dilution, this formula inactivated 99.95 and 99.97% of the virus within 1 min and 2 min, respectively. A 1:5000 dilution killed 99.78% and 99.97% of the virus within 1 min and 2 min, respectively. Both dilutions killed > 99.99% of PRRSV within 5 min. In addition, 1:5000 dilution was found to be non-cytotoxic to cell cultures, e.g., ST (swine testicle), PK-15 (porcine kidney) and MARC-145 (monkey kidney). These results indicate that modified MCFAs can inactivate PRRSV in cells and could be a valuable option for swine industry. To validate the efficacy of modified MCFAs in vivo, further studies are in progress to determine the effect of this formula in PRRSV-infected pigs.

Investigating the role and mechanism of host cell death in PRRSV infection.

M. Steyn1*, S.Lawson2, S. Aftab1, X. Wang1, G. Imre1

1Department of Biology and Microbiology, 2Department of Veterinary and Biomedical Sciences, Dakota State University, Brookings, SD-57007, USA.

Lytic forms of regulated cell death have not been investigated in detail in the context of PRRSV infection. Detection of pathogen-associated molecular patterns, including viral RNA and viral proteins, might trigger pyroptosis and necroptosis. In pyroptosis, this event leads to the activation of a protein complex called the inflammasome, resulting in selective processing/cleaving of substrate proteins, such as gasdermins that oligomerize and form transmembrane pores throughout the cell membrane. This leads to the release of pro-inflammatory molecules and culminates in the destruction of the cell. Caspases are a family of cysteine proteases that play a central role in apoptosis regulation, and members of this group have a role in inflammatory processes and pyroptosis. In necroptosis, the initial stimulus results in the formation of a protein complex called necrosome, leading to the oligomerization of mixed lineage kinase domain like pseudokinase (MLKL). MLKL molecules form transmembrane pores in the cytoplasm membrane resulting in cell death. All these cell death mechanisms are regulated and therefore can be manipulated, opening novel opportunities to change the course of the disease. Elucidating these pathways may facilitate the development of treatments for this disease. The objective of this study is to identify the mechanism of lytic cell death and identify novel elements of the pathways involved in host cell death signaling in response to PRRSV infection.

To achieve this objective, cell death and infection in MARC145 and Porcine Alveolar macrophages were simultaneously characterized within a single sample by a newly established method for flow cytometry. This enabled a novel quantitative way to distinguish between infected live and infected dead cells. In this method, the cells were labeled with a FITC-conjugated antibody for a highly conserved epitope on the nucleocapsid protein of PRRSV and were stained by a Live/Dead stain to determine the loss of membrane integrity of the cells. To determine which type of cell death modality takes place in response to infection, pharmacological inhibitors of various cell death pathways were employed. Cells were treated with apoptotic caspase inhibitor zVAD, necroptosis inhibitor necrosulfonamide, and NLRP-3 inhibitor to block pyroptotic cell death.
By employing the newly established double staining flow cytometry method, cells treated with zVAD showed increased cell death and infectivity after viral infection as compared to cells without caspase inhibitor. Treatment with MLKL inhibitor alone did not reduce cell death of infected cells. Intriguingly cells treated both with zVAD and MLKL inhibitor efficiently reduced the cell death rate of infected cells. This data indicates that the cell death induced by the PRRSV infection is primarily caspase dependent, however upon caspase inhibition, the cell death is not inhibited, instead it is switched to an alternative cell death route. Since this cell death can be blocked by MLKL inhibitor necrosulfonaminde, we hypothesise that this cell death form is necroptotic.

To summarize, our preliminary data suggested that the lytic form of cell death induced by PRRSV infection is due to necroptosis and double treatment of caspase and necroptosis inhibitors might prevent PRRSV infection induced cell death and reduce viral infection.

A novel method for evaluating environmental and pig-derived targets in pen-based oral fluids

G. Tarasiuk1*, M. Remmenga2, K.C. O'Hara2, M. Rotolo3, P. Zaabel4, Gimenez Lirola1, J.J. Zimmerman1

1Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA; 2USDA, Veterinary Services, Strategy and Policy, Center for Epidemiology and Animal Health, Fort Collins, CO, 80526; 3PIC North America, Hendersonville, TN 37075; 4National Pork Board, Clive, IA 50325

Introduction: Oral fluids (OF) are widely used as a surveillance sample for infectious diseases of swine. The detection of specific pathogens is widely researched from the perspective of detection in the lab, but we have an incomplete understanding of the source of the target (pig vs environment) in the oral fluid sample. For example, porcine epidemic diarrhea virus (PEDV) is readily detected in OF, despite the fact that it is not shed orally. This study presents a novel approach, using the detection of fluorescence from food dye, to explore individual and environmental contributions to OF samples.

Experimental Design: To avoid the complications of working with an infectious agent in the field, FD&C Red dye was used as a surrogate. FD&C Red dye is approved by the FDA for use in drugs, cosmetics, and foods, and has measurable fluorescence that can be quantified using standard laboratory equipment. Pilot studies evaluated: 4 different dye concentrations, treatment volumes, the addition of sugar to entice pigs to interact with the treatment, treatment to sampling intervals, and sample handling requirements, to consistently detect fluorescence in OF samples. The final protocol, used for both pig and environmental "treatments", included a dye solution composed of distillated water (50 ml), white sugar (25 g), and red food dye (50 ml) (McCormick & Co., INC., Hunt Valley, MD), and an extended centrifugation step during processing.

