PROGRAM AND PROCEEDINGS FOR THE

CHICAGO
2018

North American PRRS Symposium

CHICAGO MARRIOTT MAGNIFICENT MILE
December 1-2, 2018
www.vet.k-state.edu/na-prrs/

EXECUTIVE DIRECTORS
Bob Rowland and Ying Fang

KANSAS STATE UNIVERSITY | College of Veterinary Medicine

The 2018 North American PRRS Symposium
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Welcome to 2018 NA PRRS Symposium

Dear Friends,

The North American PRRS Symposium was initiated in November 2003 as the "International Workshop on PRRS". During the last 15 years, the conference has retained a focus on presenting the latest developments and research directed at the control of PRRS, the most costly viral disease to ever face a global swine industry.

Over the years, the program offerings have evolved to include contemporary emerging diseases along with African swine fever virus (ASFV) and classical swine fever virus (CSFV), two important foreign animal disease threats. Conference activities and outcomes that result in dissemination of new scientific knowledge, animated scientific discussions, and the formation of new research collaborations, will place the swine industry in a better position to respond to endemic, emerging and future disease threats.

The executive directorship, scientific committee, and conference staff hope this symposium will provide all attendees with an informative and scientifically rewarding experience.

Enjoy the beautiful city of Chicago!

Raymond (Bob) Rowland and Ying Fang
Executive Directors of the 2018 NAPRRS Symposium

The 2018 North American PRRS Symposium is dedicated to the memory of Dr. Michael P. Murtaugh

Dr. Michael P. Murtaugh

September 18, 2018

The animal health and veterinary virology communities suffered a tremendous loss with the recent passing of Dr. Michael P. Murtaugh, a long-time faculty member in the University of Minnesota's College of Veterinary Medicine. Dr. Murtaugh, 67, was internationally recognized as a leader in the battle against swine diseases, including porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus-associated disease (PCVAD). Mike authored more than 225 peer-reviewed journal articles and was the primary advisor for 30 Master's and PhD students. Many of his former students occupy leadership roles in animal health. He successfully completed nearly 160 sponsored projects as a Principal Investigator (PI) or co-PI.

Earning a BS in Biology at the University of Notre Dame, he went on to serve as a Peace Corps volunteer in Venezuela. He earned a PhD in Entomology at The Ohio State University followed by a postdoc at the University of Texas Medical School in Internal Medicine and Pharmacology. In 1985, Mike joined the Department of Veterinary and Biomedical Sciences at the University of Minnesota, St Paul campus.

Mike was one of the original PRRS researchers. He was the first to report on the genetics of PRRSV, which has laid the groundwork for investigations into antigenic variation, virus gene evolution, and vaccine development. He is generally considered "The PRRS Immunologist". Mike possessed the unique gift of translating his benchtop successes to the field. As a scientist, Mike maintained a healthy skepticism, which kept the PRRS scientific community honest. He held us all to the highest standards in research and ethical conduct.

Mike is the founder of the conference we now know as the North American PRRS Symposium. It began in November 2003 as the "International Workshop on PRRS", held in conjunction with CRWAD. The format for the conference, including topics, location, and timing remain relatively unchanged. Mike is best known for his leadership in the PRRS community. He was director of the first PRRS USDA Coordinated Agricultural Project, or PRRS CAP-1. His vision for collaboration in research as a means to address producer's needs is the same standard of practice applied today. Mike's ideas of the 1990's continue to ripple through the scientific community. His passing has created a gap in animal disease infectious disease research that can never be filled. He will be missed.

A graduate fellowship is established in his name: https://crowdfund.umn.edu/Murtaugh

Dr. Bob Morrison Travel Scholarship
Sponsored by Boehringer Ingelheim Animal Health

The Dr. Bob Morrison Travel Scholarship, sponsored by Boehringer Ingelheim Animal Health is given in memory of Dr. Bob Morrison, who was a professor in the Department of Veterinary Population Medicine at the University of Minnesota.

Dr. Morrison graduated with a BS in 1974 followed by a DVM in 1979; both from the University of Saskatchewan. In 1984, he received his PhD from the University of Minnesota. The title of his dissertation was, "An epidemiological investigation into enzootic pneumonia of swine." In 1994, Dr. Morrison went on to receive his MBA from the University of Minnesota.

His research focused on the control of PRRS and other diseases on the farm. He translated science into practical solutions. Dr. Morrison was part of the team that discovered PRRSV and, more recently, was the creator of the Swine Health Monitoring Program, an industry-wide effort designed to help the industry prevent the next "PRRSV". Between 2008 and 2013, he directed the extension efforts for the PRRS Coordinated Agricultural Project (PRRS CAP).

As a leader, he demonstrated the feasibility of designing, developing, implementing, and managing regional elimination projects. His efforts resulted in the recognition that PRRS elimination is feasible and should be a stated goal for the swine industry. Perhaps his greatest strength was his unique ability to convince farmers to share data and information to the benefit of the entire swine production community.

In addition to educating producers on disease control methods, he was a good friend and mentor to many in the PRRS research and outreach communities. Everyone was affected by his enthusiasm and passion.

This Travel Scholarship, organized by Dr. Reid Philips and Dr. Jens Kjaer, Boehringer Ingelheim Animal Health, will provide travel support for future scientists who wish to follow in his footsteps.

2018 Dr. Bob Morrison Travel Scholarship Recipients	
Pengcheng Shang	Kansas State University
Aleksandra Wozniak	Warsaw University of Life Sciences
Fangfeng Yuan	Kansas State University

The Dr. David A. Benfield Student Travel Fellowship

Dr. David A. Benfield received his BS and MS degrees from Purdue University and a PhD from the University of Missouri-Columbia. He has a distinguished 25 years in research related to viral diseases of food animals. In 1990, he was the co-discoverer of the cause of "mystery swine disease" or porcine reproductive and respiratory syndrome virus (PRRSV). He has remained a role model and mentor to many of those who are currently in the PRRS field. He is a member of the American Society for Virology, American Association of Veterinary Laboratory Diagnosticians, and Honorary Diplomat of the College of Veterinary Microbiologists, American Association of Swine Veterinarians and the American Association for the Advancement of Science. Currently, he is an Associate Vice President of Agricultural Administration and Director of the College of Food, Agricultural and Environmental Sciences Wooster Campus a part of The Ohio State University and a Professor in the Food Animal Health Research Program in the College of Veterinary Medicine. Dr. Benfield's generous donation initiated this fellowship program. It is his hope that these fellowships provide students with the experience of attending the PRRS Symposium to present their work on PRRS. This Travel Fellowship is possible through generous donations from Dr. David Benfield and others.

2018 Dr. David A. Benfield Student Travel Fellowship Recipients	
Nicholas Black	The Ohio State University
Yulu Chen	Iowa State University
Laura Constance	Kansas State University
Qian Dong	Iowa State University
Andrew Kick	North Carolina State University
Yanhua Li	Kansas State University
Carolina Maciel Malgarin	University of Saskatchewan
Dagmara Milek	Warsaw University of Life Sciences
Naemi Shadipeni	Kansas State University
Vlad Petrovan	Kansas State University
Ana Stoian	Kansas State University
Yin Wang	Kansas State University
Xingyu Yan	Kansas State University

2018 North American PRRS Symposium Organizing Committees

2018 NA PRRSS Planning Committee

Executive Directors, NA PRRSS:	Dr. Raymond R.R. Rowland *Kansas State University*
	Dr. Ying Fang *Kansas State University*
Executive Director, CRWAD:	Dr. David Benfield *The Ohio State University*
Industry Liaisons:	Dr. Lisa Becton *National Pork Board*
	Dr. Paul Sundberg *Swine Health Information Center*
NA PRRSS Publicist:	Joe Montgomery *Kansas State University*
NA PRRSS Internal Coordinator and Proceedings Editor:	Francine Rowland *Kansas State University*
NA PRRSS Audio Visual Speaker Coordinator:	Maureen Sheahan *Kansas State University*
NA PRRSS Onsite Coordinator:	Jennifer Stalley *Midwest Solutions*

NA PRRSS and NC229 Joint Scientific Committee:

Dr. Kay Faaberg:	USDA Agricultural Research Service
Dr. Montserrat Torremorell:	University of Minnesota
Dr. Hiep Vu:	University of Nebraska
Dr. Leyi Wang:	University of Illinois
Dr. Dongwan Yoo:	University of Illinois
Dr. Yanjin Zhang:	University of Maryland

NA PRRSS Travel Fellowship Selection Committee:

Dr. Joan Lunney:	USDA, ARS-BARC
Dr. Derrald Holtkamp:	Iowa State University
Dr. Daniel C.L. Linares:	Iowa State University

2018 North American PRRS Symposium

GENERAL CONFERENCE INFORMATION

Chicago Marriott Downtown Magnificent Mile Room Locations:

- **Plenary Sessions:** Sessions 1, 2 and 3 are located on the **7th Floor** in **Salon II**. **Note: Session 4 is located on the 5th Floor – Chicago Ballroom**.
- **Poster Setup and Evening Reception**: The Sunday evening reception and joint poster session is held between 6:30 PM and 8:30 PM on the **7th Floor** in **Salon III**. Posters can be set up beginning at 12:00 PM Saturday, December 1, 2018 and *must be removed* by 8:30 PM on Sunday, December 2, 2018. **Presenters are encouraged to set up posters as early as possible so that attendees can view the posters during the breaks.**
- **Refreshment breaks** for **Sessions 1, 2 and 3** are located in the **foyer** area immediately outside of the **7th Floor – Salon II meeting room.** A buffet lunch for *registered attendees* will be held on Saturday, December 1, 2018 in the same area.

Registration:

- Symposium onsite registration and badge/program pickup for pre-registered attendees:
  - Friday, November 30, 2018 - 4:00 PM to 6:00 PM – 4th Floor – Waveland Foyer
  - Saturday, December 1, 2018 - 7:00 AM to 11:30 AM – 5th Floor – Registration Desk/Kiosk
- **A lunch ticket is included with the Symposium badge along with one drink ticket (beer, wine, or soda) for the Sunday evening reception.**

Continuing Education Credits:

The NA PRRS Symposium may qualify for continuing education credits in the jurisdiction where you are licensed. Please check with your licensing board to determine if this program qualifies for continuing education credit. If you need verification of participation on the Symposium, please visit the Symposium Registration Desk Kiosk.

Name Badges:

Please display your **2018 NA PRRS Symposium** name badge at all times. This is essential for admittance to all Symposium sessions and refreshment breaks, the Saturday Symposium luncheon, the NC-229 session on Sunday afternoon (Session 4), and the Sunday evening reception. Conference attendees wearing a CRWAD badge are welcome to attend all Symposium Plenary Sessions.

2018 North American PRRS Symposium

GENERAL CONFERENCE INFORMATION

Food and Beverage Service:

Symposium registered attendees receive refreshments at the three breaks (Saturday, December 1 – morning and afternoon and Sunday, December 2, morning) and one luncheon ticket for the Saturday lunch, December 1, 2018. One drink ticket (beer, wine, or soda) is also included for the Sunday evening reception.

Refreshment breaks and the buffet lunch are located on the **7th Floor – Salon II foyer area**.

The Sunday Evening Reception and Poster Session in conjunction with CRWAD reception (with small bites) is located on the **7th Floor - Salon III**.

Internet and Computer Access:

Wireless internet access is available in the meeting rooms and foyer areas during the Symposium. Attendees are advised to bring their own computers/tablets to access the internet. If additional computers are needed, attendees may use the business center located in the hotel.

Use the following to connect: (please note capitalization)

Network ID: CRWAD

Password: crwad18

Cameras and Video Recorders:

All presentations for this conference are considered the sole intellectual property of the abstract authors. Photography of posters or presentations is not allowed. Copyright laws PROHIBIT any copying, redistributing, retransmitting, or repurposing protected material.

Elevator Access:

Elevators are available on all meeting floors. Please ask the hotel or Symposium staff for the most direct route to your destination.

Restrooms:

Restrooms are conveniently located near all meeting locations.

2018 North American PRRS Symposium Final Program

Chicago Marriott Downtown Magnificent Mile

FRIDAY, NOVEMBER 30, 2018

4:00-6:00 pm **Registration, 4th Floor – Waverly Foyer**

SATURDAY, DECEMBER 1, 2018

7:00-11:30 am **Registration, 5th Floor Registration Desk Kiosk**

8:00-12:00 pm **Session 1 - 7th Floor – Salon II**

Session 1: Nidoviruses: Emerging Coronaviruses and PRRSV

Moderators: *Ying Fang-Kansas State University, Hiep Vu-University of Nebraska*

8:00 am **Michael P. Murtaugh Memorial Lecture**

Michael Rahe-Iowa State University, **"Characterizing the memory B cell response to PRRSV**"

8:30 am *Tomasz Stadejek-Warsaw University of Life Sciences,* "**Origin and diversity of PRRSV-1 in Europe**"

9:00 am *Yaowei Huang-Zhejiang University*, **"Receptor usage and potential cross-species transmission of porcine deltacoronavirus"**

9:30 am *Kay Faaberg-USDA Agricultural Research Service*, **"A fresh look at PRRSV evolution: US field strains from 2013-2017"**

10:00 am **Refreshment Break**
7th Floor - Foyer – Salon II

10:30 am *Qiuhong Wang-The Ohio State University*, **"Emerging and re-emerging coronaviruses in pigs"**

11:00 am *Pengcheng Shang-Kansas State University*, "**Emerging PRRSV variants: molecular signatures in nsp2 region**"

11:20 am *Kimberly VanderWaal-University of Minnesota***, "Forecasting outbreaks of PRRS and PED in swine movement networks"**

11:40 am Late Breaking News

Kristin Whitworth-University of Missouri, "**Genetic modification of ANPEP in pigs and resistance to coronavirus infection**"

12:00 pm **Lunch – 7th Floor - Foyer – Salon II**

Important Note:

*Registration to the NA-PRRS Symposium**, which includes a lunch ticket,** is required.*

12:00 pm **Poster set-up opens - 7th Floor – Salon III**

1:00-5:00 pm **Session 2 - 7th Floor – Salon II**

Session 2: Global Challenges in Infectious Disease-Update on ASF in China

***Moderators**: Paul Sundberg-SHIC, Daniel Rock-University of Illinois, Bob Rowland-Kansas State University*

Session supported in part by a grant from USDA NIFA (**2018-67015-27893**)

1:00 pm *Paul Sundberg-SHIC*, "**Setting priorities for disease threats and strategies: Is ASF coming closer?"**

1:20 pm *Alex Morrow-STAR-IDAZ,* **"Emerging diseases: Addressing the unmet vaccine needs"**

1:40 pm Raymond (Bob) Rowland - Kansas State University, "Host genetics: Bringing the pig to the vaccine"

2:10 pm *Daniel Rock-University of Illinois***, "African swine fever vaccine development … perhaps the end of the beginning."**

2:40 pm *Johnny Callahan-Tetracore*, "**The evolution of ASFV real-time PCR, assuring fit-for–purpose as disease spreads worldwide"**

3:00 pm **Refreshment Break**
7th Floor - Foyer – Salon II

3:30 pm *Alexey Zaberezhnyy-Y.R.Kovalenko and K.I.Skryabin Federal Research Center,* **"Progress in the control of CSF and ASF in Russia"**

4:00 pm *Scott Dee-Pipestone, Diego Diel-South Dakota State University, Megan Niederwerder-Kansas State University*, **"Risk for transmission by feed ingredients and potential mitigation strategies for ASF and other transboundary diseases"**

4:40 pm *Douglas Gladue-Plum Island Animal Disease Center, ARS, USDA***, "Rapid development of experimental live attenuated vaccines for outbreak strains of African swine fever virus"**

5:00 pm **End of Saturday session**

SUNDAY, DECEMBER 2

8:00-12:00 pm **Session 3 - 7th Floor – Salon II**

Session 3: PRRSV Vaccines, Immunity and Host Response to Infection

Moderators: *Bob Rowland-Kansas State University, Jay Calvert-Zoetis*

8:00 am **Opening Keynote Address**

Michael Roof-Boehringer-Ingelheim, "**Lessons and opportunities learned from 25 years of PRRSV MLV**"

8:30 am *Enric Mateu-IRTA-UAB, Spain*, **"PRRSV vaccines and vaccination: present and future"**

9:00 am *Waithaka Mwangi-Kansas State University*, "**Vectored vaccine strategies for ASF**"

9:30 am *Bob Rowland-Kansas State University***, "PRRSV at the level of the population: Implications for vaccine development"**

10:00 am **Refreshment Break**
7th Floor - Foyer – Salon II

10:30 am *Nick Serao-Iowa State University*, **"Genomic analysis of total antibody response to Porcine Reproductive and Respiratory Syndrome (PRRS) Modified Live Virus (MLV) vaccination in commercial replacement gilts"**

11:00 am *Kyu-Sang Lim-Iowa State University***, "Blood transcriptome in healthy piglets as a potential biomarker to improve disease resilience"**

11:20 am *Andrew Kick-North Carolina State University***, "Deciphering immunosuppression and immunological memory to homologous and heterologous strains of porcine reproductive and respiratory syndrome virus"**

11:50 am **Lunch (on your own)**

1:00-4:30 pm **Session 4 - 5th Floor - Chicago Ballroom**
Beginning of CRWAD meeting

Session 4: NC-229-PRRSV and Emerging Respiratory Diseases of Pigs

Moderators: *Daniel Rock-University of Illinois,*
Sheela Ramamoorthy-North Dakota State University

1:00 pm	*David Benfield-The Ohio State University, and Daniel Rock - University of Illinois*, "**Opening remarks and tribute to Michael P. Murtaugh**"
1:15 pm	**Featured Speaker**
	Montserrat Torremorell-University of Minnesota, "**Transmission and control of influenza: the role of the piglet**"
2:00 pm	*Jack Dekkers-Iowa State University*, "**Genetically improving resistance of pigs to PRRS virus infection**"
2:15 pm	*Levon Abrahamyan-University of Montreal,* **"Global picture of Nidovirus - Host cell interactions revealed by comparative proteomics"**
2:30 pm	**Break**
3:00 pm	*Janice Telfer-University of Massachusetts*, "**Swine WC1 genes are a multi-genic array with bacterial binding capacity**"
3:15 pm	*Hiep Vu-University of Nebraska*, "**Correlates of cross-protective immunity to porcine reproductive and respiratory syndrome virus**"
3:30 pm	*Yanhua Li-Kansas State University*, "**Hyperphosphorylation of PRRSV nsp2-related proteins regulate viral subgenomic RNA accumulation**"
3:45 pm	*Laura Constance-Kansas State University*, "**Fecal microbiota transplantation shifts microbiome composition and reduces morbidity and mortality associated with PCVAD**"
4:00 pm	**NC-229 Business Meeting**
4:30 pm	**End of session**
6:30 pm	**Sunday Evening: Reception and Poster Session, in conjunction with CRWAD, Salon III, 7th Floor**
8:30 pm	**Remove all posters**

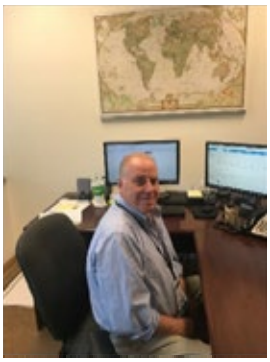
2018 North American PRRS Symposium Speakers

December 1-2, 2018, Sessions One through Three

Keynote Speaker

Mike Roof
Boehringer-Ingelheim

Mike Roof earned his M.S. and Ph.D. at Iowa State University in 1991 with emphasis on virulence mechanisms of Salmonella and swine immunology that ultimately led to ISU patents on vaccine technology. This technology was licensed by NOBL Laboratories, a company focused on swine diseases, and Mike joined the company as Scientist to further develop and commercialize the technology (Product SC-54). During his tenure at NOBL, the "mystery pig" disease occurred and Mike collaborated with Boehringer Ingelheim, SDSU, University of Minnesota, and IDDLO to be part of the initial PRRS investigations and research. He subsequently was involved in the research, development, licensure, and launch of the first PRRS MLV (Ingelvac PRRS) in the USA and has subsequently been licensed in over 26 countries across the globe. NOBL was acquired by Boehringer Ingelheim and Mike played various leadership roles leading Swine R&D, leading US R&D sites (Ames, St Joseph, Fort Dodge), and through 2016 a global role coordinating Bio-Vaccine efforts across all major species. Throughout this time he has remained active in the area of PRRS R&D with additional product licenses in the US (3FLEX, and Ingelvac PRRS ATP) as well as EU registration of a type 1 MLV (PRRS FLEX EU). He has also been involved in numerous internal and collaborative efforts investigating new and different PRRS solutions such as chimeric vaccines, infectious clones, KV/MLV combinations, and alternate routes and formulations. In recent years Mike has had the opportunity to work for some extended period of time in Ingelheim Germany, Hannover Germany, and Shanghai China which gives him a good global perspective on animal health and swine diseases. Mike and his wife (Jill) have 2 children (Kevin and Kayla) who are successful professionals in Mechanical Engineering and Physical Therapy PhD, respectively. The Roof family enjoys anything outdoors and active – hiking, biking, skiing, boating, hunting, fishing as well as good food and wine!



Johnny Callahan
Tetracore

Dr. Johnny Callahan is the Manager of Veterinary Diagnostics Business Development and the USDA/CVB Regulatory Affairs Liaison for Tetracore, Inc. in Rockville, MD. He received his BS degree in Medical Technology from the University of South Carolina in Charleston and then a MS and PhD in Medical Pathology from the University of Maryland in 1994 and 2005 respectively. His professional career spanning 33+ years has been devoted to laboratory medicine where he has kept pace with the expansion of technology for the detection and characterization of public health, veterinary and zoonotic pathogens. It is his philosophy that the classical laboratory methods are not entirely replaced by modern molecular methods and that the laboratory should make use of all of the tools available to provide the most accurate laboratory diagnosis. To understand the status of an infection for any given disease within an infected host, a laboratorian needs to consider how the host-disease interaction determines the outcome of disease. With this underlying knowledge the most appropriate test can be selected and used on the most appropriate sample matrix that will best illustrate the disease status for a given patient or animal. The journeyman laboratory worker understands the immunopathogenisis of disease; the advantages and limitations of each laboratory test and will be able to explain the significance of the laboratory results to the public health or veterinary clinician.

Scott Dee
Pipestone Applied Research

Scott Dee earned his DVM, MS and PhD from the University of Minnesota. He is a board certified veterinary microbiologist and a past President of the AASV. After working in swine practice for 12 years, Scott was a Professor at the University of Minnesota College of Veterinary Medicine where he focused his research on the transmission and biosecurity of PRRSV for a 12-year period. This effort culminated in the development and validation of a nationally-applied air filtration system for reducing the introduction of airborne diseases to swine facilities. In 2011, Scott joined Pipestone Veterinary Services in Pipestone, MN where he currently serves as Director of Pipestone Applied Research (PAR), a business unit which conducts collaborative research efforts with production companies across North America comprising approximately 1.5 million sows. Scott has been awarded > 9M in research funds, has published 146 papers in peer reviewed journals (including the initial publication providing proof of concept of PEDV transmission in feed) and is currently studying the transboundary risk of pathogen spread through feed ingredients. He has received the AASV Practitioner of the Year award, the Leman Science in Practice award and the AASV Howard Dunne Memorial award. Scott and his wife Lisa have 2 children (Nicholas and Ellen) and live in Alexandria, MN along with their Scottish terrier, Abigail.

Diego Diel
South Dakota State University

Dr. Diego Diel received his DVM degree from Federal University of Santa Maria (UFSM), Brazil in 2004. He conducted a MS in Virology at the same institution and then came to University of Illinois at Urbana-Champaign to conduct the research of his PhD degree, which was completed in 2010. Dr. Diel joint the USDA Southeast Poultry Research Laboratory in 2011 for post-doctoral training and then returned to the University of Illinois in 2013 for a second post-doc. In August, 2014 Dr. Diel joined the Department of Veterinary and Biomedical Sciences at the South Dakota State University as an Assistant professor, in 2016 he became the section leader of the Virology section in the Animal Disease and Research Diagnostic Laboratory (ADRDL)

Kay Faaberg
USDA Agricultural Research Service

Dr. Kay Faaberg, PhD, is a Research Microbiologist and the Lead Scientist for the project Intervention Strategies to Control Endemic and New and Emerging Viral Diseases of Swine" of the National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture. A molecular virologist studying porcine reproductive and respiratory syndrome virus (PRRSV) and porcine coronaviruses, Dr. Faaberg and her collaborators use reverse genetics to modify viral genomes in order to better understand the pathogenesis of swine nidoviruses. She is also interested in PRRSV evolution and recombination. Her laboratory was the first to assemble an infectious clone of the Type 2 PRRSV prototype strain, VR-2332, has since developed reverse genetic systems for several other PRRSV strains, PRRSV chimeras and coronaviruses, described the first evidence of high frequency viral recombination in PRRSV, and led the initiative to deposit more than 8500 PRRSV ORF5 and 47 complete genomes into GenBank, the United States National Institutes of Health Genetic Sequence Database. Dr. Faaberg's team and collaborators are presently engaged in the study of the contribution of viral enzymes to nidoviral interferon inhibition and virulence, producing a vaccine for swine coronaviruses and a DIVA vaccine for newer strains of PRRSV, and in implementing the new US Swine Pathogen Database.