Treatments: In brief, the study was conducted in pens holding ~30, ~65, and ~125, 12- to 14-week old pigs (32 pens per size). Oral fluid samples were collected twice from each pen: one day prior to treatment (negative control) and again immediately after treatment. The negative control sample was used to provide a baseline fluorescence value, i.e., to account for naturally occurring fluorescent substances (feces, feedstuffs, etc). Pens received one of 2 treatments. For pig exposure, a convenience sample of 10% of the pigs in the pen were orally administered 3 ml of the dye solution. For environmental exposure, 20 ml of the dye solution was placed on the floor in the center of the pen. Immediately following treatments, ropes were hung for 30 minutes in pens of ~30 pigs, 45 minutes in the pens of ~65 pigs, and 60 minutes in the pens of 125 pigs. Pre- and post-treatment samples were tested for fluorescence at 530/570 nm (SpectraMax i3x, Molecular Devices LLC, San Jose, California) and the results analyzed for evidence that the dye was transferred from the environment and/or from pigs to the pen-based oral fluid sample.

Results and conclusions: For both treatments (environmental vs pig exposure) and regardless of pen size, specific fluorescence was consistently detected in pen-based oral fluid samples, successfully demonstrating that food dye can be used as a surrogate to evaluate environmental and pig-derived OF contributions.

Exploring the Emergence, Antigenicity, and Immune-Mediated Evolution of PRRSV-2 Variants through Bioinformatics

Kimberly VanderWaal, Maxim Cheeran, Cesar Corzo, Albert Rovira, Declan Schroeder, Julia Baker, Jing Huang, Dennis Makau, Nakarin Pamornchainavakul, Igor Paploski

College of Veterinary Medicine, University of Minnesota

Porcine Reproductive and Respiratory Syndrome Virus 2 (PRRSV-2) continues to pose significant challenges to the swine industry worldwide. One for the foremost challenges to PRRSV control is its genetic and antigenic diversity that has resulted from its exceptionally high rate of evolution. The consequence of the rapid diversification of this virus is that successful PRRSV control often seems like a moving target. Here, we highlight recent work aimed at unraveling the dynamic landscape of PRRSV-2 genetic variants and lineages, shedding light on their emergence, antigenicity, immune-mediated evolution and potential immune escape, and patterns of spread. Our findings illuminate mechanisms driving the continual evolution of PRRSV-2, including selection pressures from host immune responses that drive changes at viral epitopes. Additionally, we examine the potential role of immunity in shaping genetic diversity, highlighting the intricate interplay between the virus and host immune system. This work underscores the importance of large-scale sequence analysis in elucidating the complex dynamics of PRRSV-2, offering valuable insights for the development of strategies to mitigate its impact on the swine industry and animal health.

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

K.E. Walker1*, A. Pasternak2, A. Jones3, M.K. Mulligan3, A. Van Goor4, J.C.S. Harding5, J.K. Lunney1

1. USDA, ARS, BARC, ANRI, APDL, Beltsville, MD; 2. Department of Animal Sciences, Purdue University, West Lafayette, 3. School of Veterinary Medicine, St. George's University, Grenada, West Indies; IN; 4. USDA, NIFA, Division of Animal Systems, Kansas City, MO; 5. Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, SK, Canada.

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the costliest diseases to pork producers worldwide. Since its discovery there have been multi-pronged initiatives aimed at addressing this virus. The pregnant gilt PRRSV infection model has emerged as an essential design for facilitating research into vertical transmission and the fetal response to infection. The goal of this study is to identify critical tissues and genes that forecast fetal resilience or susceptibility following PRRSV infection.

In this study pregnant gilts (N=30) were infected with PRRSV at day 85 of gestation. At 21 days post infection, the gilts and fetuses were euthanized, and fetal tissues collected for further investigation. The fetuses in this study were characterized based on PRRS viral load in fetal serum and thymus as well as meconium staining status (i.e., viable or meconium stained) and then three distinct subsets selected: uninfected (UNIF; <1 log per µL or mg PRRSV RNA), resilient (>5 log virus but viable) and susceptible (>5 log virus and meconium stained). Total RNA was extracted and purified from fetal liver, heart, and kidney (N=10/group). These samples were analyzed using Qubit for RNA concentration and overall quality (RIN #). NanoString transcriptomics analyzed expression of 58 genes, based on 5 pathways hypothesized to be involved in fetal resilience or susceptibility including inflammatory, and metabolic pathways and 7 housekeeping genes.

Results herein focus on immune related genes and indicate a significant ($P<0.05$) response between fetal groups in chemokines CCL2, CCL5, and CXCL10 expression, indicating robust recruitment of immune cell types to infected tissues. Differences in chemokine gene expression was generally increased as fetal preservation declined across tissue types. These results provide insight into targeted tissue responses and clues to the mechanism of fetal protection following maternal infection. This data will inform treatment options and swine facility management strategies.

Support: Lab work supported by USDA ARS Project: 8042-32000-117-00D. Sample collection funding from Genome Canada (Project 2014LSARP_8202) and Genome Prairie (Saskatchewan Ministry of Agriculture, Project #346143) with administrative support provided by Genome Alberta. Jones stipend supported by USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003.

Survey of Intraspecific Variation within Swine ANP32A/B and Effects on Host Permissiveness to Avian Influenza Virus

L.R. Walker1*, D.C. Ciobanu1

1 Animal Science Department, University of Nebraska, Lincoln, United States of America.

Recent research has demonstrated the importance of host acidic leucine-rich nuclear phosphoprotein 32 (ANP32) proteins in avian influenza virus (AIV) zoonosis and the unique pro-viral capacity of swine ANP32A compared to other mammalian orthologues. Despite extensive characterization of interspecific differences, no studies have been reported that assess intraspecific variation in swine ANP32A/B and impact on AIV permissiveness. Given the immense role that domestic swine play in the generation of mammalian-adapted AIV, identification of ANP32A/B variants that affect host permissiveness would provide a plausible avenue for limiting the emergence of novel influenza strains. The overall goal of this research is to identify variants within swine ANP32A/B that alter AIV pro-viral capacity. First, we will conduct high-throughput parallel sequencing for discovery of ANP32A/B variants and isoforms across diverse domestic pig populations. Second, we will employ in vitro gene editing, co-immunoprecipitation, and influenza minigenome reporter assays to characterize the functional effect of selected variants and isoforms on AIV polymerase activity and affinity. The outcomes of this research have the potential to not only benefit swine producers directly by improving animal welfare, but human health as well by limiting the ability of swine to serve as AIV reservoirs and emergence of novel pathogenic strains with potential for human pandemic transmission.

The "Tricks" of African Swine Fever Virus Escaping Host Anti-Viral Innate Immune Responses

Changjiang Weng11,*

1 National African Swine Fever Para-reference Laboratory, State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Harbin 150069 , China.

African swine fever is an acute, highly contagious infectious disease caused by African swine fever virus (ASFV). The virus can infect domestic pigs or wild boars. In the past four months, more than 16,000 ASF cases have been reported in 27 countries. Therefore, prevention and control of ASF is still the toughest problem in the world. Upon ASFV infection, host cGAS sensed ASFV genomic DNA to promote cGAMP production. cGAMP binds to the ER-localized adapter protein STING, which then recruits TBK1. The activated TBK1 phosphorylates IRF3 and promotes it to translocate to the nucleus, inducing of type I IFN production. During the processing, RNF128 promotes ubiquitination of TBK1, which is necessary for TBK1 activity. ASFV I215L recruits RNF138 and enhances the interaction between RNF138 and RNF128, which promotes RNF138 to degrade RNF128, leading to reduce RNF128-mediated K63-linked ubiquitination of TBK1 and type I IFN production.
In this study, we also found that host Vimentin can sense ASFV genomic DNA to activate NLRP3 inflammasome. The active caspase-1 cleaves GSDMD to produce GSDMD-N fragment, which could form a hole on the cell membrane to release inflammatory cytokines. In one way, ASFV MGF505-7R interacts with NLRP3 to block NLRP3 inflammasome activation. In another way, ASFV S273R protein cleaves GSDMD-N fragment to produce two small fragments, which could not form hole on the cell membrane to release inflammatory cytokines. Recently, we found that pS273R cleaved GSDMD to inhibit inflammatory responses to promote ASFV replication. Taken together, during the past five years, our lab found that many ASFV proteins are involved in a regulating cGAS-STING signaling pathway, IFN-JAK-STAT signaling and inflammatory response signaling.

Keywords: African swine fever, innate immune, inflammatory response, CRISPR, attenuated live vaccine

Efficacy evaluation of a bivalent ready-to-use protein subunit vaccine against PRRSV and *Mycoplasma hyorhinis* challenge

F.C. Chang[1], M.J. Chen[1], G.J. Lin[1], C.-I. Chen[2], Z.-W. Chen[2], P.-Y. Wu[1*]

[1]Department of Research and Development, Reber Genetics Co. Ltd., Taoyuan County, [2]Animal Technology Research Center, Agriculture and Technology Research Institute, Miaoli County, Taiwan (R.O.C.)

Mycoplasma hyorhinis (Mhr) and porcine respiratory and reproductive syndrome virus (PRRSV) are pathogens contributing to porcine respiratory disease complex (PRDC). A strong correlation between Mhr and PRRSV infection was reported in Korea and this correlation has also been observed in Taiwan and other Asian countries. In the present study, the efficacy of a bivalent ready-to-use protein subunit vaccine composed of antigens derived from Mhr and PRRSV was evaluated in pigs.

Specific-pathogen-free pigs were vaccinated with one dose of PRRS/Mhr bivalent subunit vaccine or normal saline at 3 and 6 weeks old. At 9 weeks of age, pigs were challenged with either *Mycoplasma hyorhinis* or a virulent NA PRRSV strain. For Mhr challenge, pigs were observed daily for clinical symptoms including anorexia and joint swelling. At 21 days post challenge, animals were sacrificed and examined for polyserositis (peritonitis, pleurisy, pericarditis) and arthritis and scores were assigned according to the severity of lesion. For PRRSV challenge, blood samples were collected for viremia and antibody analysis. At 21 days post challenge, animals were sacrificed and examined for lung lesions.

After Mhr challenge, vaccinated pigs had lower clinical scores and fewer pigs exhibited swollen joints than the unvaccinated pigs. The average lesion score in peritonitis, pleurisy, pericarditis, and arthritis in vaccinated pigs was also lower than in unvaccinated pigs. Vaccinated pigs were PRRSV antibody positive on the day of PRRSV challenge and had lower PRRSV viremia 10 days after challenge. In addition, vaccinated pigs had lower lung lesion scores in apical lobes, cardiac lobes, and diaphragmatic lobes.

Vaccination of pigs with one dose of a bivalent ready-to-use PRRS/Mhr subunit vaccine at 3 and 6 weeks old can reduce clinical symptoms and tissue lesions associated with PRRSV and Mhr, suggesting that it can be an efficient method to control *Mycoplasma hyorhinis* and PRRSV infection in swine population and reduce the economic loss.