Douglas Gladue
Plum Island Animal Disease Center, ARS, USDA

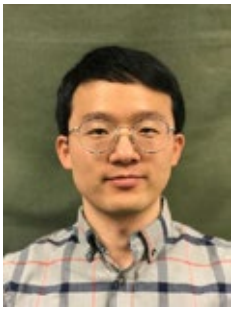
Dr. Douglas Gladue received his Ph.D. in Molecular Genetics and Microbiology from Stony Brook University and is currently a Senior Scientist at the Foreign Animal Disease Research Unit, Agricultural Research Service at Plum Island Animal Disease Center. For over a decade, his research has focused on the molecular mechanisms of viral pathogenesis and virus-host protein interactions and applying these discoveries to the design of rational vaccines for foreign animal viral diseases. He has discovered over one hundred host-viral protein interactions and has used this discovery combined with a custom computational pipeline involving both bioinformatic and functional genomic data, to identify critical domains in viral proteins. Deletion or mutation of these domains has been used as a basis to develop rationally designed vaccines for both classical swine fever virus (CSFV) and foot-and-mouth disease virus (FMDV). With no commercial vaccine and recent outbreaks of ASFV affecting the Caucasus and Eastern Europe, Dr. Gladue has focused his research on developing a novel rationally designed ASFV vaccine. His recent accomplishments include the functional characterization of ASFV proteins and the development of new methodology allowing for rapid development of recombinant ASFV with multiple deletions in the ASFV genome, allowing for safer ASFV vaccine design strategies. Dr. Gladue has authored numerous peer-reviewed scientific publications, served on multiple scientific committees, editorial boards, and holds multiple patents in the field of foreign animal diseases.

Yaowei Huang
Zhejiang University

Dr. Huang received his B.S. degree in Biology from Nanjing University in China and his Ph.D. degree from Department of Biomedical Engineering of Zhejiang University in China. He then joined College of Veterinary Medicine, Virginia Tech, as a Postdoctoral Associate and later as a Research Assistant Professor, focusing on molecular mechanism of replication and pathogenesis of hepatitis E virus (HEV), PRRSV and Torque teno sus virus (TTSuV). Dr. Huang accepted a professor position in Department of Veterinary Medicine, College of Animal Sciences of Zhejiang University, China, in 2013. Dr. Huang has authored or co-authored 62 publications in peer review journals such as mBio, Journal of Virology and Emerging Infectious Diseases. In 2017, his lab discovered a novel swine enteric alphacoronavirus (SeACoV; also referred as SADS-CoV by another lab) derived from the bat coronavirus HKU2 from diarrheic piglets in Southern China. His current research is mainly focused on epidemiology, biology and vaccine development of swine enteric coronaviruses including PEDV, PDCoV and SeACoV/SADS-CoV.

Andrew Kick
North Carolina State University

Lieutenant Colonel Andrew Kick is a military intelligence officer in the United States Army. Andrew obtained his MS from North Carolina State University (NCSU) in 2010 with Dr. Glen Almond and studied the effects of husbandry stress on the porcine adaptive immune system. Andrew taught chemistry and human physiology at the United States Military Academy (USMA) from 2010-2013. Andrew is currently pursuing a PhD at NCSU and researching the adaptive immune response to PRRSV with Dr. Tobias Kaeser and Dr. Almond. His follow-on assignment is teaching at USMA. Andrew has deployed to Iraq, Afghanistan and served multiple tours in the Republic of Korea.



Kyu-Sang Lim
Iowa State University

Dr. Kyu-Sang Lim grew up in South Korea and received B.S., M.S. and Ph.D. degrees from Korea University. He did his first post-doctoral research at the National Institute of Animal Science, South Korea, on meat quality in pigs, imprinting in cattle and heat stress in ducks using RNA-sequencing data. In 2017, he joined the Animal Breeding and Genetics group at Iowa State University as a post-doctoral research associate. Current research focuses on developing methods to select for disease resilience in pigs based on blood gene expression profiles of young healthy pigs by applying quantitative genetic analysis to population-level of transcriptome data prior to exposure to a natural polymicrobial disease challenge.

Enric Mateu
IRTA-UAB, Spain

Enric Mateu is professor of infectious diseases at the Veterinary Faculty of Barcelona and researcher at CReSA-IRTA. Earned his Veterinary Medicine Degree in 1989, got a PhD from the Universitat Autònoma de Barcelona in 1993 and is (European) Diplomate in Porcine Health and Management. He post-doc researcher at the University of Illinois in 1994-95. His research focuses on viral diseases of swine, particularly PRRS. He authored or co-authored numerous papers on the immune response of pigs to viral agents.

Alex Morrow
STAR-IDAZ

Dr. Alex Morrow, BA, MVB, PhD, MRCVS is veterinary surgeon with eighteen years' experience in research working on the pathogenesis and control of Amblyomma variegatum-associated dermatophilosis, followed by four years in a research support capacity at Edinburgh University and fourteen years in his current position in research programme management with Defra where he is International Evidence Lead Animal Health and Welfare. He established and coordinated for 10 years the European Collaborative Working Group (CWG) on Animal Health and Welfare research, under the EU Standing Committee on Agriculture Research, and led the associated EU-funded EMIDA ERA-NET on Emerging and Major Infectious Diseases of Animals. He currently leads the STAR-IDAZ global network, "Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses", and the associated International Research Consortium (IRC), with a higher level of commitment to collaboration, which was launched by the European Commission in January 2016. He now also heads the EU-funded IRC secretariat.

Waithaka Mwangi
Kansas State University

Dr. Waithaka Mwangi received his BS from the University of Nairobi, Kenya in 1990 and his PhD from Washington State University, College of Veterinary Medicine, where he also did postdoctoral training for two years and then accepted a non-tenure track Assistant Professor and Graduate Faculty position. He joined Texas A&M University, College of Veterinary Medicine in July 2005 as a tenure track Assistant Professor and Graduate Faculty. He was promoted to a tenured Associate Professor in 2014. In 2016, he was recruited to Kansas State University, College of Veterinary Medicine as a tenured Associate Professor/Graduate Faculty and Director of the flow cytometry lab. Current research efforts are focused on the development of prototype live-vectored vaccines for protection of pigs against African Swine Fever Virus and development of broadly protective Bovine Viral Diarrhea Virus subunit vaccines. His third area of research is on exploitation of novel cow antibodies that have unique antigen binding structures capable of binding unique/cryptic markers to develop potent broadly neutralizing antibodies against HIV and other pathogens. He is a member of the American Association of Veterinary Immunologists and American Association of Immunologists (AAI) where he is currently serving as a member of the AAI-Veterinary Immunology Committee.

Megan Niederwerder
Kansas State University

Dr. Megan Niederwerder is an Assistant Professor at the Kansas State University College of Veterinary Medicine in the Department of Diagnostic Medicine/Pathobiology. She received her DVM from Kansas State University and after 3 years as a practicing veterinarian, Dr. Niederwerder returned to Kansas State where she completed her PhD in viral diseases of swine. Dr. Niederwerder's research is focused on understanding the risks of virus introduction and transmission in feed and feed ingredients as well as identifying tools to mitigate this risk. The Niederwerder laboratory also investigates the gut microbiome as an alternative tool for reducing the effects of polymicrobial respiratory disease on the health and growth of commercial swine. Her work has included ASFV, CSFV, PRV, PRRSV, PEDV and PCV2. Dr. Niederwerder serves as the course coordinator and instructor of Veterinary Virology to second year veterinary professional students.

Michael Rahe
Iowa State University

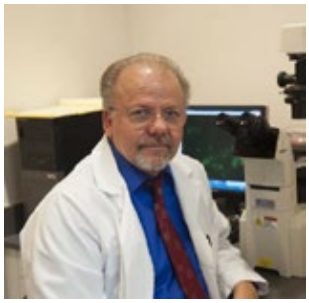
Michael P. Murtaugh Memorial Lecture

Michael Rahe is a post-doctoral research associate in the Veterinary Diagnostic Laboratory at Iowa State University. He received both his Bachelor's degree and his DVM degree from Iowa State University while concurrently achieving his Master of Public Health from the University of Iowa. He then joined Michael Murtaugh's lab at the University of Minnesota where he worked on characterizing the memory B cell response to PRRSV infection. After graduating with his PhD in 2017, he transitioned to his current role at the ISU Veterinary Diagnostic Laboratory where he is in his second year of training as an anatomic pathology resident and diagnostician trainee.

Daniel L. Rock
University of Illinois

Dr Rock's research has focused on animal infectious disease with an emphasis on molecular mechanisms underlying viral virulence and host range of high-consequence viral diseases such as African swine fever, classical swine fever, foot-and-mouth disease, rinderpest and exotic poxviruses. His laboratory has used comparative and functional genomic approaches together with animal disease models to define and characterize the role of specific viral and host genes in disease.

Dr. Rock obtained a BSE degree from Drake University in Des Moines and a Ph.D. in Veterinary Microbiology from Iowa State University in 1981. His Postdoctoral research was at the Wistar Institute in Philadelphia in molecular virology. He held faculty positions at North Dakota State University and the University of Nebraska-Lincoln before joining the Agricultural Research Service as Research Leader of Exotic Viral Diseases at the Plum Island Animal Disease Center in New York in 1989. There he developed and led major research initiatives on foreign animal diseases, pathogen functional genomics and rapid pathogen detection. In March 2005 he joined the faculty of the Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana-Champaign.



Bob Rowland
Kansas State University

Raymond R. R. "Bob" Rowland is a Professor in the Department of Diagnostic Medicine and Pathobiology of Kansas State University's College of Veterinary Medicine. Rowland's current research interests center on addressing fundamental problems in the detection and control of infectious diseases caused by emerging and foreign pig viruses. A historical focus has been on the molecular mechanisms of diseases caused by porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Since 2008, the Rowland lab has been actively involved in understanding the role of host genetics in the response of pigs to viral infection, including the first characterization of a line of pigs that are SCID; i.e. lack an immune system. The extension of the genetics approach is the use of genetic modification of PRRSV receptors to make pigs resistant to disease. Related research includes the control of viruses in the field and the development of novel detection methods for domestic and foreign animal diseases, such as classical swine fever virus (CSFV) and African swine fever virus (ASFV). Rowland is actively involved in the research training of graduate, undergraduate and DVM students. Rowland is co-director of the PRRS Host Genetics Consortium (PHGC) and the Executive Director of the North American PRRS Symposium, an annual meeting held in Chicago. Rowland serves on advisory boards related to PRRS and other infectious diseases.

Nick Serão
Iowa State University

Nick Serão is an Assistant Professor of Swine Genetics in the Department of Animal Science at Iowa State University. Nick Serão research program focuses on the genetic and genomics bases of swine health, reproduction, and mortality, and on statistical methodologies for genomic and experimental analyses. Nick Serão grew up in Brazil and received BSc. (Animal Science; 2007) and MSc. (Breeding and Genetics; 2009) degrees from the Federal University of Viçosa, and a PhD in Animal Science (focused in Statistical Genomics) from University of Illinois at Urbana-Champaign. After 2.5 years as a Postdoctoral Research Associated at Iowa State University, working on genetics and genomics of swine health, he was on the faculty at North Carolina State University from 2015 to 2017, working on swine and cattle health, and statistical methodologies. Since March 2017, he is an Assistant Professor of Swine Genetics at Iowa State University.

Tomasz Stadejek
Warsaw University
of Life Sciences

Professor Dr. Tomasz Stadejek graduated from the Faculty of Veterinary Medicine at the University of Life Sciences in Lublin, Poland in 1990. From 1991 to 2011 he worked at the Department of Swine Diseases of the National Veterinary Research Institute in Pulawy, Poland. He obtained his Ph.D. in 1996 and D.Sc. in 2002. He worked as a guest researcher at the National Animal Disease Centre (NADC) and National Veterinary Services Laboratories in Ames, Iowa, USA, National Veterinary Institute in Uppsala, Sweden, Veterinary Laboratories Agency - Weybridge, Addlestone, UK and National Veterinary Institute, Lindholm, Denmark. His main research topics were molecular diagnosis and epidemiology of pestiviruses, arteriviruses and circoviruses. In 2007 Prof. Stadejek was appointed by the World Organization for Animal Health (OIE) as an expert for PRRS, and in 2007-2011 he was the head of the OIE. Reference Laboratory for PRRS. He is a member of Arterivirus Study Group of the International Committee for Taxonomy of Viruses. In 2008 he obtained diploma of the European College of Porcine Health Management (ECPHM) and from 2011 to 2013 he was a board member and the secretary of the college. Since 2012 he is full professor at the Faculty of Veterinary Medicine at the Warsaw University of Life Sciences. His current research is focused on diagnostic and epidemiology of emerging viral pathogens of swine and their impact on production performance.

Paul Sundberg
Swine Health Information Center

Dr. Paul Sundberg is the Swine Health Information Center's Executive Director. The mission of the Swine Health Information Center is to protect and enhance the health of the United States swine herd through coordinated global disease monitoring, targeted research investments that minimize the impact of future disease threats, and analysis of swine health data. Dr. Sundberg is responsible for implementing the Center's mission and objectives.

Dr. Sundberg was named to this position in July 2015. Before leading the Swine Health Information Center, he was a Vice President with the National Pork Board and responsible for the programs and personnel of the Science and Technology Department.

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

Dr. Sundberg is a member of the American Veterinary Medical Association, the Canadian Veterinary Medical Association, the American Association of Swine Veterinarians and the Iowa Veterinary Medical Association.

Paul and his wife Debra live in Ames, Iowa.

Qiuhong Wang
The Ohio State University

Dr. Wang, Associate Professor, FAHRP, Department of Veterinary Preventive Medicine, OARDC, at The Ohio State University has received extensive training in preventive medicine, virology, veterinary sciences, and cell biology. Her background includes studying rotavirus, astrovirus, calicivirus and coronavirus. She has studied porcine epidemic diarrhea virus (PEDV) since its US emergence in spring, 2013 and is a corresponding/co-corresponding author for more than ten peer-reviewed journal articles and several review articles/book chapters on PEDV. Her laboratory is one of a few labs in the US to isolate and passage genetically diverse PEDV strains in cell culture, and to study PEDV pathogenesis in germfree piglets, cesarean-derived colostrum-deprived (CDCD) piglets, and conventional nursing piglets and the sows. Currently, her team is studying the genetic factors related to PEDV virulence using reverse genetics technologies, and testing the virulence of recombinant viruses in pigs.

Kimberly VanderWaal
University of Missouri

Dr. Kimberly VanderWaal is an assistant professor in the Department of Veterinary Population Medicine at the College of Veterinary Medicine, University of Minnesota. She is a disease ecologist and epidemiologist who has worked extensively on understanding transmission of pathogens in animal populations. Her research combines tools from data science, infectious disease ecology, network analysis, computational modeling, and animal movement data to improve surveillance, prevention, and control of infectious pathogens of cattle and swine. She has spearheaded projects related to the use of animal movement data to understand the spread of diseases at regional and national levels. These models have been used to develop targeted control strategies for bovine tuberculosis and to understand the spread of porcine epidemic diarrhea virus (PED) and porcine reproductive and respiratory syndrome virus (PRRS) in the US.

Kristin Whitworth
University of Missouri

Kristin Whitworth is a research scientist and Interim Project Director at the University of Missouri National Swine Resource and Research Center (NSRRC). She completed her B.S. in agriculture from Illinois State University and a M.S. and PhD in animal science with an emphasis in reproductive biology from the University of Missouri. Kristin focuses her research efforts on the use of gene editing tools such as CRISPR/Cas9 to create both agricultural and biomedical pig models. Kristin's role as Project Director for the NSRRC includes providing and distributing high quality pig models to the scientific community.

Alexei D. Zaberezhny
K.I.Skryabin and Y.R.Kovalenko Federal Research Center "All-Russian Research Institute of Experimental Veterinary Medicine", Russian Academy of Science

Alexei D. Zaberezhny is a Science Director of K.I.Skryabin and Y.R.Kovalenko Federal Research Center "All-Russian Research Institute of Experimental Veterinary Medicine", Russian Academy of Science, located in Moscow (since 2012). He received MS degree in Molecular Biology at Moscow Engineering Physics Institute (1983), Candidate of Science (Ph.D.) in Biochemistry at Y.R.Kovalenko All-Union Research Institute of Veterinary Medicine, USSR Academy of Agricultural Sciences, Moscow (1988), Doctor of Science in Virology, D.I.Ivanovski Virology Institute, Russian Academy of Medical Science, Moscow (2004), Professor in Virology since 2010. He received additional training as post-doctoral scientist (1990-1993) at Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames IA, at laboratory of Prof. Prem Paul and in collaboration with Dr.W. Mengeling at NADC. Since 1997 he supervises a laboratory at D.I.Ivanovski Virology Institute (Moscow). His work has always been focused on swine viruses, including classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV). He is also Assistant to Editor-in-Chief of Voprosy Virusologii (Problems of Virology) since 2006.

2018 North American PRRS Symposium, Sessions 1 through 3 Invited Speaker Abstracts

Abstract #	Speaker Name	Institution
S001	Michael Rahe	Iowa State University
S002	Tomasz Stadejek	Warsaw Univeristy of Life Sciences
S003	Yaowei Huang	Zhejiang University
S004	Kay Faaberg	USDA Agricultural Research Service
S005	Quihong Wang	The Ohio State University
S006	Pengcheng Shang	Kansas State University
S007	Kimberly WanderWaal	University of Minnesota
S008	Kristin Whitworth	University of Missouri
S009	Paul Sundberg	Swine Health Information Center
S010	Alex Morrow	STAR-IDAZ
S011	Raymond Rowland	Kansas State University
S012	Daniel Rock	University of Illinois
S013	Johnny Callahan	Tetracore
S014	Alexey Zaberezhnyy	Y.R.Kovalenko and K.I. Skryabin – Federal Research Center
S015	Scott Dee	Pipestone
S016	Diego Diel	South Dakota State University
S017	Megan Niederwerder	Kansas State University
S018	Douglas Gladue	Plum Island Animal Disease Center, ARS, USDA
S019	Michael Roof	Boehringer-Ingelheim
S020	Enric Mateu	IRTA-UAB Spain
S021	Waithaka Mwangi	Kansas State University
S022	Nick Serao	Iowa State University
S023	Kyu-Sang Lim	Iowa State University
S024	Andrew Kick	North Carolina State University

S001

Characterizing the memory B cell response to PRRSV

Michael C. Rahe[1], Abby Patterson[3], Cheryl M. Dvorak[2], Diem Gray[2] Michael Roof[3] and Michael P. Murtaugh[2]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa 50011 [2]Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Avenue, St. Paul, MN 55108 [3]Boehringer Ingelheim Vetmedica, 2412 S Loop Dr, Ames, IA 50010

Porcine reproductive and respiratory syndrome virus (PRRSV) is the most important pathogen of swine health and wellbeing worldwide largely due to an insufficient understanding of the adaptive immune response to infection. The memory and anamnestic response to infection are critical gaps in knowledge in PRRSV immunity. The lack of effective tools for the evaluation of the memory response previously hindered the ability to effectively characterize the porcine memory response to infection. However, the creation and validation of a PRRSV nsp7-specific B cell tetramer (four protein antigens linked together with a fluorescent marker) now facilitates the ability to detect very rare memory B cells and thus describe the memory response of the pig. Here, we describe the nsp7-specific B cell response in six key secondary lymphoid organs. PRRSV MLV viremia was detected at day 7 and lasted until day 28. This resulted in anti-PRRSV IgG seroconversion starting at day 28 and increasing in intensity at day 56. An expanded population of nsp7-specific B cells was identified at day 28 in the inguinal, tracheobronchial, and mesenteric lymph nodes which contracted by day 56. An antigen specific B cell response was noted in the blood and spleen of immune animals at day 56 which was maintained in the blood out to 113 days post-vaccination. This definition of memory B cell kinetics to PRRSV will answer key questions involved in regional specialization of the immune response following intramuscular inoculation of PRRSV MLV.

S002

The origin and diversity of PRRSV-1 in Europe

Tomasz Stadejek

Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

Porcine reproductive and respiratory syndrome is caused by PRRSV-1 and PRRSV-2 viruses, which are currently classified as two species within genus Porarterivirus, family Arteriviridae. PRRSV-1 is genetically more diverse than PRRSV-2, and its strains belong to several genetic subtypes. However, this classification is mostly based on the analysis of ORF5 and ORF7 sequences. The globally distributed PRRSV-1 strains belong to a single genetic subtype 1, while subtypes 2, 3 and 4 were discovered only in the countries formerly being part of the Soviet Union, such as Russia, Ukraine, Belarus, Lithuania and Latvia. The most common recent ancestor of all PRRSV-1 viruses is thought to exist around 1946-1967, but the globally spread subtype 1 seemed to emerge more recently (TMRCA dated on 1979).

The lack of known natural reservoirs and vectors of PRRS viruses, other than *suide*, proves that the trade of live pigs or semen, was, and still is the main factor of long-distance transmission. An example from Central Eastern European countries, such as Czech Republic, Hungary, Poland or Slovakia, and others, in which after the collapse of the dominance of the former Soviet Union in 1989, and later, after joining the European Union in 2004, significant changes in pig production occurred. It involved intensive import of breeding animals and semen, as well as in recent years, piglets for fattening. It is commonly believed that the imported animals were vectors of PRRSV and its emergence in early 1990-ties in this region. Indeed, many highly similar strains were detected nearly simultaneously across Europe in the early 1990-ties. The western origin of PRRSV in Central Eastern Europe is further supported by the lack of subtypes 2, 3 and 4, common in the countries formerly being part of Soviet Union, where they most likely emerged. However, the earliest evidence of PRRSV antibodies in Europe came from the former German Democratic Republic (GDR), from 1988, when this part of Germany was under Soviet dominance (Ohlinger, 1992). Although the genetic analyses prove the existence of subtype 1 viruses since 1970-ties, the area of their emergence remains unknown.

Balka et al. (2018) have proposed to classify PRRSV-1, subtype 1 ORF5 sequences in three genetic lineages. Lineage 1 contains globally spread viruses, lineage 2 contains mostly Danish viruses and lineage 3 contains viruses mostly from Italy, Poland, Czech Republic and Slovakia, including the earliest examples of PRRSV detection in those countries. The simultaneous emergence of lineage 3 viruses in Central Eastern Europe and Italy cannot be explained by official live pig trade. Single findings of lineage 3 sequences in Germany, Denmark and Belgium make it unlikely that such viruses emerged in Western Europe before being spread to Central Eastern Europe and Italy. Anecdotal information from Italian veterinary practitioners says that in the late 1980-ties, when the Soviet Bloc was already falling apart, some Italian traders brought to Italy piglets of rather poor health status from former GDR and Czechoslovakia. In theory they could harbour PRRSV-1 subtype 1 viruses already endemic in those countries, that later evolved into so called “Italian-like” strains.

The emergence and evolution of PRRSV viruses will likely never be explained. However, considering very low number of PRRSV-1 sequences from Europe available for analysis, which still show the level of genetic diversity and geographic clustering unseen in PRRSV-2, it can be speculated that some coordinated action on pan-European PRRSV sequencing may help to better understand the emergence of at least PRRSV-1.

S003

Receptor usage and potential cross-species transmission of porcine deltacoronavirus

Yao-Wei Huang

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

Interspecies transmission of CoV continues to pose a serious risk to human and animal health. In the current study, we first demonstrated that porcine aminopeptidase N (pAPN) acts as a cross-genus CoV functional receptor for both enteropathogenic porcine DeltaCoV (PDCoV) and AlphaCoV (transmissible gastroenteritis virus, TGEV). The soluble S1 protein of PDCoV efficiently bound to surface of target porcine cell lines known to express pAPN as TGEV-S1 did, which could be blocked by soluble pAPN pre-treatment. Either PDCoV-S1 or TGEV-S1 physically recognized and interacted with pAPN. Exogenous expression of pAPN in refractory cells conferred susceptibility to PDCoV-S1 binding and for PDCoV entry and productive infection. PDCoV-S1 appeared to have a lower pAPN-binding affinity and likely consequent lower infection efficiency in pAPN-expressing refractory cells as compared to TGEV-S1. Furthermore, overexpression of orthologues of APN from multiple species can permit infection by PDCoV in porcine APN-knockout small intestine epithelial cells. Mutagenesis analysis revealed that two glycosylation sites and four aromatic amino acids within domain B of the S1 subunit (S1B) are critical for APN binding. In addition, the S1 subunit of the PDCoV spike glycoprotein, and the S1A and S1B domains in particular, elicited potent neutralizing antibodies against PDCoV infection. PDCoV was also capable of binding to sialic acid of various species, which might account for the ability of S1A to elicit neutralizing antibodies. Finally, PDCoV was able to experimentally infect chickens and mice *in vivo*, distributed mainly in enteric and lymphoid tissues, suggesting that these animals may serve as intermediates and reservoirs of PDCoV for interspecies transmission. Our study reveals the tremendous potential of PDCoV for interspecies transmission and the molecular mechanisms of APN binding of PDCoV as well as the probable connection between them, providing a theoretical basis for development of intervention strategies.

S004

A fresh look at PRRSV evolution: US field strains from 2013-2017

Kay S. Faaberg[1], Tavis K. Anderson[1], Blake Inderski[1], Diego G. Diel[2], Ganwu Li[3], Phillip C. Gauger[3], Jianqiang Zhang[3], Karen M. Harmon[3], Jianfa Bai[4], Elizabeth Paulson Porter[4], Lance Noll[4]

[1] National Animal Disease Center, ARS, USDA, Ames, IA, USA, [2] Animal Disease Research & Diagnostic Laboratory, South Dakota State University, Brookings, SD, USA, [3] Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, USA, [4] Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA

The US Swine Pathogen Database (USSPD) initiative, funded by the National Pork Board and USDA-ARS-NADC-VPRU, has established a new and robust database of nucleotide sequences for significant swine pathogens. The database presently houses porcine reproductive and respiratory syndrome virus (PRRSV), Seneca virus A, and swine coronaviruses, and will eventually include circoviruses and others, except for swine influenza which is housed at The Influenza Research Database (flu.db). Accessible to all at swinepathogendb.org, the USSPD has now incorporated all available GenBank files that have been fully annotated, along with convenient tools to display and mine the sequences.

Working with scientists at the 4 major veterinary diagnostic laboratories (SDSU, ISU, KSU, UMN), we have now assembled field sequence data along with the US State and Year of sample collection (and other criteria) for 2013-2017. The presentation will highlight the major findings from the first look at these new US field sequences of PRRSV. Notably, the NADC30-like viruses recently detected in China, whether looking at the full genome or nonstructural protein 2, group separately into their own subgroup and are more distantly related to all other MN184-like sequences. Most other full genomes clustered in other MN184-like groups or in a separate major branch containing SDSU73, P129 and Ingelvac-ATP Type 2 strains. When looking at the PRRSV ORF5 region only, the Chinese NADC30-like sequences only grouped with one of 3600 recent US ORF5 sequences. However, several new virus groups have emerged in the US, including the RFLP 1-7-4, 1-8-4, 1-3-2, 1-26-2, 1-26-4, and 1-37-2 strains. Sequence alignments will outline the major differences between the emerging RFLP types. However, analyses from ORF5 to full genome suggest, for the most part, that US viruses continue to evolve differently from other parts of the world.

S005

Emerging and re-emerging coronaviruses in pigs

Qiuhong Wang

The Ohio State University

Porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV) and swine acute diarrhea syndrome-coronavirus (SADS-CoV) are emerging/re-emerging coronaviruses (CoVs). They cause acute gastroenteritis in neonatal piglets. Sequence analyses suggest that PEDV and SADS-CoV may have originated from bat CoVs and PDCoV from a sparrow CoV, reaffirming the interspecies transmission of CoVs. The clinical signs and pathogenesis of the three viruses are similar. Necrosis of infected intestinal epithelial cells occurs, causing villous atrophy that results in malabsorptive diarrhea. The severe diarrhea and vomiting may lead to dehydration and death of piglets. Natural infection induces protective immunity, but there is no cross-protection among the three viruses. Besides strict biosecurity measures, individual vaccines are needed for each virus for disease prevention and control.

S006

Emerging PRRSV variants: molecular signatures in nsp2 region

Pengcheng Shang[1], Xingyu Yan[1], Yanhua Li[1], Fangfeng Yuan[1], Andrew E. Firth[2], Chase Stahl[3], Mark Wagner[3], Giselle Cino[1], Ying Fang[1]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, Kansas, USA; [2]Department of Pathology, University of Cambridge, Cambridge, UK; [3]Fairmont Veterinary Clinic, Fairmont, Minnesota, USA.

The PRRSV nsp2 is the largest (mature) viral protein, which contains at least four distinct domains: the N-terminal papain-like proteinase (PLP2) domain, a central hypervariable region (HVR), a putative transmembrane domain, and a C-terminal region of unknown function. In our previous studies, a -2/-1 programmed ribosomal frameshifting (PRF) signal was identified in the nsp2 region, which generates two novel proteins, nsp2TF and nsp2N. Both proteins shared the N-terminal PLP2 domain that was previously determined as a member of the ovarian tumor domain family of deubiquitinating (DUB) enzymes. In addition to PLP2, these nsp2-related proteins stand out for their HVR region, in which deletions and insertions have occurred. Unique deletions/insertions in the HVR region appeared to be molecular signatures of emerging new variants, such as the hallmark of discontinuous 30 amino acids deletion in high pathogenic PRRSV from South-East Asia. Although its interactions with the immune system remain to be elucidated, a cluster of immunodominant B cell epitopes and potential T-cell epitopes is associated with the deletion/insertion, suggesting certain HVR regions that are non-essential for replication may play an important role in PRRSV pathogenesis *in vivo.* Besides the shared PLP2 and HVR regions, nsp2TF contains a unique C-terminal TF domain, while nsp2N is expressed as a truncated nsp2 due to a stop codon encountered immediately by -1 PRF. Recently, we identified unique PRF signal sequences in a group of emerging PRRSV variants, which were isolated from pigs experienced PRRSV outbreaks in swine farms at Midwest of US since the end of 2016. Full-length genome sequence analysis revealed that the PRF signal region of these new PRRSV variants has the most difference from historical PRRSV strains. These viral isolates contain mutations disrupting the -1 PRF stop codon; therefore, extending the translation of nsp2N to generate additional 16 or 23 amino acids at its C-terminus. Using reverse genetics, recombinant viruses carrying specific mutations in PRF signal were generated, which showed attenuated pathogenicity in infected piglets. Taken together, sequence variation in certain regions of nsp2-related proteins maybe correlated with increased pathogenicity of emerging PRRSV variants; and manipulating the expression of nsp2-related products provides basis for MLV vaccine development against newly emerging PRRSV strains.

S007

Forecasting outbreaks of PRRS and PED in swine movement networks

Rahul Bhojwani[1], Amy Kinsley[1], Cesar Corzo[1], Igor Paploski[1], Carles Vilalta[1], Andres Perez[1], Meggan Craft[1], Gustavo Machado[2], Kimberly VanderWaal[1]

[1] University of Minnesota, St. Paul, MN, USA
[2] North Carolina State University, Raleigh, NC, USA

Objectives

Porcine reproductive and respiratory syndrome virus (PRRS) and porcine epidemic diarrhea virus (PED) are two of the most important endemic swine pathogens in the U.S., yet risk factors that drive viral circulation at regional levels are poorly understood partly because large-scale datasets are lacking. Using data on swine movements and environmental factors, our objective is to forecast and report the risk of PRRS and PED infection at the farm-level in order to promote data-informed and targeted disease management and prevention measures.

Methods

We utilize a data set available through the Morrison Swine Health Monitoring Project that contains weekly PRRS and PED infection status for ~30% of the U.S. breeding sites. We first perform a dynamic network analysis on swine production systems to quantify the vulnerability of swine movement networks to disease spread and identify key farms that are potential super-spreaders. Second, using data on pig movements, geolocations of farms, environmental, and weather factors, we applied predictive machine-learning algorithms that forecast the probability that a sow farm will become infected in a bi-weekly period.

Results

Our results revealed a high amount of variability in movement patterns across production systems. Furthermore, we show that targeting surveillance and control interventions towards farms based on their mean infection potential, a metric that captured the temporal sequence of movements, substantially reduced the potential for transmission of an infectious pathogen in the contact network. In addition, the most important factor predicting outbreaks of PRRS and PED was the number of pigs moved into neighboring farms (within 10 km of the focal farm), followed by environmental characteristics of the surrounding neighborhood. The machine learning algorithms resulted in farm-level outbreak predictions that had sensitivity and high positive predictive values.

Conclusions

The ability to identify and target key super-spreader farms within swine networks help us understand how to prioritize resources necessary to control disease spread in the event of an epidemic. In addition, our outbreak prediction algorithms form the foundation for near real-time disease prediction and mapping, which will advance disease surveillance and control for endemic swine pathogens in the United States.

S008

Genetic modification of ANPEP in pigs and resistance to coronavirus infection

Kristin M Whitworth[1], Vlad Petrovan[2], Raymond RR Rowland[2], Kevin D Wells[1], Randall S Prather[1]

1 Division of Animal Sciences, University of Missouri, Columbia, MO 65211,
2 Department of Diagnostic Medicine, Kansas State University, Manhattan, KS, 66506

The entry mediator for some coronaviruses is thought to be amino peptidase N (ANPEP). Coronaviruses with agricultural and zoonotic significance include porcine deltacoronavirus, porcine respiratory coronavirus, transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus (PEDV). Both TGEV and PEDV are high sources of morbidity and mortality in neonatal pigs as a consequence of dehydration caused by viral infection and necrosis of enterocytes. To determine the biological relevance of ANPEP as an entry mediator for TGEV and PEDV, pigs were created with a knockout of *ANPEP* by using the CRISPR/Cas9 system. In vivo challenges revealed that the *ANPEP* null pigs were susceptible to infection by PEDV, but not TGEV. While TGEV and PEDV possess similar clinical signs and tropism, their receptor requirements are distinct. The different receptor requirements for TGEV and PEDV have important implications in the development of new genetic tools for the control of enteric disease in pigs. Entry mediators for other coronaviruses remain to be confirmed. Editing the genome is a powerful tool to determine entry mediators and create animals resistant to viruses that have significant impacts on animal welfare, sustainability, and food security. Since some coronaviruses have zoonotic importance, control of these viruses goes beyond the impact on swine.

S009

Setting priorities for disease threats and strategies: Is ASF coming closer?

Paul Sundberg[1], Eliana Paladino[2], Auguste Brihn[2], Sol Perez[2], Andres Perez[2], John Deen[2]

[1]Executive Director, Swine Health Information Center. [2]University of Minnesota.

The Swine Health Information Center was funded by a National Pork Board grant in July of 2015. Its mission is to protect and enhance the health of the United States swine herd through coordinated global disease monitoring, targeted research investments that minimize the impact of future disease threats, and analysis of swine health data.

Funded by SHIC in 2017, a program for systematically monitoring swine diseases around the world was developed at the University of Minnesota using a private-public-academic partnership including collaboration with the USDA/APHIS Center for Epidemiology and Animal Health (USDA-CEAH). The aim of these reports is to have a support system for near real-time identification of hazards that will contribute to the mission of assessing risks to the industry and ultimately, early detect, identify, or prevent occurrence of events, in partnership with official agencies, and with our international network of collaborators.

Monthly reports are created based on the systematic screening of multiple official data sources, such as government and international organization websites, and soft data sources like blogs, newspapers and unstructured electronic information from around the world that then are curated to build a raw repository. Afterward, a group of experts uses a multi-criteria rubric to score each event, based on novelty, potential direct and indirect financial impacts on the US market, credibility, scale and speed of the outbreak, connectedness, and local capacity to respond average is calculated. The output of the rubric is a final single score for each event which is then published in the report.

On November 16, ASF was reported in Yibin City in the southeastern area of Sichuan province. Sichuan and Henan are the two provinces with the highest pig production in China, representing over 30% of total production. Sichuan province slaughtered 69 million of the approximately 700 million pigs produced by China in 2016.

The outbreak in Sichuan continues a southwestward trend in the epidemic. With 64 outbreaks reported in 18/34 provinces across China, the concern is that the disease may have steadily spread, and that major impact to pork suppliers may be seen in coming months.

Also on November 16 the Chinese Ministry of Agriculture confirmed the first case of ASF in a wild boar (Jilin province, northeastern China). This significantly increasing concerns around the effectiveness of control strategies implemented by authorities since last August.

One of the major concerns is that commercial feed may become ASF-contaminated. In a statement to the Shenzhen Stock Exchange, the Tangrenshen Group reported that ASF was detected in feed samples in one of their units. The feed was manufactured by their 51 percent owned subsidiary, Bili Meiyingwei Nutrition Feedstuff. Following this statement, on November 13, Tangrenshen Group

followed up stating that test made by one of the units has actually ruled out the presence of ASF in feed. Subsequently, the operation resumed work as normal.

According to a study by Cristina Jurado, et. al, doi: 10.1111/tbed.12996, 2018, on average 8,000 pork derived products are annually confiscated by Customs and Border Protection at the United States (US) ports of entry such as international airports, harbors or mail offices. Their study aimed at analyzing the risk of African swine fever (ASF) and classical swine fever (CSF) being introduced into the US through prohibited swine products carried by air passengers and identifying locations and time periods at higher risk where and when preventive and mitigation measures should be implemented.

Focusing on ASF, the the overall mean annual probability of ASF entry was estimated as 0.061 at 95% confidence interval (CI) [0.007, 0.216]. July and May were the months at highest risk for entry. For ASF, the origin countries of those air passengers that represented the highest risk (above 70% of the total risk) were Ghana, Cape Verde, Ethiopia, and the Russian Federation.

The study used international travel and USDA Customs and Border Protection data from January of 2010 to March of 2016. Thus, the results were based on data prior to the August 2018 notification of the beginning of the ASF outbreak in China. In addition, World Bank data on air transport in and from China shows a 13% increase from 2016 to 2018. The combination of the 2018 ASF epidemic in China and the increased travel in and from China may change the estimate of the risk of ASF entry through US airports. The Swine Health Information Center and the National Pork Board are co-funding a project with the University of Minnesota to update this risk estimate.

On September 5, 2018, U.S. Department of Agriculture (USDA) and Food and Drug Administration (FDA) officials met with U.S. pork sector groups – including the American Association of Swine Veterinarians, the National Pork Board, the National Pork Producers Council and the Swine Health Information Center – to evaluate additional measures to prevent the spread to the United States of African swine fever (ASF) currently active in China and some European nations.

The industry organizations and USDA agree that prevention could be enhanced by Customs and Border Protection (CBP) and Plant Protection and Quarantine (PPQ) ensuring flights from China and Russia get enhanced passenger and cargo inspection attention. USDA has made a request to that effect to CBP. Sampling and monitoring of imported products that might pose a risk of ASF transmission is also being considered.

Collaboration can help to address concerns about potential risk associated with feed and feed component imports. We know from research conducted by Dr. Scott Dee, et al. that certain feedstuffs are able to support ASF during a simulated trans-Pacific shipment. A validated method to test bulk feed products and applying it to monitoring shipments for pathogen contamination has been funded by SHIC and is currently being researched. Also, currently being researched with results expected soon are feed pathogen mitigation options. Feed additives, component holding time and temperature before processing, Hazard Analysis and Risk-Based Preventive Controls and blockchain are being investigated with urgency.

The USDA Center for Epidemiology and Animal Health's Risk Identification Unit (RIU) is monitoring diseases around the world, including China. Increased, regular communication through contact or reports is another action that could enhance prevention through raising awareness. This provides a

vehicle for two-way communication to provide information from industry sources to RIU and for RIU to provide the most up-to-date information they have back to industry.

The Swine Health Protection Act gives USDA-APHIS regulatory authority for compliance inspections for licensed food waste feeders and searches for non-licensed waste feeding facilities. The pork industry organizations are communicating to USDA their request for increased inspections and searches.

The inspection and compliance processes for imported pork casings and other food products was another topic of discussion. While USDA does not allow the import of casings originating in ASF positive regions, the risk of US origin casings that have been sorted in China and returned to the US is being considered.

Response initiatives include a survey of the National Animal Health Laboratory Network (NAHLN) for their surge capacity should the industry need ASF testing to identify issues or shortcomings. USDA is already conducting the survey.

A project to validate a PCR test for ASF – and FMD and CSF – on oral fluids has been underway for over a year. Only whole blood and tonsil are currently USDA-validated for ASF surveillance via PCR testing. Diagnostic capabilities need to be updated to enable spleen and lymph nodes, tissues likely to be submitted during routine animal health event investigations, to be USDA-validated for ASF detection and surveillance. And response capability needs to be able to be applied at the herd level through plans for USDA accredited veterinarians being integrated into national response should federal resources become overwhelmed during an outbreak.

ASF-specific emergency response exercises are being planned, including allied industry, NAHLN and state and federal animal health officials' participation. And response and communication coordination with Canada and Mexico are additional items being discussed and have already been initiated by USDA.

ASF control in China will be extremely difficult, if even possible. The Chinese pork industry has had difficulties in controlling FMD and CSF and has relied heavily on the use of vaccines. As a vaccine is not available for ASF, the industry is thus reliant on heightened biosecurity, rapid diagnosis, complete isolation, and then elimination of infected pigs and contaminated materials. US plans for prevention and response have to be considered to be long-term adjustments to the biosecurity of our national herd.

S010

Emerging diseases: Addressing the unmet vaccine needs

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Animal disease risks have increased over recent decades, especially as a result of the increased globalisation of trade and animal product movements, and the consequent transfer of associated fast evolving pathogens. Since the emergence of PRRSV in the 1980s the swine industry has been hit by a number of new disease challenges including major outbreaks of PMWS, Porcine Epidemic Diarrhoea and African Swine Fever, all with substantial mortalities. These disease outbreaks have highlighted the inadequacy of the available measures to respond rapidly and successfully to emerging disease challenges. New pathogens are constantly being discovered including the recent identification of porcine deltacoronavirus in the US, swine acute diarrhoea syndrome corona virus (a novel HKU2-related corona bat virus), PCV3 and Porcine Circovirus-like P1in China.

Vaccines offer the most cost effective option for the control of infectious diseases. However pathogen diversity and emergence and re-emergence of pathogens will remain a challenge for vaccine development and effective rapid disease control. For none of the diseases mentioned above was there an effective vaccine available to deploy at the time of the early outbreaks and current response times for vaccine delivery are widely seen as being far too slow. New technology platforms including vectored vaccines, virus-like particles and self-amplifying RNA technology offer the hope of shortening the innovation pathway and approval process so that vaccines are available more rapidly for emerging pathogens in the not too distant future.

S011

Host genetics: Bringing the pig to the vaccine

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Attenuated live-based vaccines can deliver good protection. However, the response of vaccinated pigs can be variable, with some pigs showing adverse clinical signs after vaccination or limited protection after vaccination and challenge. The diverse outcomes suggest a strong genetic component, which contributes to protection following vaccination. The next generation of vaccines, which will depend on a vectored delivery system need to activate protective immune response pathways while avoiding immunopathogenesis. A genome-wide RNA-Seq approach was used to characterize differentially expressed genes (DEGs) during infection with the live attenuated OUR T88/3 (OURT) vaccine strain and the highly pathogenic Georgia 2007/1 (GRG). Whole blood RNA was collected at day 0 and at euthanasia day (ED) or 7 to 10 days after infection with GRG, and on days 3, 7, 14 and 28 days for OURT. The highest number of highly upregulated DEGs was found in the GRG-infected pigs, consistent with the response of a host to a pathogenic virus. The twenty most highly upregulated genes in infected pigs could be placed in four groups: 1) genes associated with macrophage functions; 2) genes associated with known ASFV-associated cell responses; 3) genes associated with NK and T cell effector functions; and 4) other genes. For OURT, the response to infection was dominated by genes associated with the activation of NK cells. This study identifies potential innate and adaptive immune response pathways, which can be exploited for the design of future vaccines.

S012

Challenges for African swine fever vaccine development - " . . . perhaps the end of the beginning."

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African swine fever (ASF), an acute, viral hemorrhagic disease in domestic swine with mortality rates approaching 100%, is arguably the most significant emerging disease for the swine industry worldwide. Devastating ASF outbreaks and the continuing epidemic in the Caucasus region, Russia, eastern Europe and now China (2007 – to date) highlight significance of this disease threat. There is no vaccine for ASF, thus leaving depopulation the only effective disease control option. Vaccination is possible as protection against reinfection with the homologous strain of African swine fever virus (ASFV) has been clearly demonstrated. However, vaccine development is hindered by large gaps in knowledge concerning ASFV infection and immunity, the extent of ASFV strain variation in nature and the identification of viral proteins (protective antigens) responsible for inducing protective immune responses in the pig. The development of broadly-effective and cross-protective ASFV vaccines requires these critical knowledge gaps be successfully bridged. Current challenges and opportunities for ASF vaccine design and development will be discussed.

S013

The evolution of ASFV real-time PCR, assuring fit-for–purpose as disease spreads worldwide

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Beginning in 2004 work began to validate a real-time PCR for ASFV in collaboration with ARS at Plum Island which resulted in a 2005 publication by Zsak et al. In 2007, an urgent request from ARS was received to manufacture tests to support an outbreak investigation in the Republic of Georgia where ≈2500 dried assays where manufactured and hand-carried to Georgia approximately one week after the initial request. The assay was further evaluated by APHIS and adopted for use by the NAHLN laboratories in 2012. As viruses evolve they often mutate which may impact the efficacy of PCR assay designs should a significant mutation occur. To ensure that the established assay designs remain fit for purpose, AAVLD guidelines suggest that emerging strain sequences should be compared in silico to the assay primer/probe sequences at least annually to ensure that the test remains fit-for-purpose. The original ASFV assay published by Zsak, et al, was updated in 2015 by adding additional primers and probes for a second target that considers new ASFV sequences that were unknown at the time of the original design. In 2016 the design was field tested in Uganda where the virus is endemic. In Uganda, Blood and/or tissue samples were collected from domestic pigs during outbreak investigations in five districts of Uganda. Sample preparations included either simple dilution in PBS or magnetic bead nucleic acid extraction. A dried-down ASFV PCR kit with internal control (IC) (Tetracore Inc., Rockville, Maryland) was used on a portable real-time PCR thermocycler T-COR 8™ (Tetracore Inc.), performed on-site in the affected villages and in a simple lab setting. As a reference, the OIE recommended UPL assay (Fernández-Pinero et al 2013) was performed on a Stratagene Mx3000P at NADDEC. Pigs from two of the five suspected outbreak sites investigated were positive for ASFV using the ASF kit on the T-COR 8™ and these results matched those of the reference method in the lab at NADDEC. For blood diluted in PBS, inhibition was prevalent in 20-fold diluted and present in some 40-fold diluted samples. Archived samples were also tested and in total samples for twenty-two pigs were positive for ASFV out of sixty-nine tested. Overall, the portable platform performed with very good correlation to the reference method. This study showed that confirmation of an outbreak can be performed on-site in ≤ 2 hours and then appropriate actions can be taken. The experience of performing the PCR assays in remote areas highlighted several factors that need to be carefully considered, including biosafety issues, simplicity and effectiveness of sample preparation and turn-around time. In light of the current situation in China, the test should be empirically tested with current Chinese ASFV positive and negative samples to make sure that the test is fit for purpose for Chinese ASFV strains.

S014

Progress in the control of ASF and CSF in Russia

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African Swine Fever (ASF) is an infectious viral disease that causes high economic losses due to necessity of depopulation of pigs in affected areas, sanitary measures, trade restrictions etc. The virus (ASFV) is relatively stable in the meat products and environment, thus, large territories are at risk due to free movement of people and products. The ASFV does not affect people and animals except wild and domestic pigs. Some ticks can become infected and carry the virus for years. Adaptation of the virus by changing into less virulent form would mean the threat of an endemic situation in the area. The disease is endemic in domestic and wild pigs in most of sub-Saharan Africa and Sardinia/Italy. There is no treatment for ASF, and no vaccine has been developed. In case of infection with less virulent ASFV strains, recovered pigs could spread the virus as long as thy live. By clinical sympthoms, ASF is very similar to Classical Swine Fever. Methods of laboratory diagnostics are well developed and efficient for identification of ASFV and virus-specific antibodies. Experience of eradication of ASF in Spain suggests the importance of serological monitoring of pigs. In the spring of 2007 the ASF was detected in Caucasus region. Same virus was detected in Georgia, Armenia, Azerbaijan, and Russia. The ASFV circulating in the Caucasus and the Russian Federation is a highly virulent virus. By the year of 2018, the ASF appeared in Western Russia, Poland and in the Baltics. Despite the virus was of same genotype as the first introduction in 2007, its virulent properties have reportedly changed. Domestic pigs play important role in ASFV spread in Russia; they transfer the virus to wild boars. The virus circulates in the population of wild boars depending on their density in the area. Occasionally, the disease is spread from wild to domestic pigs. There is no evidence of ticks being involved in the process. Thus, human activity in raising pigs is largely responsible for continuous spread of the disease. Despite vigorous monitoring and sanitary measures, the disease has not been stopped. The control strategy for ASF should consider international (especially Spanish) experience and local situation. The strategy is based on number of important steps including rapid localization of the disease by trained specialists, setting up buffer zones, constant monitoring of swine population and farms, improvement of diagnostic facilities, training of veterinary personnel, development of system of information and international collaboration.

Classical Swine Fever Virus (CSFV, Hog Cholera Virus) is a pestivirus related to Bovine Viral Diarrhea Virus and Border Disease Virus of sheep. Vaccination of pigs against Classical Swine Fever (CSF) in Russia is mandatory. It has been performed for decades with live attenuated vaccines derived from viral strains LK, CS, LKK and developed in the USSR. Vaccination practice has decreased the

yearly incidence of CSF in Russia to single cases, however, the disease remains endemic. The virus circulates in swine population that has CSFV-specific antibodies due to vaccination. The CSFV contains positive single stranded RNA of 12.3 kb that is infectious. After electroporation of the virus target cells the RNA replicates and translates using IRES mechanism into a single polypeptide precursor which is further processed. This is sufficient for development of infectious virus. We have worked with Russian vaccine strain CS as a backbone for new modified viruses. Molecular and reverse genetics methods were used to characterize Russian vaccine strain CS. Recombinant baculovirus-based E2 glycoprotein of the CS strain was used for subunit vaccination, production of monoclonal antibodies and epitope mapping. Ten hybridomas have been obtained using native E2 protein, and eighteen - with baculovirus-produced recombinant protein. Analysis of their specificity has revealed 8 different antigenic epitopes in the E2 protein. Five new previously not described non-overlapping linear B cell epitopes have been mapped using a set of 32 overlapping synthetic peptides. The region of 834-856 a.a. that contains 2 linear epitopes, is structurally different between different pestiviruses. The monoclonal antibodies that have been obtained using native E2 protein, can be used in sandwich ELISA for antigen detection. Blocking ELISA for antibody detection was developed based on rE2 and monoclonal antibodies. Molecular tests were developed for CSFV detection and CSF/ASF differentiation. A collection of historic CSFV isolates were sequenced and analysed. Majority of sequenced field isolates from 1981-1984 belonged to subgroup 1.2, while isolates from 1995-2002 belonged to 1.1, and from 2004-2007 – to 2.3. Eradication of CSF requires a complex approach. In order to contribute to potential eradication programs, new tools, such as markered vaccines, immunochemical and molecular diagnostic tools have being developed. More strict biosecurity measures currently taken against ASF will help to control CSF.

S015, S016, S017

Risk for transmission by feed ingredients and potential mitigation strategies for ASF and other transboundary diseases

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In 2013, the U.S. pork industry became infected with porcine epidemic diarrhea virus (PEDV). Several epidemiological studies revealed feed and feed ingredients as a possible vehicle for the transboundary movement of pathogens. Through a series of experiments, we demonstrated transmission to naïve pigs through the consumption of PEDV-contaminated feed, the ability of feed additives to reduce this risk, and evaluated survival of the virus in different feed ingredients. Building on this theme, we developed a Trans-Pacific model to simulate the movement of PEDV as well as 10 additional viruses in contaminated feed ingredients from China to the U.S. Additionally, the project was expanded to include African swine fever virus in a Trans-Atlantic model to simulate shipment from Eastern Europe to the U.S. The transboundary models assess variability across feed ingredients, pathogens, transport times between countries, and environmental conditions over land and sea. Through this work, we have studied the effect of certain feed ingredients on viral survival and introduced the concept of a “High Risk Combination”. We have demonstrated that some ingredients, such as soybean meal, have the ability to promote survival of several transboundary viruses. Our collaborative work has expanded to investigate mitigation strategies, such as storage time in different environmental conditions and chemical feed additives, to reduce the risk of transboundary viral introduction and spread in feed and feed additives. Furthermore, we are actively investigating the oral infectious dose of viruses in feed to further define this risk. Data from these studies can provide a basis for the platform known as “Responsible Imports”, a science-based plan to reduce the risk of transboundary viral introduction through feed and feed ingredients.