Molecular Mechanism of Porcine Epidemic Diarrhea Virus Evading from Host Interferon Immune Responses by Inhibiting Histone Deacetylase 1

Jidong Xu1*, Weihuan Fang1, Xiaoliang Li1

1Department of Veterinary Medicine, College of Animal Sciences, Institute of Preventive Veterinary Medicine, Zhejiang University, Hangzhou, Zhejiang, China,

Porcine epidemic diarrhea (PED) is a highly contagious acute disease of pigs caused by porcine epidemic diarrhea virus (PEDV) that imposes significant threat to the swine industry. Recent studies suggest that PEDV has evolved to deploy complicated antagonistic strategies to escape from interferon (IFN) immune responses. However, the specific mechanism of PEDV disrupting the IFN signaling remains to be explored. In this study, as histone deacetylases (HDACs) are considered crucial regulators of IFN signaling pathway, we found that PEDV infection led to reduced expression of HDACs, especially HDAC1, in porcine IPEC-J2 cells.

We first demonstrated that HDAC1 acted as a negative regulator of PEDV replication in IPEC-J2 cells, as shown by chemical inhibition, gene knockout, and overexpression. Of the viral proteins that were overexpressed in IPEC-J2 cells, the nucleocapsid (N) protein was more inhibitory to HDAC1 transcription. PEDV N protein could be localized in the nucleus through the putative nuclear localization sequence 261-PKKNKSR-267. As transcription factor Sp1 regulates the transcription of HDAC1 by binding to the GC-box within its promoter region, PEDV N protein interacted with Sp1 and interfered with its binding to the promoter region, thereby inhibiting its transcriptional activity for HDAC1 expression and inducing enhanced protein acetylation.

Signal transducer and activator of transcription 1 (STAT1) is a critical transcription factor regulating ISGs expression and anti-PEDV immune responses, as shown by overexpression, chemical inhibition, and gene knockdown in IPEC-J2 cells. We further showed that PEDV infection and its N protein overexpression, though upregulated STAT1 expression, could significantly block poly(I:C)- and IFN-lambda-induced STAT1 phosphorylation and nuclear localization. Western blotting revealed that PEDV and its N protein promoted STAT1 acetylation via downregulating HDAC1, thus impaired STAT1 phosphorylation. It indicated that STAT1 acetylation negatively regulated its activation. Consequently, PEDV inhibited transcription of at least two ISGs, ISG15 and OAS1, in favor of its replication.

In summary, PEDV manipulates the host Sp1-HDAC1-STAT1 signaling axis to inhibit transcription of ISG15 and OAS1 to facilitate its replication. This novel immune evasion mechanism is realized by suppression of STAT1 activation through preferential modulation of STAT1 acetylation over phosphorylation as a result of HDAC1 expression inhibition. Our findings contribute to a better understanding of the PEDV-host interaction as part of the pathogenetic mechanisms of coronaviruses**.**

Current status of PRRS in China: diversified strains of PRRSV and complicated clinical conditions

Jun Han, Lei Zhou, Hanchun Yang*

Key Laboratory of Animal Epidemiology of the Ministry of Agriculture and Rural Affairs, College of Veterinary Medicine, China Agricultural University, Beijing 100193, People's Republic of China

It has been almost 30 years since the introduction in early 1990s of porcine reproductive and respiratory syndrome virus (PRRSV) into China, where it has remained a prominent threat, leading to colossal economic losses to the Chinese swine industry. There are at least four milestone turning points along the trajectory of PRRSV evolution in China, including the first introduction of classic PRRSV strains in 1995, sudden emergence of the HP-PRRSV strains in 2006, appearance of NADC30-like strains around 2013, and NADC34-like strains in 2017, and the recent emergence of L1C (1-4-4) like strains in several provinces following the initial outbreak in the US. This presentation will update the recent status in China of the PRRSV genetic diversity and recombination of PRRSV-2, and the issues regarding PRRSV MLV vaccination. In facing the diversity and complexity of PRRSV-2 in China, this presentation will also discuss the strategies on PRRS control in China, including the biosecurity measures and immunization strategies to push forward the elimination of PRRSV on breeding pig farms and boar studs, constructing more PRRSV-free breeding herds/farms at national level.

Double mutations in nsp1 and N genes of PRRSV downregulate inflammatory cytokine expressions and confer clinical attenuation in pigs during bacterial co-infection

D. Yoo1*, C-M Su1, F. A. Zuckermann1, J. Tang1, Y. F. Hung1, R. Husmann1 , P. Roady2 , J. Kim3, Young-Min Lee3

1Department of Pathobiology, 2Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, 3Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT.

PRRSV infection triggers the negligible response of type I interferons (IFNs) and the upregulation of proinflammatory cytokine expressions during infection. Such unusual host responses lead to undesirable innate and adaptive immune responses and cause enhanced clinical outcomes, especially during co-infection with a secondary pathogen, resulting in the porcine respiratory disease complex (PRDC). We previously constructed a double-mutant PRRSV by reverse genetics to remove the IFN suppression function from the nsp1 protein and the NF-kB activation function from the nucleocapsid (N) protein. The double-mutant virus induced a higher level of IFN response and lower levels of cytokine expressions in macrophages in vitro. To examine the clinical consequence of the double-mutant PRRSV, 40 piglets were randomly allotted to 6 groups (5 test groups, 7 animals per test group, and 5 animals for mock infection), and co-infected with double-mutant PRRSV and Streptococcus suis. At 7 days post-infection with double-mutant PRRSV, pigs were co-infected with Strep. suis, and at 7 days of co-infection, all animals were sacrificed. While co-infection with wild-type PRRSV and Strep. suis exhibited enhanced clinical severity, co-infection with double-mutant PRRSV and Strep. suis resulted in reduced clinical symptoms, indicating the clinical attenuation of double-mutant PRRSV. Alveola macrophages prepared from bronchoalveolar lavages showed reduced expressions of proinflammatory cytokines and chemokines, including the markers for cytokine storms in COVID-19 patients. Our data demonstrate that the virulence of double-mutant PRRSV is attenuated, possibly due to the increased production of IFNs and decreased production of proinflammatory cytokine during co-infection. Our study demonstrates the potential of reprogramming viral immune evasion for developing a novel vaccine platform for PRRS.