S018

Rapid development of experimental live attenuated vaccines for outbreak strains of African swine fever virus

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African swine fever virus (ASFV) causes a highly contagious disease called African swine fever (ASF), currently causing outbreaks in both Europe and Asia. This disease is often lethal for domestic pigs, causing extensive losses for the swine industry. Currently there is no commercially available treatment or vaccine to prevent this devastating disease, current outbreaks are controlled by culling of infected animals, and have already caused extensive losses to the swine industry in outbreak areas. Development of recombinant ASFV for producing live-attenuated vaccines against emerging outbreak strains is essential to effectively control outbreaks of ASFV. Development of live-attenuated vaccines currently relies on deleting ASFV genes that are involved in virulence. Deletion of ASFV genes has been a complex and very time consuming process that relies only on rare events of homologous recombination in primary swine macrophages followed by a lengthy and tedious process of purifying recombinant virus from wild-type parental ASFV. Here we will present a rapid process using CRISPR/Cas9 in combination with fluorescent markers that allows multiple deletions in emerging outbreak strains that can facilitate rapid production of new live-attenuated ASFV vaccines against emerging outbreak strains. A process that historically has taken years has now been reduced to weeks.

S019

Lessons and opportunities learned from 25 years of PRRS vaccination

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Since the initial reports of “Mystery Pig” disease in the early 1990’s, the swine industry has been searching for tools to help control and eliminate the PRRS virus. Since 1994, commercial and autogenous vaccines have been tools available to help address this economically important and clinically devastating disease. Despite the widespread application of these vaccine-based tools, PRRS disease and its global impact remain important today. The presentation will discuss some of the key factors that impact vaccine development and optimal use in the field.

- Initial expectations, insufficient diagnostic tools, and improper vaccine use led to industry frustration and confusion in attempts to control PRRS virus.
- Since its discovery, the PRRS virus has continued to change and evolve. These changes have happened at an unexpectedly fast rate and led to eight different lineages of PRRS within North America alone. These changes have led to the recognition that PRRS isolates have dramatically different phenotypes and isolates can vary in their virulence and in vivo dynamics. Attempts to develop vaccines based on genomic sequence, lineage, date of isolation, or geography have not led to significant improvements in the field situation.
- The swine industry and research community have used the terms “homologous” and “heterologous” immunity in various contexts that have led to some level of confusion. As a virus that can exist as a quasispecies, homologous immunity becomes biologically irrelevant in most cases. Secondly, most controlled experiments using replicating PRRS virus vaccine in naive animals have successfully demonstrated statistically significant reductions of important clinical parameters using even the most diverse and genetically heterologous isolates.
- Many research studies and valid conclusions have been made using specific PRRS isolates. However due to the genetic variation between isolates and the associated diversity in clinical phenotype, conclusions in some cases may be isolate specific (or biased) and so caution must be applied to broad statements and this applies to vaccination protocols and isolates as well. Not all PRRS isolates are created equal!!
- Live virus inoculation (LVI) has been a tool applied in the field as an attempt to improve herd immune status based on the belief that homologous immunity can be achieved and such immunity is important. Use of LVI and its value is ill-defined, but risks and negative consequences of using a wild-type virus in large pig populations require careful risk assessment.
- Despite dramatic improvements in reagents, assays, and tools, the in vivo bio-assay remains the most sensitive tool to determine the presence of viable PRRS virus in a sample. Use of this tool in mixed virus samples may help focus on isolates of most biologic significance.

- Use of vaccines, bio-assay, and improved diagnostic tools, including Next Generation Sequencing (NGS), may be a new and improved set of tools to control the PRRS virus, especially if applied in a regional control program.
- Challenges in the development of next generation PRRS vaccines include genomic stability of some potential research tools, PRRS genetic change of the virus in the field, and economic benefit of new tools (is the cost of development sufficient given marginal improvements over current tools).

In conclusion, the research community and swine industry have made huge strides in the area of PRRS virus knowledge and tools to apply to clinical challenges in the field. Numerous commercial vaccines are sold and used in the industry, however these tools provide the most value when used in combination with solid management practices (internal & external biosecurity protocols, pigflow; etc), state of the art diagnostics (oral fluids, NGS, PCR, Serology), understanding of PRRS virus epidemiology/spread, and defined PROGRAMS that address the PRRS virus challenge at a whole herd or regional effort. We have the ability to control or even eliminate PRRS from a herd, but the approach requires the application of all our tools into a complimentary and comprehensive program for success.

S020

PRRSV vaccines and vaccination: Present and future

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Vaccines for porcine reproductive and respiratory virus (PRRS) have been available in the market since the mid-1990s. They are useful tools in the control of PRRS by reducing the impact of the disease and helping the process of stabilizing a herd. Most of the commercially available vaccines for PRRSV are made of modified live viruses (MLV) (with or without adjuvants) but some inactivated vaccines are marketed as well. In any case, current vaccines present serious limitations. For example, they do not provide sterilizing immunity against heterologous strains and they do not allow the differentiation of infected from vaccinated pigs.

Those limitations arise in a great part from two factors: the uncomplete understanding of the correlates of protection and the extremely high genetic diversity of PRRS viruses. Several studies have shown that passively transferred neutralizing antibodies may protect against the homologous challenge. However, heterologous protection is still highly unpredictable. In recent years, it was shown that broadly neutralizing antibodies (bNA) may exist but it is still unclear to what extent the development of such antibodies depends on the viral isolate, the idiosyncrasy of the individual pig, and the time course of the immune response. The identification of the bNA-inducing epitopes would be of major interest for the development of new vaccines. The role of cell-mediated immunity in the protection against PRRSV has been also a subject of debate for many years. A recent paper suggested that CTL responses could be present in convalescent animals. The confirmation of this finding and the identification of the involved epitopes would also be of major importance for the development of newer vaccines. Recent studies showed that a synthetic consensus virus was able to induce a broadly protective response although still only partial.

As stated above, most of the available vaccines are MLV. The balance between attenuation and immunogenicity is not a minor issue since the immune response depends on the antigenic load but can also be affected by immunologically undesirable effects of viral replication. Furthermore, a vaccine virus replicates in the recipient animal and is shed, with the consequent potential to recombine with field strains. Alternative approaches such as vectored or subunit vaccines would overcome this problem. Similarly, mRNA vaccines might be worth exploring.

The present talk will review all these aspects emphasizing the need for international collaborative research to boost the understanding of immunity against PRRSV and the development of newer vaccines.

S021

Vectored vaccine strategies for African Swine Fever

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African Swine Fever Virus (ASFV) is a complex high-consequence transboundary animal disease pathogen for which there is no vaccine or treatment. Protection against ASFV can be induced with attenuated virus and there is evidence that ASFV-specific cytotoxic T lymphocytes (CTLs) are required for viral clearance. However, there are safety issues associated with attenuated ASFV vaccine and on-going efforts are geared towards development of safe and efficacious mutants, but none has been approved for deployment. Thus, it is rational to develop vectored ASF subunit vaccines. However, protective ASFV antigens and a suitable delivery vector needed for subunit vaccine development have not yet been identified. In proof-of-concept studies, we tested immunogenicity and efficacy of several adenovirus-vectored prototype immunogens containing multiple rationally selected ASFV antigens. Immunization of pigs with one of the prototypes induced significant and unprecedented antigen-specific CTL responses that were detectable in peripheral blood mononuclear cells (PBMCs). Significant IFN-γ-secreting cells were also detected in PBMCs. In addition, the immunogen primed antibody responses that were recalled upon boost. Importantly, following intranasal challenge with the ASFV Georgia 2007/1 isolate, 5/9 vaccinees were healthy 17 days post-challenge, whereas 2/5 negative controls were alive, but they were moribund. This outcome will be validated in a larger animal study.

S022

Genomic analysis of total antibody response to Porcine Reproductive and Respiratory Syndrome (PRRS) Modified Live Virus (MLV) vaccination in commercial replacement gilts

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Previous studies proposed the use of total antibody response to PRRS, measured as sample-to-positive (S/P) ratio using a commercial ELISA, as a potential genetic selection tool to improve reproductive performance of sows infected with the PRRS virus (PRRSV). However, these previous results have not been validated. Moreover, these studies have not directly evaluated the genomic response of animals to PRRS MLV vaccination as an alternative strategy for the generation of S/P ratio data, as opposed to requiring a PRRS outbreak to collect such data. Therefore, the objective of this work was to characterize the genomic basis of total antibody response to PRRS MLV vaccination in replacement gilts. A total of 900 F1 gilts (Landrace x Large White), from 2 commercial farms (639 in farm 1 and 266 in farm 2), were sourced from the same high-health multiplier herd and vaccinated with a commercial PRRS MLV vaccine when entering the farms. Blood samples were taken at 52 (farm 1) and 44 (farm 2) days post-vaccination (around peak PRRS antibody response) for subsequent PRRS serum ELISA analysis (IDEXX PRRS X3 Ab Test) and high-density SNP genotyping (~50K SNPs). Heritability of S/P ratio due to MLV vaccination was estimated using Bayes-GBLUP. Genomewide association (GWA) and genomic prediction analyses for S/P were performed using BayesB (pi = 0.99). For genomic prediction analysis, a 5-fold cross-validation strategy was used, in which 5 groups of 180 animals were randomly selected, and then SNP effects were estimated using 4 groups whereas the remaining group was used for validation. This process was repeated until all 5 groups were used for validation. Accuracy of genomic prediction was calculated as the average correlation between adjusted phenotype and genomic estimated breeding values across the 5 folds, divided by the square root of heritability. The statistical models included the fixed effect of farm and SNP markers as random effects. All analyses were performed in GenSel. The heritability estimate for S/P ratio due to vaccination was of 0.38±0.03. The GWA analysis identified a major quantitative trait locus (QTL) at megabase (Mb) 25 on chromosome 7, which explained 29% of the total genetic variance for S/P ratio. This region harbors the major histocompatibility complex (MHC), the most important genomic region in vertebrates that control the immune response. The average accuracy of genomic prediction of S/P ratio was of 0.69±0.08 when using all SNP markers. When only using SNPs within 5 Mb of the MHC region, the average genomic prediction accuracy was 0.45±0.03. When using all SNP markers but those within 5 Mb of the MHC region, the average accuracy of genomic prediction decreased to 0.30±0.09. These results validate previous hypotheses that total PRRS Ab response to vaccination is heritable, the MHC region is the major genomic region controlling this trait, and high-density SNP markers can be used to identify animals with greater immune response to PRRS. Additional studies are needed to validate the use of S/P ratio from vaccinated animals as a selection tool to improve reproductive performance in PRRSV-infected animals.

S023

Blood transcriptome in healthy piglets as a potential biomarker to improve disease resilience

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Improving disease resilience is an issue of considerable importance in swine breeding programs. However, evaluation of resilience after exposure to disease conflicts with the high-health conditions required in pig nucleus breeding herds. Hence, developing blood biomarkers of resilience that can be obtained at the nucleus herd level can help to overcome this limitation. The aim of this study was to investigate the blood transcriptome of young healthy pigs as early disease resilience predictors. Seven batches of weaned barrows (n = 441 Yorkshire x Landrace) from healthy multiplier farms were entered into the experimental facility in Québec, Canada. They were acclimated in a healthy quarantine nursery and blood samples were collected at ~27 days of age for quantifying gene expression levels using 3'mRNA sequencing. Two weeks after bleeding, the pigs were moved to a nearby natural disease challenged nursery-finisher, where several resilience phenotypes were measured, including average daily gain in the challenge nursery (NurADG) and finisher (FinADG), mortality, and the number of treatments per 180 days (Trt180). The expression of 3,523 genes in blood at a young age was significantly associated with mortality (FDR<0.05). Genes related with protein catabolic processes, metabolic processes, and immune response pathways showed lower expression in pigs that subsequently died during the natural disease challenge. Only two genes were significantly (FDR < 0.05) associated with NurADG and there were no significant associations with FinADG and Trt180. In conclusion, gene expression patterns in young healthy pigs showed significant associations with mortality following exposure to disease and are, therefore, promising as early disease resilience predictors for use in nucleus breeding programs. Funding from USDA-NIFA, Genome Canada, PigGen Canada.

S024

Deciphering immunosuppression and immunological memory to homologous and heterologous strains of Porcine Reproductive and Respiratory Syndrome Virus

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Objectives: Developing a vaccine protecting against heterologous Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) strains remains a critical objective for the pork industry worldwide. Despite the availability of commercial PRRSV vaccines and specific corporate vaccination strategies, PRRSV outbreaks continue to cause significant economic loss and degrade animal health. The efficacy of a PRRSV vaccine depends on whether it induces immunological memory towards the virus and across strains. Current *in-vitro* vaccine evaluation methods do not adequately determine induction of immunological memory. The objective of this study was to develop a system to precisely evaluate vaccine- and infection-induced immunological memory to homologous and heterologous PRRSV strains. In addition, regulatory T cells (Tregs) were monitored to evaluate the induction of immunosuppression by a modified live virus (MLV) PRRSV vaccine, a low path (1-3-4) and a high path (1-7-4) PRRSV strain. Understanding the establishment of immunosuppression and immunological memory responding to homologous and heterologous PRRSV strains will significantly improve vaccine development and testing by providing superior predictive determinants of vaccine efficacy.

Materials and Methods: Four-week old PRRSV naïve piglets were divided into four groups: MOCK, MLV vaccinated, or infected with a LP or HP PRRSV strain. Blood was collected over nine weeks to study systemic anti-PRRSV IgG levels, viremia, neutralizing antibodies, and the isolation of PBMC. Regional immune responses were studied in cells from draining lymph nodes. PBMC were *in vitro* re-stimulated with homologous and heterologous PRRSV strains to determine and functionally describe cross-reactive immune memory cells via multi-color flow cytometry and cytokine multiplex.

Results: PRRSV infection and vaccination resulted in viremia and a strong humoral immune response. Clinical disease was moderate with fever and lethargy, and only occurred in animals infected with LP and HP strains. Our *in vitro* system to determine homo- and heterologous memory immune cells revealed that PRRSV vaccination and infection led to the induction of Tregs. Currently ongoing are the analyses if these Tregs play an active or passive role in PRRSV immunosuppression. In regard to immunological memory, LP infection induced the strongest overall memory immune response followed by HP infection and MLV vaccination. About 20% of the MLV vaccination-induced memory cells also reacted to the LP and HP strains. LP and HP strain-induced memory cells had about 20% cross-reactivity to the MLV PRRSV strain VR-2332 and about 80% cross-reactivity to the HP and LP strain, respectively.

Conclusions: This study provides a novel reference for better understanding the porcine immunological memory response to homologous and heterologous PRRSV strains and also induction of Tregs and immunosuppression. Future studies will address correlating specific characteristics of these memory cells with protection against homo- and heterologous PRRSV strains. These so-called immune correlates of protection will facilitate and improve vaccine development and the selection of protective PRRSV vaccines for emerging PRRSV strains.

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PRRS 02	Li, Yanhua	Interactome analysis of nsp1beta protein of porcine reproductive and respiratory syndrome virus	Kansas State University
PRRS 03	Black, Nicholas	Assessment of internal personnel movements on a U.S. swine farm using innovative technology	The Ohio State University
PRRS 04	Yan, Xingyu	Genetic characterization of emerging variants of porcine reproductive and respiratory syndrome virus (PRRSV) in the United States: new features of -2/-1 programmed ribosome frameshifting in nsp2-coding region	Kansas State University, College of Veterinary Medicine
PRRS 05	Zhang, Yanjin	PRRSV inhibits interferon-activated STAT2 signaling via nsp11	University of Maryland
PRRS 06	Balka, Gyula	Genetic diversity of PRRSV 1 in Central Eastern Europe in 1994–2014: origin and evolution of the virus in the region	University of Veterinary Medicine, Department of Pathology, Budapest, Hungary
PRRS 07	Balka, Gyula	SWINOSTICS – Swine diseases field diagnostics toolbox	University of Veterinary Medicine, Department of Pathology, Budapest, Hungary
PRRS 08	Paploski, Igor	Spatiotemporal patterns of different genetic subtypes of PRRS in pig movement networks	University of Minnesota, Department of Veterinary Population Medicine
~~**PRRS 09**~~	~~Lalonde, Christian~~ **WITHDRAWN**	~~Genomic characterization of porcine reproductive and respiratory syndrome virus (PRRSV) Quebec strains within clinical samples using whole genome sequencing~~	~~University of Montreal, Swine and poultry infectious diseases research center, Faculty of veterinary medicine~~
PRRS 10	Goss, Nikki	Biosecurity Risk Associated with Electrostatically Charged Synthetic Media Filters	AAF Flanders
PRRS 11	Bernal, Guillermo	Field evaluation of a modified-live PRRS Type II vaccine in PRRS control and reproductive performance in a positive herd in Northwest Mexico	MSD Salud Animal
PRRS 12	Constance, Laura	Fecal microbiota transplantation shifts microbiome composition and reduces morbidity and mortality associated with PCVAD	Kansas State University, College of Veterinary Medicine
PRRS 13	Kaeser, Tobias	Prediction of vaccine-induced long-term protection via advanced analysis of PRRSV-specific memory immune cells	North Carolina State University
PRRS 14	Madapong, Adthakorn	Interleukin-10 (IL-10) production and protective efficacy against concurrent infection with types 1 and 2 following intramuscular and intradermal vaccination with UNISTRAINÂ® PRRS in experimental pigs	Chulalongkorn University, Department of Veterinary Microbiology, Bangkok, Thailand
PRRS 15	Liu, Wenjun	Synthetic B- and T-cell epitope peptides of porcine reproductive and respiratory syndrome virus with Montanide ISA as adjuvant induced humoral and cell-mediated immunity	CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
PRRS 16	Chen, Yulu	The Genetic Basis of Natural Antibody Titers and Relationships with Disease Resilience in Pigs	Iowa State University
PRRS 17	Dong, Qian	Polymorphisms in the CD163, CD169, RGS16, and TRAF1 genes associated with host response to PRRS vaccination and co-infection with PRRSV and PCV2b in nursery pigs	Iowa State University
PRRS 18	Dunkelberger, Jenelle	Clinical signs of infection can be used to predict mortality and growth rate following PRRSV and PCV2b co-infection	Topigs Norsvin USA
PRRS 19	Pasternak, Alex	Measurement of soluble CD163 in pig serum to assess host responses following PRRSV infection	University of Saskatchewan, Western College Veterinary Medicine

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PRRS 20	Maciel Malgarin, Carolina	PRRSV-infected fetuses display metabolome alterations	University of Saskatchewan, Western College Veterinary Medicine
PRRS 21	Mora Diaz, Juan Carlos	Characterization of the immune response against porcine hemagglutinating encephalomyelitis virus in grow-finisher pigs	Iowa State University
PRRS 22	Wang, Xiuqing	Porcine reproductive and respiratory syndrome virus (PRRSV) interferes with the formation of stress granules induced by sodium arsenite and dithiothreitol	South Dakota State University
PRRS 23	Stoian, Ana	PRRSV-1 and PRRSV-2 recognition of peptide sequences in CD163 SRCR5	Kansas State University, College of Veterinary Medicine
PRRS 24	Stoian, Ana	SRCR4-5 inter-domain region is important for PRRSV-2 infection	Kansas State University, College of Veterinary Medicine
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PRRS 27	Fleming, Damarius	Pieces of a dream: A study in mapping transfer RNA fragments (tRFs) within the porcine genome	USDA/ORISE
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PRRS 29	Lunney, Joan	Evaluating the fetal and placental response to congenital PRRSV infection using NanoString transcriptomics	USDA ARS BARC Animal Parasitic Diseases Laboratory
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PRRS 31	Olcha, Matthew	Models for understanding African swine fever virus entry into cells	Kansas State University, College of Veterinary Medicine
PRRS 32	Frossard, Jean-Pierre	Evolution of the genetic diversity of PRRSV-1 strains in Britain occurs at a steadily increasing rate	Animal and Plant Health Agency, Virology Department, Weybridge, U.K.
PRRS 33	Henao-DÃaz, Alexandra	PRRSV oral fluid ELISA performance comparison among three commercial kits	Iowa State University
PRRS 34	Henao-DÃaz, Alexandra	Susceptibility of collared peccary (Pecari tajacu) to PRRSV	Iowa State University
PRRS 35	Rauh, Rolf	A Cost Effective Method for Surveillance of Influenza Viruses A, B, C and D in Swine Oral Fluids Using a Newly Developed Multiplex rRT-PCR.	Tetracore
PRRS 36	Wang, Yin	A Luminex multiplex assay for the detection of PRRSV, PCV2 and PCV3 and for PRRSV vaccine differentiation in the US	Kansas State University, College of Veterinary Medicine
PRRS 37	Wozniak, Aleksandra	Porcine circovirus type 3 (PCV3) and porcine circovirus type 2 (PCV2) detection in pig farms vaccinated and non-vaccinated against PCV2	Warsaw University of Life Sciences, Warsaw, Poland
PRRS 38	Yuan, Fangfeng	Isolation and characterization of a divergent strain of porcine sapelovirus from swine farm in US	Kansas State University, College of Veterinary Medicine
PRRS 39	Zheng, Wanglong	A multiplex real-time PCR assay for the detection and differentiation of five bovine pinkeye pathogens	Kansas State University, College of Veterinary Medicine
PRRS 40	Petrovan, Vlad	Diagnostic application of monoclonal antibodies against African swine fever virus	Kansas State University, College of Veterinary Medicine

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PRRS 41	Anderson, Tavis	The United States Swine Pathogen Database: Integrating diagnostic sequence data of emerging pathogens of swine.	National Animal Disease Center, US-DA-ARS, Virus and Prion Research Unit
PRRS 42	Jang, Guehwan	Sequence analysis and pathogenicity of porcine epidemic diarrhea virus isolates from the endemic outbreaks on Jeju Island, South Korea	Kyungpook National University, School of Life Sciences, Animal Virology Laboratory
PRRS 43	Jang, Guehwan	Molecular characterization of a novel recombinant enterovirus G inserting a torovirus papain-like protease gene associated with diarrhea in swine in South Korea	Kyungpook National University, School of Life Sciences, Animal Virology Laboratory
PRRS 44	Kimpston-Burkgren, Kay	AlphaLISA platforms for rapid and sensitive detection of PEDV antibody	Iowa State University
PRRS 45	Milek, Dagmara	Association between porcine reproductive and respiratory syndrome virus (PRRSV) and porcine parvoviruses 1-7 (PPV1-7) viremia in pigs	Warsaw University of Life Sciences, Warsaw, Poland
PRRS 46	Shang, Pengcheng	Characterization and application of a panel of monoclonal antibodies against the capsid protein of porcine circovirus	Kansas State University, College of Veterinary Medicine

PRRS 01

Global Picture of Nidovirus-Host Cell Interactions Revealed by Comparative Proteomics

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The porcine reproductive and respiratory syndrome virus (PRRSV) and the porcine epidemic diarrhea virus (PEDV) are responsible for severe economic losses and considered as the primary emerging livestock pathogens worldwide. Host-virus interactions are highly dynamic and may involve multiprotein complexes. Earlier, we and others showed that the composition of virus-host multiprotein complexes is controlled by viruses through a direct recruitment of the host proteins. Growing evidence indicates that extracellular microvesicles (EMV) play an important role in viral pathogenesis and modulation of host immune responses to infection. Consequently, the characterization of the molecular composition of the EMV of virus-infected cells and identification of the host proteins that are specifically encapsidated into or bound to virions are important for our further understanding of virus-host interactions. To accomplish this objective, we produced and purified PRRSV and PEDV virions and EMV using both the simian cell cultures and natural target cells (of porcine origin). We hypothesized that alterations in the proteomic profiles of PRRSV and PEDV virions and EMV will reflect changes in the environmental conditions (e.g., pH, cell-type). Furthermore, we hypothesized that the tight interactions between host and viral proteins defines the fate of infection and pathogenesis.

We examined the composition of progeny virions in order to identify cellular proteins that are associated with virions or EMV using state-of-the-art mass spectrometry (MS) strategies, including a high-resolution hybrid Quadrupole-Orbitrap MS. The present study has demonstrated the incorporation of numerous cellular proteins into PRRSV and PEDV virions. We found that the PRRSV and PEDV infections affected the abundance levels of numerous host proteins associated with EMV. More specifically, our proteomic data showed that the abundance of proteins involved in immune responses and metabolic processes was dramatically affected by PRRSV infection. The abundance of proteins involved in immune responses was also changed in PEDV infected cells. Interestingly, in PEDV infected cells, host proteins involved in cell cycle regulation and cytoskeletal system were affected in abundance, which is not surprising because several investigators have reported that cytoskeletal proteins are actively participating in moving the viral components to the assembly site, and that many viruses manipulate DNA repair and cell cycle in order to achieve a cellular environment favorable for their replication.

Further investigations are needed to evaluate the role of individual cellular proteins in the nidoviral replication, assembly, and pathogenesis.

PRRS 02

Interactome analysis of nsp1beta protein of porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economic important pathogens to pigs. As a replicase subunit involved in viral replication, PRRSV nsp1β is a major antagonist of host innate immune responses, as well as a key factor in the activation of -2/-1 programmed ribosomal frameshifting to translate PRRSV nsp2TF and nsp2N. Nsp1β also suppresses the expression of cellular factors by blocking the nuclear exporting of mRNA. To fulfill its multifunctionality, nsp1β interacts with a variety of cellular partners. In this study, we identified the interacting partners of nsp1β by employing affinity purification method and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Immunoprecipitation was performed to pull down the protein complex interacted with nsp1β using the cell lysate of HEK-293T cells expressing 3xFLAG-tagged nsp1β. A total of 813 proteins were identified by LC-MS/MS in the protein complex and among them 245 proteins were defined as the interacting partners of nsp1β. Bioinformatics analysis was performed to categorize these cellular factors. Based on KEGG pathway analysis, enriched pathways include ribosome, RNA transport, ribosome biogenesis in eukaryotes, spliceosome, RNA degradation, and mRNA surveillance pathway. Of note, in the enriched pathway of RNA transport, 13 proteins are components of nuclear pore, which are key factors in the process of mRNA nuclear exporting. Protein interaction networks were generated based on the STRING analysis; and we validated the interactions between nsp1β and several specific host factors involved in RNA transport. This study comprehensively profiled the interactome of PRRSV nsp1β in host cells, which may provide novel cellular target proteins for development of therapeutics and disease control strategies.