PRRS virus nsp1 protein downregulates TRIM19 expression and promotes viral replication

D. Yoo1*, C.M Su1, J. Tang1, Y.F. Hung1, M. Han1, R. Everett2.

1Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, 2MRC-University of Glasgow Center for Virus Research, Glasgow, Scotland, UK.

Porcine reproductive and respiratory syndrome virus (PRRSV) is known to inhibit type I IFN production and signaling during infection, and PRRSV nsp1 protein has been identified as the major IFN antagonist. TRIMs are member proteins of the tripartite motif family of E3-ubiquitin ligase, and TRIM19 is an IFN-stimulated gene (ISG) product playing a role in antiviral response. TRIM19 is known as promyelocytic leukemia protein (PML) which is an archetype of membrane-less organelles within the nucleoplasm. PML is the main organizer of PML nuclear bodies, and the PML nuclear bodies participate in a wide range of biological processes including antiviral defense. In the present study, the role of TRIM19 in PRRSV replication was examined. We found that in PRRSV-infected cells, the number of PML nuclear bodies were reduced, and PRRSV nsp1-beta protein was determined as the negative regulator for PML nuclear body formation. The knockdown of PML expression by siRNA gene silencing increased PRRSV replication, and the overexpression of all 6 isoforms of PML (I through VI) restricted PRRSV replication, confirming the inhibitory role of PML for PRRSV replication. Among the isoforms, PML-II and PML-IV were the most potent suppressor for viral replication. The downregulation of PML was post-translational and was mediated via ubiquitination-dependent proteasomal degradation. PRRSV nsp1-beta bound to PML and induced ubiquitination and degradation. PRRSV nsp1-beta protein contained 4 small ubiquitin-like modifier (SUMO)-interactive motif (SIM), and by site-directed mutagenesis, SIM1 and SIM4 were determined to be the PML binding motifs. Double mutations at SIM1 and SIM4 completely abolished the nsp1-beta binding to PML. When examined the role of nsp1 for other member viruses of the Arteriviridae family, the PML degradation was found to be common for equine arteritis virus, simian hemorrhagic fever virus, and lactate dehydrogenase-elevating virus. Our study reveals a novel strategy of arteriviruses for immune evasion, which promotes the viral replication.

Effects of maternal vaccination on the hippocampal transcriptome and intestinal microbiome of neonatal piglets infected by influenza A virus

Fangfeng Yuan1#, Lufan Yang1#, Shih-Hsuan Hsiao2, Adrienne M. Antonson3,4, Christopher A. Gaulke1,4*, Ying Fang1,4*

1Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana Champaign, Urbana, IL 61802, USA
2Veterinary Diagnostic Laboratory and Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana Champaign, Urbana, IL 61802, USA
3Department of Animal Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois at Urbana Champaign, Urbana, IL 61802, USA
4Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana Champaign, Urbana, IL 61802, USA

Influenza A virus (IAV) causes respiratory disease with systemic complications. IAV can infect a variety of avian and mammalian hosts, including humans and pigs. Severe cases of influenza can result in neurological complications especially in early life, when the brain is still growing and developing. Maternal influenza vaccination is known to reduce the risk of IAV infection in infants, but it is unknown whether passive transfer of anti-IAV antibodies can also protect against the downstream complications of infection. In this study, we established a pregnant sow-neonatal piglet model to study the effects of maternal vaccination on the hippocampal transcriptome and intestinal microbiome of neonatal piglets during influenza virus infection. Sows were vaccinated with an influenza experimental vaccine at 75 days gestation, while control sows were unvaccinated. Neonatal piglets born from vaccinated and unvaccinated sows were challenged with a pathogenic IAV isolate at 6 days post-farrowing. Bulk RNA sequencing of hippocampal tissue identified 1,406 differentially expressed genes (DEG) between groups. While IAV-infected piglets from vaccinated sows showed increases in genes related to immune activation and response, IAV-infected piglets from unvaccinated sows showed increases in genes related to cognition disorders (e.g., TANC2, MAP2, and others). As there is growing evidence that the gut microbiome plays a significant role in brain health, we next determined whether differences in piglet microbiota relate to influenza-associated patterns of hippocampal gene expression. Indeed, the abundance of upregulated hippocampal genes involved with immune function and cognition disorders positively correlated with several gut microbial taxa, including microbes belonging to the genera Prevotella, Lachnospiraceae, Actinobacillus, and Turicibacter. Together, our results indicate that maternal vaccination partially protects neonatal piglets against influenza virus infection and may reduce the risk of neurological complications during early life. Maternal vaccination may also provide protection via neonatal gut microbiome-brain axis signaling, although this remains to be tested.

An early warning tool for PRRS virus occurrence in the U.S. swine breeding herds

X. Yue1*, M. Kikuti1, C.M. Melini1, E. Geary1, P. Fioravante1, C.A. Corzo1

1Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) generates a huge cumulative burden on pig herds. To effectively prevent and control PRRS outbreaks, producers have actively engaged in understanding the regional risk of PRRS introduction. The Morrison Swine Health Monitoring Project (MSHMP) is a voluntary initiative launched in 2011, inviting participating producers to share their farms' information and health status to facilitate a timely response to emerging pathogens. MSHMP currently monitors the PRRSv, PEDv, and PDCoV in 38 swine production systems, accounting for more than half of the U.S. industry breeding herd. With the continuous development of MSHMP over the years, a tool communicating health events (e.g., PRRS breaks) to production systems at the regional level will help understand the regional risk and translate this information into actionable interventions to reduce risk. Therefore, the objective of this study is to develop an automated near-real-time report to alert participating systems of current regional PRRS occurrences related to their farms.

Methods

MSHMP-participating production systems were invited and enrolled in this study, which currently includes 187 breeding herds representing approximately 55.4 thousand sows. Upon voluntary weekly reporting from each MSHMP participant, an algorithm was developed to calculate the total number of PRRS-positive sites within the neighborhood of each enrolled farm. The results were compiled into a report, which was shared weekly with all the participants in the study. In order to balance the epidemiological relevance and confidentiality, an individual reporting radius for each studied farm was determined in 3 steps. First, the initial reporting radius is set to 25 miles for the farms in pig-dense states (e.g., MN, IA) and to 50 miles for farms in other states. Secondly, we generated four variables to count the farms within the initial reporting radius: variable A) Total number of farms that are PRRS positive (AASV PRRS status 1); B) Total number of farms from other production systems that are PRRS positive; C) Total number of farms participating in this study, and D) Total number of MSHMP monitored farms. Subsequently, we automatically increased the reporting radius when there is a confidentiality concern, thus preventing participants from knowing which specific farms from other production systems are PRRS positive. Additionally, changes in the total number of positive farms (variable A) compared to the previous week were indicated by a “trend” variable with an up, down arrow or dash.

Results and Conclusion

The early PRRS occurrence warning tool developed in this study enhances producers' ability to effectively communicate and provides the opportunity to quickly respond to health threats in the form of infectious diseases to mitigate regional PRRS transmission, ultimately resulting in lower regional disease pressure. Furthermore, together with the MSHMP project, the developed tool can be particularly useful in the emergence of existing and imminent health threats to the U.S. swine industry allowing stakeholders to timely tailor control programs to effectively contain these pathogens.

Evaluation of protective efficacy of six commercial PRRSV-2 MLV vaccines against the emergent PRRSV 1-4-4 L1C variant strain in weaned pigs

Gaurav Rawal1, Marcelo Almeida1, Phillip Gauger1, Fangshu Ye2, Chris Rademacher1, Ethan Aljets1, Loni Schumacher1, Derald Holtkamp1, Alyona Michael1, Patrick Halbur1, Jianqiang Zhang1

1Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University
2Department of Statistics, College of Liberty Arts and Science, Iowa State University

Currently, six commercial PRRSV-2 modified live virus (MLV) vaccines are available in the USA to combat PRRS: Ingelvac PRRS MLV and Ingelvac PRRS ATP from Boehringer Ingelheim, Fostera PRRS from Zoetis, Prime Pac PRRS RR from Merck, Prevacent PRRS from Elanco, and PRRSGard from Pharmgate. However, the protective efficacy of these vaccines against the recently emergent PRRSV L1C variant strain has not been thoroughly evaluated and compared. The objective of this study was to compare the protective efficacy of these six PRRSV MLV vaccines against the PRRSV L1C variant strain in a weaned pig model under experimental conditions.
Seventy PRRSV naïve pigs at 3-4 weeks of age were divided into 7 groups with 10 pigs per group, one group per room. At 0 day post vaccination (DPV), Group 1 received virus-negative medium as a negative control and Groups 2 through 7 were intramuscularly vaccinated with Prime Pac PRRS, Ingelvac PRRS ATP, Fostera PRRS, Ingelvac PRRS MLV, Prevacent PRRS, and PRRSGard vaccines, respectively, using the doses labeled by the vaccine manufacturers. At 35 DPV, all pigs were challenged with PRRSV L1C variant isolate MN/01775GA/2021 grown in ZMAC cells via intramuscular (2ml/pig) and intranasal (2ml/nostril) inoculation at a dose of 10^6 TCID50/pig. All surviving pigs were euthanized and necropsied at 49 DPV (14 days post challenge [DPC]). Clinical signs, microchip temperature, weight, and various samples were monitored or collected over time. Serum, lung, and brain samples were tested by three quantitative PRRSV real-time RT-PCR assays: 1) a commercial PRRSV screening PCR; 2) each vaccine-specific PCR; and 3) L1C variant challenge virus-specific PCR. Gross and microscopic lesions and PRRSV IHC in lung tissues were examined. PRRS X3 ELISA and virus neutralizing antibody were determined.
During 0-35 DPV before challenge with the PRRSV L1C variant strain, pigs in G1-G7 did not develop fever, anorexia, or lethargy. The average weights among G1-G7 were similar at each of the time points (0, 7, 14, 21, 28 and 35 DPV).
After challenging pigs with the PRRSV L1C variant strain at 35 DPV through the end of the study at 49 DPV (14 DPC), Vaccines A-F showed varying degrees of protection against the L1C variant challenge. The parameters such as mortality, anorexia and lethargy scores, body temperature, average daily weight gain, viremia level, gross lung lesions, histopathological lung lesions, viral loads in lung and brain tissues, and PRRSV IHC scores in lung and brain tissues were evaluated and compared. Veterinarians can choose the vaccine(s), based on their assessments of these parameters, to protect against the PRRSV L1C variant strain.

IL-1beta and TNF-alpha released from highly pathogenic porcine reproductive and respiratory syndrome virus-infected pulmonary alveolar macrophages enhance pulmonary vascular permeability by dysregulating claudin-8 and claudin-4

Weifeng Sun1, 2, 3, Weixin Wu1, 2, Xinyu Fang1, 2, Xinna Ge1, 2, Yongning Zhang1, 2, Jun Han1, 2, Xin Guo1, 2, Lei Zhou1, 2* and Hanchun Yang1, 2.