PRRS 03

Assessment of internal personnel movements on a U.S. swine farm using innovative technology

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Porcine reproductive and respiratory syndrome (PRRS) is an endemic viral disease of swine that leads to major economic consequences for the swine industry worldwide. Within-farm PRRS virus (PRRSv) transmission via fomite plays a major role for the spread and persistence of the virus in the herd, thus a strong biosecurity protocol is integral to the control and elimination of the virus. The amount of within-farm staff movement may be associated with time-to-stability for PRRS, but information for movements inside a swine farm is currently unavailable in the literature. This study aims to utilize beacon-sensing technology to estimate the quantity of between-room movement within a U.S. swine farm, particularly the frequency of movements that are known to facilitate PRRS virus dissemination; and to investigate whether there would be a difference in the amount of such "risky" movements before and after a study information session with farm employees.

A 4,400-sow farrow-to-wean commercial swine farm located in a high swine dense area with history of PRRS outbreaks was enrolled and an internal biosecurity system (B-eSecure®, PigCHAMP Pro Europa) was installed. Internet services were optimized throughout the farm and sensors (n=25) were placed in each room, including farrowing rooms, nursery areas, gilt development areas, gestation barns, load out areas, supply rooms, and the office. These sensors were set up to detect Bluetooth-based beacon devices, which were individually distributed to farm employees. Movement data was collected and sent to a central database. A movement was defined when an employee spent at least two minutes in one room from another room in the farm. A "risky" movement was defined when an employee moved from a shipping point or a nursery to other parts of the farm. The difference between the amount of "risky" movements pre- and post-information session was tested using the non-parametric Wilcoxon rank test in Stata-IC 14.

Across the 15-week study period, there was an average of approximately 1,841 (SD=352.4) and 263 (SD=50.3) total movements per week and per day, respectively. Movements categorized as "risky" occurred on average approximately 176 (SD=74.4) times per week and accounted for approximately 9.3% (SD=2.5%) of weekly movements. Frequency (P=0.64) and proportion (P=0.41) of "risky" movements did not differ significantly before and after the information session. However, there was a numerical reduction in the weekly average frequency and proportion of risky movements by approximately 15.2% and 13.1%, respectively. In conclusion, this study provided baseline information for the amount of overall and traditionally considered "risky" movements in a large-scale commercial swine farm in the U.S. Next steps include adding a larger period of time and two other farms to the analysis. The capability of capturing movement information will help veterinarians and producers to focus their efforts in PRRS control and elimination from swine herds.

Merck Animal Health provided funding for this study. PigCHAMP Pro Europa provided technological support for B-eSecure; and the means for all data collection and processing.

PRRS 04

Genetic characterization of emerging variants of porcine reproductive and respiratory syndrome virus (PRRSV) in the United States: new features of -2/-1 programmed ribosome frameshifting in nsp2-coding region

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PRRSV is one of the most economically important swine pathogens worldwide. Recent emergence of PRRSV variants caused increased economic loss in the US. In this study, we characterized emerging PRRSV variants isolated from swine farms in Midwest of US, in which pigs experienced PRRSV outbreaks since the end of 2016. Over 40% mortality in growing pigs and 20-30% reproductive failure in sows have been observed. Two viruses, KS 17-C1 and KS 17-C2 were isolated from serum samples of PRRSV-infected pigs. Full-length genome sequencing analysis showed that KS 17-C1 and KS 17-C2 have GP5 RFLP cutting pattern of 1-7-4 and 1-18-2, respectively; and they belong to two distinct phylogenetic clades, in which each associated with a group of emerging PRRSV strains that have been reported from pigs with severe clinical manifestations. Further in-depth sequence analysis revealed that the -2/-1 programmed ribosome frameshifting (PRF) signal located within nsp2 is the region where KS 17-C1 and KS 17-C2 differ the most from historical PRRSV strains. Our previous studies demonstrated that -2/-1 PRF generates two frameshifting products, nsp2TF and nsp2N, respectively. In the genome of historical PRRSV strains, the -1 ribosome frameshifting immediately encounters a stop codon, which terminates the translation of -1 reading frame to produce nsp2N. However, KS 17-C1 and KS 17-C2 isolates contain mutations disrupting the -1 PRF stop codon; therefore, extending the translation of nsp2N to generate additional 16 or 23 amino acids at 3'-end (nsp2N+16aa, nsp2N+23aa). The emergence of -1 PRF stop codon mutants was traced back in the PRRSV sequences published in the Genbank since 2011 and percentage of the mutants has quickly increased afterward (up to 69% of the sequences). More importantly, these -1 PRF stop codon mutants were all reported from swine farms experiencing PRRSV outbreaks with increased mortality/morbidity. Since the PRF products, especially nsp2N, have been identified as strong innate immune antagonists in our previous studies, current research is directed to determine whether nsp2N extension correlates with the increased pathogenicity of these viruses. Recombinant virus with restored -1 PRF stop codon was generated and compared with wild-type (WT) virus. Results showed that the mutant with restored -1 PRF stop codon induced higher levels of innate immune response, suggesting a possible link between nsp2N extension and pathogenicity of this group of newly emerging PRRSV variants in the US.

Key words: PRRSV, emerging variants, -2/-1 PRF, nsp2TF and nsp2N.

PRRS 05

PRRSV inhibits interferon-activated STAT2 signaling via nsp11

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Interferons (IFNs) play a crucial role in the antiviral response of the host innate immunity. IFNs activate JAK/STAT (Janus kinase/signal transducer and activator of transcription signaling) signaling pathway to induce expression of myriad antiviral genes. STAT2 is a key player in the IFN-activated JAK/STAT signaling. The objective of this study was to investigate the effect of porcine reproductive and respiratory syndrome virus (PRRSV) on STAT2 signaling. Here, we report that PRRSV downregulates STAT2 to inhibit the IFN-activated signaling. Both type 1 and type 2 PRRSV strains reduced STAT2 protein levels, whereas they had minimal effect on its transcript. The PRRSV infection led to STAT2 reduction in a dose-dependent manner as the STAT2 level decreased, along with incremental amounts of PRRSV inoculum. PRRSV infection shortened STAT2 half-life significantly. Treatment of the PRRSV-infected cells with the proteasome inhibitor MG132 restored STAT2 level, which indicates that the STAT2 reduction was through the ubiquitin-proteasome pathway. The PRRSV nsp11 was identified to reduce STAT2 level and the N-terminal domain of nsp11 interacted with STAT2 to promote its degradation. Together, these results demonstrate that PRRSV antagonizes IFN signaling by accelerating STAT2 degradation via the N-terminal domain of nsp11, in addition to the other mechanisms previously identified. This study provides further insight into the PRRSV interference with the IFN signaling and the consequent perturbation of the host immune response.

PRRS 06

Genetic diversity of PRRSV 1 in Central Eastern Europe in 1994–2014: origin and evolution of the virus in the region

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More than 20 years after the first outbreaks, the phylogenetic picture of PRRSV is still incomplete and full of gaps, especially in regards of PRRSV 1. Due to the exceptional diversity observed at the eastern borders of Europe and the low number of available sequences from Central Eastern European countries, the authors collected and analyzed both recent as well as already submitted sequences comparing them to a large backbone set of available ORF5 sequences representing the full spectrum of PRRSV 1 Subtype 1 diversity to conduct a systematic phylogenetic analysis and reclassification elucidating the diversity of the virus in these countries. Moreover, further analyses of the EUROSTAT data regarding the live pig movement trends revealed their influence of virus diversity evolution. The results indicate that besides the effect of local, isolated divergent evolution and the use of modified live vaccines, the most important factor influencing a given country's virus diversity is the transboundary movement of live, infected animals.

They have identified Subtype 1 strains only in the region. Even though all the countries were members of the USSR-led Council for Mutual Economic Assistance, the trade and movement of live pigs, pork and pork meet products were rather centripetal and directed towards the Soviet Union. This phenomenon seems to have limited the exceptional PRRS 1 diversity within the borders of USSR.

How did PRRSV 1 enter into Western Europe? The very first outbreaks of PRRS observed in the early 1990's coincided with an enormous increase of the wild boar population in Eastern Germany, that started after World War II. culminating in 1988. The situation was so dramatic that livestock keepers had to patrol their facilities to protect them from interlopers spreading diseases to their animals. The time and location of the very first PRRSV seropositive samples coincided both geographically (German Democratic Republic) and historically (1987) with this event. These events suggest a wild boar related link between Eastern Germany and the hypothetical location of PRRSV origin. Later evidences suggest that only the ancestors of Subtype 1 strains survived the bottleneck effect, and they established the whole PRRSV 1, Subtype 1 population in other parts of Europe and the world.

PRRS 07

SWINOSTICS – Swine diseases field diagnostics toolbox

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The increased population density in modern animal production systems has made them vulnerable to various transboundary infectious agents that threaten productivity of the meat industry. Early diagnosis and establishment of reliable countermeasures to infectious disease outbreaks is essential to limit severe biophysical and socio-economic consequences. The need for the development of mobile diagnostic systems, as point-of-care (POC) units has recently been recognized. Reliable and simple diagnostic testing directly on site would enable rapid local decision making which is crucial to prevent further spread of the disease.

SWINOSTICS addresses these challenges and needs, by developing a novel field diagnostic device, based on advanced, proven, bio-sensing and photonics technologies to tackle emerging and endemic viruses causing epidemics in swine farms in Europe that lead to relevant economic damages. The diagnostic device will allow immediate threat assessment at the farm level, with the analytical quality of commercial and institutional laboratories. The device will be portable and will provide results in 10 minutes for 5 samples simultaneously, making it highly suitable for use in the field. The modular construction of the device would allow future upgrades to increase capacity if so desired. The technology will depend on antigen recognition by the antibodies directed against selected swine viruses. The antibodies will be immobilized on the nano-photonic structure and the binding of the specific analyte (antigen) to them leads to a change in the chemical and physical properties of the photonic resonating structure, expressed as a variation of their refractive index. The amount of shift in the transmission response can be related to the concentration of the target antigen.

SWINOSTICS is being developed by a multi-disciplinary team, coordinated by CyRIC, Cyprus Research and Innovation Center Ltd, in the framework of EU's Horizon 2020 Programme. The project has just been launched (1st November 2017) and will run for three and a half years, to allow enough time for the development and real-world validation of the technology.
The overall concept underpinning the project is that of a device for early, field-based detection of important swine diseases (ASFV, PRRSV, H1N1, PPV, PCV2 and CSF). The device will use swine oral fluid samples as its main input, even though, it will be compatible with the use of other types of samples, such as faeces, blood or nasal swabs. The use of oral fluids as the main input diminishes the time needed for the analysis and simplifies the sample collection, allowing also the collection of wild boar samples.

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PRRS 08

Spatiotemporal patterns of different genetic subtypes of PRRS in pig movement networks

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Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most important diseases affecting the swine industry, with an annual economic impact in the U.S. exceeding $660 million. Despite this, factors associated with the occurrence and dynamics of specific genetic subtypes of PRRSV are not fully determined and could provide insights to transmission routes and possible interventions that could diminish the impact of PRRS in the United States swine industry. By utilizing a dataset of 1901 PRRS sequences voluntarily provided by Morrison Swine Health Monitoring Project (MSHMP) participants over 3 recent years, we described spatiotemporal patterns in the occurrence of different genetic subtypes of PRRSV and investigated the extent to which the network of pig movement between farms determines the occurrence of PRRS from similar genetic subtypes. We showed that PRRS genetic subtypes occurred at different frequencies across geographically overlapping production systems. The relative frequency in which specific genetic subtypes occur seem to be increasing (and others decreasing), and the rate at which that occurs seem to be system-specific. Some genetic subtypes were also more common in farms of specific production types (i.e. sow farm or nursery). As expected, farms that were connected via pig movements were more likely to share the same genetic subtype than expected by chance across all years. These findings suggest that system-specific characteristics at least partially drive PRRS occurrence over time and across farms of different production types. Our results also indicate that animal movement between farms is a driver of PRRS occurrence, strengthening this hypothesis of viral transmission. Additional research is needed to quantify risks and develop mitigation measures related to animal movement. Altogether, our analysis of complex PRRS sequence datasets can provide novel insights into the dynamics of PRRS transmission.

PRRS 10

Biosecurity Risk Associated with Electrostatically Charged Synthetic Media Filters

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Specific Objective

Electrostatically charged filters have been reported to lose efficiency while in service in swine barn environments. ASHRAE method 52.2 measures the overall efficiency of a filter by assigning a Minimum Efficiency Reporting Value (MERV) value. If a filter is non-uniform in charge or has a physical defect, the AHSRAE 52.2 method may not address these types of local inefficiencies as they will not be significant on the overall efficiency of the filter. This, however, leads to a false security of pathogen barrier efficiency based on a perceived MERV rating and barn breaks may occur. Leakage in the filters in the frame or media packs in actual practice are of concern for entry of pathogenic agents into swine barns.

Methods

In surface charged mapping, a Fieldmeter, like the Simco Electrostatic Fieldmeter FMX-004, is used to determine the variation of a charge across a filter. For synthetic filters, the charge is proportional to the local efficiency (spot efficiency). The filter frame can also carry a charge based on its composition. Two competitor final filters, one in a V-bank form and one in a box form, were analyzed using the surface charged mapping and the efficiency shifts when fully discharged.

Results

The V-bank filter was non-uniform with the lowest charge measured at the center of the media packs and surprisingly showed a very high charge on the plastic frame components. The box-type filter had a more uniform charge (than the V-bank media packs) although there was a charge bias across the pack. The discharge treatment efficiency dropped from 90% removal to 60% removal on both filters. Charge mapping was also carried out after the discharge, which indicated that the low charge regions in the V-bank filter were providing low efficiency filtration, putting the herd at risk.

Conclusions

Air filtration is a front-line defense against airborne transmission of PRRS and other pathogens. Effective air filtration utilizing the proper type of media reduces biosecurity risk and significantly impacts swine health, production and operating costs.

PRRS 11

Field evaluation of a modified-live PRRS Type II vaccine in PRRS control and reproductive performance in a positive herd in Northwest Mexico

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Introduction

For more than two decades-since 1992, Porcine Reproductive and Respiratory Syndrome virus (PRRSv) has caused severe economic losses to the Mexican pork industry. Efforts to eradicate the disease have yielded mixed results and acute outbreaks are still common. The preferred approach for PRRSv control in Northwest Mexico has been to maintain a uniform level of immunity through a combination of vaccination and live virus inoculation. However, these practices have not uniformly stopped PRRSv circulation and disease outbreaks still occur.

Objective

To evaluate the efficacy of a recently approved PRRSv vaccine (Prime Pac® PRRS, MSD Animal Health, Boxmeer, Netherlands) in sows and gilts in a highly PRRSv challenged operation located in Northwest Mexico by evaluation of reproductive performance and PRRSv farm status. The farm previously vaccinated with a different PRRS MLV vaccine.

Material and Methods

The study was conducted in a 1,600 sow, breed-to-wean farm. Monthly reproductive data over a 28 month period (January 2016 through April 2018) were evaluated. During this period, mass-vaccinations (1 mL by I.M. route) of the sow herd were done in months 5, 9 and 14 and 1,057 gilts were vaccinated (1 mL by I.M. route) prior to herd entry and then introduced in 3 batches in months 6, 10 and 12. To monitor PRRSv herd status by rtPCR, pre-suckle newborn and weaned pigs were bled during months 10 to 23. Sentinel, non-vaccinated gilts were introduced in month 19 and were tested for PRRS antibody by ELISA through month 24. Reproductive parameters included farrowing rate, total born, live born, mummies, stillborn and pre-weaning mortality per litter, non-productive days and pigs weaned per month.

Results

Reproductive parameters were compared before (months 1 to 14; Period 1) and after (months 15 to 28; Period 2) the third mass vaccination when sow mass-vaccinations, gilt vaccinations and replacement strategies were considered complete. The number of pigs weaned per month improved from 2,214 in Period 1 to 3,029 in Period 2 (+36.8%) due to increased total born (+11.5%) and weaned (+10.7%) pigs per litter, increased farrowing rate (+9.2%) and fewer non-productive days (-31.5%). All pre-suckle samples were negative and all weaned pig samples were negative except for months 12 and 19 where 4/12 and 3/36 pools, respectively, were positive. Sentinel gilts were ELISA negative at all time points after introduction.

Conclusion

The Prime Pac® PRRS vaccination strategy (mass-vaccination of sows and vaccination of gilts prior to entry) implemented in this sow herd was highly effective based on the significantly improved weaned pig output due to increased weaned pigs per litter, increased farrowing rate and reduced non-productive days, along with achieving provisional negative, stable herd status.

PRRS 12

Fecal microbiota transplantation shifts microbiome composition and reduces morbidity and mortality associated with PCVAD

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Porcine circovirus associated disease (PCVAD) is a term used to describe the multifactorial disease syndromes caused by porcine circovirus type 2 (PCV-2), which can be reproduced in an experimental setting through the co-infection of pigs with PCV-2 and porcine reproductive and respiratory syndrome virus (PRRSV). The resulting PCVAD affected pigs represent a subpopulation within the co-infected group. In co-infection studies, the presence of increased microbiome diversity is linked to a reduction in clinical signs. In this study, fecal microbiota transplantation (FMT) was investigated as a means to prevent PCVAD in pigs co-infected with PRRSV and PCV-2d. The sources of the FMT material were high-parity sows with a documented history of high health status and robust litter characteristics. The analysis of the donated FMT material showed the absence of common pathogens along with the presence of diverse microbial phyla and families. One group of pigs (n = 10) was administered the FMT while a control group (n = 10) was administered a sterile mock-transplant. Fecal microbiomes of the transplanted and control groups were analyzed before and after FMT or mock-transplantation by a pan-microbial array (LLMDA) and 16S rDNA sequencing. Compared to the control pigs, transplanted pigs had reduced species diversity in the families *Spirochaetaceae* and *Vibrionaceae* coupled with an increase in the relative abundance of the families *Veillonellaceae*, *Lachnospiraceae* and *Ruminoccaceae*. Over the 42-day post infection period, the FMT group showed fewer PCVAD-affected pigs, as evidenced by a significant reduction in morbidity and mortality in transplanted pigs, along with increased antibody levels. Overall, this study provides evidence that FMT leads to shifts in microbiome composition and abundance of several bacterial families that are associated with a reduction in clinical signs of PCVAD following co-infection with PRRSV and PCV-2.

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PRRS 13

Prediction of vaccine-induced long-term protection via advanced analysis of PRRSV-specific memory immune cells

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During the activation of an immune response to a pathogen like PRRSV, the immune system develops long-living memory cells to protect the host against the homologous and heterologous strains of that pathogen. Vaccination relies on the induction of these memory cells, but current testing of the vaccine-induced immune response in swine mostly relies on measuring immune effector molecules as antibodies or interferon gamma. Since non-memory cells also produce these effector molecules, their predictive value for long-term protection is limited. To overcome this limitation, we developed an *in vitro* cell culture system combined with multi-color flow cytometry to directly detect and differentiate various pathogen-specific memory immune cells. We also studied their production of effector molecules and homing patterns on a single cell level to predict how protective these memory cells are. This combination also enables us to estimate the duration of protection. So far, we deciphered the immune memory response of PRRSV vaccinated and infected animals to homologous and heterologous PRRSV strains using the MLV strain VR-2332, the low-pathogenic 1-3-4 strain, and the high-pathogenic 1-7-4 strain.

This system has immense potential to improve the pig as a large animal model, research on porcine host-pathogen interactions, and vaccine development. It has additional direct implications for the swine industry. In this presentation, I will give an overview of the methodology and the outcomes of the performed studies. The second focus of the presentation will be an outlook on our future studies and how this system can benefit academia, vaccine industry and swine producers. I will address how a collaborative effort between these three branches can use the system to limit the impact of PRRSV by advancing vaccine development and by predicting the most protective vaccine for newly emerging PRRSV strains.

PRRS 14

Interleukin-10 (IL-10) production and protective efficacy against concurrent infection with types 1 and 2 following intramuscular and intradermal vaccination with UNISTRAIN® PRRS in experimental pigs

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Abstract

Modified-live porcine reproductive and respiratory syndrome virus (PRRS MLV) vaccines has been used to control PRRSV infection worldwide, although incomplete protection is recognized. The administration of PRRSV MLV vaccines can yield undesired outcomes including delayed immune response and the induction of suppressive cytokines, in particular, IL-10. These undesired outcomes potentially resulted in the slow induction of effective immunity following vaccination. The administration through intradermal (ID) route using intradermic applicator has increasingly been used. Therefore, the study was conducted to evaluate the induction of IL-10 following intramuscular and ID routes. In addition, vaccinated pigs were co-challenged with types 1 and 2 to evaluate the protective efficacy. Eighteen-, 3-week-old, PRRS-free pigs were allocated into 3 separated groups (n = 6) including the UnVac, IM, and ID groups, respectively. Pigs in the UnVac group was left non-vaccination. Two groups were vaccinated with a modified-live PRRSV vaccine. The IM group was vaccinated intramuscularly with one dose (2 ml) of UNISTRAIN® PRRS (Laboratorios HIPRA S.A., Spain) and the ID group was intradermally vaccinated with one dose (0.2 ml) of UNISTRAIN® PRRS. Peripheral blood mononuclear cells (PBMC) were isolated at 0, 7, 14, 21, 28 and 35 days post vaccination (DPV). PBMC was stimulated *in vitro* with vaccine virus. Cell culture supernatant from stimulated PMBC were collected and quantified for porcine interleukin-10 (IL-10) using ELISA kit (Quantikine® ELISA porcine IL-10, R&D systems, USA). All pigs were intranasally challenge with types 1 and 2 at 35 DPV and necropsies at 42 DPV, 7 days post challenge (DPC). PRRSV induced pneumonic lung lesion was evaluated. The results demonstrated that both IM and ID vaccinated groups exhibited significantly higher IL-10 levels than that of the UnVac group at 7 and 14 DPV. The ID group showed relatively lower IL-10 level than that of the IM group. Following co-challenge with types 1 and 2, both vaccinated groups had significantly lower level of PRRSV induced pneumonic lung lesion compared to the UnVac group. The ID group had relatively lower lung lesion compared to the IM group. These findings demonstrated that ID vaccination with PRRS MLV induce lower IL-10 following vaccination and provide better protection compared to IM vaccination as demonstrated by lower lung lesion score.

PRRS 15

Synthetic B- and T-cell epitope peptides of porcine reproductive and respiratory syndrome virus with Montanide ISA as adjuvant induced humoral and cell-mediated immunity

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Abstract:

Current evidence suggests that either inactivated or attenuated vaccine only provided a limited protection against PRRSV infection. In the past decades, the synthetic peptide approach has been attracted great attention for vaccine development. A growing number of cell-epitope of PRRSV has been identified and closely related to the clearance of PRRSV infection. In the present study, two B- cell-epitope (BCE) and seven T- cell-epitope (TCE) of PRRSV and a Pan DR T-helper cell epitope were synthesized and mixed with Montanide ISA 61 VG as adjuvant, and the humoral and cell mediated immunity elicited by these peptides were evaluated in mice and piglets, respectively. In the mouse model, ELISA and serum-virus neutralization assay showed a high level of ELISA-antibody and neutralizing antibody (NA) induced by the BCE peptides, while Montanide ISA 61 VG could increase both for about 3-folder. Furthermore, lymphocyte proliferation and IFN-gamma /ELISPOT assays demonstrated that each TCE peptide could induce significantly peptide-specific cellular immune responses in the splenocytes of vaccinated mice. In the piglet model, no NA was detected even with a low detectable ELISA-antibody. However, virus-specific and TCE peptide-specific lymphocyte proliferative responses were detectable. More importantly, we found that Montanide ISA 61 VG could improve the production of IL-12 and TNF-alpha, decrease IL-4 and IL-10 in the serum of peptides-vaccinated piglets, showed a predominant Th1 type of immune response. These evidences may provide important information for the development of PRRSV epitope-based synthetic peptide vaccines and Montanide ISA may be used as an attractive immunomodulator in swine.

PRRS 16

The Genetic Basis of Natural Antibody Titers and Relationships with Disease Resilience in Pigs

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Disease resilience is the ability of an animal to maintain performance under pathogen exposure but is difficult to select for because breeding animals are typically raised in a high-health bio-secure environment. Selective breeding for resilience requires a trait that is heritable, easy to measure on healthy animals at a young age, and genetically correlated to general disease resilience. Natural antibodies (NAb) are an important part of the innate immune system and have been found to be heritable and associated with disease in dairy cattle and poultry. The objective of this study was to investigate NAb as potential predictors of disease resilience in pigs. Data used were from a natural disease challenge facility (multiple bacterial and viral pathogens, including PRRS), in which batches of 60 to 75 weaned Large White by Landrace barrows were sourced every three weeks (total of 28 batches and 1799 pigs) from healthy multiplier farms of PigGen Canada members. NAb were evaluated by indirect ELISA in blood samples collected at ~35 days of age, prior to entering the natural disease challenge. Levels of IgG and IgM NAb that bind four common antigens were obtained: lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan (PDG), and keyhole limpet hemocyanin (KLH). Disease resilience data were collected until the pigs reached market age (181±9 days of age). After quality control, genotypes on 498,714 single nucleotide polymorphisms (SNPs) were available on all pigs from the 650k Affymetrix chip. Genetic parameters were estimated by univariate and bivariate analyses in ASReml4, using an animal model with batch and entry age as fixed effects and litter as a random effect. Both single-marker regression (GenABEL) and Bayesian variable selection (GenSel) methods were used for genome-wide association studies (GWAS). Heritability estimates were lower for IgG NAb (0.03 to 0.22) than for IgM NAb (0.24 to 0.42) but maternal effects were much larger for IgG NAb (0.49 to 0.58) than for IgM NAb (0.04 to 0.12). Phenotypically, IgM titers correlated with each other (r = 0.26 to 0.71), as did IgG titers (r = 0.40 to 0.81), but correlations between IgM and IgG titers were low (r = 0.00 to 0.13). Genetic correlations showed similar patterns; they ranged from 0.44 to 0.99 among IgG, from 0.45 to 0.84 among IgM, and from -0.30 to 0.25 between IgG and IgM. Genetically, higher levels of NAb tended to be associated with greater disease resilience, measured by lower day-to-day fluctuations in feed intake under challenge, but had low genetic correlations with number of individual medical treatments, although standard errors were large. Both genetically and phenotypically, however, pigs that survived to market age had higher levels of several NAb than pigs that died prematurely. GWAS identified several genomic regions associated with NAb levels. In conclusion, levels of NAbs in blood of healthy young piglets are potential predictors of multifactorial disease resilience. Further study will focus on the biological significance of the identified genomic regions. This project was funded by Genome Alberta (ALGP2), Genome Canada, PigGen Canada, Swine Innovation Porc, and USDA-NIFA with grant number 2017-67007-26144.