1National Key Laboratory of Veterinary Public Health Security, College of Veterinary Medicine, China Agricultural University, Beijing, P.R. China, 2Key Laboratory of Animal Epidemiology of Ministry of Agriculture and Rural Affairs, College of Veterinary Medicine, China Agricultural University, Beijing, China, 3Current address: China Institute of Veterinary Drug Control, Beijing, China

The pulmonary endothelium is a dynamic, metabolically active monolayer of squamous endothelial cells that internally lines blood vessels and mediates key processes of lung homeostasis. The endothelial barrier dysfunction is central to acute lung injury/ acute respiratory distress syndrome in virus-induced pneumonia. The tight junction proteins are closely correlated with this barrier function and their dysregulation leads to the disruption of the endothelial barrier, resulting in leakage. The highly pathogenic PRRSV (HP-PRRSV) infection can cause severe pneumonia, manifested as the destruction of lung structure with extensive hemorrhage and a large number of inflammatory cells infiltration, which is an ideal model to study the pathological process of ALI. Here, the alveolar lavage fluid of pigs infected with HP-PRRSV, as well as the supernatant of HP-PRRSV infected pulmonary alveolar macrophages (PAMs), were respectively collected to treat the pulmonary microvascular endothelial cells (PMVECs) in Transwell culture system to explore the mechanism of pulmonary microvascular endothelial barrier leakage caused by viral infection. By testing the trans-endothelial electrical resistance (TEER) and FITC-Dextran permeability of treated PMVECs, the factors released from HP-PRRSV-infected PAMs, but not virus particles, were found to enhance the permeability. According to the cytokine screening and adding/ blocking experiment, it was found that IL-1beta and TNF-alpha released from HP-PRRSV-infected PAMs, can compromise the integrity of the pulmonary vascular barrier by dysregulating tight junction protein claudin-8 and claudin-4, meanwhile, the downregulation of claudin-8 and upregulation of claudin-4 synergistically contribute to the compromise of the pulmonary endothelial barrier. Furthermore, the transcriptional promoters of claudin-4 and claudin-8 were first identified to use for DNA pulldown and followed with mass spectrum (MS) analysis to identify the regulating transcription factors. Then three transcription factors interleukin-2 enhancer binding factor 2 (ILF2), general transcription factor III C subunit 2 (GTF3C2), and thyroid hormone receptor-associated protein 3 (THRAP3) were identified to accumulate into the nucleus of PMVECs to regulate the transcription of claudin-8 and claudin-4. Last, through miRNA target prediction and regulating effect validation, the up-regulated ssc-miR-185 was also found to suppress claudin-8 expression via post-transcriptional inhibition. This study does not only reveal the molecular mechanism that HP-PRRSV infection caused endothelial barrier leakage in acute lung injury, but it can also provide some novel insights into the tight junctions' function and regulation on vascular homeostasis.

Achieving effective and sustainable regional surveillance

G. Trevisan1, P. Morris2, C. Wang1,2, R. Main1, G.S, Silva1, J. Zimmerman1*

1Department of Veterinary Diagnostic and Production Animal Medicine and 2Department of Statistics, Iowa State University, Ames Iowa.

Introduction Typically, we think of surveillance in terms of individual farms. That is, we test to establish a farm as negative (or positive). Thus, in the PRV eradication program, farm status was determined by collecting and testing 29 blood samples from each "segregated" group of ≥ 1000 pigs.1 When all the farms in a defined area are considered negative, then the region is negative.

When dealing with the detection of FADs, history has repeatedly shown that surveillance based on clinical signs does not work - clinical signs are neither pathognomonic nor diagnostic. We need test-based surveillance, but the classic approach based on testing to determine individual farm status is too expensive and slow. In this study, we explored a surveillance design based on testing a few samples from each of many farms in a defined region. Based on the design, farm personnel would collect samples from live but poor-doing pigs (n = 10), pool into two samples (5 pigs each), and submit them for testing (antibody or PCR) in a NAHLN laboratory.

Methods The USDA Animal Disease Spread Model (ADSM)2 was used to simulate the introduction and spread of a pathogen in a population of 17,521 farms (51,515,699 pigs) in a large geographic region (1,615,246 km2). Using the spread data generated in ADSM, we estimated the probability of detecting ≥ 1 positive farms in the region as a function of farm-level detection probability (10%, 20%, 30%, 40%, 50%), percent of farms participating in the program (20%, 40%, 60%, 80%, 100%), and farm-level prevalence in the region. Thereafter, the cost of the program was estimated in terms of cost per farm and cost per pig in inventory. The cost analysis assumed that the farm would provide the labor and materials to collect, process, package, and ship samples.

Results The results showed that this design was highly effective. For example, the system achieved ≥ 90% probability of detection at 0.1% prevalence (18 infected farms among 17,521 farms) when producer participation was ≥ 40% and farm-level detection probabilities ≥ 30%. Based on PCR at $25.00 and ELISA at $7.50 per sample, the cost per pig in the region was estimated at $0.03 and $0.02 per pig, respectively.

Conclusions This approach is very different from traditional surveillance: 1) The goal is to determine the region's status - not the status of individual farms. Thus, fewer samples per farm are required. 2) Samples are collected from poor-doing pigs because "targeted" sampling is more efficient than random sampling at low prevalence. 3) "Alternative" specimens will facilitate sampling, e.g., blood swabs, oral swabs, nasal swabs, fecal swabs, oral fluids. The use of these specimens is supported by published research. 4) Sampling is performed by farm personnel (producer, staff, herd veterinarian), i.e., the people in the best position to determine the pigs to sample.