PRRS 17

Polymorphisms in the CD163, CD169, RGS16, and TRAF1 genes associated with host response to PRRS vaccination and co-infection with PRRSV and PCV2b in nursery pigs

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Single nucleotide polymorphisms (SNPs) in immune response candidate genes *CD163*, *CD169*, *TRAF1* and *RGS16* were previously identified to be associated with response to PRRSV or PCV2 (*RGS16* only) infection. In addition, deletion of the scavenger receptor cysteine-rich 5 domain in the extracellular portion of *CD163* has been shown to cause complete resistance to PRRSV infection in pigs. Objectives of this study were to evaluate associations of nineteen naturally occurring SNPs in *CD163*, four in *CD169*, two in *RGS16*, and two in *TRAF1* with host immune response to PRRS vaccination and to PRRSV and PCV2b co-infection. Large White/Landrace crossbred nursery barrows from one genetic supplier in two trials of the PRRS Host Genetics Consortium were pre-selected for genotype at the WUR10000125 (WUR) SNP (n=184 AA and n=212 AB pigs), which has been shown to be associated with PRRSV viral load (VL, area under the curve) following infection. Pigs were randomly assigned to one of two rooms. Pigs in one room were vaccinated using a PRRS modified lived virus vaccine and four weeks later, all pigs were challenged with field isolates of PRRSV and PCV2b and followed for 42 days post infection (dpi). Blood samples were collected to quantify PRRSV VL post vaccination (VxPRRS) and PRRSV and PCV2b VL post co-infection (PostPRRS and PostPCV). Average daily gain (ADG) post vaccination/prior to co-infection (PreADG) and post co-infection (PostADG) were also evaluated. SNP genotyping was done using the Agena Mass Spec platform. Haploview 4.2 was used to quantify linkage disequilibrium (LD) between SNPs in the same gene. Two groups of four SNPs in *CD163* and two SNPs in *TRAF1* were in complete LD. Three other SNPs in *CD163* were fixed. Bivariate animal models were used to analyze PostPRRS, PostPCV, PreADG, and PostADG of vaccinated (Vx) versus non-vaccinated (Non-Vx) pigs as two separate traits using ASReml 4.1. PRRS VL following first exposure to PRRSV, either as a vaccine (VxPRRS) or through co-infection Non-Vx PostPRRS was also analyzed using a bivariate analysis. In all bivariate models, WUR genotype, genotype for one of the 17 candidate gene SNPs, and their interaction were fitted as fixed effects, along with trial, and the covariates of weight and age at -28 dpi and of PCV2b viremia at 0 dpi. An animal genetic effect, litter, and pen were fitted as random effects. Several SNPs were found to be significantly ($p<0.1$) associated with PRRSV and PCV2b VL, PreADG, and PostADG, the effects of some depended on previous vaccination for PRRS or WUR SNP genotype. Interestingly, depending on WUR genotype, five SNPs in *CD163* (four had $p<0.001$), three SNPs in *CD169* (one $p<0.001$), all SNPs in *TRAF1* and *RGS16* (two $p<0.001$) had a significantly ($p<0.1$) different effect on PRRS VL following first exposure to the virus. The identified SNPs can serve as genetic markers to select for increased natural resistance to PRRSV and PCV2. This project was funded by USDA-NIFA grant 2013-68004-20362. We also acknowledge contributions from members of the PRRS Host Genetics Consortium.

PRRS 18

Clinical signs of infection can be used to predict mortality and growth rate following PRRSV and PCV2b co-infection

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The ability to identify animals expected to have superior host response to PRRS, or co-infection of PRRS virus (**PRRSV**) with another pathogen, based on clinical signs of infection, would be an asset to management staff when making decisions regarding treatment intervention and animal care, as well as for selection and culling. The objectives of this study were to identify clinical signs recorded during the early phase or throughout the entire co-infection period that best predict mortality and growth rate following co-infection with PRRSV and porcine circovirus type 2b (**PCV2b**).Two groups of 200 commercial nursery pigs from the PRRS Host Genetics Consortium trials were used for this study. Shortly after weaning, pigs were randomly sorted into two rooms and all pigs in one room were vaccinated with a PRRS modified live virus vaccine. Pigs in the other room were not. Four weeks later, all pigs were co-infected with PRRSV and PCV2b and followed until 42 days post-infection (**dpi**). For each pig, incidence of each clinical sign, defined as the % of days that the clinical sign was observed, of the number of days that the pig was alive, was computed for veterinary treatment, pallor/jaundice, attitude, body condition (**BC**), lameness, joint effusion, dyspnea, open mouth breathing, nasal discharge, blue ear, muscle wasting (**MW**), coughing, and diarrhea. Weekly body weights were used to calculate average daily gain (**ADG**) as the regression of weight on day from 0 to 42 dpi. Multiple regression animal genetic models with backwards stepwise ($P<0.05$) selection were used to identify the set of clinical signs that best predicted: 1.) mortality between 0 and 21 dpi (**M0_21**), 2.) between 22 and 42 dpi (**M22_42**), and 3.) between 0 and 42 dpi (**M0_42**), and 4.) ADG from 0 to 42 dpi (**ADG0_42**). For incidence of clinical signs from 0 to 21 dpi, attitude best predicted M0_21 ($P=0.002$) while dyspnea and blue ear best predicted M22_42 ($P\leq0.02$). For incidence of clinical signs from 0 to 42 dpi, pallor/jaundice, attitude, BC, lameness, and MW best predicted M0_42 ($P\leq0.04$); these same signs, as well as joint effusion, open mouth breathing, nasal discharge, and coughing, best predicted ADG0_42 ($P\leq0.04$). Compared to a model fitting no clinical signs, final models for M0_21, M22_42, and M0_42 showed an increase in the area under the curve of the prediction model of 25.1%, 1.6%, and 11.1%, respectively. For ADG0_42, clinical signs fitted in the final model explained 37.5% of the total variation in ADG. For each analysis, all effects were in the expected direction (increased incidence was associated with increased probability of mortality or lower ADG), except pallor/jaundice for analysis of M0_42, and attitude, BC, lameness, MW, and coughing for analysis of ADG0_42. These unexpected effect directions resulted from high correlations with other clinical signs simultaneously fitted in the model. In conclusion, results show that clinical signs of infection can be used to predict mortality and growth rate following co-infection with PRRSV and PCV2b. Each clinical sign that best predicted M0_42 was also included in the set of clinical signs that best predicted ADG0_42, and therefore, may be used to evaluate pigs and facilitate management decisions and treatment intervention following PRRSV and PCV2b infection. This research was supported by USDA-NIFA grants 2012-38420-19286 and 2013-68004-20362.

PRRS 19

Measurement of soluble CD163 in pig serum to assess host responses following PRRSV infection

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The obligate role of CD163 in PRRSV infection has been definitively proven through the challenge of knockout pigs, however, should commercialization of such transgenic pigs prove difficult, alternative methods of controlling the PRRS virus will be essential. CD163 is a scavenger molecule found primarily on the surface of antigen presenting cells which play host to the virus, but under inflammatory conditions the protein is enzymatically cleaved releasing the extracellular region in soluble form. As the soluble form includes the PRRSV binding domain we hypothesize that the natural variation in this shedding mechanism, either at rest or in response to infection, will correlate with viral susceptibility either directly through interaction with the viral capsid or indirectly as an indicator of reduced cell surface abundance.

To quantify sCD163 levels in a population of sufficient size to effectively evaluate this hypothesis, a highly specific and low-cost assay was required. To this end, an array of commercially available monoclonal antibodies was tested to identify a matched pair with high affinity for porcine CD163 which were subsequently used to develop a quantitative ELISA protocol. The initial utility of the assay was demonstrated through the quantification of sCD163 in cell culture supernatant following PRRSV infection of alveolar macrophages. Prior to *in vivo* application, the impact of sample quality and storage was evaluated and found to have limited impact provided the serum was effectively separated prior to freezing. Finally, the specificity of the assay was verified using serum from CD163 wildtype and knockout pigs generously provided by Dr. R. Rowland (Kansas State University).

In order to establish the temporal dynamics and degree of natural variation in serum sCD163 levels among commercial swine, serum was collected from animals across the production cycle on three high health farms. A significant temporal effect was found in feeder pigs with sCD163 levels decreasing between 3-4 weeks (pre-weaning) and 8 weeks of age, after which levels appeared to stabilize. In gestating females, no significant difference in average sCD163 levels over parity was identified; however, an increase in variation at higher parity (3-5) driven by the presence of “high shedders” was noted. Interestingly, once age effects were accounted for, no significant difference in sCD163 levels was found between farm despite the presence of different genetic lines.

Finally, to evaluate the relationship between serum sCD163 levels and response to PRRSV infection, a time course of archived sera samples from past respiratory (PRRS Host Genome Consortium) and reproductive (Pregnant Gilt Model) challenges was assessed. Within both challenge experiments significant variation in serum sCD163 levels at rest and in response to infection were identified. In the respiratory model, levels of sCD163 appear to decrease over the 11-day time course following infection. In contrast, levels appear to initially increase among pregnant gilts out to day 6 post infection and decrease thereafter in a pattern congruent with viral load. Analysis of sCD163 levels in relationship to susceptible, resistant and resilient phenotypes identified in these experiments is currently underway.

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PRRS 20

PRRSV-infected fetuses display metabolome alterations

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PRRSV infection in pregnant sows can lead to fetal death and abortions, although the mechanisms triggering these effects are not well understood. Since resistant and susceptible fetuses can coexist in the same litter, we propose that there may be differential mechanisms used by some fetuses to evade infection and/or disease progression. Metabolomic studies are used to identify alternations in the biochemical pathways that are occurring in animals of divergent phenotypic groups and help elucidate pathophysiological mechanisms of disease. Our objectives were to investigate possible differences in the metabolome of PRRSV-infected and non-infected fetuses to elucidate possible causes of fetal death following PRRSV infection.

Serum samples collected from fetuses on gestation day 106, twenty-one days post PRRSV-2 infection, were processed at The Metabolomics Innovation Centre, University of Alberta, by direct flow injection mass spectrometry (DI-MS) and nuclear magnetic resonance (NMR) techniques. Twenty-four fetuses were selected from each of four phenotypic groups as follow: 1) fetuses from non-inoculated gilts (CON); 2) fetuses from inoculated gilts that escaped infection (UNINF); 3) infected high viral load viable fetuses (HVL); and 4) infected high viral load meconium-stained fetuses (MEC). Univariate (ANOVA followed by Tukey) and multivariate statistical analyses (PCA and PLS-DA) were performed on log transformed, normalized data to determine group differences and the most relevant metabolites associated with disease progression.

In total, 140 metabolites were detected. Significant differences in the metabolome were observed, especially between PRRSV-negative fetuses (CON and UNINF) and MEC fetuses, while HVL fetuses appear to span both groups. The two metabolites with highest variable importance in projection (VIP) scores were alpha-aminoadipic acid (alpha-AAA) and kynurenine (KYN), having the highest concentration in MEC and HVL fetuses, respectively, compared to CON and UNINF. Alpha-AAA belongs to the lysine degradation pathway and, when in high concentration, can lead to acidosis and possibly death. While KYN belongs to the tryptophan metabolic pathway and increased levels are associated with chronic neurological diseases in humans, it is not yet clear how this metabolite might cause fetal death in pigs. The high concentration of KYN in HVL and MEC fetuses, may in fact be the biproduct of a fetal immune response to PRRSV infection, since a precursor of the KYN conversion, indoleamine 2,3-dioxygenase (IDO1), which converts tryptophan into KYN, is induced by IFN-gamma, TNF-alpha, and IFN-alpha. Another enzyme, kynurenine aminotransferase (KAT II), which is present in both pathways and involved with the degradation of alpha-AAA and KYN, is also being investigated. A deficit of this enzyme could explain the higher concentrations of both metabolites and may indicate one factor of fetal PRRSV susceptibility. Pathway analyses are ongoing as well as other assays to confirm the differences between infected and non-infected fetuses cited above.

In conclusion, there are significant differences in metabolomes of PRRSV-infected and non-infected fetuses. How these differences potentially lead to fetal death during PRRSV infection are being evaluated and the most significantly affected metabolic pathways are being deeply investigated. More conclusive results will be available in the next few months. This research was funded by Genome Canada and Genome Prairie (Sask. Ministry of Agriculture).

PRRS 21

Characterization of the immune response against porcine hemagglutinating encephalomyelitis virus in grow-finisher pigs

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Introduction

Porcine hemagglutinating encephalomyelitis virus (PHEV) is the only known neurotropic coronavirus of pigs. PHEV can infect naïve pigs of any age, but clinical disease is age-dependent. In growing pigs and adults, PHEV infection is subclinical, but acute outbreaks of vomiting and wasting syndrome and encephalomyelitis may be seen in neonatal pigs born from naïve sows, resulting in mortality of up to 100%. In this study, we characterized the viral dynamics and immune response in 7-week-old pigs over the course of PHEV infection.

Material and Methods

The study included a PHEV inoculated group (n=12) and mock inoculated negative group (n=12). Viral shedding was evaluated daily using pen-based (6 pens, 2 pigs per pen) oral fluids and feces throughout the study. Serum samples were collected at -7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42 days post-inoculation (DPI) to evaluate both viremia and humoral immune response by real-time RT-PCR and a protein-based indirect ELISA, respectively. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood on 0, 3, 7, 10, 14 and 21 DPI to evaluate cellular immune response.

Results

Mild neurological signs, including tremor and generalized muscle fasciculation, were reported in 2/12 pigs at 4-6 DPI. Virus shedding was consistently detected by real-time RT-PCR assay in pen oral fluids (DPI 1-28) and feces (DPI 1-10). Viremia was not detected throughout the observation period. Isotype-specific antibody responses in serum showed a strong IgM response at 7 DPI that declined quickly after 14 DPI. Strong IgA and IgG responses were detected by DPI 10 and declined gradually after 28 DPI. Flow cytometry analysis revealed an increase on both monocytes (DPI 10) and cytotoxic T cell (DPI 21) populations in response to PHEV infection.

Conclusions

This study describes the humoral and cellular immune responses and PHEV shedding patterns in PHEV-naive pigs. This information will be immediately useful in developing approaches for detecting and monitoring PHEV infections and in understanding immunity against the virus.

PRRS 22

Porcine reproductive and respiratory syndrome virus (PRRSV) interferes with the formation of stress granules induced by sodium arsenite and dithiothreitol

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Stress granules are dynamic membrane-less cytoplasmic ribonucleoprotein complexes formed under stress conditions such as virus infections to regulate translation and cell signaling. However, some viruses evolved strategies to inhibit formation of stress granules to facilitate virus replication. Ras GTPase-activating protein-binding protein 1(G3BP1) is a nuclear RNA binding protein and an essential structural component of stress granules. Many viruses target G3BP protein to suppress stress granule formation. Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-sense single-stranded RNA virus and belongs to the *Arteriviridae*. PRRSV transiently induces stress granules in some infected MARC-145 cells during early infection. PRRSV modulates the expression of G3BP through unknown post-transcriptional mechanisms. Furthermore, PRRSV inhibits the formation of stress granules induced by treatment of cells with either sodium arsenite or dithiothreitol. Our data suggest that PRRSV may interfere with stress granule formation by manipulating the expression of G3BP, a key component of stress granules.

PRRS 23

PRRSV-1 and PRRSV-2 recognition of peptide sequences in CD163 SRCR5

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CD163, a receptor for PRRSV, is a protein composed of nine scavenger receptor cysteine-rich (SRCR) domains anchored by a single transmembrane segment and followed by a short cytoplasmic domain. Work by us and others, showed that deletion of the SRCR5 domain is sufficient to prevent infection of CD163-transfected cells with PRRSV-1 and PRRSV-2 isolates. The overall goal of this research is to identify the minimum changes in CD163 sufficient to make HEK293T cells expressing modified CD163 receptors resistant to infection with PRRSV-1 and PRRSV-2. The approach was to conduct insertional mutagenesis along the peptide sequence by the insertion of proline-arginine (PR) dipeptides about every 10 amino acids along the 101 amino acid SRCR5 peptide sequence. When placed in the same reading frame, the SacII sequence, CCG CGG, codes for a proline-arginine. The model system consisted of transfecting HEK cells with a plasmid that expressed modified CD163 SRCR5 as an EGFP fusion protein. Cells were infected with either PRRSV-1 (Lelystad) or PRRSV-2 (P129-RFP). For the infections using PRRSV-1, the results were visualized by IFA staining using an antibody recognizing PRRSV-N protein. For the PRRSV-2 infections, a positive result was visualized as cells expressing both green and red fluorescence. The results for both viruses showed a wide range of infection rates; from mutations that had little effect compared to others that almost completely blocked infection. When tested for PRRSV-2 infection, three PR insertions, located at positions 9, 48, 55 and 100 of SRCR5, produced the greatest reduction in infection, with only a small percentage of transfected cells showing infection after 48 hrs. Growth curves and limited dilution titration studies confirmed the negative impact of the PR insertions. Computer modeling showed that the PR insertions affecting PRRSV-2 infection appeared to form a well-defined binding pocket consisting of antiparallel β4 and β7 strands along with two opposing loop structures, located between β1/β2 and β4/β5 of SRCR5. On the other hand, the results from the PRRSV-1 infection identified only two PR insertions, located at positions 58 and 100, which produced the greatest reduction in infection. The PR insertion at position 100 was shared by both viruses. Overall, the infection results show differences and similarities in the recognition of CD163 SRCR5 by PRRSV-1 and PRRSV-2 and can be applied to the further characterization of contact regions and structural requirements in CD163 involved in PRRSV infection.

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PRRS 24

SRCR4-5 inter-domain region is important for PRRSV-2 infection

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CD163, a receptor for PRRSV, is a protein composed of nine scavenger receptor cysteine-rich (SRCR) and two proline-serine-threonine (PST) domains. Previous studies by our lab and others demonstrated that deletion of the SRCR5 domain prevents infection. However, the analysis of several CD163 modifications showed that some SRCR5 deletion constructs retained the ability to sustain a low-level of infection. The unique aspect of these deletion infection-positive constructs was the retention of the peptide sequence AHRK, which forms the SRCR4-5 inter-domain region. The purpose of this study was to explore the potential role of the SRCR4-5 inter-domain region in PRRSV-2 infection. Mutations were placed in a CD163 cDNA sequence fused to EGFP and expressed in pCDNA3.1. HEK cells were transfected with the CD163-EGFP constructs and expression confirmed by EGFP fluorescence microscopy. The permissiveness of each mutation was assessed by infection with a PRRSV-RFP virus. At 2 days after infection, a positive result was visualized as the expression of both green and red fluorescence. The results showed that the deletion of the inter-domain region or replacement with AAAA made transfected cells resistant to infection. Alanine scanning showed that the constructs AAAK and AARA produced a negative effect on infection, suggesting that histidine in combination with an arginine or proline were required for infection. However, the insertion of alanine to form the constructs, AHARK and AHRAK also showed a reduction in infection. Even though it appears that histidine, arginine and lysine are important, the exact structural requirements that contribute to how the SRCR4-5 inter-domain region participates in infection remains unclear.

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PRRS 25

Survival of African swine fever virus (ASFV) in feed ingredients under transboundary shipping conditions

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African swine fever virus (ASFV) causes a highly contagious disease in swine that threatens the pork industry worldwide. Since 2007, ASFV has been detected in Eastern Europe and the Caucus region, increasing the risk of spread globally. The goal of this study was to evaluate the survival of ASFV in animal feed ingredients that are imported daily into the United States under simulated transboundary shipping conditions. Virus survival was evaluated using a Trans-Atlantic transboundary model involving 11 representative feed ingredients, transport times and environmental conditions, with samples tested by polymerase chain reaction (PCR), virus isolation (VI) and/or swine bioassay. Controls included complete feed (positive and negative controls) and a stock virus positive control (virus only, no feed matrix). Briefly, 5g of each ingredient were inoculated with 10^5 $TCID_{50}$ of the contemporary strain, ASFV Georgia/07. The PCR data showed consistent inoculation and nucleic acid stability across all feed ingredients during the 30-day transboundary model, along with PCR-negative results for the negative controls. Viable ASFV was detected by VI at 30 days post-inoculation (DPI) in 8 tested ingredients as well as both positive controls, with mean titers ranging between 10^2 and 10^3 $TCID_{50}$. Both VI and swine bioassay failed to demonstrate infectivity of ASFV in 3 ingredients, including dried distillers' grains, lysine and vitamin D. Our data support the conclusion that ASFV maintains viability in varying environmental conditions, even in the absence of a protective feed matrix. This study provides additional information supporting the hypothesis that feed ingredients may play a role in the transboundary movement of foreign animal diseases, such as ASFV.

PRRS 26

Co-infection between porcine epidemic diarrhea virus and porcine deltacoronavirus enhances severity of clinical disease in neonatal piglets

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Abstract

Swine enteric coronavirus disease is caused by coronavirus in family *Coronaviridae* including porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV) and transmissible gastroenteritis coronavirus (TGEV). The three viruses cause similar clinical abnormalities including diarrhea and mortality of which are difficult to visually differentiate. Infected piglets display watery diarrhea leading to severe dehydration and death. The virus infects epithelial cells resulting in villi atrophy and malabsorption. Since the emergence of PEDV in 2007, the Thai swine industry has experienced repetitive outbreaks of PEDV. The emergence of PDCoV in 2016 rather complicate disease outbreaks in a herd of which PEDV and PDCoV are concurrently detected. Understanding of cohabitation of PEDV and PDCoV and outcomes following co-infection with the two viruses in piglets becomes interesting questions. Therefore, we investigated severity of clinical diseases in pigs experimentally co-infected with of PEDV and PDCoV. 21 genes associated with immune modulation and expression of cytokine were additionally evaluated. In the study, 3-day-old pigs were orally inoculated with PDCoV, and PEDV G1 or G2. Fecal score, clinical diseases and fecal shedding were daily monitored. Pigs in all groups were necropsied at 3 and 5 days post inoculation (dpi). All infected piglets displayed watery diarrhea. Co-infection exacerbates clinical severity compared to single infection. Intestinal walls in all infected pigs were thin and transparent, and severe villous atrophy in all parts of small intestine was observed on 3 and 5 dpi. Moreover, we found that co-infected piglets demonstrated slower recovery of villous height/crypt depth (V/C) ratio of middle jejunum than single infection. The PEDV shedding was not statistically difference between single PEDV-infected and co-infected groups. However, PDCoV shedding was significantly decreased when piglets were co-infected with either PEDV varinat. Gene expression demonstrated that infected piglets in all groups, excepted PDCoV and G1 PEDV co-infection group, up-regulated interferon alpha (IFN-α) expression at 3 dpi. Single PDCoV and G1 PEDV infection group could induced IFN-α expression at 5 dpi. PDCoV and G2 PEDV co-infection statistically significant induced tumor necrosis factor-α (TNF-α) expression at 3 dpi. However, infected piglets in all groups could be induced expression of TNF-α at 5 dpi. Co-infection groups showed lower expression level of interleukin 6 (IL-6) and IL12 than single expression groups at 5 dpi according to lower level expression of NF-κB. In conclusion, concurrent infection with PEDV and PDCoV can develop and can exacerbate the severity of clinical diseases compared to infection with either one. In addition, the concurrent infection down-regulated the expression of IFN-α.

PRRS 27

Pieces of a dream: A study in mapping transfer RNA fragments (tRFs) within the porcine genome

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Advancements in the study of porcine immunogenetic responses to highly pathogenic porcine reproductive and respiratory virus (HP-PRRSV) infections have served to uncover a litany of small non-coding RNAs involved in fostering tolerance or susceptibility to illness. Small non-coding RNAs (sncRNA) function as regulators of gene expression and epigenetic modifications. Some well-studied sncRNAs, such as miRNAs and tRNAs, have emerged as important modulators of the immune system and homeostatic regulation during HP-PRRSV infections. Unfortunately, there is little known about another class of sncRNA related to tRNAs referred to as tRNA fragments (tRFs) that can be similar in both size and function to miRNAs. It is possible that these tRFs, like miRNAs, also have modulating effects on the immune response in virally infected livestock. Regrettably, to date no list of expressed tRF molecules have been mapped for healthy or sick pigs; making it difficult to interrogate their expression during HP-PRRSV infections. Therefore, a study was undertaken to identify and quantify tRF expression in HP-PRRSV infected pigs.

The study was conducted using porcine whole blood of 24 pigs split into two treatment groups (control/infected) and three time points (1, 3, and 8 dpi) to examine tRF differential expression. Discovery and mapping of possible porcine tRFs from the S.scrofa 10.2 reference genome were conducted using a comparative genomics approach.

The results of the study showed that all five tRF classes (5', 3', 5'-halves, 3'-halves, i -tRFs) could be identified in both healthy and infected porcine blood and that expression levels differed between treatments.

The results from this study highlights changes in tRF expression that has the potential to unlock new targets for understanding the effect HP-PRRSV has on the porcine immune response. Future studies will look to apply these methods to the updated porcine reference genome.