1. USDA APHIS. 2003. Pseudorabies Eradication Program Standards. APHIS 91-55-071.
2. Harvey et al. 2007. The North American Animal Disease Spread Model: A simulation model to assist decision making in evaluating animal disease incursions. Prev Vet Med 82:176-197.

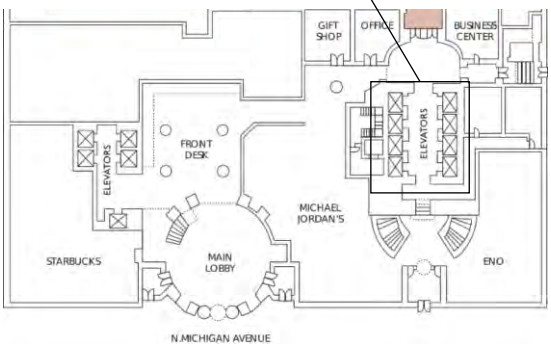
A broadly protective vaccine against swine influenza A virus based on the matrix protein 2 (M2)

F. A. Zuckermann[1*], Y. Grinkova[2], R. Husmann[1], Pires-Alvez M., S. Storms[1], S. G. Sligar[2],

Department of Pathobiology[1] and Department of Biochemistry[2], University of Illinois at Urbana-Champaign, Urbana, IL.

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

Intercontinental Hotel Map



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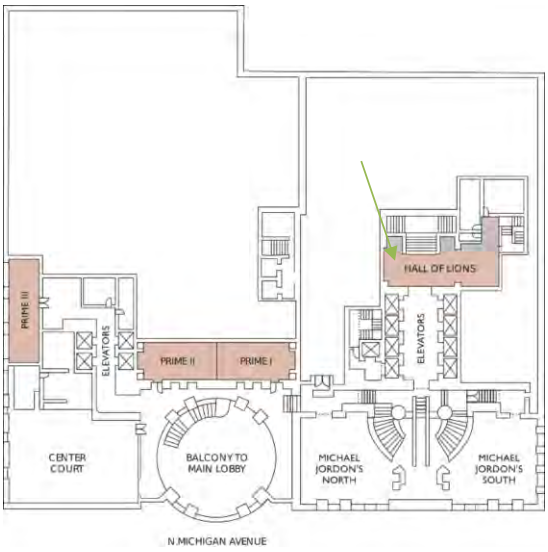
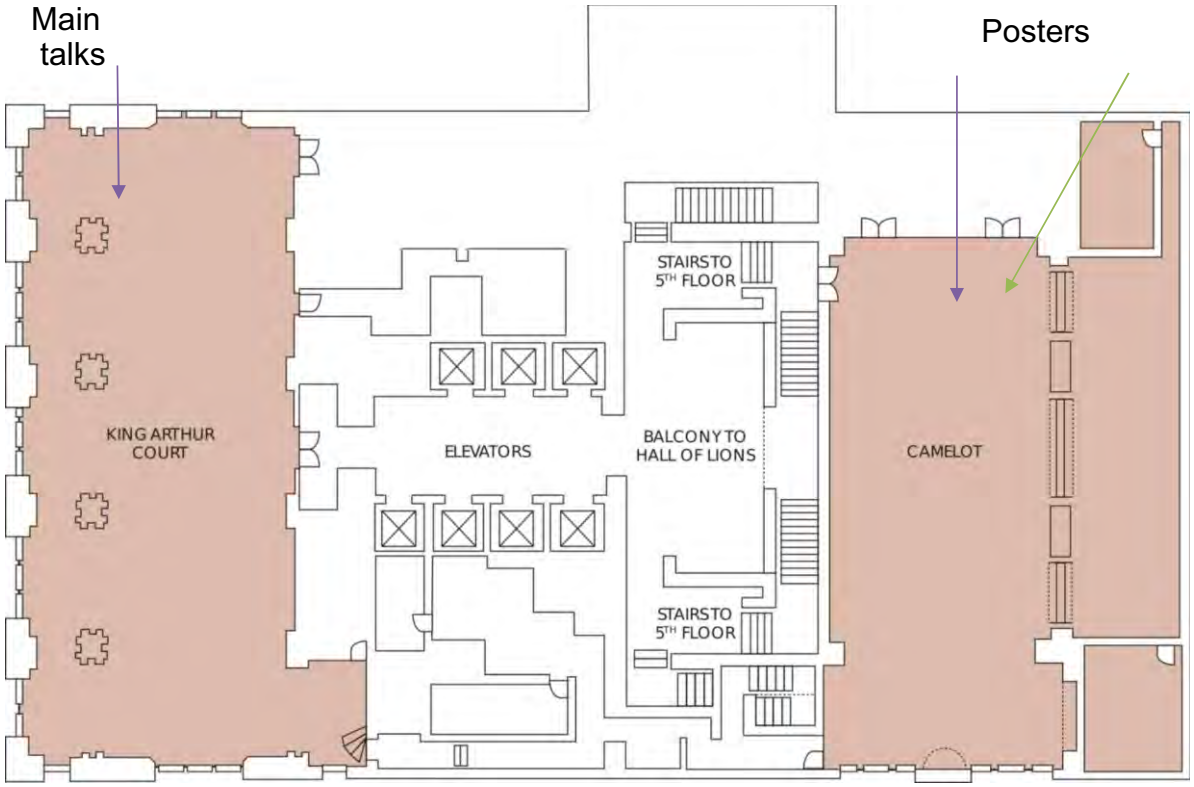


2ND FLOOR

3rd Floor



MEETINGS & EVENTS