PRRS 28

Essential role of mitogen-activated protein kinase (MAPK) signaling pathways in porcine deltacoronavirus replication

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The mitogen-activated protein kinase (MAPK) cascade pathways are central building blocks in the intracellular signaling network. The extracellular signal-regulated kinase (ERK), p38 MAPK and JNK1/2 are three major signaling pathways that play important roles in the regulation of various cellular processes. Since viruses are obligate intracellular parasites, they have developed several strategies to manipulate a variety of host cell signal transduction pathways for successful virus survival. To date, however, little is known about the intracellular signaling mechanisms involved in porcine deltacoronavirus (PDCoV) replication. The present study was conducted to determine whether such signaling pathways are involved in PDCoV replication. We found that PDCoV infection induces the activation of ERK1/2 by 9 h post-infection (hpi). In particular, UV-irradiated inactivated PDCoV, which is capable of allowing viral attachment and internalization but incapable of pursuing viral gene expression, was sufficient to trigger ERK1/2 phosphorylation, suggesting that PDCoV-cell interaction is responsible for its activation. Direct inhibition of ERK activation by chemical inhibitors, PD98059 and U0126, significantly suppressed PDCoV replication by affecting viral RNA synthesis, viral protein expression, and progeny release. Furthermore, PDCoV infection was found to triggers phosphorylation of both p38 MAPK and JNK1/2 pathways at 9 hpi. Pharmacological suppression of p38 and JNK1/2 activation by each specific inhibitor, SB202190 for p38 and SP600125 for JNK1/2, reduced PDCoV replication. We previously demonstrated that PDCoV induces caspase-dependent apoptosis through the activation of cytochrome *c* (cyt c)-mediated intrinsic mitochondrial pathway to facilitate viral replication *in vitro*. Next, it was investigated whether MAPK activation is associated with the PDCoV-induced cyt c-mediated apoptosis pathway. Results of experiments to assess the correlation between PDCoV-induced MAPK and apoptotic cell death pathways will be discussed. Taken together, our data suggest that the MAPK signaling pathways play critical roles in post-entry steps of the PDCoV life cycle and beneficially contributes to virus replication.

PRRS 29

Evaluating the fetal and placental response to congenital PRRSV infection using NanoString transcriptomics

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Purpose: A persistent question in the study of reproductive PRRS is whether fetal mortality is the result of viral disruption of placental function or aberrant fetal immune response to infection, subsequently leading to placental disfunction or disruption of normal fetal homeostasis. To better understand this, we have evaluated transcriptomic responses in fetal and placental tissues following maternal PRRSV challenge.

Methods: Landrace gilts were inoculated with PRRSV at day 86 of gestation along with 5 control gilts. Gilts (3 infected and 1 control) were euthanized at 2, 5, 8, 12 and 14 days post infection (dpi) and tissues collected from the gilts and every fetus. Tissues were frozen, and PRRS viral load quantitated in thymus and in placenta (PLC) that were separated from the endometrium. RNA was extracted from homogenized tissue using Qiagen RNA Isolation kit and integrity affirmed using an Agilent 2200 Bioanalyzer. Differential expression (DE) of genes was evaluated using a 230 gene NanoString array (designed on biomarkers previously predicted to alter PRRS resistance and susceptibility). Based on log viral load PLC samples were assigned to 3 experimental groups: ND (none detected) (PLC=0, N=12), LOW (PLC >1 & <3.9, N=30) and HI (PLC >3.9, N=34). For fetal thymus (THY) equivalent groups were selected based on a combination of THY and Serum (SER) viral load: ND (THY & SER virus=0, N=22), LOW (THY=0, serum >1 & < 5, N=22) and HI (THY>4 and SER>5, N=11). The resulting data was normalized using a combination of NanoString positive control probes and 9 housekeeping genes, and a univariate analysis conducted using a Generalized Least Squares (GLS) model with false discovery rate (FDR) correction.

Results: In the PLC, while no genes were found to be significantly altered between the ND and LOW group, a total of 52 genes were found to be significantly upregulated between ND and HI. In the THY, a total of 197 and 84 genes were found to be differentially expressed between ND-LOW and ND-HI, respectively. In comparing the response between PLC and THY, 35 genes were found to be commonly differentially regulated showing a clear transcriptomic response to type 1 interferons (IFNs) including upregulation of STAT1-3 and IFN response genes including IFIT1-3, IFIHI, IRF1&5 and GBP5 among others.

Conclusions: Gene selection for the NanoString array was effective in distinguishing PLC and THY immune responses to PRRSV infection. Efforts are continuing to assess the impact of viral load in PLC and thymuses to distinguish the effect of viral infection and cross placental transmission on fetal survival and local immune responses.

PRRS 30

Adaptation of PRRSV-2 to cells expressing mutant CD163 receptors

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CD163, a receptor for PRRSV, is a protein composed of nine scavenger receptor cysteine-rich (SRCR) domains anchored by a single transmembrane segment and followed by a short cytoplasmic domain. CD163 on macrophages is important for the removal of hemoglobin in the blood and participates in the regulation of the inflammatory response by M2 macrophages. One goal of our research is to identify the minimum changes in CD163 sufficient to make CD163-modified pigs resistant to PRRSV while retaining all of the important biological functions of the CD163 protein. However, one concern is that PRRSV, an RNA virus can rapidly mutate and as a result readily adapt to mutant CD163 receptors. Mutations in CD163 were prepared by inserting proline-arginine (PR) dipeptides along SRCR5. When placed in the same reading frame, the SacII sequence, CCG CGG, codes for a proline-arginine. Permanent CD163-expressing cell lines were constructed by fusing the mouse kappa light chain signal sequence to residues 47 to 1115 of porcine CD163 (NCBI Accession AAZ50616). Mutant variants of the CD163 cDNA were generated by inserting proline arginine dipeptides sequence after CD163 residues 484 (PR-9), 530 (PR-55), 575 (PR-100), 497 (PR-22), 507 (PR-32), 513 (PR-38), 517 (PR-42), 529 (PR-58), or 538 (PR-67). All mutations were within the SRCR5 domain of CD163. A wild-type CD163 was included as a positive control. The synthesized DNA segments were subcloned into a mammalian expression vector that contained a puromycin selection cassette. To increase expression of the CD163, the vector was modified to contain HS4 chromatin insulators and Piggybac transposon ITR elements flanking the CD163 expression cassette. Plasmids were co-transfected into HEK 293 cells along with a plasmid expressing Piggybac transposase. After transfection, the cells were maintained in selection medium containing puromycin and cells were passaged in selection medium for 2 to 3 weeks. CD163 expression was verified by immunostaining with anti-CD163 monoclonal antibody followed by flow cytometry. After 3 weeks, all cell lines expressed CD163 on their surface to equivalent levels. Confluent monolayers were infected with a PRRSV-2 isolate that expressed a red fluorescent protein (RFP) cDNA as a separate subgenomic mRNA fragment. Results showed that all cell lines supported PRRSV-RFP infection. Three CD163 constructs, PR-22, PR-58, and PR-67, which supported infection at 2 logs below the wild-type CD163 were selected for further study. Viruses derived from each cell line were designated PRRSV-22, -58, -67 and -WT. Viruses were successfully passaged at least 3 times. ORFs 2-6 of each virus are being sequenced to identify mutations associated with adaptation to each cell line.

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PRRS 31

Models for understanding African swine fever virus entry into cells

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In the absence of a vaccine, control measures that target African swine fever virus (ASFV) replication steps are being actively pursued. A significant body of work identified CD163 as required for ASFV cell entry; however, macrophages lacking CD163 and CD163 knockout pigs support infection. ASFV also utilizes macropinocytosis, a non-specific cellular uptake pathway, to enter the cell, which may explain why CD163 KO pigs can be infected with ASFV. The purpose of this study is to evaluate the effect of macropinocytosis (non-specific uptake) and receptor-mediated endocytosis inhibitors on ASFV replication using a Vero-adapted isolate, BA71V. For the purpose of comparison, the same compounds were tested on BHK-21 cells infected with vaccinia virus (VV). Macropinocytosis inhibitors included EIPA, cytochalasin D, and wortmannin. Inhibitors of endocytosis included chlorpromazine, dynasore, ML-7, and nocodazole. Cells were treated with different concentrations of drug and infected with virus. The media was changed after 6 hours and 24 hours later the plates were fixed, stained with antibody, and counted under a fluorescence microscope. The results are presented as percent reduction in infection compared to untreated controls. The inhibitory effects of the various compounds are summarized in the table below. The results show that the response of VV and ASFV to inhibitors share several similarities. Together, the results support previous studies in the literature on the effects of the same inhibitors and provide model systems for understanding the roles of receptor and non-receptor mediated mechanisms involved in the ASFV infection of macrophages. Future work is focused on evaluating the effect of compounds on macrophages lacking different surface proteins.

Compound	Target(s)	Concentration and percent reduction in infection				
		Vaccinia			ASFV	
		uM	%		uM	%
EIPA	Na+/H+ antiport	25.0	80		25.0	42
Cytochlalasin D	Actin polymerization	12.5	51		10.0	62
Wortmannin	Phosphatidylinositol-3 kinase	10.0	41		10.0	72
Chlorpromazine	Adaptor protein-2	25.0	81		15.0	81
Dynosore	dynamin1, 2, DRP1	25.0	23		100	67
ML-7	Myosin	12.5	69		12.5	54
Nocodazole	Microtubule formation	50.0	22		50.0	71

PRRS 32

Evolution of the genetic diversity of PRRSV-1 strains in Britain occurs at a steadily increasing rate

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PRRS was first seen in Great Britain (GB) in 1991, since when it has become one of the most important endemic diseases of GB pigs targeted for control. To date only PRRSV-1 has been detected in GB pigs, and only vaccines derived from PRRSV-1 strains are licensed for use. Molecular epidemiology is now used by some veterinarians and pig farmers to inform epidemiological investigations and control strategies, so ORF5 sequencing is often carried out following positive PRRSV PCR results. Surveillance funding from Government has also allowed periodic sequencing of PRRSV from disease outbreaks, and both are sources of sequence data from 1991 to 2018, allowing the diversity of the PRRSV-1 populations circulating in GB over a 27 year period to be characterised.

A total of 803 complete ORF5 sequences derived from PRRSV-1 strains detected in serum and tissue samples of GB pigs have been analysed. These were aligned using the ClustalW algorithm, and the pairwise distances were determined using MEGA7. These were also aligned with 542 published non-British PRRSV-1 ORF5 sequences and a representative PRRSV-2 sequence, followed by phylogenetic analysis using the Neighbor-Joining method with evolutionary distances computed using the Kimura 2-parameter method. Inferred GP5 amino acid sequences for the British viruses were also examined and compared.

The phylogenetic analysis showed no evidence of distinct novel PRRSV-1 incursions into Great Britain since the mid-1990s. Rather, it appears that the viral populations currently circulating result from continued evolution of the initial virus strains present in the country since the 1990s. The mean percentage similarity of ORF5 sequences of viruses found between 1991 and 1994 was 97.2% (±2.04, n=16), decreasing to 91.6% (±3.19, n=178) in 2010, 90.3% (±3.22, n=171) in 2013, 89.7% (±3.34, n=263) in 2016, and just 89.4% (±3.42, n=175) in mid-2018. The greatest number of nucleotide differences observed between strains in 2010 was 97, versus 118 in 2018. These results indicate that the genetic diversity of the PRRSV-1 populations in Britain has been increasing over time, as expected. The maximum level of nucleotide substitutions observed between the early 1990s and the present corresponds to a molecular clock value of 4.67×10^{-3} substitutions per site per year, which falls within the previously published estimates of 3.8×10^{-3} to 5.8×10^{-3}. The corresponding amino acid sequences were shown to vary similarly, with the most variable sites showing greater diversity in more recent years.

These data show that a PRRSV-1 population circulating in a defined geographic area continues to evolve and become more diverse over time at a predictable, steadily increasing rate, through a combination of mutation and genetic recombination, even in the face of vaccine use, and without the introduction of novel virus strains. Given Britain's island nature and the relatively small numbers of live pig imports, this represents a useful setting in which to characterise the natural evolution of PRRSV-1 in a pig population over a period of more than 25 years. These data demonstrate the importance of continuing to monitor PRRSV-1 diversity to inform ongoing vaccine and diagnostic test developments.

PRRS 33

PRRSV oral fluid ELISA performance comparison among three commercial kits

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Introduction

Continuous and reliable monitoring/surveillance of PRRSV is required to track the success of control/elimination programs. Oral fluid-based ELISAs are the most cost-effective method for routine antibody detection in commercial swine herds[1]. Several commercial PRRSV OF ELISA kits are available for this purpose, but their diagnostic performance had not been compared or reported.

Objective

Compare the diagnostic performance of three commercial PRRSV OF ELISAs using samples collected from pigs of known status.

Materials and methods

Twelve (12) individually-housed pigs were vaccinated with a PRRSV MLV vaccine (Ingelvac® PRRS MLV) and then sampled for 42 days post-vaccination, resulting in 132 serum samples and 564 OF samples. Serum samples were tested using the IDEXX PRRS X3 Ab ELISA (ELISA 'S'). Oral fluid samples were tested on each of 3 commercial PRRSV oral fluid ELISAs (1) IDEXX PRRS OF Ab®; (2) HIPRA CIVTEST® SUIS PRRS A/S PLUS; (3) QIAGEN Pigtype® PRRSV Ab OF. The diagnostic performance (sensitivity and specificity) of the PRRS serum and oral fluid ELISAs was estimated and compared by ROC analyses. The rate of positivity for serum and OF ELISA results was estimated and compared for differences using Cochran's Q test.

Results

Individual pig temporal serum antibody responses demonstrated that all animals responded immunologically to PRRSV vaccination. Comparisons of diagnostic sensitivity and specificity detected no difference between ELISAs 1 and 3 ($p > 0.05$), although ELISA 3 produced 23 presumed false negative results. ELISA 2 was significantly different from ELISAs 1 and 3 ($p < 0.05$), producing 143 presumed false negative results.

Comparisons between ELISA 'S' and ELISA 1 or 3 found no difference in the proportion of positive results ($p > 0.05$), whereas ELISA 2 was significantly different ($p < 0.002$). Comparisons in the proportion of positives among oral fluid ELISAs showed significant differences between ELISA 2 and ELISAs 1 or ELISA 3 ($p < 0.05$).

Discussion and Conclusion

In terms of overall test performance, the three commercial PRRSV OF ELISAs were ranked as: ELISA 1 ≥ ELISA 3 > ELISA 2. That is, not all commercial PRRSV OF ELISAs demonstrate the same level of diagnostic performance.

1. Ramirez A, et al., 2012. Efficient surveillance of pig populations using oral fluids. Prev Vet Med 104:292–300.

PRRS 34

Susceptibility of collared peccary (*Pecari tajacu*) to PRRSV

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Introduction and objective

Collared peccary (*Pecari tajacu*) and pigs (*Sus scrofa*) are two members of superfamily *Suoidea* that coexist in the Americas and share susceptibility to certain parasitic, bacterial, and viral infections. Collared peccary are known to be susceptible to classical swine fever virus, foot-and-mouth disease virus, porcine circovirus type 2, pseudorabies virus, and other viral pathogens of swine[1]. Therefore, the objective of this study was to determine whether collared peccaries are susceptible to PRRSV.

Materials and methods

Four collared peccaries and eight PRRSV-naïve domestic pigs were included in the experiment. One pig (positive control) and three peccaries were exposed to wild-type PRRSV by intramuscular inoculation using serum from a PRRSV-viremic pig. Four pigs (contact controls) were placed in pens contiguous to the pens holding the inoculated peccaries on day post inoculation (DPI) 3. The remaining peccary and pigs (n = 2) served as negative controls. Serum samples collected on DPI 0, 3, 7, 10, 15, and 23 were tested by ELISAs for the presence of PRRSV IgM, IgA, and IgG, and by rtRT-PCR for the presence of PRRSV nucleic acid.

Results

Inoculated peccaries were PRRSV rtRT-PCR-positive from DPI 3 to 23. ELISA cutoffs have not been established for peccaries, but a marked antibody S/P response was observed on 10 and 15 DPI for IgM and IgG, respectively, with a slight increase in IgA. Pigs exposed to infected peccaries via nose-to-nose contact tested negative by PRRSV rtRT-PCR and PRRSV ELISAs.

Discussion and conclusion

Although PRRSV is among the most impactful pathogen of swine worldwide, the susceptibility of other members of superfamily *Suoidea* has not been reported previously. The development of viremia (DPI 3 to DPI 23) and a PRRSV-specific humoral immune response (≥ DPI 10) supported the conclusion that collared peccary are susceptible to PRRSV. The results raise questions regarding the natural history of PRRSV in non-Sus members of superfamily Suoidea and their role in the evolution and ecology of PRRSV**.**

1. Molina-Barrios R, Luevano-Adame J, Henao-Díaz YA, et al. Collared peccary (Pecari tajacu) are susceptible to porcine reproductive and respiratory syndrome virus (PRRSV). Transbound Emerg Dis. 2018;00:1–8. https://doi.org/10.1111/tbed.12944.

PRRS 35

A Cost Effective Method for Surveillance of Influenza Viruses A, B, C and D in Swine Oral Fluids Using a Newly Developed Multiplex rRT-PCR

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Influenza viruses evolve rapidly by undergoing antigenic drift and shift and can “jump species” to new hosts. The ecology of the four influenza genera known as influenza A, B, C and D (IAV, IBV, ICV and IDV) is only partially understood and characterized. Better surveillance tools are needed to identify and recover viruses for further study. Here we describe the development of a multiplex real-time RT-PCR for the simultaneous detection of all four influenza subtypes. Primers and probes were designed to specifically detect and differentiate matrix gene sequences associated with the four subtypes of influenza. All assays have been tested for specificity and sensitivity with several In Vitro Transcripts (IVTs) that were derived from homologous sequence regions representing each of the four influenza genotypes. The PCR assays were further tested with 224 samples collected from 35 ferrets IBV, 45 guinea pigs ICV and 144 guinea pigs IDV that had been experimentally infected, and the results were compared and analyzed with the viral titers expressed in 50% tissue culture infective dose (TCID50/ml) obtained by virus isolation. All 35 IBV infected ferret samples were positive by the multiplex RT-PCR for IBV. Of the 45 ICV infected guinea pigs, 15 (33.3%) were positive by virus isolation but negative by PCR. For IDV, a total of 144 guinea pig samples were tested and 65.28% (94/144) tested positive in the multiplex RT-PCR assay, however 32.63% (47/144) of the samples yielded Ct’s <36 (RT-PCR positive) in the absence of a measurable virus titer. Additionally, of the IDV samples tested, 2.08% (3/144) of the samples were positive by virus isolation but undetected in RT-PCR assay. It is suspected that the ICV samples may have degraded before testing therefore, the ICV PCR was retested with 10-fold dilutions of a viral stock and the PCR showed an analytical sensitivity at least the 100 RNA copy level. Overall, there is a strong correlation between the described multiplex RT-PCR assay and traditional cell culture-based TCID50 assay. Ninety oral swine fluid field samples were also tested and 21.11 % (19/90) demonstrated detectable Ct values for IAV, while IBV, ICV and IDV remained undetectable for these samples. As influenza viruses have a high rate of mutation and can readily adapt to new hosts, the overall ecology of the four influenza genera in terms of how they exist in nature, their reservoirs and potential for human infection are not fully understood. To efficiently conduct surveillance, molecular based, rapid and portable laboratory tools are necessary.

PRRS 36

A Luminex multiplex assay for the detection of PRRSV, PCV2 and PCV3 and for PRRSV vaccine differentiation in the US

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Objective:
To develop a molecular assay for the detection of PRRSV, PCV2 and PCV3 viruses, and for differentiation of the four US vaccine strains using the Luminex platform.

Methods:
The Luminex xTAG assay that can hypothetically analyze more than 100 different targets in a single reaction was applied. It is a color-coded, bead-based nucleic acid detection method that hybridizes to pre-amplified specific target to generate detection signal. In primer design, all available sequences were used in the bioinformatics analysis to ensure high coverage of different genotypes and strain variations.

Results:
Two pairs of primers targeting the M and N genes of PRRSV were designed based on 694 PRRSV-2 full or near-full genomes. The design has a high detection coverage of 98.1%. Four pairs of primers targeting on the *nsp*2 gene of vaccine strains were designed for differentiation. Analytical sensitivity of this Luminex assay for PRRS is one half log to one log lower than that of a typical real-time PCR assay. Testing on the vaccine strains and 472 PRRS field strains indicated that the Luminex PRRS assay we have developed could detect 94% of the field strains, and could differentiate majority of the vaccine-like strains. Furthermore, the assay included PCV2 and PCV3 detections and the primer coverage to GenBank sequences were 98.6% (1852/1878) for PCV2 and 98.9% (86/87) for PCV3 strains. Analytical sensitivity result showed that detection limits were similar to that generated by real-time PCR assays. Testing on clinical sample indicated that the assay can detect PRRSV, PCV2, and PCV3 individually and in combination, and selected samples were verified by Sanger sequencing or other validated PCR assays.

Conclusion:
Although Luminex assays do not provide quantification data as these Ct values generated by real-time PCR assays, it provides a cost-effective way of comprehensive detection of different pathogens divergent virus strains of a given pathogen. In this study, the important viruses circulating in swine production systems, PRRSV, PCV2 and PCV3, can be detected and differentiate by the new Luminex assay.

PRRS 37

Porcine circovirus type 3 (PCV3) and porcine circovirus type 2 (PCV2) – detection in pig farms vaccinated and non-vaccinated against PCV2

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Introduction: PCV2 is very common worldwide and causes economically important swine diseases (PCVD) as well as subclinical infections. PCV3 seems to be highly prevalent in pig farms globally, but its true role in swine health remains unknown.
The aim of the study was to assess the presence of PCV3 and PCV2 in 11 Polish pig farms of different PCV2 vaccination status.

Materials and method: Serum samples (n=701) were obtained from 3-21-week-old pigs from 7 farms vaccinated against PCV2 (VF) and 4 non-vaccinated farms (NVF). Samples were pooled by 4-6 before DNA extraction and tested with real-time PCR for PCV3 and PCV2. Serums from pools, where concurrent infections of these viruses were detected, were tested individually. Samples with Ct<37 were considered as positive.

Results: VF: In total, PCV3 and PCV2 were detected in 30.0% (Ct=33.2±2.1) and 2.2%(Ct=33.6±2.3) of all tested serum pools from vaccinated farms, respectively. PCV3 viremia was found in 30.0% (Ct=33.2±5.2), 16.7% (Ct=32.7±3.6) and 35.7% (Ct=33.0±2.4) of serum pools collected from piglets, weaners and fatteners, respectively. PCV2 was detected only in fatteners (3.6% of serum pools, Ct=33.6±2.3). No concurrent infection of PCV3 and PCV2 was found in 448 of serum samples from vaccinated farms.

NVF: Overall, 21.2% (Ct=32.2±0.7) and 63.5% (Ct=29.0±2.6) of all tested serum pools were positive for PCV3 and PCV2, respectively. PCV3 was detected only in weaners (10.0% of serum pools, Ct=33.5) and fatteners (29.4% of serum pools, Ct=31.7±1.8). PCV2 viremia was found in 12.5% (Ct=34.2), 40.0% (Ct=29.1±5.3) and 82.4% (Ct=28.6±4.5) of serum pools collected from piglets, weaners and fatteners, respectively. Concurrent PCV3/PCV2 infection was detected only in fatteners in 3 out of 253 serum samples (1.2%) from non-vaccinated farms.

Discussion and Conclusion: Our results show that both, PCV3 and PCV2, are most common in finishers. As expected, vaccination against PCV2 significantly limits the incidence and level of PCV2 viremia (2.2% positive pools in VF vs. 63.5% in NVF). Interestingly, the occurrence of PCV3 viremia in VF is higher than in NVF (30.0% vs. 21.2%, respectively). PCV2 vaccination has no impact on PCV3 infection. On the other hand, PCV3 infection does not seem to compromise efficacy of PCV2 vaccination. Concurrent infections of PCV3 and PCV2 are rare, but more frequent in non-vaccinated farms. Further studies are needed to establish correlation between the presence of PCV3 and other common pathogens in pig farms.

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NCN (National Science Centre, Poland) No.2017/25/N/NZ7/02810

PRSS38

Isolation and characterization of a divergent strain of porcine sapelovirus from swine farm in US

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Porcine sapelovirus (PSV) can cause both symptomatic and asymptomatic diseases. Recently, it has been reported to be the etiologic agent for pigs with polioencephalomyelitis. However, little is known about the viral strains causing asymptomatic infection in animals. Specific diagnostic agent and assays are needed for epidemiological surveillance and disease control. In this study, a divergent PSV strain (designated KS18-01) was identified through next-generation sequencing from a healthy piglet in the US swine farm. *De novo* assembly generated a full-length genome of 7407bp, encoding a polyprotein of 2324 amino acids (AA). Phylogenetic analysis of full-length genome nucleotide (nt) sequence illustrates that the virus is closely related to two US strains reported so far. Compared to other PSV strains, KS18-01 genome has 79.66-86.44% nt identity. The 5'-untranslated region (5'-UTR) is most conserved region of the genome while the most variable region is the VP1 gene with 75.75-86.25% nt identity (79.7-95.1% AA identity) to the other PSV strains. We further isolated the virus and produced a panel of monoclonal antibodies (mAb) against VP1 and VP2 proteins for viral detection. Using these mAbs, immunohistochemical analysis identified the viral antigen in a colon tissue from the infected pig. The availability of PSV isolate (KS18-01) and specific mAbs provide powerful tools for development of rapid diagnostic assays for disease control and prevention.

PRRS 39

A multiplex real-time PCR assay for the detection and differentiation of five bovine pinkeye pathogens

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Objective: Infectious bovine keratoconjunctivitis (IBK), also known as pinkeye, is the most common eye disease of cattle. A variety of pathogens have been associated with cases of IBK, but most frequent ones include *Moraxella bovis*, *Moraxella bovoculi*, *Mycoplasma bovis*, *Mycoplasma bovoculi* and bovine herpesvirus (BHV). Therefore, the objective of this study was to develop a multiplex real-time PCR assay for the detection and differentiation of *Moraxella bovis*, *Moraxella bovoculi*, *Mycoplasma bovis*, *Mycoplasma bovoculi* and BHV from cattle.

Methods: Species-identifying gene targets were used for the assay design, and regions flanking the molecular targets were amplified and cloned to serve as positive amplification controls. Concentrations of each primer and probe were optimized with plasmid DNA extracted from the target-carrying clones. The sensitivity of the assay was determined using serially diluted plasmid DNA and bacterial culture.

Results: In pure culture, the limit of detection, based on standard curve data for each target, was Ct of ~37; correlation coefficients for each target were all > 0.99 and PCR efficiencies were between 90 and 110%. The assay specifically detected targeted pathogens without cross-detection of other targets. A total of 113 bovine ocular swab samples were collected from cattle with positive-clinical IBK and subjected to the multiplex real-time PCR testing. Percentage of samples positive for *Moraxella bovis*, *Moraxella bovoculi*, *Mycoplasma bovis*, *Mycoplasma bovoculi* and BHV were as follows: 49.6% (56/113), 63.7% (72/113), 1.8% (2/113), 70% (79/113), 7.8% (8/113), respectively. The analytical limit of detection (LOD) for *Moraxella bovis, Moraxella bovoculi, Mycoplasma bovis, Mycoplasma bovoculi* and BHV were 19, 23, 25, 24 and 26 copies per reaction, respectively.

Conclusion: This assay detects and differentiates five major IBK pathogens with high sensitivity and specificity. It can potentially serve as a high-throughput method for detecting IBK in cattle.

Keywords: Infectious bovine keratoconjunctivitis (IBK); Multiplex PCR; *Moraxella* spp; *Mycoplasma* spp; bovine herpesvirus; pinkeye.

PRRS 40

Diagnostic application of monoclonal antibodies against African swine fever virus

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African swine fever is one of the most important foreign animal diseases threatening the US swine producers. Recent outbreaks in Europe and China emphasize the need for diagnostic reagents and assays for disease control and prevention. The etiologic agent is African swine fever virus (ASFV), a large DNA enveloped virus and currently the only member of the *Asfarviridae* family. The structural protein, p30, is localized on the virus inner membrane and abundantly expressed during early infection, which is a good inducer of humoral immune responses.

The purpose of this study was to develop ASFV-specific diagnostic reagents and asssays by generation and application of monoclonal antibodies (mAbs) against p30. A panel of mouse mAbs was prepared against the recombinant p30 protein expressed in *E.coli*. Hybridomas were initially screened using recombinant p30 expressed in Vero cells, and the result was confirmed on ASFV-infected cells. Three clones were isolated and used for further characterization.

Epitope mapping was performed against recombinant p30 polypeptides. One epitope, recognized by mAb #47-3, is located in a conserved region between residues 60-101 amino acid (aa). In contrast, mAb #62-35 and #142-4, only recognized the C-terminal half of p30, suggesting the presence of a large conformational epitope. However, both antibodies retained the ability to react with the C-terminus of the protein in a western blot, a property maybe associated with intrinsically disordered regions (IDR). In addition, computer analysis showed that the C-terminal region 91-137 aa, is highly hydrophilic, enriched in glutamic acid residues, which is a property also associated with IDR.

Based on their reactivity, these three mAbs were applied in developing different diagnostic tests, including immunofluorescence (IFA), immunohistochemistry (IHC) and blocking Enzyme Linked Immunosorbent Assay (bELISA). As a result, mAb #47-3 recognized ASFV antigen in different paraffin-embedded tissues, mAb #62-35 recognized ASFV infected cells in an IFA assay and mAb #142-4 was used to develop a blocking ELISA assay using convalescent swine serum.

Taken together, a panel of anti-p30 mAbs were developed, which provide useful tools for ASFV control and prevention.

PRRS 41

The United States Swine Pathogen Database: Integrating diagnostic sequence data of emerging pathogens of swine.

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Veterinary diagnostic laboratories annually derive partial nucleotide sequences of thousands of isolates of porcine reproductive and respiratory syndrome virus (PRRSV), Senecavirus A, and swine enteric coronaviruses. In addition, next generation sequencing has resulted in the rapid production of full-length genomes. Presently, sequence data are only released to diagnostic clients, as data are associated with sensitive information. However, this information can provide information to: objectively design field-relevant vaccines; determine when and how pathogens are spreading across the landscape; and identify virus transmission hotspots. In tandem with the USDA-ARS Big Data initiative, we have developed a centralized sequence database at the National Animal Disease Center. We implemented the Tripal toolkit, using Drupal and the Chado database schema. Hosting is via Amazon Web Services (AWS) for Federal Government with resource scaling, and dedicated support for prevention of data theft and database vulnerabilities. Sequences housed in the database contain at a minimum four data items: genomic information; date of collection; collection location (state level); and a unique identifier. Additionally, because the bulk of the database are PRRSV sequences, custom curation and annotation pipelines have determined PRRSV genotype (Type 1 or 2), the location of open reading frames and nonstructural proteins, generated amino acid sequences, and identified putative frame shifts. Other swine pathogens will be annotated with similar tools to facilitate data mining and hypothesis generation. Following the creation of a user account, access to all data is possible. The resource will provide researchers timely access to sequences discovered by highly qualified veterinary diagnosticians, allowing for biological data mining and epidemiological studies. The result will be a better understanding concerning the emergence of novel viruses in the United States, how these novel isolates are disseminated in the US and abroad, and discovering new patterns of biological consequence.

PRRS 42

Sequence analysis and pathogenicity of porcine epidemic diarrhea virus isolates from the endemic outbreaks on Jeju Island, South Korea

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Porcine epidemic diarrhea virus (PEDV) is an epizootic and deadly swine coronavirus that affects the economies of countries with established swine industries. Since the incursion of the virulent G2b PEDV pandemic strains in South Korea during 2013–2014, moderate-scale outbreaks have recurred regionally. In particular, areas with extensive swine production on Jeju Island have faced repeated epidemics since the re-emergence in 2014. The present study reports the complete genome sequences and molecular characterization of the representative PEDV strains responsible for the endemic outbreaks in 2018 on Jeju Island. The full-length genome sizes of four isolates were found to differ from the G2b epidemic field strains; the KNU-1816 genome was 28,050 nucleotides (nts) in length (12-nt longer) due to a 4-amino acid (KSGL) insertion in the spike (S) protein, the KNU-1807 and -1815 genome sequences were 28,017 and 28,029 nts long, resulting from the presence of a continuous 21- or 9-nt deletion (DEL) within open reading frame 1a that encodes nonstructural protein 3 (nsp3), respectively, and the remaining KNU-1818 virus possessed the genome of 28,035 nts in size because of a 3-nt DEL in nsp6. The 2018 Jeju isolates shared 1.3–3.4% and 0.6–1.5% variations at the S gene and whole-genome levels, respectively, when compared to global G2b PEDV strains. Genetic and phylogenetic analyses indicated that the 2018 isolates were most close to the 2014 G2b re-emergent Jeju strains but appeared to have undergone substantial independent evolution in this geographic area. In addition, an nsp3-DEL strain, KOR/KNU-1807/2018, was isolated successfully on Vero cells and subjected to genomic sequence analysis. Compared to the original KNU-1807 sample, 8-nt DEL arose in the C-terminal region of the S gene of KNU-1807 at the 4th passage (P4), resulting in a premature termination of S by 9-aa residues (EVFE**KVHVQ**) that contain a potential ER retrieval signal KxHxx motif. Furthermore, increased sizes of syncytia were observed in KNU-1807 virus-infected cells, suggesting that loss of the signal can facilitate transportation of S proteins onto the cell surface and enhance cell fusion activity. In order to determine association of the ER retention signal of S with viral pathogenesis, the pathogenicity of KNU-1807 virus was examined in neonatal piglets. Results of animal inoculation studies to assess the virulence of the PEDV KNU-1807 strain will be discussed.

PRRS 43

Molecular characterization of a novel recombinant enterovirus G inserting a torovirus papain-like protease gene associated with diarrhea in swine in South Korea

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Enteroviruses (EVs) are small, non-enveloped viruses and belong to the genus *Enterovirus* within the family *Picornaviridae* of the order *Picornavirales*, which contains 15 species (EV A–L and rhinovirus A–C). Enterovirus species G (EV-G) comprises a highly diversity of 20 genotypes that is prevalent in pig populations, with or without diarrhea. In the present study, a novel EV-G strain (KOR/KNU-1811/2018) that resulted from cross-order recombination was discovered in diagnostic fecal samples from neonatal pigs with diarrhea that were negative for swine enteric coronaviruses and rotavirus. The recombinant EV-G genome possessed an exogenous 594-nucleotide (198-amino acid) sequence, flanked by two viral $3C^{pro}$ cleavage sites at the 5′ and 3′ ends in its 2C/3A junction region. This insertion encoded a predicted protease similar to the porcine torovirus papain-like cysteine protease (PLCP), which was recently found in the EV-G1, -G2, and -G17 genomes. The PLCP sequence of Korean EV-G only shared 75.0−89.3% aa sequence identity with other recombinant EV-G1, -G2, and -G17 strains, exhibiting the highest identity with the US EV-G17 strain 08/NC_USA/2015. Subsequent phylogenetic analysis based on the PLCP genes of EV-Gs and nidoviruses revealed that the foreign PLPC gene of KNU-1811 is most closely related to that of the USA EV-G17 strain, forming a well-supported cluster with PLPCs of other EV-Gs, but is only distantly related to those of porcine, bovine, and equine toroviruses, showing lower sequence identities (41.0−51.3% in the aa sequence). The complete KNU-1811 genome shared 73.7% nucleotide identity with a prototype EV-G1 strain, but had 83.9–86.7% sequence homology with the global EV-G1-PLCP strains. Genetic and phylogenetic analyses demonstrated that the Korean recombinant EV-G's own VP1 and inserted foreign PLCP genes are most closely related independently to contemporary chimeric G1-PLCP and G17-PLCP strains, respectively. These results implied that the torovirus-derived PLCP gene might have undergone continuous nucleotide mutations in the respective EV-G genome following its acquisition through naturally occurring recombination. Our results advance the understanding of the genetic evolution of EV-G driven by infrequent viral recombination events, by which EV-G populations laterally gain an exotic gene encoding a virulence factor from heterogeneous virus families, thereby causing clinical disease in swine.

PRRS 44

AlphaLISA platforms for rapid and sensitive detection of PEDV antibody

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Objective:

Develop AlphaLISA platforms for rapid and sensitive detection of PEDV antibody.

Methods:

The N-terminal portion of PEDV spike protein (S1) was identified as a target for antibody-based diagnosis of PEDV infections. S1 provided the best diagnostic sensitivity for PEDV strains, with no cross-reactivity with other porcine coronaviruses. AlphaLISA is a bead-based luminescent proximity homogenous, no-wash immunoassay platform with high sensitivity and wide dynamic ranges. Donor and acceptor beads are coupled with target proteins. With donor and acceptor beads in close proximity, an energy transfer occurs, producing a chemiluminescent signal, which activates a fluorophore on the bead.

Platform 1 is an ultra-rapid, 1-step, 1-well, no wash assay where both donor and acceptor beads are coupled to PEDV S1 protein, and the beads are drawn together by the presence and co-recognition of PEDV antibody. Platform 2 is a 2 hour, 2 steps, 2 well, no wash isotype specific confirmatory assay where PEDV IgG or IgA can be detected separately by using a second acceptor bead coupled to either anti-pig IgA or anti-pig IgG antibody.

Both platforms were evaluated using longitudinal serum samples (n=360) collected weekly from a PEDV positive wean-to-finish production site for 12 weeks, and experimental serum samples of known PEDV positive (n=132) and negative (n=132) immune status collected on day post-infection (dpi) -7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42.

Results:

The rapid assay detected total PEDV antibody responses within 10 minutes. The first antibodies were detected by 7 dpi under experimental and field conditions. The second AlphaLISA platform was used to describe PEDV serum IgG and IgA antibody kinetics. Both serum IgG and IgA were detected between 7-14 days post-exposure. The serum IgA response provided better diagnostic performance. Serum IgG antibodies declined slowly over the monitoring period while IgA antibodies were persistently detected throughout the study.

Conclusion:

The AlphaLISA is a versatile, fast, and user-friendly alternative to high throughput immunoassay platforms such as ELISA and Luminex.

PRRS 45

Association between porcine reproductive and respiratory syndrome virus (PRRSV) and porcine parvoviruses 1-7 (PPV1-7) viremia in pigs

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Introduction

Previous reports confirmed that pigs could be co-infected with PRRSV and PPV3 or PPV6. However, the importance of such co-infections for pig health remains unknown. There is no data about co-infections with PRRSV and other PPVs.

The aim of the study was to investigate the correlation between the presence of PRRSV and PPV1-7 in serum of pigs from conventional farms in Poland.

Material & Methods

The serum samples (n = 820) were obtained from 3-21-week-old pigs, from 12 farms with known PRRSV status. From each age group 6-10 samples from random pigs were collected and DNA was extracted after pooling by 3-5. Overall, 164 serum pools were tested. Real-Time PCR was performed to detect PRRSV and PPV1-7.

Results

In total, 69.5% of serum pools tested negative for PRRSV (NEG-PRRSV). The positive pools (POS-PRSSV) were divided into LOW-PRRSV (16.5%), MEDIUM-PRRSV (9.8%) and HIGH-PRRSV (4.3%) (Ct≥30, Ct=25-30 and Ct≤25, respectively). Overall, PPV1, PPV2, PPV3, PPV4, PPV5, PPV6 and PPV7 DNA was detected in 6.1%, 50.6%, 18.3%, 11.6%, 18.3%, 20.7% and 28.0% of serum pools, respectively. The probability that the pool was positive for PPV2, PPV5 or PPV7 was significantly greater for POS-PRRSV compared with NEG-PRRSV (72.0% vs. 41.2%; 26.0% vs. 14.9%; 46.0% vs. 20.2%, respectively). The presence of PPV1 or PPV6 was similar in NEG-PRRSV or POS-PRRSV (6.0% vs. 6.1%; 20.2% vs. 22.0%, respectively). Detection rate of PPV3 and PPV4 was slightly higher in NEG-PRRSV than in POS-PRRSV (20.2% vs. 14.0%; 14.9% vs. 4.0%, respectively). PPV2 and PPV7 were the most prevalent in LOW-PRRSV (81.5% and 48.1%, respectively), less in MEDIUM-PRRSV (68.8% and 43.8%, respectively) and least prevalent in HIGH-PRRSV (42.9% and 28.6%, respectively). There were no significant differences between presence of other PPVs types in different category of PRRSV positive serum pool based on Ct value.

Conclusion

In our study the prevalence of PPV2, PPV5 and PPV7 was higher in POS-PRRSV than in NEG-PRRSV serum pools. Further analysis showed that the detection rate of PPV2 and PPV7 was the highest in LOW-PRRSV pools and the lowest in HIGH-PRRSV pools. This indicates that there could be some association between PPVs and PRRSV infections. The striking differences between the levels of PRRSV viremia and different parvoviruses warrant further studies on the possible role of such co-infections for pig pathology.

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PRRS 46

Characterization and application of a panel of monoclonal antibodies against the capsid protein of porcine circovirus

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[2]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS;
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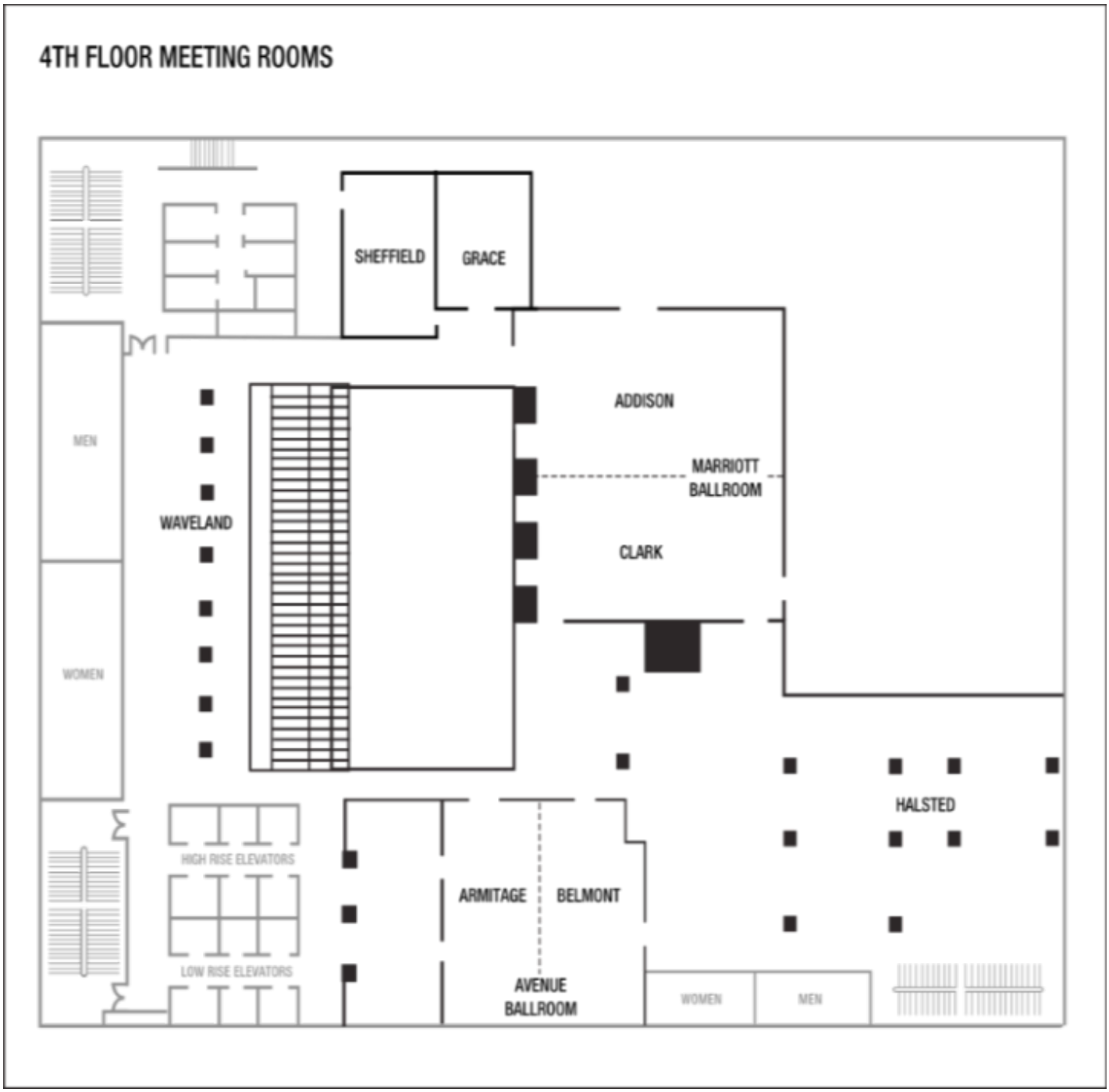
Porcine circovirus (PCV)-associated disease is clinically manifested by postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome. Understanding the antigenicity of the viral capsid protein and development of serological test are important in disease control and prevention. In this study, we produced and characterized a panel of monoclonal antibodies (mAbs) against the nucleocapsid protein of different PCV genotype/species, including the classical genotype-PCV2b, the newly shifted genotype-PCV2d, and the novel species-PCV3. Antigenic mapping of the nucleocapsid protein showed that the epitope recognized by mAb of PCV2b nucleocapsid is located at C-terminus of the protein [210–233 amino acids (aa)], while three antigenic regions (57–83 aa, 183–209aa, and 131–163aa) were recognized by mAbs against PCV2d nucleocapid; mAbs for PCV3 nucleocapsid recognize two antigenic regions (140–170 aa and 90–109aa) in the protein. Antibody cross-reactivity analysis revealed that all the PCV2d mAbs recognize PCV2b; no antibody cross-reactivity was observed between the genetically distant PCV2 and PCV3 capsid proteins. A blocking ELISA is established using specific mAbs for differentiating different genotypes/species of PCV in serological assays. The availability of this panel of mAbs and differential ELISA test provides valuable diagnostic tools for epidemiological survaillence and disease control.

Key words: Porcine circovirus, monoclonal antibody, epitope mapping, blocking ELISA

Maps

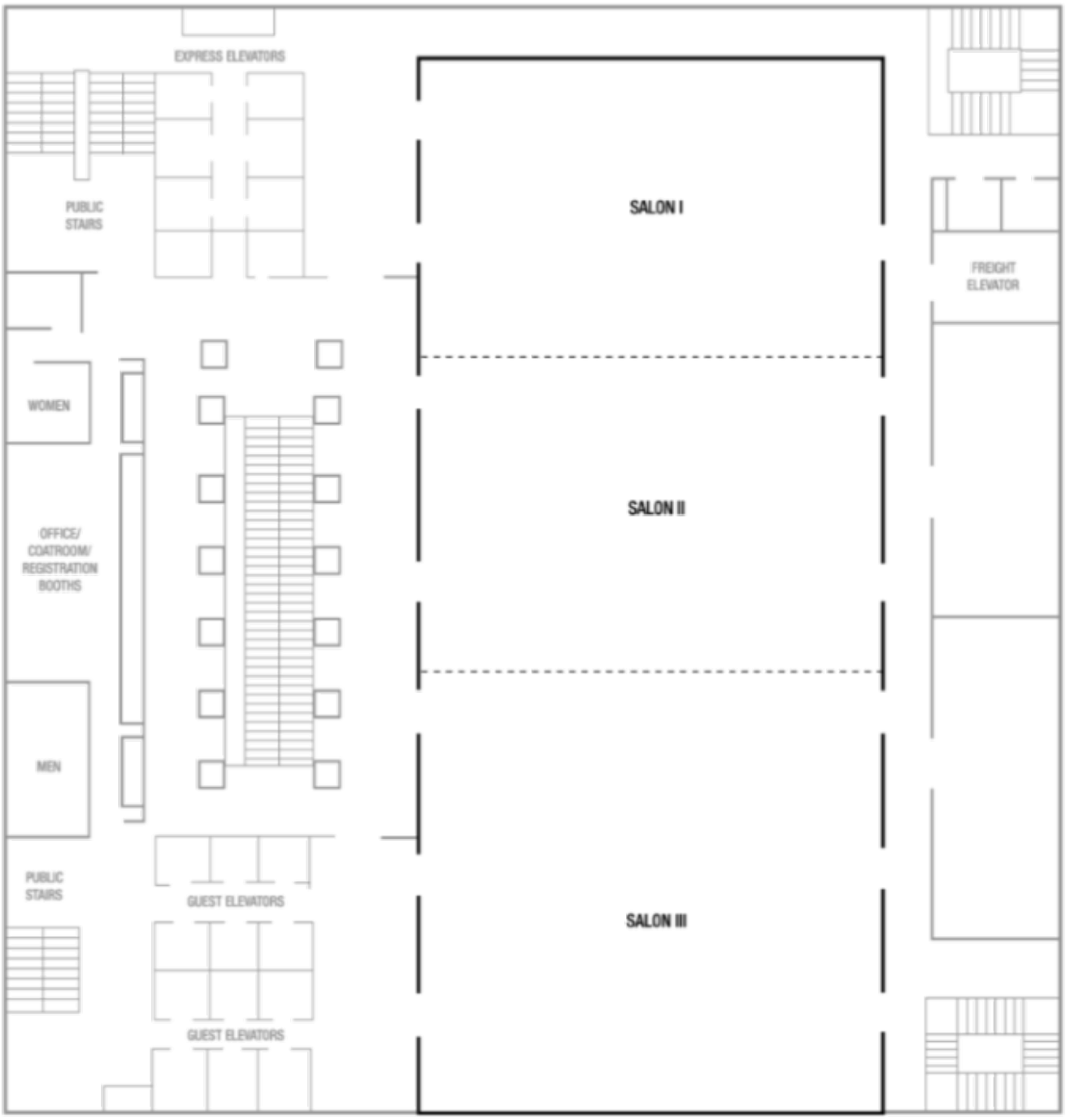
CHICAGO MARRIOTT MAGNIFICENT MILE



Maps

CHICAGO MARRIOTT MAGNIFICENT MILE

7TH FLOOR MEETING ROOMS



2018 North American PRRS Symposium
Program At A Glance

Friday, November 30, 2018

4:00-6:00 PM	Onsite registration – Preregistered badge/program pickup *4th Floor – Waverly Foyer*

Saturday, December 1, 2018

07:00 AM - 11:30 AM	Onsite registration – Preregistered badge/program pickup *5th Floor – Registration Desk/Kiosk*
8:00 AM – 12:00 PM	Session 1 – Nidoviruses – Emerging Coronaviruses and PRRSV 7th Floor – Salon II
10:00 AM – 10:30 AM	Session 1 Refreshment Break 7th Floor – Salon II Foyer
12:00 PM – 1:00 PM	Buffet Lunch (Symposium lunch ticket is required) 7th Floor – Salon II Foyer
12:00 PM	Poster set-up begins 7th Floor – Salon III
1:00 PM – 5:00 PM	Session 2 – Global Challenges in infections Disease Update on ASF in China 7th Floor – Salon II
3:00 PM – 3:30 PM	Session 2 Refreshment Break 7TH Floor – Salon II Foyer

Sunday, December 2018

08:00 AM – 12:00 PM	Session 3 – PRRSV Vaccines, Immunity and Host Response to Infection 7th Floor – Salon II
10:00 AM – 10:30 AM	Session 3 Refreshment Break 7th Floor – Salon II Foyer
12:00 PM – 1:00 PM	Lunch on your own
1:00 PM – 4:30 PM	Beginning of CRWAD Meeting and Session 4 Session 4 – NC229-PRRSV and Emerging Respiratory Diseases of Pigs 5th Floor – Chicago Ballroom
4:00 PM – 4:30 PM	CRWAD Business Meeting
6:30 PM – 8:30 PM	Reception and Poster Session in conjunction with CRWAD 7th Floor – Salon III